Biomarkers in Disease:
Methods, Discoveries and Applications
Series Editors: Vinood B. Patel
Victor R. Preedy

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Rajkumar Rajendram · Victor R. Preedy
Vinood B. Patel
Editors

Biomarkers in Trauma, Injury and Critical Care

Springer

Biomarkers in Disease: Methods, Discoveries and Applications

In the past decade there has been a major sea change in the way disease is diagnosed and investigated due to the advent of high throughput technologies, such as microarrays, lab on a chip, proteomics, genomics, lipomics, metabolomics etc. These advances have enabled the discovery of new and novel markers of disease relating to autoimmune disorders, cancers, endocrine diseases, genetic disorders, sensory damage, intestinal diseases etc. In many instances these developments have gone hand in hand with the discovery of biomarkers elucidated via traditional or conventional methods, such as histopathology or clinical biochemistry. Together with microprocessor-based data analysis, advanced statistics and bioinformatics these markers have been used to identify individuals with active disease or pathology as well as those who are refractory or have distinguishing pathologies. New analytical methods that have been used to identify markers of disease and is suggested that there may be as many as 40 different platforms. Unfortunately techniques and methods have not been readily transferable to other disease states and sometimes diagnosis still relies on single analytes rather than a cohort of markers. There is thus a demand for a comprehensive and focused evidenced-based text and scientific literature that addresses these issues. Hence the formulation of Biomarkers in Disease. The series covers a wide number of areas including for example, nutrition, cancer, endocrinology, cardiology, addictions, immunology, birth defects, genetics and so on. The chapters are written by national or international experts and specialists.

Rajkumar Rajendram • Victor R. Preedy • Vinood B. Patel
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Biomarkers in Trauma, Injury and Critical Care

With 132 Figures and 101 Tables

Springer

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Series Preface

In the past decade, there have been major changes in the way diseases are diagnosed and investigated due to the advent of high-throughput technologies and advances in chemistry and physics. This has led to the development of microarrays, lab-on-a-chip, proteomics, genomics, lipomics, metabolomics, and other new platforms. These advances have enabled the discovery of new and novel markers of disease relating to autoimmune disorders, cancers, endocrine diseases, genetic disorders, sensory damage, intestinal diseases, and many other conditions too numerous to list here. In many instances, these developments have gone hand in hand with the analysis of biomarkers elucidated via traditional methods, such as histopathology, immunoassays, and clinical biochemistry. Together with microprocessor-based data analysis, advanced statistics, and bioinformatics, these markers have been used to identify individuals with active disease as well as those who are refractory or have distinguishing pathologies.

Unfortunately, techniques and methods have not been readily transferable to other disease states, and sometimes diagnosis still relies on a single analyte rather than a cohort of markers. Furthermore, the discovery of many new markers has not been put into clinical practice partly because of their cost and partly because some scientists are unaware of their existence or the evidence is at the preclinical stage. There is thus a demand for a comprehensive and focused evidenced-based text that addresses these issues. Hence the book series Biomarkers in Disease: Methods, Discoveries and Applications. It imparts holistic information on the scientific basis of health and biomarkers and covers the latest knowledge, trends, and treatments. It links conventional approaches with new platforms. The ability to transcend the intellectual divide is aided by the fact that each chapter has:

- **Key Facts (areas of focus explained for the lay person)**
- **Definitions of Words and Terms**
- **Potential Applications to Prognosis, Other Diseases, or Conditions**
- **Summary Points**

The material in **Potential Applications to Prognosis, Other Diseases, or Conditions** pertains to speculative or proposed areas of research, cross-transference to

other diseases, or stages of the disease, translational issues, and other areas of wide applicability.

The series is expected to prove useful for clinicians, scientists, epidemiologists, doctors and nurses, and also academicians and students at an advanced level.

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Preface

In recent years, there have been major changes in the way diseases are diagnosed and investigated due to the advent of high-throughput technologies and advances in chemistry and physics. This has led to the development of microarrays, lab-on-a-chip, proteomics, genomics, lipomics, metabolomics, and other new platforms. These advances have enabled the discovery of new and novel markers of disease relating to autoimmune disorders, cancers, endocrine diseases, genetic disorders, sensory damage, intestinal diseases, and many other conditions too numerous to list here. In many instances, these progressions have gone hand in hand with the analysis of biomarkers elucidated via traditional methods, such as histopathology, immunoassays, and clinical biochemistry. Together with microprocessor-based data analysis, advanced statistics, and bioinformatics, these markers have been used to identify individuals with active disease as well as those who are refractory or have distinguishing pathologies.

Unfortunately, techniques and methods have not been readily transferable to other disease states, and sometimes diagnosis still relies on a single analyte rather than a cohort of markers. Furthermore, the discovery of many new markers has not been put into clinical practice partly because of their cost and partly because some scientists are unaware of their existence or the evidence is at the preclinical stage. There is thus a demand for a comprehensive and focused evidenced-based text that addresses these issues. Hence the book Biomarkers in Disease: Methods, Discoveries and Applications – *Biomarkers in Trauma, Injury, and Critical Care*. It imparts holistic information on the scientific basis of health and biomarkers and covers the latest knowledge, trends, and links with treatments. It links conventional approaches with new platforms.

In the present book, *Biomarkers in Trauma, Injury, and Critical Care*, we have sections on:

1. **General Overviews: Circulating and Body Fluid Biomarkers in Organ Injury and Trauma**
2. **Biomarkers in Critical Care and Illness**
3. **Biomarkers in Trauma**
4. **Biomarkers in Specific Conditions**
5. **Specific Components**

6. **Biomarkers in COVID-19**
7. **Physical Platforms and Physiology**
8. **Scoring Systems, Models, and Indirect Measures**
9. **Resources**

The ability to transcend the intellectual divide is aided by the fact that each chapter has:

- **Key Facts (areas of focus explained for the layperson)**
- **Definitions of Words and Terms**
- **Applications to Prognosis, Other Diseases, or Conditions**
- **Summary Points**

The material in **Applications to Prognosis, Other Diseases, or Conditions** pertains to speculative or proposed areas of research, cross-transference to other diseases, or stages of the disease, translational issues, and other areas of wide applicability.

The editors recognize the difficulties in assigning chapters to parts of the book, as some chapters can fit into more than one section. Nevertheless, the book has enormously wide coverage and is well indexed.

The chapters are written by national and international experts. This book is designed for health scientists specializing in trauma, surgeons, toxicologists, pharmacologists, clinical biochemists, epidemiologists, researchers, doctors, and nurses, from students to practitioners at the higher level. It is also designed to be suitable for lecturers and teachers in health care and academic libraries as a reference guide.

Riyadh, Saudi Arabia — Dr. Rajkumar Rajendram
London, UK — Professor Victor R. Preedy
London, UK — Dr. Vinood B. Patel
March 2023 — The Editors

Contents

Volume 1

About the Editors

Dr. Rajkumar Rajendram, AKC BSc (Hons) MBBS (Dist) MRCP (UK) EDIC FRCP Edin FRCP Lon CCT Internal Medicine (Acute), Anaesthesia and Intensive Care, is a Clinician Scientist who has a focus on anesthesia, intensive care, and peri-operative medicine. Dr. Rajendram graduated in 2001 with a distinction from Guy's, King's, and St. Thomas Medical School, in London. As an undergraduate, he was awarded several prizes, merits, and distinctions in pre-clinical and clinical subjects.

Dr. Rajendram began his post-graduate medical training in general medicine and intensive care in Oxford. He attained membership of the Royal College of Physicians (MRCP) in 2004 and completed specialist training in acute and general medicine in Oxford in 2010. Dr. Rajendram also trained in anesthesia and intensive care in London. During this training, he obtained extensive experience in the management of trauma, toxicology, and sepsis at the internationally renowned major trauma centers of the Royal London Hospital and the Oxford University Hospitals. He was awarded fellowship of the Royal College of Anaesthetists (FRCA) in 2009 and fellowship of the Faculty of Intensive Care Medicine (FFICM) in 2013. He was awarded the European Diploma in Intensive Care Medicine (EDIC) and completed specialist training in anesthesia and intensive care in 2014. In 2017, he became a fellow of the Royal College of Physicians (FRCP), Edinburgh and then became an FRCP, London in 2019.

Dr. Rajendram returned to Oxford as a Consultant in the Department of Acute General Medicine at the John Radcliffe Hospital, Oxford. The UK National Spinal Cord Injury Centre is based at Stoke Mandeville Hospital, Aylesbury where Dr. Rajendram subsequently thrived as a Consultant in the Department of Anaesthesia and Intensive Care. He is currently a Consultant in the Department of Medicine at King Abdulaziz Medical City, National Guard Heath Affairs, Riyadh, Saudi Arabia. This is the leading major trauma center in Saudi Arabia. To improve the outcomes of victims of major trauma, Dr. Rajendram recently developed a novel medical-surgical co-management service (i.e., peri-operative medicine) for this complex cohort that is at high risk of morbidity and mortality.

As a clinician scientist, Dr. Rajendram has also devoted significant time and effort to research and education. He is a joint appointment Assistant Professor in the College of Medicine, King Saud bin Abdulaziz University for Health Sciences,

Riyadh, Saudi Arabia and has published over 300 textbook chapters, review articles, peer-reviewed papers, and abstracts many of which are related to the pathogenesis, investigation, and treatment of disease which includes critical care and trauma. He is lead editor of the books *Diet and Nutrition in Critical Care*, *The Neuroscience of Traumatic Brain Injury*, and *The Neuroscience of Spinal Cord Injury.*

Victor R. Preedy, BSc, PhD, DSc, FRSB, FRSPH, FRCPath, FRSC, is Professor of Clinical Biochemistry and Pathology at King's College Hospital, Emeritus Professor of Nutritional Biochemistry at King's College London, and Visiting Professor at the University of Hull. Professor Preedy graduated in 1974 with an Honors Degree in Biology and Physiology with Pharmacology. He gained his University of London PhD in 1981. In 1992, he received his Membership of the Royal College of Pathologists, and in 1993 he gained his second doctoral degree, for his outstanding contribution to protein metabolism in health and disease. Professor Preedy was elected as a Fellow to the Institute of Biology in 1995 and to the Royal College of Pathologists in 2000. Since then, he has been elected as a Fellow to the Royal Society for the Promotion of Health (2004) and The Royal Institute of Public Health (2004). In 2009, Professor Preedy became a Fellow of the Royal Society for Public Health and in 2012 a Fellow of the Royal Society of Chemistry. In his career, Professor Preedy has carried out research at the Cardiothoracic Institute, National Heart Hospital (part of Imperial College London), The School of Pharmacy (now Part of University College London), and the MRC Centre at Northwick Park Hospital. He has collaborated with research groups in Finland, Japan, Australia, USA, and Germany. He is a leading expert on the science of health and has a long-standing interest in biomarkers, especially related to tissue pathology. He has lectured nationally and internationally. To his credit, Professor Preedy has published over 750 articles, which includes peer-reviewed manuscripts based on original research, abstracts and symposium presentations, reviews, and numerous books and volumes.

Vinood B. Patel, BSc, PhD, FRSC, is currently Reader in Clinical Biochemistry at the University of Westminster. He presently directs studies on metabolic pathways involved in liver disease, particularly related to mitochondrial energy regulation and cell death. Research is being undertaken to study the role of nutrients, antioxidants, phytochemicals, iron, alcohol, and fatty acids in the pathophysiology of liver disease. Other areas of interest are identifying new biomarkers that can be used for the diagnosis and prognosis of liver disease and understanding mitochondrial oxidative stress in Alzheimer's disease and gastrointestinal dysfunction in autism. Dr. Patel graduated from the University of Portsmouth with a degree in Pharmacology and completed his PhD in Protein Metabolism from King's College London in 1997. His postdoctoral work was carried out at Wake Forest University Baptist Medical School studying structural-functional alterations to mitochondrial ribosomes, where he developed novel techniques to characterize their biophysical properties. Dr. Patel is a nationally and internationally recognized researcher and

has several edited biomedical books related to the use or investigation of active agents or components as well as biomarkers. These books include *Handbook of Nutrition, Diet, and Epigenetics*, *Biomarkers in Cancer*, *Biomarkers in Cardiovascular Disease*, and *Biomarkers in Liver Disease*. In 2014, Dr. Patel was elected as a Fellow to The Royal Society of Chemistry.

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Part I

General Overviews: Circulating and Body Fluid Biomarkers in Organ Injury and Trauma

A Synopsis of Routine Blood Biomarkers in Trauma, Injury Critical Care and Recovery: General Overview

1

Jelena Milic and Dunja Stankic

Contents

Abstract

Biomarkers are characteristic biological indicators that are used to identify (indirect changes) physical damage or disorders of physiological processes in humans or animals. The potentials they have for detection, diagnostics, prognostics, and intervention direction is crucial in the field of trauma, injury critical care,

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and recovery. From the last decade of the twentieth century onwards, researchers and clinicians have identified and introduced an increasing number of biomarkers into everyday use. This chapter presents various definition, classifications, and utilization of biomarkers in trauma, injury critical care, and recovery. The current conceptual status of biomarkers both in the field of clinical and diagnostic assessment, more specifically in trauma, injury critical care, and recovery, is presented through the roll of both tools and outcomes in clinical practice and research with the final aim of providing a valid frame for interpretation of clinical states and research questions that rely significant on such biomedical measures. As modern biomedicine uses biomarkers and their presence both as primary outcomes in clinical trials and practice, and this is now a widely accepted and highly appreciated trend, this chapter also covers the overlaps and differences between clinical outcomes and biomarkers. Finally, the chapter presents the role of biomarkers in the process of management of disease that is of highest importance for trauma, injury critical care, and recovery. Although we use biomarkers extensively in clinical practice, our knowledge in this field is quite limited. Thus, further research in this area is needed to confirm the current findings and detect more useful biomarkers.

Keywords

Biomarkers · Trauma · Injury critical care · Recovery · Biological indicators · Biomonitoring · Biological system

Abbreviations

AFP	Alpha fetoprotein
ALI	Acute lung injury
ALP	Alanine aminotransferase
ALS	Alkaline phosphatase
AST	Aspartate aminotransferase
BMP-2	Bone morphogenetic protein 2
BNP	B-type natriuretic peptide
CA 125	Cancer antigen 125
CC16	Club cell secretory protein 16
CEA	Carcinoembryonic antigen
CK-BB	Creatine kinase brain biomarker
CK-MB	Creatine kinase myoglobin binding
CRP	C-reactive protein
CSF	Cerebrospinal fluid
cTnI	Cardia Troponin I
cTnT	Cardia Troponin T
ESR	Erythrocyte sedimentation rate
FABP	Fatty acid-binding proteins
GGT	Gamma-glutamyl transferase
IGFBP7	Insulin-like growth factor-binding protein 7
IL-10	Interleukin-10

IL-18	Interleukin-18
KIM-1	Kidney injury molecule-1
LDH	Lactic acid dehydrogenase
MDSCs	Myeloid-derived suppressor cells
NF-L	Neuro-filament light chain
NGAL	Neutrophil gelatinase-associated lipocalin
NT-proBNP	N-terminal proBNP
PCT	Procalcitonin
PSA	Prostate-specific antigen
S100A8/9	Calprotectin
SPD	Surfactant protein D
TIMP-2	Tissue inhibitor of metalloproteinase 2
VEGF	Vascular endothelial growth factor
WBC	White blood cell

Introduction

Biomarkers are measurable changes in the parameters of biochemical systems (built of organic and inorganic compounds) that are monitored by biomonitoring. Also, biomarkers present characteristic biological indicators that are used to identify (indirect changes) physical damage or disorders of physiological processes in humans or animals. Thanks to biomarkers, many changes in animals and humans are successfully monitored (identified) today, using minimally invasive techniques. One of the earliest biomarker that was discovered in 1948, when Henry Bence Jones (1813–1873), a British physician, discovered a chain of immunoglobulins present in serum in urine in 75% of patients with certain diseases, especially multiple myeloma, is known as Bence-Jones protein (Sewpersad and Pillay 2020). Bence-Jones protein is a monoclonal globulin protein or immunoglobulin light chain found in the urine, with a molecular weight of 22–24 kDa. Detection of Bence-Jones protein may be suggestive of multiple myeloma or Waldenström's macroglobulinemia. Then, in the 1960s, AFP and CEA were discovered as biomarkers for cancer. Furthermore, in the eighties of the twentieth century, the biomarker CA-125 was discovered as a marker in ovarian tumors and also PSA as a specific antigen for prostate (Diamandis et al. 2013). From the last decade of the twentieth century onwards, researchers and clinicians have identified and introduced an increasing number of biomarkers into everyday use.

Characteristics of Biomarkers in Trauma, Injury Critical Care, and Recovery

In order for a medical biomarker (biochemical indicator) to be successful, it must meet some of the criteria: 1. that its concentration is relatively high in the corresponding tissue and negligible in other tissues; 2. that the overall distribution is approximately equal, so that after tissue damage it can quickly be in the circulation; 3. to remain in the circulation long enough to monitor its concentration; 4. that

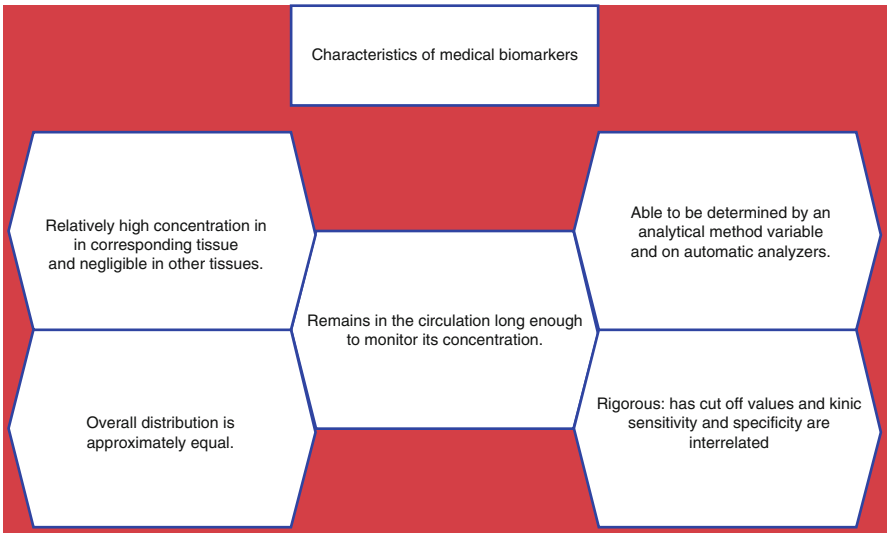

Fig. 1 Characteristics of medical biomarkers

it can be favorably determined by a suitable and sensitive analytical method variable and on automatic analyzers; 5. rigorous, cut off values have been established for it, taking into account that clinic sensitivity and specificity are interrelated. (Fig. 1).

Various Definitions of Biomarkers

Biomarkers are measurable biological parameters that change in response to exposure to xenobiotics and other environmental or physiological stressors and serve as indicators of exposure to adverse effects. Biomarkers are also defined as sensitive molecular, cellular, and organic indicators of changes in environmental protection and health of the organism. They also present physiological or biochemical measure, such as the concentration of cholinesterase in the blood, which can be an indicator of exposure to pollution. Further, biomarkers can be observed as indicators of differences in biochemical or cellular elements or processes in the structure or function of biological systems or samples. Generally speaking, biomarkers are a large group of small methodologies or methods that include metabolic, genotoxic, immune, and other approaches to assessing the toxicological effects on living organisms. Via biomarkers we are able to detect whether an event occurs in a biological system or sample and show the level of exposure or the effect of sensitivity. They also present parameters that can be used to identify the effect in an individual's body and can be used to extrapolate risk assessment between species. If we take all these definitions into consideration, we can say that from the point of view of biomedicine, a biomarker, or biological marker, is an indicator of some biological state or condition that can be detected and measured. In more precise words, a biomarker is a naturally occurring molecule, gene, or characteristic by which a particular pathological or

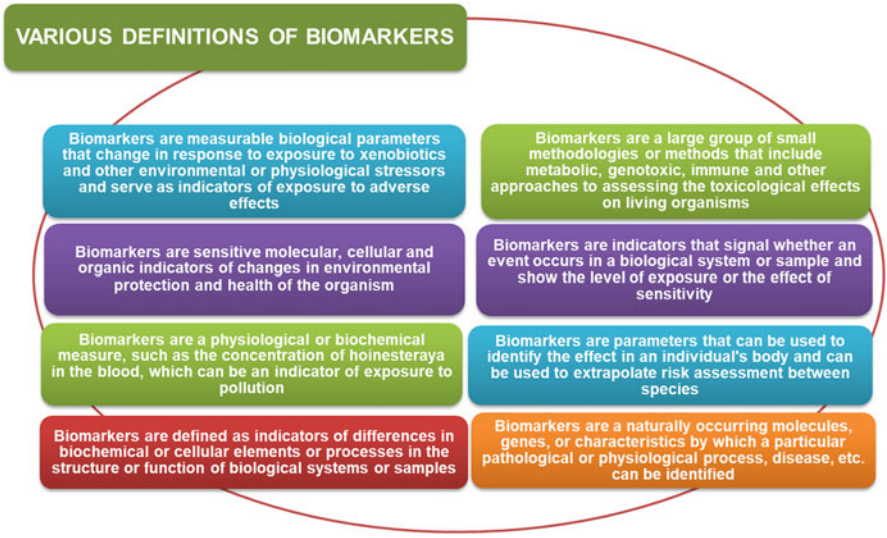

Fig. 2 Various definitions of biomarkers

physiological process, disease, etc. can be identified. Thus, a biomarker is defined as an indicator of a bio-physiological state of a living human organism. Furthermore, the most common way to asses a biomarker, to measure and evaluate it, is through easily obtainable material, such as blood, urine, or soft tissue (Hirsch and Watkins 2019). This material is used to examine vital functioning of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Siderowf et al. 2018). Biomarkers are not used only in biomedicine. We can find their usage and application in many scientific fields. Most importantly, biomarkers also help in pathogenic processes or pharmacologic responses to a therapeutic intervention (Strimbu and Tavel 2010). They help develop targeted therapies for diseases, screening interventions and prognostics of a disease progression or forecast studies in the field of research. As they also respond to a treatment as mentioned, they help to monitor the patient prior to diagnosing, while on treatment or afterwards (Visser et al. 2018). This chapter focuses on the role of biomarkers in modern medicine diagnostics (Fig. 2). In the next subsection of this chapter we will focus on biomarkers in emergency medicine diagnostics with in the field of trauma, injury critical care, and recovery.

Significance of Medical Biomarkers in Trauma, Injury Critical Care, and Recovery

The magnitude of hazards and risks of certain substances in nature and the environment is characterized not only by their concentration but also by their bioavailability. So far, the most reliable way to determine the risk of certain substances in the environment and nature is transpiration and interpolation of biomonitoring results in

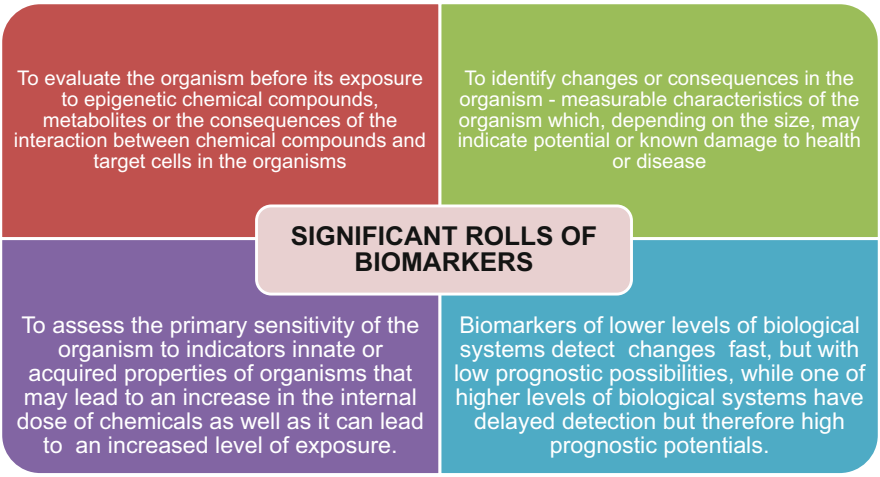

Fig. 3 Significant rolls of biomarkers

conjunction with other classical forms of monitoring. Biomonitoring based on biomarkers reliably confirms or refutes, and qualifies hazards and risks specific to each environment (Paustenbach and Galbraith 2006). Thus, the significance of biomarkers is of a high relevance for detection and further disease development, but most of all the main significance is related to medical biomarkers in trauma, injury critical care, and recovery where proper diagnostics and good timing is of vital importance (Fig. 3). This significance can be observed in these four different types of activities:

1. To evaluate the organism before its exposure to epigenetic chemical compounds, metabolites, or the consequences of the interaction between chemical compounds and target cells in the organisms.
2. To identify changes or consequences in the organism – measurable characteristics of the organism which, depending on the size, may indicate potential or known damage to health or disease (Mayeux 2004; Mitrea et al. 2020).
3. To assess the primary sensitivity of the organism to indicators innate or acquired properties of organisms that may lead to an increase in the internal dose of chemicals or an increased level of exposure (Torrealba et al. 2019).
4. Speed change of detection in body changes – biomarkers of lower levels of biological systems are characterized by speed of detection of changes but low prognostic possibilities, while biomarkers of higher levels of biological systems are characterized by delayed detection but therefore high prognostic potentials.

Biomarkers in Critical Care

Monitoring biomarkers is considered to be very useful in intensive care units. Biomarkers have been found that predict mortality as well as morbidity and complications (Abidi et al. 2011). Biomarkers present a way to monitor progress and

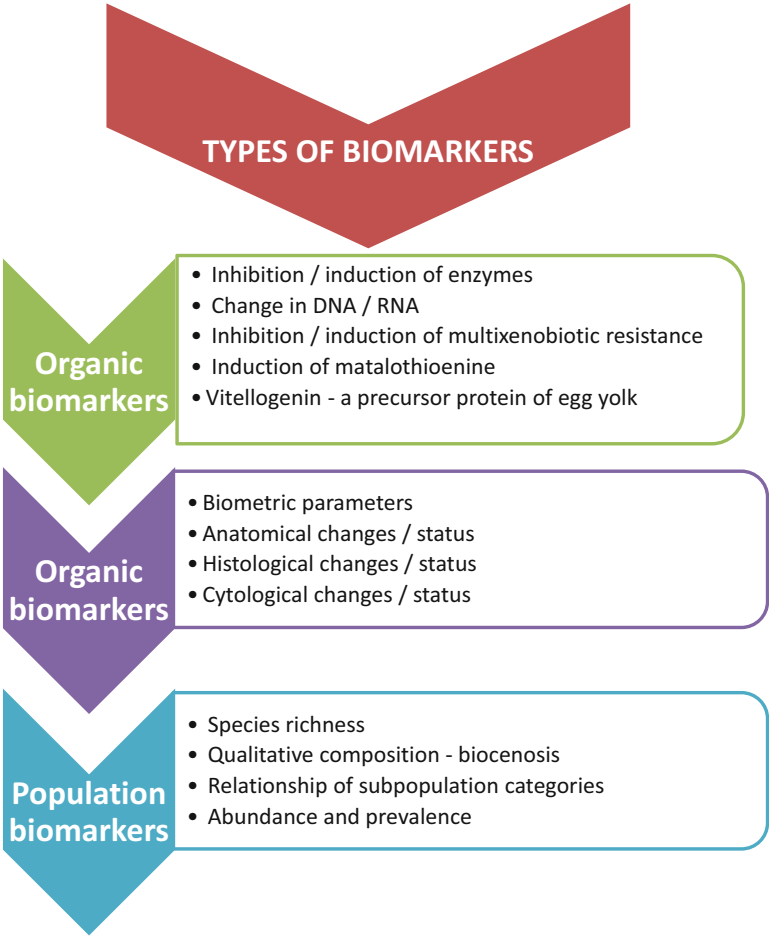

Fig. 4 Types of biomarkers in in trauma, injury critical care and recovery

severity of conditions in way that puts relatively little stress on an already vulnerable patient. Because of this, there is a constant search for new, sensitive biomarkers for a wide spectrum of diseases that put people in ICU, from acute kidney injury to the COVID-19 virus (Cooper et al. 2020), as well as biomarkers for conditions that are a result of a stay in ICU (Parker et al. 2021). In the next few subsections the overview of the relevant biomarkers in acute injuries presented per organ for type of injury will follow (Fig. 4).

Established Blood Biomarkers in Orthopedic Trauma

An overview of the literature on biomarkers in acute orthopedic trauma indicates the big spectrum of different biomarkers (Table 1). Routine ones are familiar to many, for example, C-reactive protein (CRP) is one of the routine infection markers in

Table 1 Established biomarkers in orthopedic trauma with emphasis on traumatic brain injury[a]

Blood biomarkers	Clinical use	Clinical use potential	References
C-reactive protein (CRP)	Yes	Diagnosis and monitor of postoperative infections. Preoperative marker for risk stratification (for potential complications). Independent fracture-risk-factor. Basic inflammatory parameter in orthopedic surgery	Neumaier et al. (2015)
White blood cell (WBC)	Yes	WBC is a reliable biomarker of neurohumoral activation and can help in identifying patients with major injuries. A significant elevation in WBC in a blunt trauma patient, even with minimal initial signs of severe injury, should heighten suspicion for occult injury	Santucci et al. (2008)
Erythrocyte sedimentation rate (ESR)	Yes	Measure of the inflammatory response is elevated in 90% of patients who have serious orthopedic infections	Schulak et al. (1982)
Myeloid-derived suppressor cells (MDSCs)	Yes	Negatively correlated with functional bone regeneration as early as 1 week post-treatment	Kimura et al. (2010)
Immunosuppressive cytokine interleukin-10 (IL-10)	Yes	Negatively correlated with functional bone regeneration as early as 1 week post-treatment	Lord et al. (2014)
Bone morphogenetic protein 2 (BMP-2)	Yes	Delivered 8 week after initial creation of the bone defect (delayed treatment)	Kolambkar et al. (2011)
Neuro-filament light chain (NF-L)	Yes	Detect brain injury and predict recovery in multiple groups, with acute or chronic concussions and clinic-based patients with mild, moderate, or severe traumatic brain injury	Shahim et al. (2017)
CEREBROSPINAL & BLOOD MARKERS (can be detected in blood serum when compromised blood–brain barrier) Neuron-specific enolase, S100 calcium binding protein B, Myelin basic protein, Creatine kinase brain isoenzyme, Glial fibrillary acidic protein, Plasma desoxyribonucleic acid,	Yes	Markers for cell damage in central nervous system	Giacoppo et al. (2012)

(continued)

Table 1 (continued)

Blood biomarkers	Clinical use	Clinical use potential	References
Brain-derived neurotrophic factor, Ubiquitin carboxy-terminal hydrolase-L1			

[a]Traumatic brain injury is the number one cause of disability and mortality in cluster of sub-populations aged 1 to 45 (Ahmed et al. 2017)

blood serum of patients, used in all medical departments. However, in orthopedics CRP is of significant help in infections diagnostics and monitoring process. CRP remains superior to conventional and newer infection-related parameter and it is considered to be a basic parameter for inflammation. The previous study identifies excellent prognostic abilities that CRP has in chances of development of postoperative infection (Neumaier et al. 2015). The study reveals the evolution in CRP concentrations stating that the peak level occurred on the second or third postoperative day and it is also reflected the extent of surgical trauma. Following that, a second rise of CRP in the postoperative course indicates a complication. Finally, the authors claim that the highest levels are reached in bacterial infection after the fourth postoperative day with a cut-off level about 10 mg/dl (Neumaier et al. 2015). Can white blood cells be a good biomarker in orthopedic trauma? Indeed, so as it has been hypothesized that an increase of a trauma patient's white blood cell (WBC) count may be an alternative marker of neurohumoral activation and be valuable in identifying patients with major injuries. It is already marked that research in blunt trauma patients have shown higher WBC counts in more severely injured patients. Also, there is a markable increase in WBC in a blunt trauma patient, even when there is a minimal initial sign of severe injury. Thus, WBC does present a reliable biomarker for occult injury, as well (Santucci et al. 2008). Next, the erythrocyte sedimentation rate (ESR), a sensitive measure of the inflammatory response, is elevated in 90% of patients who have serious orthopedic infections, e.g., discitis, septic arthritis, and hematogenous osteomyelitis (Schulak et al. 1982). Further, the immunologist managed to raise the awareness that surgical and traumatic injury is strongly influenced by the inherent and adaptive immune responses, and that a noticeable suppression in cell-mediated immunity that is always accompanying an exorbitant, inflammatory response. This is highly important due to the fact that the mentioned suppression increases sensitivity to subsequent sepsis. Among such biomarkers, one that is in the routine use is myeloid-derived suppressor cells (MDSCs) (Kimura et al. 2010), and immunosuppressive cytokine interleukin-10 (IL-10) (Lord, Midwinter, Chen, Belli, Brohi, Kovacs, et al., 2014) which both negatively correlate with functional bone regeneration as early as 1-week post-treatment. Moreover, we also have a biomarker that can predict the speed of the recovery process, namely bone morphogenetic protein 2 (BMP-2). BMP-2 is one of the many bones' morphogenetic proteins. This specific protein plays a crucial part in the growth of bone and cartilage. Thus, it presents a reliable biomarker in the process of orthopedic trauma recovery (Kolambkar et al. 2011).

Recent basic and clinical studies have elucidated the biomarkers of surgical and traumatic injury on the trauma severity, pre−/postoperative infections and immune system response. The future research studies of interest may address more to prognostic biomarkers of orthopedic trauma recovery.

Biomarkers for Mild Traumatic Brain Injury: Unresolved Issues

As modern medicine is prognostic both in prognostics and treatments, with the special emphasis in intensive care units diagnostic imaging model that these units most frequently use, there is a markable reduction in deaths and disability resulting from traumatic brain injuries (Giacoppo et al. 2012). Traumatic brain injury is usually caused by a blast or other traumatic injury to the head or body. The severity of harm can rely on multiple factors, including the etiology of the injury and the force of impact. Trauma of the brain is the most frequent cause of hospitalization in the population of young people. The prevalence is observed higher in mail gender. Traumatic brain injury is the number one cause of disability and mortality in cluster of subpopulations aged 1 to 45 (Ahmed et al. 2017). Nonetheless, the knowledge we have about the underling mechanisms of pathophysiological damage in brain trauma are still limited and it is still very hard to make any prognostic approximation and quantify the severity of the primary and consequent pathological development. The further destruction of initial injury might occur and that is one additional reason why is it very important to have effective therapeutic measures and even more so to effectively predict the potential outcome. This is the exact motivation that inspired multiple researchers and clinicians in recent years to dedicate their main attention to detection of specific biomarkers of brain injury to improve the diagnosis and the prognostics of outcome. As the previous study indicates, the proteins synthesized in the astroglia cells or in the neurons, such as neuron-specific enolase, S100 calcium-binding protein B, myelin basic protein, creatine kinase brain isoenzyme, glial fibrillary acidic protein, plasma desoxyribonucleic acid, brain-derived neurotrophic factor, and ubiquitin carboxy-terminal hydrolase-L1, have been proposed as potential markers for cell damage in central nervous system (Giacoppo et al. 2012) (Table 1). It is normally the case that the levels of mentioned proteins increase following brain injury and therefore the concentrations in the cerebrospinal fluid are also increased upon exploration, this all depending on the severity of injury, as it can also be the case that they are detected in blood stream because of a compromised blood–brain barrier. Another study suggested that neurofilament light chain as a blood biomarker can detect brain injury and predict recovery in multiple groups, with acute or chronic concussions and clinic-based patients with mild, moderate, or severe traumatic brain injury (Shahim et al. 2017). Once that a person has suffered traumatic brain injury the mentioned neurofilament light chain breaks away from neurons in the brain and further collects in the cerebrospinal fluid (CSF), as well as it collects in the blood in level that correlate closely with the level that can be at the same time detected in the CSF. Therefore, one can carefully infer that neurofilament light chain in the blood can detect brain injury and predict recovery across all stages

of traumatic brain injury in a valid and reliable way (Table 4). However, so far detected biomarkers in trauma injuries especially the biomarkers in mild, moderate, and severe brain injury remain uncomprehensive in regard to multiple issues. (Zetterberg et al. 2013; Papa et al. 2015; Ramamurthy 2020; Manivannan et al. 2018; Adrian et al. 2016; Bogoslovsky et al. 2016; Kawata et al. 2016; Thelin et al. 2017). The recent review (Hier et al. 2021) focuses on several main unresolved issues related to the use of blood biomarkers to diagnose and manage traumatic brain injury, and according to authors they are: 1. How do biomarkers enter and exit the blood?; 2. What are the kinetics of blood biomarkers?; 3. What is the optimal sampling time for blood biomarkers?; 4. How long do the blood biomarker levels remain elevated?; 5. What are the confounding factors for blood biomarker levels?; 6. Can blood biomarkers differentiate between subjects with concussions or sub-concussive hits to the head from healthy controls?; 7. Can blood biomarkers predict CT scan positivity?; 8. Can blood biomarkers predict outcome or severity of traumatic brain injury?

These abovementioned issues remain unclear and further research is necessary to elucidate the mechanism underlying the activity and prognostic potential of these biomarkers.

Established Blood Biomarkers in Acute Cardiac Disease

Nowadays, cardiovascular emergencies and symptoms are one of the most common reasons for patients' attendance in any emergency department (Anjum 2018). The detection and validation of cardiac biomarkers has contributed a very valuable understanding to clinicians into the condition of the myocardium. Admittedly, cardiac biomarkers like myoglobin (Aydin et al. 2019) and creatine kinase-myoglobin binding (CK-MB) nowadays represent an essential criterion in the definition of acute myocardial infarction. The previous study emphasized that the various combinations of creatine kinase-MB, myoglobin, and cardiac troponin I or T (cTnI/cTnT) can detect patients with suspected acute coronary syndrome (Hachey et al. 2017). On top of that, the authors present the current recommendations that suggest the usage of 99th percentile of cTnI/cTnT as the sole marker for diagnosis of acute myocardial infarction. Generally speaking, in regard of biomarkers, in their relevancy to the cardiovascular diseases, there has been an overwhelming progress of usage of both efficient and reliable assays to detect biomarkers in the serum (Table 2). If we observe simultaneously the patient history and electrocardiographic analysis, the indispensable data gathered from serum cardiac biomarkers underpin diagnosis, choice of therapeutic option and have prognostic abilities. Routine blood biomarkers like troponin and CK-MB have already been in the spotlight of cardiovascular clinicians' attention due to their capability to identify myocardial ischemia (Shafiq et al. 2013; Korff 2006). The current practice in field of cardiovascular diseases uses biomarkers to identify not only ischemia but also other acute cardiovascular clinical states. For instance, B-type natriuretic peptide or brain natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) serve in diagnosing heart

Table 2 Established biomarkers in acute cardiac disease

Blood biomarkers	Clinical use	Clinical use potential	References
Myoglobin	Yes	Evaluating infarct size and reperfusion	Aydin et al. (2019)
Cardia Troponin I (cTnI) and Cardia Troponin T (cTnT); Ratio of Cardia Troponin I	Yes	Highly sensitive troponin assays show great potential in earlier detection of acute myocardial infarction and better risk prediction in patients with acute coronary syndrome. The current recommendation* is to use the 99th percentile of cTnI/cTnT as the sole marker for diagnosis of acute myocardial infarction	Korff (2006), Hachey et al. (2017)
Creatine kinase-myoglobin binding (CK-MB)	Yes	Increased several-fold in hypoxic myocardium: Higher than normal CK-MB enzymes may indicate an inflammation of the heart muscle or patients are having or recently had a heart attack	Shafiq e tal. (2013)
Creatine kinase-brain biomarker (CK-BB)	Yes	Increased CK-BB enzymes may indicate that patients is having or have had a stroke or brain injury	Capocchi et al. (1987)
B-type natriuretic peptide or brain natriuretic peptide (BNP); *N*-terminal proBNP (NT-proBNP)	Yes	Diagnosing heart failure, including diastolic dysfunction	Long et al. (2019), Cao et al. (2019)
Lactic acid dehydrogenase (LDH)	Yes	Late marker of myocardial infarction	Manonmani and Kavitha (2021)

failure, including diastolic dysfunction (Long et al. 2019; Cao, Jia & Zhu, 2019). Further, lactic acid dehydrogenase (LDH) is a nonspecific marker for myocardial infarction, and more precisely it is considered to be a marker of myocardial infarction (Manonmani and Kavitha 2021), and its concentration can be elevated also in hemolytic anemia, stroke, pancreatitis, ischemic cardiomyopathy, and a variety of other diseases present markers except for the last one (LDH) are highly sensitive and specific to myocardial injury. The future challenges lie in the attempt to discover biomarkers that would also be able to identify the method of injury.

Established Blood Biomarkers in Acute Lung Injury

Potential biomarkers of acute lung injury (ALI) are not specific or sensitive enough to be used in the decision-making process for people suffering from ALI. Researching such biomarkers has, however, given insight in the pathophysiology

Table 3 Established blood biomarkers in acute lung injury

Blood Biomarkers	Clinical use	Clinical Use Potential	References
Surfactant protein D (SPD)	Yes	Indicator of alveolar damage – Pneumocytes type I and II	Takahashi et al. (2000)
Club cell secretory protein 16 (CC16)	Yes	Indicator of airway conductive damage, possibly not in acute lung injury	Lin et al. (2017)
Vascular endothelial growth factor (VEGF)	Yes	Promotes angiogenesis and increases vascular permeability	Lin et al. (2019)
Procalcitonin (PCT)	Yes	Antibiotic reduction	Feldman (2018)
Eosinophilic granulocyte	Yes	Reduction of corticosteroid use	Singh et al. (2014)
Mitochondrial DNA	No	Indicator of inflammation or infection	Lee et al. (2017)

of injury and reparation of the lungs. Biomarkers for ALI can currently only be used to research said processes of injury and repair.

We present an overview of blood biomarkers currently used in the study of the mechanisms of injury and repair of the lungs in patients suffering ALI (Table 3). A number of blood biomarkers are used as indicators for conditions related to ALI. Firstly, surfactant protein D (SPD) points to alveolar damage to pneumocytes type I and II (Takahashi et al. 2000). Secondly, mitochondrial DNA is used as an indicator of inflammation or infection in the lungs (Lee et al. 2017). Thirdly, club cell secretory protein 16 (CC16) indicates airway conductive damage, although it is possibly not a good indicator in case of acute lung injury (Lin et al. 2017). Vascular endothelial growth factor (VEGF) promotes angiogenesis and increases vascular permeability and is shown to mediate fat embolism-induced acute lung injury (Lin, Lin, Huang, Shi, Yang, Yang). Two blood biomarkers can potentially reduce the use of certain medications in people suffering from ALI. Procalcitonin (PCT) guidance proves useful in reducing antibiotics use (Feldman). Finally, eosinophilic granulocyte can help do the same with corticosteroid use (Singh et al. 2014).

Established Blood Biomarkers in Acute Kidney Injury

The acute kidney injury is defined as sudden and often reversible reduction in the kidney function, as measured by increased creatinine or decreased urine volume. This state was previously known as acute renal failure. Pathophysiology of acute kidney injury is divided into three categories: prerenal, renal, and post-renal. Each of the categories has different etiopathogenesis (Sharples 2017).

Numerous biomarkers of acute kidney injury have been identified and in use. These biomarkers can be traced both in urine and blood and signal systemic damage to the kidney. Clinically, they are recognized as adjunct diagnostics to serum creatinine and urinary output to improve the early detection, differential diagnosis,

Table 4 Established blood biomarkers in acute kidney injury

Blood biomarkers	Clinical use	Clinical use potential	References
Neutrophil gelatinase-associated lipocalin (NGAL)	Yes	Bacteriostatic function in the innate immune system, iron delivery to mammalian cells	Goetz et al. (2002)
Kidney injury molecule-1 (KIM-1)	Yes	Tubular regeneration by mediating phagocytosis of apoptotic bodies	Ichimura et al. (2008)
Liver-type fatty acid-binding protein (L-FABP)	Yes	Regulation of fatty acids uptake and the intracellular transport	Chmurzyńska (2006)
Interleukin-18 (IL-18)	Yes	Proinflammatory effect	Cheung et al. (2005)
Insulin-like growth factor-binding protein 7 (IGFBP7)	Yes	It has been used as an early diagnosis and prognostic marker for acute renal insufficiency	Wang et al. (2021)
Tissue inhibitor of metalloproteinase 2 (TIMP-2)	Yes	Earlier than conventional tests can detect subclinical acute kidney injury	Schrezenmeier et al. (2016)
Calprotectin (S100A8/9)	Yes	Polarization of M2 macrophages that promote the repair after injury	Dessing et al. (2015)

and prognostic assessment of acute kidney injury. Here (Table 4), we present an overview of the most important biomarkers of acute kidney injury found in blood: neutrophil gelatinase-associated lipocalin (NGAL) that is related to bacteriostatic function in the innate immune system and iron delivery to mammalian cells (Goetz et al. 2003), kidney injury molecule-1 (KIM-1) indicating tubular regeneration by mediating phagocytosis of apoptotic bodies (Ichimura et al. 2008), liver-type fatty acid-binding protein (L-FABP) that regulates the fatty acids uptake and the intracellular transport (Chmurzyńska 2006), interleukin-18 (IL-18) that has the proinflammatory effect (Cheung et al. 2005), insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase 2 (TIMP-2) that also have the potentials of detecting the subclinical acute kidney injury earlier than conventional tests can (Schrezenmeier et al. 2016), and calprotectin (S100A8/9) that has the biomarker potential in indicating the polarization of M2-type of macrophages Polarization of M2 macrophages that promote the repair after injury (Dessing et al. 2015). We also acknowledge that there are still various gaps and perspectives for future studies.

Established Blood Biomarkers in Acute Medical Condition of Gastrointestinal Track and/or Liver

In this subsection an overview of established blood biomarkers in acute injury of gastrointestinal track and liver diseases is presented (Table 5). The mentioned biomarkers differ in regard to indication or on inflammatory, angiogenetic, or fibrotic stage. The quantification of the biomarker needs to be higher if grading and staging

Table 5 Established blood biomarkers in acute medical condition of gastrointestinal tract and/or liver

Blood biomarkers	Clinical use	Clinical use potential	References
Alanine aminotransferase (ALT)	Yes	Markers of hepatocellular injury; sensitive and relatively specific test for hepatocyte damage. Its activity in serum rises even in a small damage of the liver cell, caused by increased permeability of the cell membrane	Jeschke (2009)
Aspartate aminotransferase (AST)	Yes	Markers of hepatocellular injury, not specific for the liver tissue, it can be elevated also in damage of skeletal muscle and myocardium – in blood rises in acute myocardial infarction (heart stroke) and following heart surgery, but also due to a long-lasting strenuous physical exercise	McGill (2016)
Alkaline phosphatase (ALP)	Yes	Markers of cholestasis; elevations occur as a result of both intrahepatic and extrahepatic obstruction to bile flow	Brennan et al. (2021)
Gamma-glutamyl transferase (GGT)	Yes	Markers of cholestasis; play a role in secretory and absorptive events in the hepatobiliary system	Aithal et al. (2011)
Serum bilirubin	Yes	Markers of liver dysfunction	Sticova (2013)
D-lactate	Yes	By-product of bacterial fermentation, with only a small amount being produced by human cells. It can be found in the circulation after ischemic injury, increased intestinal permeability, or bacterial overgrowth	Ewaschuk et al. (2005)
Fatty acid-binding proteins (FABP)	Yes	Cytosolic proteins involved in the uptake and intracellular transport of fatty acid, expressed by enterocytes located at the tips of the intestinal mucosal villi, the anatomical region that is first affected by ischemic injuries	Gollin et al. (1993)
Citrulline	Yes	Circulating citrulline is mainly produced by enterocytes of the small bowel. For this reason, plasma or serum citrulline concentration has been proposed as a biomarker of remnant small bowel mass and function	Crenn et al. (2008)

of liver injury is more severe. Early grades biomarker is known to be not reliable, as they may not remain same or even indicative at the end stage of the disease. On the other hand, inflammatory biomarkers have a tendency to be very reliable in regard to livery injury pattern: necrotic, cholestasis, drug-induced, or apoptotic liver injury pattern. In acute liver disease, also the fibrotic biomarkers at end stage of the liver disease hold the tendency to have the different deception. The next step in research of the gastrointestinal and liver biomarkers should be aimed toward the therapeutic options targeting specific level of response/injury. If we would be able to understand the nature of the gastrointestinal tract or liver injury by clinical biomarker (pathogenesis and pathophysiology), it would be highly beneficial in diagnostic, prognostic, and therapeutic purposes.

Conclusion

Biological markers (biomarkers) can be defined as a biochemical, molecular, or cellular alternation that is measurable in biological media such as tissues, cells, or fluids. However, the most important thing connected to biomarkers is the fact that they have a significant role and use in precision medicine of today. We can conclude that clinical utilization of biomarkers in so far most applicable fields of clinical medicine develops in a way that we can classify the goals of their application into the following categories: diagnosis, risk stratification, goals of therapy, targeting of therapy, drug development, evaluation and registration. The most spoken about next field of application of biomarkers can be in acute kidney injury. Further, in prostate cancer and psychiatric diseases (primarily depression) and many other fields of medicine, which are today almost impossible to imagine without using without the help of biomarkers involved in all stages in the process of diagnosis and curing. Although we use biomarkers extensively in clinical practice, our knowledge in this field is quite limited. We need to dedicate ourselves to additional research in this area.

Mini-Dictionary of Terms

- Bioavailability – a measure of how much a substance is able to access the circulation and reach the target area and it depends on absorption (how much we get it) and secretion (how much we get out).
- Biomarkers – a biomarker (short for biological marker) is an objective measure that captures what is happening in a cell or an organism at a given moment.
- Biomonitoring – the measurement of chemical compounds or their metabolites (versions of the compounds that are transformed in the body) in biological specimens
- Human organism – schematic of the human organism as an evolving complex network of dynamical interactions between organ systems.
- Pathogenic processes – pathogenesis is the process by which a disease or disorder develops; it can include factors which contribute not only to the onset of the disease or disorder, but also to its progression and maintenance.

Key Facts of Biomarkers in Trauma, Injury Critical Care, and Recovery

- We use biomarkers extensively in clinical practice, and our knowledge in this field is quite limited. We need to dedicate ourselves to additional research in this area.
- There are three types of biomarkers, also in trauma, injury critical care, and recovery: molecular, organic, and population biomarkers.

- Biomarkers are measurable biological parameters that change in response to exposure to xenobiotics and other environmental or physiological stressors and serve as indicators of exposure to adverse effects.
- According to their main clinical application, we can divide biomarkers in trauma, injury critical care and recovery in the following groups: diagnostic, monitoring, pharmacodynamics/response, predictive, prognostic, susceptibility or risk biomarkers, and safety biomarkers.

Summary Points

- Biomarkers are measurable changes in the parameters of biochemical systems (built of organic and inorganic compounds) that are monitored by biomonitoring.
- We can measure biomarkers which can be defined as a biochemical, molecular, or cellular alternation in biological media such as tissues, cells, or fluids.
- Nowadays biomarkers involved in all stages in the process of diagnosis and curing in so many fields of medicine such as psychiatric diseases.
- Especially in the fields of trauma, injury critical care, and recovery, biomarkers have great impact on disease management.
- From the last decade of the twentieth century onwards, researchers and clinicians have identified and introduced an increasing number of biomarkers into everyday use, but there is still a need for further research.

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Author Contributions JM and DS conceived the chapter and discussed the keyword for literature search. JM was responsible for curating the data. Both authors contributed to composing of the chapter. JM wrote the first draft of the chapter and figures. Both authors assisted in revising the manuscript.

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A Synopsis of Emerging Blood Biomarkers in Trauma, Injury Critical Care, and Recovery: General Overview

2

Jelena Milic and Dunja Stankic

Contents

Abstract

In the field of biomarkers nowadays, it is possible to have valid, unbiased detection of novel biomarkers in wide range of clinical research, and from plasma. Researches dealing with biomarkers are mostly focused on detailed

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exploration of plasma proteome at novel depth and identify the detailed biological insights for emerging biomarker discovery. The emerging biomarkers should provide unbiased analysis with novel depth of many relevant proteins, they should have unmatched specificity and quantitative precision, high capacity, and be easily accessible. Further, they should provide characteristic biological indicators that are used to identify (indirect changes) physical damage or disorders of physiological processes in humans or animals. The enhanced and empowered potentials should be therefore provided in the field of detection, diagnostics, prognostics, and intervention direction should be crucial in the field of trauma, injury critical care, and recovery. Lately, researchers and clinicians have intensively worked on identifying novel biomarkers and consequently testing them for everyday clinical practice use. This chapter presents various definition, classifications, and utilization of emerging biomarkers in trauma, injury critical care, and recovery. The chapter provides an overview of the key features and benefits of next-generation biomarkers discovery solutions in the field of cardiovascular critical care, COVID-19, and biomarkers of infection in critical care. The chapter elaborates on deep level the clinical application possibilities quantification and analysis that can be performed by the emerging biomarkers. Further, the chapter presents how we identify the most promising and actionable biomarkers for research and clinical decision-making. Finally, different types of emerging biomarkers in trauma, injury critical care, and recovery are presented. Clearly, as our knowledge in evidence-based medicine is growing, it is necessary for biomarker research to grow along. Hence, the search for novel biomarkers will continue and intensify and provide new information.

Keywords

Emerging · Biomarkers · Novel biomarkers · Trauma · Injury critical care · Recovery · Clinical application

Abbreviations

ACE2	Angiotensin-converting enzyme 2
AMI	Acute myocardial infarction
cfDNA	Cell-free DNA
COVID-19	Coronavirus disease
cTnI	Cardiac troponin I
DNA	Deoxyribonucleic acid
GDF-15	Growth differentiation factor
H3Cit	Citrullinated histone 3
H-FABP	Heart-type fatty acid binding protein
HMGB1	High-mobility group box protein 1
miRNAs	MicroRNAs
MPO	DNA complexes
MT	Metallothioneins
MXR	Multixenobiotic resistance

NETs	Neutrophil extracellular traps
PD	Pharmacodynamic
RNA	Ribonucleic acid
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SIRS	Systemic inflammatory response syndrome
sST2	Soluble suppression of tumorigenicity 2
suPAR	Soluble urokinase-type plasminogen activator receptor
TREM-1	Soluble triggering receptor expressed on myeloid cells 1

Introduction

Recent research has been targeted to identify novel sensitive and specific biomarkers for the improvement in the process of diagnosing, optimizing treatment, and bettering outcomes of diseases. Here we provide an overview of the newer classification, of emerging biomarkers in the field of cardiovascular critical care, COVID-19, and biomarkers of infection in the critical care. As the future studies have the need to develop more accurate diagnostic, prognostic, and predict the clinical outcome, the novel biomarkers that could be more clinically applicable and offer greater patient acceptability than conventional biomarkers need to be classified in several different types to be better understood for future exploration purposes. In the field of biomarkers nowadays, it is possible to have valid, unbiased detection of emerging biomarkers in wide range of clinical research, and from plasma. Researches dealing with biomarkers are mostly focused on detailed exploration of plasma proteome at novel depth and identify the detailed biological insights for emerging biomarker discovery. However, we can distinguish more than one different type of emerging biomarkers and we present the overview in the upcoming subchapter.

Different Types of Emerging Biomarkers in Trauma, Injury Critical Care, and Recovery

There are many types of emerging biomarkers, but commonly used one in trauma, injury critical care, and recovery is identifying molecular biomarkers, organic biomarkers, and population biomarkers, whereas molecular biomarkers include: 1. inhibition/induction of enzymes; 2. change in DNA/RNA; 3. inhibition/induction of MXR (Fig. 1). The mechanism of multixenobiotic resistance (MXR) is present in many organisms as an important cellular detoxification mechanism. It is mediated by the activity of ABC transporters that bind and actively expel various toxic substances from cell; 4. induction of metallothionein. Metallothioneins (MT) present a family of proteins that are rich in cysteine and known for their low molecular weight that ranges between 500 and 14,000 Da. Golgi apparatus is the localization point of metallothioneins. They are highly important for many metabolic reasons but firstly for the ability to bind physiological (such as zinc, copper, and selenium) and

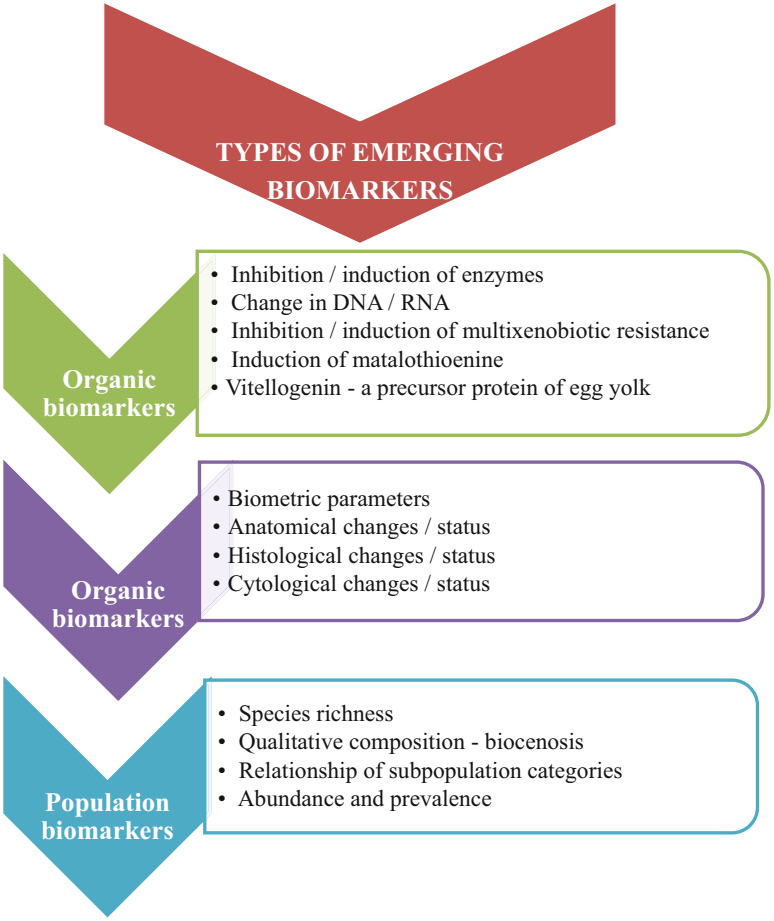

Fig. 1 Types of emerging biomarkers in trauma, injury critical care, and recovery

xenobiotic (e.g., cadmium, mercury, silver, and arsenic) heavy metals. Metallothioneins were revealed in 1957 by scientist Vali and Margoš from a purified Cd-binding protein from the equine renal cortex (Felizola et al. 2013). Metallothionein play an important role in protection against metal toxicity and oxidative stress, and participate in the regulation of zinc and copper concentrations (Wang et al. 2014); and 5. vitellogenin is a precursor protein of egg yolk. It can only be detected in females and it is used as a biomarker in vertebrates. On the other hand, the organic biomarkers are: 1. biometric parameters; 2. anatomical changes/status; 3. histological changes/status; and 4. cytological changes/status. Finally, we can go through population biomarkers, where so far identified ones are: 1. species richness; 2. qualitative composition – biocenosis; 3. relationship of subpopulation categories; and 4. abundance and prevalence. In ecology, local abundance is the relative representation of a species in a particular ecosystem. It is usually measured as the

number of individuals found per sample. The ratio of abundance of one species to one or multiple other species living in an ecosystem is referred to as relative species abundances (Preston 1948). Both indicators are relevant for computing biodiversity. A variety of sampling methods are used to measure abundance. For larger animals, these may include spotlight counts, track counts, and roadkill counts, as well as presence at monitoring stations (Wright 1991). In many plant communities the abundances of plant species are measured by plant cover, i.e., the relative area covered by different plant species in a small plot (Damgaard 2009). Abundance is in the simplest terms usually measured by identifying and counting every individual of every species in a given sector. It is common for the distribution of species to be skewed so that a few species take up the bulk of individuals collected (Verberk 2011). Relative species abundance is calculated by dividing the number of species from one group by the total number of species from all groups.

Classification of Emerging Biomarkers According to Their Main Clinical Application in Trauma, Injury Critical Care, and Recovery

According to their main clinical application, we can divide emerging biomarkers in trauma, injury critical care, and recovery in the following groups (Fig. 2): 1. diagnostic biomarkers. Biomarkers of this category are used to confirm the existence of a disease or medical condition. In this context, diagnostic biomarkers may play an important role to reach a precise diagnosis, identifying patient with a disease and facilitating the classification of patients with the same type of diagnosis to personalize drug treatments, therefore increasing the efficiency of the therapeutic response; 2. monitoring biomarkers. This category includes biomarkers measured at different time points for assessing the presence, status, or extent of a disease or medical condition. Besides this, they can be used to evaluate the effects of medical products or environmental agent exposures; 3. pharmacodynamic/response biomarkers. Pharmacodynamic/response (PD) biomarkers present a vast spectrum of applications from the early phases of the discovery research to the clinical trials and later during the clinical practice. This type of biomarker may be defined as "a biomarker used to show, that a biological response occurred in an individual exposed to a medical product and environmental agent." These biomarkers provide information about proof of mechanism, proof of concept, selection of optimal biological dosing, and understanding response/resistance mechanism; 4. predictive biomarkers. This category includes markers that identify patients more likely to experience an effect (positive or negative) after the exposure to a medical product or an environmental agent. These biomarkers are commonly used in randomized control clinical trials of new therapies as selection criteria for including patients in the study or to stratify them into intervention group; 5. prognostic biomarkers. According to its definitions, theses biomarkers identify the likelihood of a clinical event, disease recurrence, or progression in patients diagnosed with a disease or having a medical condition. In clinical trials, prognostic biomarkers allow to predict the occurrence of a clinical event in the future, such as death, disease

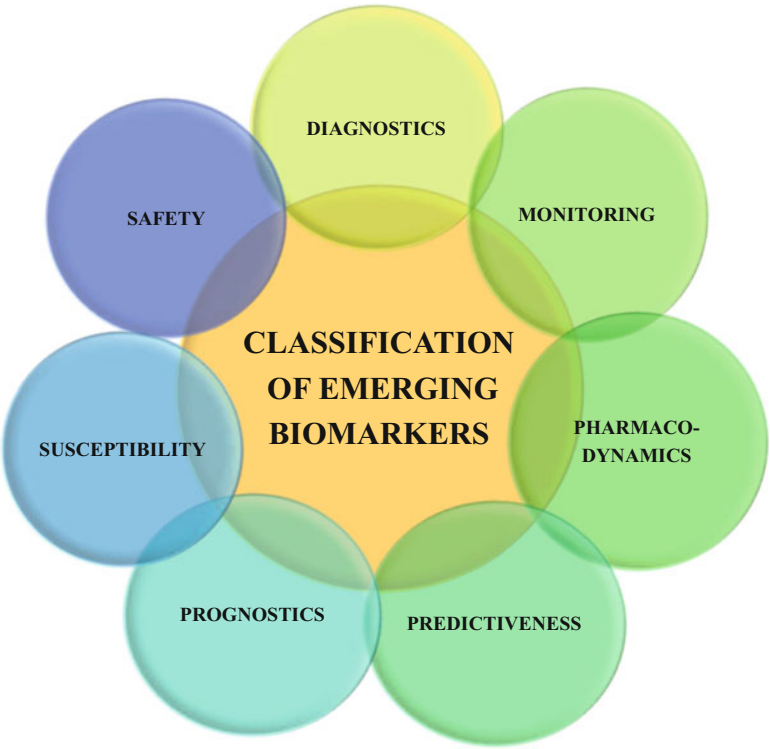

Fig. 2 Classification of emerging biomarkers according to their main clinical application in trauma, injury critical care, and recovery

progression, disease recurrence, or development of a new medical condition; 6. susceptibility or risk biomarkers. Susceptibility/risk biomarkers indicate the potential for developing a disease or medical condition in an individual not currently presenting a clinically apparent disease or medical condition. The major difference between this category of biomarkers and prognostic biomarkers lies in the fact that susceptibility/risk biomarkers are measured in individuals not presenting the disease. Thus, these biomarkers can be detected long before the appearance of a disease and are not useful to describe the response to any specific treatment; and 7. safety biomarkers (Fig. 3). The relevance of this biomarker is to predict toxic adverse events induced by drugs, medical interventions, or environmental agents' exposure. Toxicity can be reflected by the detection of the biomarker or changes in biomarker level, facilitating the necessary actions to prevent irreversible damage, such as those adjustment, treatment interaction, or initiation of a specific treatment (Davis 2017).

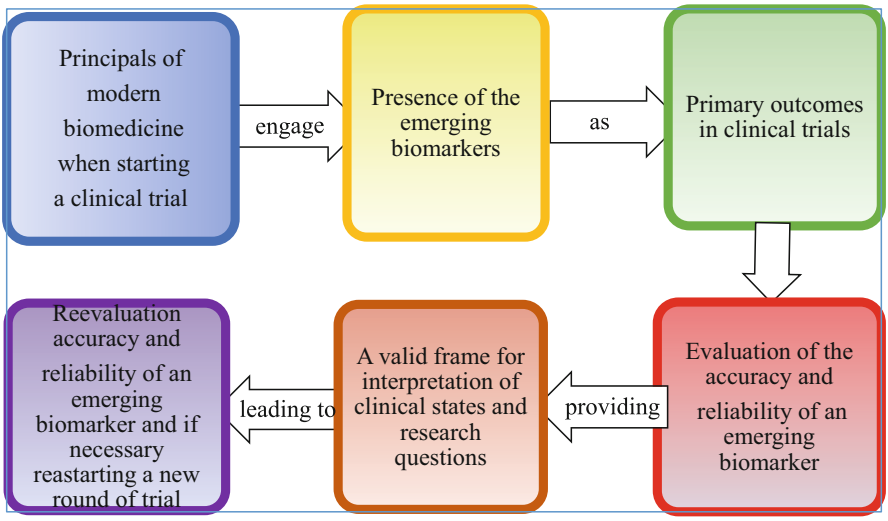

Fig. 3 Role of biomedicine and emerging biomarkers in modern medicine's trauma, injury critical care, and recovery

Fig. 4 Best treatment for all diseases

Role of Biomedicine and Emerging Biomarkers in Modern Medicine's Trauma, Injury Critical Care, and Recovery

Modern medicine and its basic and clinical research and clinical practice in trauma, injury critical care, and recovery rely on biomedicine and biomedical assessments of biomarkers more and more (Flier and Loscalzo 2017) and this applies dominantly on emerging biomarkers (Fig. 4). Biomedicine uses emerging biomarkers and their presence as primary outcomes in clinical trials and practice and this is now a widely

accepted and highly appreciated practice (Strimbu and Tavel 2010). The more one biomarker is specific the more the same biomarker is well characterized and confirmed to correctly predict relevant clinical or research outcomes across a different methodology or type of treatments and different subpopulations (Ray et al. 2010), and this applies even more to emerging biomarkers. Therefore, this kind of approach to assessment is entirely justified and appropriate. However, in many cases, the actual accuracy and reliability of a biomarker is presumed where, in fact, it needs to be further evaluated and reevaluated (Burke 2016) and that is most of all valid for the emerging biomarkers in the field of application in trauma, injury critical care, and recovery. The current conceptual status of biomarkers both in the field of clinical and diagnostic assessment, more specifically in trauma, injury critical care, and recovery, is presented through the roll of both assessment tools (biomarkers) and outcomes in clinical practice and research with the final aim of providing a valid frame for interpretation of clinical states and research questions that rely significantly on such biomedical measures. Biomedical measures derive from biomedicine. Thus, biomedicine is the branch of medicine concerned with the application of the principles of biology and biochemistry to medical research or practice focused mostly on constant identification of emerging biomarkers and other diagnostic and prognostic possibilities.

Modern Medical Diagnostics in Trauma, Injury Critical Care, and Recovery

In medical practice, laboratory diagnostics in the form of emerging biomarker testing are most often used for diagnosing and prognostics as well as monitoring of diseases. All these are used dominantly in the field of trauma, injury critical care, and recovery. This diagnostic strategy is often somewhat uncertain because it can be dishonorable due to the specificity of the emerging biomarker itself or the inaccuracy of the patient's clinical perception or interpretation of the emerging biomarker diagnostic test. The uncertainty caused by the first mentioned phenomenon is today largely reduced by the development of new, more suitable analytical procedures and by paying more attention to quality control in laboratories in regard to development and application of emerging biomarkers. Analytical laboratory methods and procedures that are mostly being developed today are emerging biomarkers of diseases targeted to certain organs that must have specific characteristics in order to meet the condition to be used as biochemical markers for detecting and differentiating certain diseases of a given organ. By applying this definition, we approach the so-called organ specificity of the human biochemical parameter, which changes in clinical enzymology, although to a limited extent. In following subchapters, an overview of some of the emerging biomarkers is presented (Fig. 5).

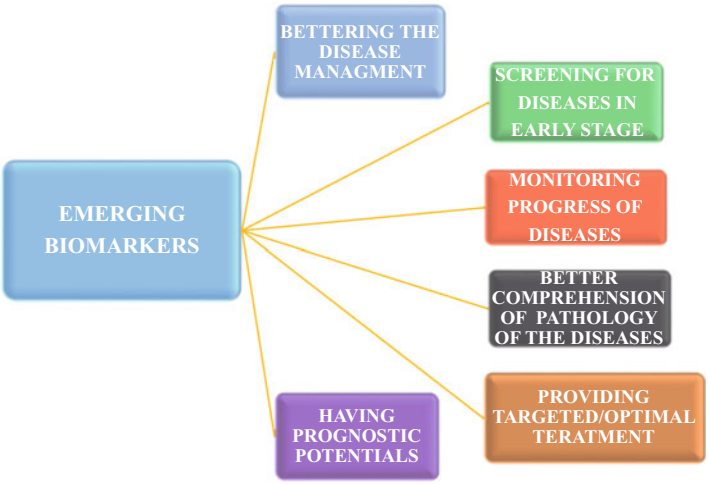

Fig. 5 The potentials of emerging biomarkers in bettering the disease management

An Overview on Emerging Biomarkers in Cardiovascular Events in Trauma, Injury Critical Care, and Recovery

The rapid assessment of patients who are in need of critical care due to cardiovascular symptoms that often are suggestive of an acute coronary syndrome is of great clinical importance. Emerging biomarkers progress toward being progressively significant in this critical care setting to help (Table 1) along with the standard diagnostics comprising electrocardiographic findings and patient history, especially since both mentioned nights sometimes be misleading. Today, cardiac troponin is still the only marker used routinely in this setting due to its myocardial tissue specificity and sensitivity, as well as its established usefulness for therapeutic decision-making. However, even current-generation troponin assays have certain limitations such as insufficient sensitivity for diagnosing unstable angina. Emerging biomarkers for cardiac state have the potential to overcome these limitations. It is inevitable to mention that further studies are needed to elucidate existing dilemmas regarding the optimal cutoffs for diagnosis and risk assessment and to provide as accurate as possible framework in which we can exclude the diagnosis of an acute myocardial infarction. It is also important to mention that some other nonmyocardial tissue-specific markers can help in regard to this diagnostic. Further studies are necessary before these emerging biomarkers can be adopted routinely in clinical practice.

Table 1 Emerging biomarkers in cardiovascular in trauma, injury critical care, and recovery

Blood biomarkers	Clinical use	Clinical use potential	References
Soluble suppression of tumorigenicity 2 (sST2)	Yes	Chronic heart failure and acute coronary syndrome have a prognostic value, while being unaffected by possible confounders such as renal failure, age, sex, and anemia. Serum soluble ST2 is a novel biomarker for neurohormonal activation in patients with heart failure. In patients with severe chronic NYHA class III to IV heart failure, the change in ST2 levels is an independent predictor of subsequent mortality or transplantation	Schernthaner et al. 2017; Li et al. 2021
Heart-type fatty acid binding protein (H-FABP)	Yes	It is found in striated muscle cells and is released to the systemic circulation as a consequence of myocardial damage, also an early indicator of myocardial infarction	Liebetrau et al. 2014
Growth differentiation factor (GDF-15)	Yes	Very useful biomarker in inflammatory processes, cardiovascular disease, and also cancer and kidney injury	Nair et al. 2017
Soluble urokinase-type plasminogen activator receptor (suPAR)	Yes	It is an inflammatory marker and is also useful for risk stratification concerning development of cardiovascular disease. Elevated levels reflect subclinical inflammation and are observed in individuals with increased alcohol consumption or smokers	Backes et al. 2012
Cystatin C	Yes	Background elevated plasma cystatin C levels reflect reduced renal function and increased cardiovascular risk	West et al. 2022
Cardiac troponin I (cTnI)	Yes	Noncausal biomarker for acute myocardial infarction – AMI	Moksnes et al. 2021
High monocyte to high-density lipoprotein cholesterol ratio (MHR)	Yes	High MHR at the time of initiation of dialysis may represent a useful predictor of cardiovascular complications	Kim et al. 2021

Emerging Biomarkers in COVID-19 Critical Care and Recovery

A pandemic of coronavirus disease 2019 (COVID-19), that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported from Wuhan, China, on 31 December 2019. It is still present and ongoing worldwide. Up to date, best to our knowledge, there is no efficient novel biomarker that would be able to

timely predict the disease progression uninfected. Therefore, all the emerging biomarkers or preexisting biomarkers now in the novel roll of having been used in last two and a half years as relevant in order to analyze the inflammatory profiles of COVID-19 patients indicate their implications in regard to the progression of COVID-19 disease and are of high importance (Table 2). Among the emerging COVID-19 biomarkers one related to coagulopathy process might be one of the most important. Coronavirus disease 2019 (COVID-19) makes patients highly vulnerable to thrombotic and thromboembolic events, provoking excessive inflammation, initiate the endothelial cell activation and injury, as well as inducing the platelet activation and hypercoagulability. Therefore, very often the patients with COVID-19 suffer a prothrombotic or thrombophilia state, with markable elevations in the levels of several emerging biomarkers of thrombosis, which are associated with disease severity and prognosis. Previously known D-dimers were mostly used as indicative early during the onset of the pandemic, but in the last year many novel biomarkers of thrombotic risk in COVID-19 have emerged. We can also notice that emerging biomarkers are found that indicate inflammation at early stage and ones that have prognostic value via respiratory function of patient. Up to date, there is still lack of strict protocol of monitoring defined set of emerging biomarkers in COVID-19. However, a thorough understanding of sensitivity and specificity of these emerging biomarkers might help risk stratification and prognostics, guide interventions, and provide a damage control intervention in future treatments. Further studies of this biomarkers are necessary.

Emerging Biomarkers of Infection in the Critical Care

Previously published evidence confirms that a share over 25% of all annual deaths in the world are due to infection (Dellinger et al. 2013). And sepsis is considered to be an infection (ongoing or threatening to happen) with consequences on the whole organism, on the systemic level, such as systemic inflammatory response syndrome (SIRS) (Dellinger et al. 2013). There is classification within the severity, and the severe sepsis is common; it occupies a significant share of critical care health unit resources, and even more importantly, this state is associated with high mortality (Angus et al. 2001). Good management of the disease and mortality prevention via timely response to infection and sepsis relay on timely diagnosis. The responses in sepsis are happening consecutive steps comprising inflammatory, humoral, cellular, and circulatory invalidities. Timely diagnosis and high-quality risk management of the disease enables quick response and targeted treatment, but are additionally demanding due to the fact that the signs and symptoms of sepsis are nonspecific and highly variable. Emerging biomarkers can be of great help and they can bring an added value to the process of valid decision-making by ruling in or out the presence of sepsis, identifying the severity of infection, and eventually in some cases determining an etiology (e.g., bacterial versus viral infection). Also, biomarkers can help differentiating systemic sepsis from local infection. In the past, a significant number of biomarkers have been already identified in infection and sepsis. However, the established biomarkers lack reliability, validity, and clinical utility confirmation and

Table 2 Emerging biomarkers in COVID-19 critical care and recovery

Blood biomarkers	Clinical use	Clinical use potential	References
Neutrophil extracellular traps (NETs)	Yes	A prothrombotic scaffold consists of neutrophil-derived chromatin associated with pro-coagulant proteins and antimicrobial proteins, such as myeloperoxidase (MPO) or neutrophil elastase. Studies have shown that NET components are present abundantly in plasma, serum, and post.mortem specimens from patients with COVID-19. Autopsy specimens from patients who died from COVID-19 have revealed NET-containing microthrombi in many cases	Zuo et al. 2020; Veras et al. 2020
Cell-free DNA	Yes	Showed a rather weak negative correlation with oxygenation parameters, but its decrease over time predicted the number of ventilator-free days	Huckriede et al. 2021
Myeloperoxidase-DNA complexes (MPO–DNA complexes)	Yes	Correlated strongly with COVID-19 severity and were also associated with thrombotic events in most studies	Guéant et al. 2020
Citrullinated histone 3 (H3Cit)	Yes	Correlated strongly with COVID-19 severity and was also associated with thrombotic events in most studies, also with requirement for respiratory support and mortality	Nicolai et al. 2020
Complement factor (C3, C5, C5a, C5b-9)	Yes	Were associated with disease severity, also studies showed increased activation of the complement system (higher levels of C5a and soluble C5b-9) in critically ill patients, suggesting prognostic utility	Ma et al. 2021
MicroRNAs	Yes	Were correlated with disease severity, and specific circulating microRNA profiles (such as miR-148a-3p, miR-451a, and miR-486-5p) seemed to have prognostic predictive utility	de Gonzalo-Calvo et al. 2021
Angiotensin-converting enzyme 2 (ACE2)	Yes	A case report found increased serum ACE2 levels in a patient with COVID-19 acute respiratory distress syndrome	Nagy et al. 2021
High-mobility group box protein 1 (HMGB1)	Yes	Elevated in the serum and plasma of patients with severe COVID-19 and is associated with an adverse prognosis	Chen et al. 2020
Progranulin	Yes	Expressed in epithelial cells, neurons, and macrophages and promotes inflammation and cell proliferation; progranulin levels were upregulated in patients with COVID-19 and were associated with adverse outcomes, suggesting prognostic utility	Rieder et al. 2020

(continued)

Table 2 (continued)

Blood biomarkers	Clinical use	Clinical use potential	References
Calprotectin	Yes	Was found to be elevated in the serum and plasma from patients with COVID-19 compared with the levels in healthy individuals, also was associated with disease severity and thrombotic risk	Bauer et al. 2021

still await further confirmations. In this subchapter, we provide an overview (Table 3) of the emerging biomarkers that are promising in diagnostics and prognostics. However, these emerging biomarkers also require further validation through more research and practice.

Clinical Outcomes and Emerging Biomarkers: Overlaps and Differences

Contrary to biomarkers, we have clinical outcomes that present type of parameters that do correlate to the health status of person who participates in a clinical trial. Namely, we observe how that participant feels, functions, or survives. Clinical outcomes are the variables that present self-perceived health-related aspects participating in the clinical trial (Fleming and Powers 2012) or objectively measured health-related aspects participating in the clinical trial. Lately with increasing number of clinical trials and both increasing number of emerging biomarkers, there is a noticeable shared approach to monitoring both emerging biomarkers and clinical outcomes when trying to understand the dynamic of the diseases and compose the optimal retreatment protocol. While some of the clinicians and researchers are prone to more trusting that clinical outcomes are the dominant and more applicable in practice, the other researchers rely on emerging biomarkers as well. Eventually, the most likely optimal approach would comprise parallel follow-up. In favor to this approach, we can encounter the situation in which after several repeated clinical trials outcome appears to be a solid indicator for certain health-related aspect, and therefore promoted to a novel biomarker. While this ability to indicate a certain aspect of health presents an overlap between an emerging biomarker and a clinical outcome, on the contra-side the aim of clinical practice is to decrease morbidity and mortality through observing clinical outcomes, while not having in focus the measurable aspects of patients' native biochemistry that are observed through biomarkers activity. Notwithstanding, we are all trying to identify optimal prevention, early detection, and best treatment for all diseases, not exclusively from the point in which we need quantifiable biomarkers that often but not always correlate with the disease but to have good direction in which we parallelly observe the somatic and mental indicators parallel to most likely outcome and through joint action set the final pathway. Thus, both emerging biomarkers and clinical outcome matter (Burke 2017). Some clinical

Table 3 Emerging biomarkers of infection in the critical care

Blood biomarkers	Clinical use	Clinical use potential	References
Soluble triggering receptor expressed on myeloid cells 1 (TREM-1)	Yes	It is a member of the immunoglobulin superfamily, and is greatly upregulated in infections, but not in noninfectious inflammatory conditions; also as an indicator of sepsis it is superior to those of CRP and PCT	Jiyong et al. 2008
Presepsin	Yes	It has a higher sensitivity and specificity in the diagnosis of sepsis as a new biomarker, and is a predictor for the prognosis of sepsis, also it seems that presepsin plays a crucial role as a supplemental method in the early diagnosis of sepsis	Zou et al. 2014
CD64	Yes	Neutrophil CD64 level was found to be an effective diagnostic biomarker for infection in patients with septic syndrome based on sepsis 2 criteria, also some studies showed that neutrophil CD64 outperformed CRP and PCT	Yeh et al. 2019
IL-27	Yes	A sepsis diagnostic biomarker in critically ill children. When used in combination with PCT, IL-27 may improve classification of critically ill adults with sepsis secondary to a non-lung source of infection	Wong et al. 2013
Cell-free DNA (cfDNA)	Yes	It can be considered a good prognostic marker of clinical outcome in septic patients. Its levels increase in case of acute kidney injury complicating sepsis, in particular if CRRT is needed, and are associated with poor outcome	Rhodes and Cecconi 2012
suPAR	Yes	A feasible biomarker for timely diagnosis and prognosis of sepsis. Compared with effective value of PCT, suPAR has similar clinical guiding value, whereas suPAR exhibits higher specificity, which can facilitate the deficiencies of PCT. It can be increased in various infectious diseases, in the blood and also in other tissues	Donadello et al. 2012
MicroRNAs (miRNAs)	Yes	It is associated with the presence and severity of sepsis. Dysregulation of several miRNAs, such as miR-146a, miR-223, miR-15a, miR-16, and miR-150, was found in the peripheral blood of sepsis patients	Wang et al. 2012

outcomes are superior than others. Outcomes that are unreliable or quantifiable give less insight; these include mitigation of not well-defined or unclear symptoms (e.g., severity of pain).

Conclusion

Emerging biomarkers can be of great aide in bettering disease management. The possibilities for these are manifold, especially in the fields of trauma, injury critical care, and recovery. The following instances in which biomarkers can be used effectively are mentioned by Jain: for a better comprehension of the path mechanics of diseases; when screening for diseases in an early stage while patients are still asymptomatic; in unambiguously diagnosing a disease; when writing up a precise description of a disease; in identifying prognoses; in laying a fundament for the development of therapeutics as well as monitoring of the disease with regard to the therapeutics being given, and by predicting which patients have a heightened probability of unwanted side effects of a treatment (Jain 2017). When monitoring patients in post-treatment period, with detection of recurrence or secondary progression of disease or complications, it is higly important to conclude that selecting the tailor-targeted therapies and drugs most likely results in favorable outcomes with a specific patient (personalized medicine).

Limitations of Biomarkers

Although the biomarkers present objective, measurable characteristic of biological activity and even supposing that the information obtained through biomarkers may be in accordance with a patient's own impression of his or her state of health and level of well-being, it is surely probable that this is incorrect. Furthermore, if no fluctuation can be detected or if the identified fluctuation has no relevant measurable effect on a patient's health, this specific aspect has no benefit of being defined as biomarker (Mayeux 2004). The exact same principle applies to many biological lineaments with markedly wide range of possible different modifications between people that minor evidence about health risks can be collected out of them.

To conclude, different types of emerging biomarkers in trauma, injury critical care, and recovery are presented. Clearly, as our knowledge in evidence-based medicine is growing, it is necessary for biomarker research to grow along. Hence, the search for novel biomarkers will continue and intensify and provide new information.

Mini-Dictionary of Terms

- Acute coronary syndrome – a term used to describe a range of conditions associated with sudden, reduced blood flow to the heart. One such condition is a heart attack (myocardial infarction) – when cell death results in damaged or destroyed heart tissue.
- Biocenosis – an association of different organisms forming a closely integrated community.
- Evidence-based medicine – medical practice or care that emphasizes the practical application of the findings of the best available current research.
- Organ specificity – characteristic restricted to a particular organ of the body, such as a cell type, metabolic response, or expression of a particular protein or antigen.
- SIRS – systemic inflammatory response syndrome (SIRS) is an exaggerated defense response of the body to a noxious stressor (infection, trauma, surgery, acute inflammation, ischemia or reperfusion, or malignancy, to name a few) to localize and then eliminate the endogenous or exogenous source of the insult.
- Sensitivity – the ability of a test to correctly identify patients with a disease.
- Sepsis – a potentially life-threatening condition that occurs when the body's response to an infection damages its own tissues.
- Specificity – the ability of a test to correctly identify people without the disease.

Key Facts on Emerging Blood Biomarkers in Trauma, Injury Critical Care, and Recovery

- Recent research has been targeted to identify novel sensitive and specific biomarkers for the improvement in the process of diagnosing, optimizing treatment, and bettering outcomes of diseases.
- Good management of the disease and mortality prevention via timely response to infection and sepsis relay on timely diagnosis.
- Although the biomarkers present objective, measurable characteristic of biological activity and even supposing that the information obtained through biomarkers may be in accordance with a patient's own impression of his or her state of health and level of well-being, it is surely probable that this is incorrect.
- We are all trying to identify optimal prevention, early detection, and best treatment for all diseases, not exclusively from the point in which we need quantifiable biomarkers that often but not always correlate with the disease but to have good direction in which we parallelly observe the somatic and mental indicators parallel to most likely outcome and through joint action set the final pathway.

Summary Points

- The emerging biomarkers should provide unbiased analysis with novel depth of many relevant proteins, they should have unmatched specificity and quantitative precision, high capacity, and easily accessibility.
- Emerging biomarkers can be of great aide in bettering disease management.
- Different types of emerging biomarkers in trauma, injury critical care, and recovery are presented day by day.
- Emerging biomarkers for cardiac state have the potential to overcome troponin's limitations.
- Very often the patients with COVID-19 suffer a prothrombotic or thrombophilia state, with markable elevations in the levels of several novel biomarkers of thrombosis, which are associated with disease severity and prognosis.
- Biomarkers can help differentiating systemic sepsis from local infection.

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Biomarkers in Neurological Injury: Fibrinogen, Fibrinogen/Fibrin Degradation Products (FDPs), and D-dimer

3

Ryuta Nakae, Yasuo Murai, and Shoji Yokobori

Contents

Abstract

Fibrinogen, fibrinogen/fibrin degradation products (FDPs), and D-dimer are important factors in neurological injury and its sequelae. When the blood–brain barrier is disrupted, fibrinogen and fibrin enter the brain. Fibrinogen, the precursor of fibrin, acts on all cellular components of the neurovascular unit and directly

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affects inflammatory, degenerative, and regenerative processes in neurological injury by binding to receptors on neuronal cells. Fibrinogen and fibrin are degraded by plasmin, reflecting primary fibrino(geno)lysis and secondary fibrinolysis, respectively. D-dimer, another important marker, is a degradation product of stabilized fibrin through secondary fibrinolysis. Fibrinolytic markers are greatly elevated in traumatic brain injury as a result of the hypercoagulable state caused by the production of large amounts of thrombin by TF released from the injured brain, subsequent consumptive coagulopathy, and a secondary hyperfibrinolytic state caused by the release of tPA from the injured brain and blood vessels. Among several randomized controlled trials, the CRASH-2 and CRASH-3 trials showed that the antifibrinolytic agent tranexamic acid reduces mortality by suppressing hyperfibrinolytic disseminated intravascular coagulation with abnormally high FDP and D-dimer levels.

Keywords

Biomarkers · Brain Injury · Coagulation · Fibrinolysis · Hemorrhage · Hemostasis · Fibrinogen · Fibrin · Fibrinogen/fibrin Degradation Products, D-dimer

Abbreviations

ACTH	adrenocorticotropic hormone
ACVR1	activin A receptor type I
α_2-PI	α_2-plasmin inhibitor
BBB	blood–brain barrier
BMP	bone morphogenetic protein
CNS	central nervous system
COVID-19	coronavirus disease 2019
DIC	disseminated intravascular coagulation
EGFR	epidermal growth factor receptor
FDP	fibrinogen/fibrin degradation product
ICH	intracerebral hemorrhage
NET	neutrophil extracellular trap
OPC	oligodendrocyte progenitor cell
PAI-1	plasminogen activator inhibitor-1
PIC	plasmin-α_2 plasmin inhibitor complex
SAH	subarachnoid hemorrhage
SCI	spinal cord injury
TAT	thrombin-antithrombin III complex
TBI	traumatic brain injury
TF	tissue factor
TGF-β	transforming growth factor-β
TNF-α	tumor necrosis factor-α
tPA	tissue-type plasminogen activator

tPA-PAI-1 complex	tissue-type plasminogen activator-plasminogen activator inhibitor-1 complex
TXA	tranexamic acid
uPA	urokinase-type plasminogen activator
XDP	cross-linked fibrin degradation products

Introduction

Fibrinogen, fibrinogen/fibrin degradation products (FDP), and D-dimer are the key biomarkers in the coagulation-fibrinolysis cascade and reflect the pathogenesis of hemorrhagic and ischemic brain injury. Fibrinogen is also reportedly involved in inflammation, degeneration, and regeneration processes of the injured central nervous system (CNS). In this chapter, we review the latest findings about fibrinogen, FDP, and D-dimer in patients with neurological injury.

Fibrinogen

Fibrinogen is a coagulation factor (coagulation factor I) in the form of a glycoprotein generated in the liver. It is present in the blood as a soluble homodimer (Tennent et al. 2007; Weisel 2005). It is composed of three polypeptide chains called Aα, Bβ, and γ linked by disulfide bridges (Mosesson 2005; Weisel 2005). Its approximate molecular weight is 340 kDa, with a biological half-life of 3–5 days. Its normal plasma concentration is 2.0–4.0 g/L (Weisel 2005). In injuries, vascular disorders, infections, and inflammatory diseases, the plasma fibrinogen concentration can increase several-fold, indicating its role as an acute-phase reactant. Plasma fibrinogen concentration increases with age toward the upper limits of normal. It has been reported that fibrinogen concentration increases by 0.25 g/L per decade, and about 81% of healthy individuals ≥65 years have fibrinogen concentrations ≥3.2 g/L (Hager et al. 1994).

The Fibrinogen and Coagulation Cascade (Fig. 1)

As fibrinogen is the only precursor of fibrin, which is indispensable for coagulation, it plays a crucial role in primary and secondary hemostasis (Mosesson 2005). When the coagulation cascade is activated, thrombin enzymatically removes fibrinopeptide A and B from fibrinogen, and exposes polymerization sites to accelerate clot formation (Mosesson 2005). Fibrinogen is then transformed by thrombin into insoluble fibrin, which is the major protein component of blood clots. Fibrin binds to activated platelets and stabilizes the platelet thrombus created in primary hemostasis, forming a fibrin clot (secondary hemostasis) (Rooney et al. 1996). Fibrin is considered to be a temporary matrix, and its rapid removal precedes tissue healing

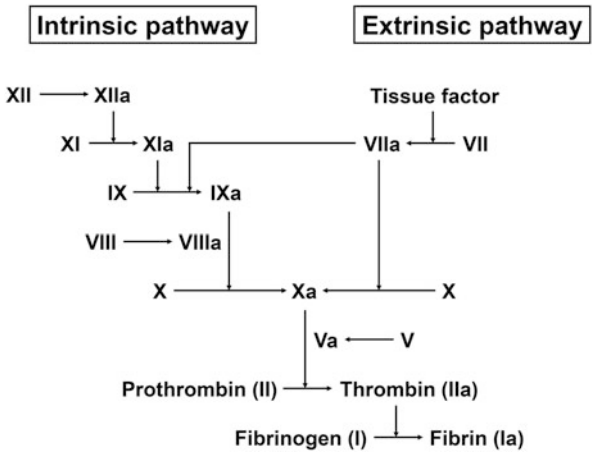

Fig. 1 Coagulation cascade. The coagulation cascade is divided into the intrinsic pathway and the extrinsic pathway

(Davalos and Akassoglou 2012). The fibrin clot is dissolved by plasmin, which is produced after tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA) activates plasminogen (Castellino and Ploplis 2005). Plasminogen activator inhibitor-1 (PAI-1) inhibits tPA and uPA, and decreases fibrinolysis (Castellino and Ploplis 2005).

Under physiologic conditions, the formation and degradation of fibrin, that is, coagulation and fibrinolysis, are in balance, but an imbalance between coagulation and fibrinolysis can lead to a variety of problems. Hypercoagulation leads to microthrombus formation that may induce ischemia, and hypocoagulation and/or hyperfibrinolysis lead to hemorrhagic lesions. Severe trauma, especially traumatic brain injury (TBI), can cause a significant decrease in fibrinogen, leading to uncontrolled or massive bleeding. The coagulation and fibrinolysis system is also involved in wound repair. In plasminogen-deficient mice, excessive or prolonged deposition of fibrin has been shown to inhibit wound healing (Romer et al. 1996). Proteolysis is important for the clearance of fibrin, and a damaged fibrinolytic system leads to excessive deposition of fibrin, resulting in impaired tissue repair.

Fibrinogen and Inflammation

While elevated plasma levels of FDP and D-dimer are useful indicators of inflammation and hypercoagulability, and are risk factors for thrombotic events (Lowe 2005), several studies have shown that fibrinogen-related proteins have additional functions, including direct cell signaling and host defense, suggesting that fibrinogen also plays other roles in the response to injury in addition to blood coagulation (Petersen et al. 2018). The pro-inflammatory function of fibrinogen and fibrin and its derivative peptides acts by binding to and activating immune cells via ligand-receptor interactions (Adams et al. 2004). Fibrinogen binds to a site different to

those involved in the coagulation cascade, transmitting signals that result in a pro-inflammatory function. A component of the perivascular extracellular matrix, fibrinogen directly affects other processes in the injured CNS by acting on all cellular components of the neurovascular unit and binding to receptors on microglia in the CNS (Davalos and Akassoglou 2012). Increased plasma fibrinogen concentration, even before extravasation into the perivascular space, is considered to be an indicator of inflammation and a high-risk marker for the development of vascular inflammatory diseases such as hypertension and atherosclerosis. The presence of fibrinopeptide B, which is cleaved from fibrinogen by thrombin, regulates the inflammatory response by inducing leucocyte chemotaxis (Skogen et al. 1988).

Fibrinogen and the CNS

The blood–brain barrier (BBB) forms a dynamic physical and metabolic barrier between the CNS and the systemic circulation. When the BBB is functioning properly, endothelial cells are linked by tight junctions and adherens junction proteins, and function as a transport system that controls the paracellular and transcellular movement of molecules and fluids between the CNS and the systemic circulation (Tietz and Engelhardt 2015). The BBB protects the CNS from hazardous substances in the blood, such as plasma proteins and immune cells, while promoting the elimination of toxins and waste substances and supplying it with essential nutrients for proper function (Zhao et al. 2015).

Fibrinogen is not present in the healthy brain and spinal cord. When the BBB is disrupted by TBI; spinal cord injury (SCI); stroke; degenerative diseases such as multiple sclerosis, Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis; infections such as encephalitis and meningitis; or psychiatric diseases such as schizophrenia and bipolar disorder, the permeability of the BBB is enhanced and fibrinogen flows into the CNS, resulting in the deposition of fibrinogen and fibrin (Hawkins and Davis 2005; Petersen et al. 2018). A sudden leak of blood into the CNS parenchyma after TBI, SCI, or intracerebral hemorrhage initiates a coagulation cascade that stops bleeding to prevent further tissue damage or death, and fibrinogen is converted to insoluble fibrin by perivascular tissue factors (TFs) and procoagulant proteins that are abundant after injury (Goodnight et al. 1974). Fibrinogen and fibrin deposition in the CNS can lead to local inflammation (Adams et al. 2004). TBI leads to ischemia of brain tissue, resulting in acute necrosis. The second phase of tissue damage is associated with the inflammatory reaction caused by TBI (Schnell et al. 1999). Interestingly, in some CNS diseases, destruction of the BBB and deposition of fibrinogen and fibrin occur prior to demyelination and other nerve damage, and a deeper understanding of fibrinogen and fibrin in the CNS has led to the development of new treatments (Zlokovic 2008). In fibrinogen knockout mice, the absence of fibrinogen and fibrin deposition markedly improved cerebral reperfusion and reduced cerebral infarction after ischemia and hypoxia (Adhami et al. 2006).

Disruption of the BBB and fibrinogen deposition in the CNS affects not only neurodegeneration and inflammation, but also the ability of damaged tissues to

regenerate. Several different cellular targets and new molecular mechanisms by which fibrinogen inhibits regeneration and repair mechanisms after CNS injury have been identified. Fibrinogen has been reported to be an important exogenous factor that inhibits neuronal regeneration and remyelination by regulating growth factor receptor signaling and inflammatory responses after CNS injury. Fibrinogen activates signaling by bone morphogenetic protein (BMP), transforming growth factor-β (TGF-β), and epidermal growth factor receptor (EGFR) in oligodendrocyte progenitor cells (OPCs), astrocytes, and neurons, respectively. This affects microglia and macrophage polarization, which promotes inflammation, and inhibits regeneration (Petersen et al. 2018). OPCs normally collect at a site of injury and differentiate into myelin-producing oligodendrocytes in a process called remyelination (Franklin and Ffrench-Constant 2008). Fibrinogen activates the BMP receptor activin A receptor type I (ACVR1) and downstream BMP-specific Smad proteins in OPCs, independently of free BMP ligands, and inhibits myelin production (Petersen et al. 2017). Fibrin also induces activation of microglia and macrophages, and may be toxic to OPCs and also inhibit remyelination (Davalos et al. 2012). In a model of demyelinating injury, fibrinogen depletion leads to decreased activation of the BMP pathway, increased numbers of mature oligodendrocytes in lesions, and accelerated remyelination (Petersen et al. 2017).

Fibrinogen also inhibits neurite outgrowth and leads to glial scarring by interfering with growth factor receptor signaling in neurons and astrocytes. Fibrinogen controls cellular functions as a ligand for several integrins and inhibits neurite outgrowth by binding to αVβ3 integrin and activating EGFR in neurons (Schachtrup et al. 2007). Fibrinogen exerts an indirect negative effect on axonal regeneration by interacting with perivascular astrocytes to form active TGF-β, which induces astrocytosis and stimulates the production of inhibitory proteoglycans that form glial scars. Fibrinogen binds to CD11b/CD18 integrin receptors, which leads to the differentiation of microglia to phagocytes in the CNS (Adams et al. 2007).

FDP and D-dimer

FDP result from fibrinogen and fibrin degradation by plasmin. Primary fibrino(geno) lysis is independent of coagulation activation and thrombus formation, in which tPA is either released from injured endothelial cells or by activation of the intrinsic fibrinolytic pathway, resulting in the development of a bleeding diathesis. Secondary fibrinolysis is caused by coagulation activation followed by the formation of a fibrin clot and the degradation of fibrin by plasmin. It occurs mainly in disseminated intravascular coagulation (DIC). FDP are measured as a marker of increased fibrinolysis, with increased fibrinogen degradation products in primary fibrino(geno) lysis and increased fibrin degradation products in secondary fibrinolysis.

Measurement of FDP is used as a screening test for fibrinolytic activity. However, it has been impossible to distinguish between primary and secondary fibrinolysis. Subsequently, a method was developed for measuring D-dimer, which is a degradation product of stabilized fibrin that reflects secondary fibrinolysis. D-dimer is also

called a cross-linked fibrin degradation product (XDP), because it represents products resulting from plasmin degradation of stabilized fibrin, which is cross-linked with unstable fibrin by the action of activated factor XIII (FXIIIa).

In healthy adults, plasma levels of FDP and D-dimer increase with age. It has been reported that mean plasma levels of D-dimer are two- to fivefold higher in subjects with age $\geq$ 60 years than in those <60 years (Cadroy et al. 1992). The higher D-dimer levels in the elderly is thought to be attributable to changes in fibrinogen catabolism, which is about 40% higher in elderly subjects (Hager and Platt 1995). In addition, since erythrocyte sedimentation rate, a marker of systemic inflammation, is significantly associated with increased D-dimer levels, higher D-dimer levels in the elderly may be due to a mild inflammatory state and increased comorbidities (Tita-Nwa et al. 2010), or due to increased production of TF in response to cytokine stimulation from endothelial cells (Pieper et al. 2000).

FDP, D-dimer and Fibrinolysis Cascades (Fig. 2)

Fibrinolysis begins (Fig. 2) when thrombin cleaves fibrinogen into a soluble fibrin monomer, which spontaneously polymerizes into a soluble fibrin polymer. Thrombin also activates FXIII, which, in the presence of calcium, cross-links the fibrin polymer, producing cross-linked fibrin polymer. Cleavage of cross-linked

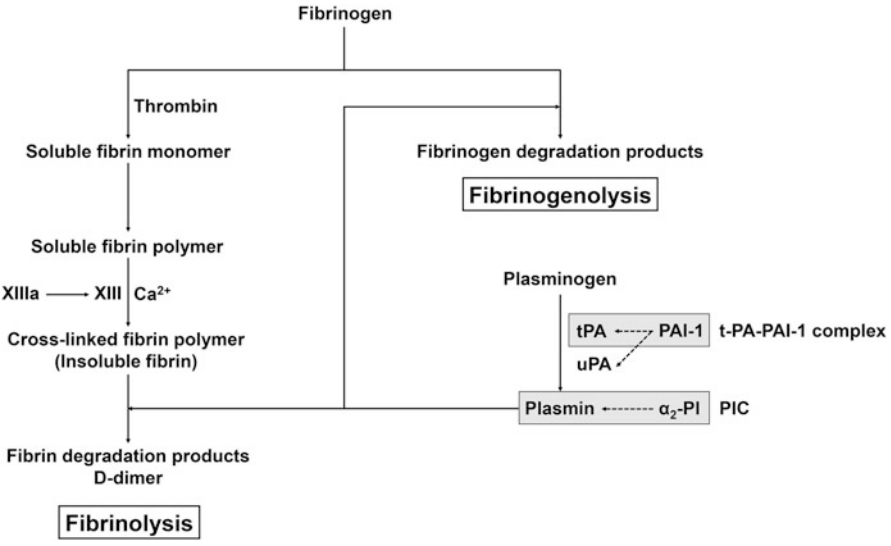

Fig. 2 Fibrinolysis cascade. The fibrinolysis cascade is divided into primary fibrinogenolysis and secondary fibrinolysis. α_2-PI, α_2-plasmin inhibitor; PAI-1, plasminogen activator inhibitor-1; PIC, plasmin-α_2 plasmin inhibitor complex; tPA, tissue-type plasminogen activator; tPA-PAI-1 complex, tissue-type plasminogen activator-plasminogen activator inhibitor-1 complex; uPA, urokinase-type plasminogen activator

fibrin polymer by plasmin results in fibrin degradation products. D-dimer, a neoantigen, is produced by the degradation of XIIIa-mediated cross-linking of fibrin polymer.

Fibrinogenolysis occurs when the fibrinolytic pathway is activated independent of the activation of coagulation. This results in the degradation of fibrinogen by plasmin, producing fibrinogen degradation products. "FDP" usually refers to a combination of fibrinogen and fibrin degradation products. Both fibrinogenolytic and fibrinolytic degradation products are detectable by the measurement of FDP, while only fibrinolytic products are detected by the measurement of D-dimer.

PAI-1 is produced by endothelial cells, smooth muscle cells, and fat cells as a 402-amino acid polypeptide. It is secreted into the circulation as a single-chain glycoprotein of 379 amino acids. PAI-1 inhibits serine protease. It binds to about 90% of the tPA produced in the blood in a 1:1 ratio, forming a tissue-type plasminogen activator-plasminogen activator inhibitor-1 complex (tPA-PAI-1 complex) that inactivates tPA. Another substance that inhibits tPA-induced fibrinolysis is α2-plasmin inhibitor (α2-PI). It also binds to circulating plasmin in a 1:1 ratio, forming a plasmin-α2-plasmin inhibitor complex (PIC) that inactivates plasmin. In addition to its primary function of fibrinolysis, plasmin also causes vascular endothelial damage, fibrinogen degradation, factor V and VIII degradation, and adrenocorticotropic hormone (ACTH) degradation. PAI-1 accumulates at the site of thrombus formation and accelerates fibrinolysis after a time lag, sparing normal vessels.

FDP, D-dimer, and the CNS

In hemorrhagic or ischemic brain injury, fibrinolytic markers such as FDP and D-dimer are elevated, reflecting the pathology of the injury. Fibrinolytic markers are also increased in cerebrovascular diseases such as subarachnoid hemorrhage (SAH), nontraumatic ICH, and cerebral infarction, but it is in TBI that fibrinolytic markers are greatly elevated (Nakae et al. 2016). In TBI, fibrinogen is also decreased, due to the hypercoagulable state caused by the production of large amounts of thrombin by TF released from the injured brain, subsequent consumptive coagulopathy, and a secondary hyperfibrinolytic state caused by the release of tPA released from the injured brain and blood vessels. In this state, fibrinolysis is strongly activated, hemostatic plugs tend to dissolve more easily, and bleeding symptoms tend to be more severe. Laboratory findings show a marked increase in both thrombin-antithrombin III complex (TAT) and PIC, as well as an increase in FDP and D-dimer (Asakura 2014). This type of DIC is called "enhanced-fibrinolytic-type DIC," in which fibrinogen degradation, known as primary fibrinogenolysis, predominates, resulting in an increased FDP/D-dimer ratio (Wada et al. 2017).

Fibrinolytic Biomarker and Treatment

Tranexamic acid (TXA) was developed in 1962 and has long been administered as a hemostatic agent after surgical procedures (Okamoto and Okamoto 1962). With a structure similar to that of lysine, it binds to the lysine-binding site of plasminogen and blocks its adsorption to fibrin, thereby exerting an antifibrinolytic effect. In other words, TXA has a hemostatic effect on bleeding associated with increased fibrinolysis. In recent years, evidence for TXA has been established in several fields in randomized control trials (CRASH-2 collaborators et al. 2010; CRASH-3 trial collaborators 2019; HALT-IT Trial Collaborators 2020; Myles et al. 2017; Post et al. 2021; Sprigg et al. 2018; WOMAN Trial Collaborators 2017) (Table 1). The most important reason for the positive or negative results may have been whether or not the disease was associated with increased fibrinolysis. In other words, trauma, especially TBI, cardiovascular surgery, and postpartum hemorrhage, may cause hyperfibrinolytic DIC with abnormally high FDP and D-dimer levels, and may benefit from the antifibrinolytic drug TXA. On the other hand, SAH and ICH do not cause DIC, so TXA may not be effective.

Applications to Prognosis, Other Diseases, and Conditions

Fibrinogen, FDP, and D-dimer are central biomarkers in the coagulation-fibrinolysis cascade; accordingly, they reflect the pathogenesis of hemorrhagic and ischemic brain injury and are reported to be prognostic factors. In TBI, a low fibrinogen concentration and high plasma level of D-dimer in the acute phase of TBI have been reported to be significantly associated with poor prognosis (Nakae et al. 2020, 2016). In cerebrovascular disease, high plasma level of D-dimer has been reported to be independent negative prognostic factor in aneurysmal subarachnoid hemorrhage (Juvela and Siironen 2006), intracerebral hemorrhage (Delgado et al. 2006), and cerebral infarction patients (Dougu et al. 2011). Other than neurological injury, it has been reported that D-dimer is a predictor of the presence of and mortality in acute aortic dissection (Mori et al. 2016; Nazerian et al. 2018), mortality in acute myocardial infarction (Zhang et al. 2021), the presence of pulmonary embolism (Righini et al. 2014), and mortality in coronavirus disease 2019 (COVID-19) pneumonia (Zhou et al. 2020).

Recently, the FDP/fibrinogen ratio or D-dimer/fibrinogen ratio has been reported to be a better indicator of the severity and activity of diseases, providing simple and quick ways for clinical diagnosis. In TBI, the D-dimer/fibrinogen ratio has been shown to be a strong predictor of the progression of hemorrhagic lesions after TBI (Xu et al. 2020). In cerebrovascular disease, the D-dimer/fibrinogen ratio has been reported to be useful in determining whether an ischemic stroke is cardioembolic or atherothrombotic (Alvarez-Perez et al. 2011). Other than neurological injury, it has been reported that the FDP/fibrinogen ratio or D-dimer/fibrinogen ratio is a novel predictor of mortality and massive transfusion in severe trauma (Lee et al. 2018), mortality in post-percutaneous coronary intervention patients with cardiovascular disease (Bai et al. 2020), and the presence of pulmonary embolism (Kucher et al. 2003).

Table 1 Summary of randomized control trials on Tranexamic acid (TXA). The CRASH-2 trial, CRASH-3 trial, ATACAS trial, and WOMAN trial showed that TXA was effective, while the ULTRA trial, TICH-2 trial, and HALT-IT trial did not show any benefit

Trial	Target disease	Analysis objects	TXA dose	Results of primary outcomes
CRASH-2 trial	Trauma	10,060 TXA group vs. 10,067 placebo group	1 g/10 min + 1 g/8 h	All-cause mortality and risk of death within 4 weeks of injury due to bleeding were significantly lower in the TXA group (14.5% and 4.9%, respectively) than in the placebo group (16.0% and 5.7%, respectively)
CRASH-3 trial	Traumatic brain injury	4613 TXA group vs. 4514 placebo group	1 g/10 min + 1 g/8 h	In mild-to-moderate head injury, risk of head injury-related death within 28 days of injury was significantly lower in the TXA group (5.8%) than in the placebo group (7.5%)
ULTRA trial	Subarachnoid hemorrhage	480 TXA group vs. 475 control group	1 g/10 min + 1 g/8 h	No significant difference in good clinical outcome at 6 months between the two groups (59.8% vs. 63.2%)
TICH-2 trial	Nontraumatic intracerebral hemorrhage	1,152 TXA group vs. 1,155 placebo group	1 g/10 min + 1 g/8 h	No significant difference in functional status at 90 days between the two groups
ATACAS trial	Coronary artery surgery	2,311 TXA group vs. 2,320 placebo group	100 mg/kg/ 30 min	Reoperation rate due to major hemorrhage and transfusion volume of red cells during hospitalization were significantly lower in the TXA group (0.8% and 3 units, respectively) than in the placebo group (2.2% and 4 units, respectively)
WOMAN trial	Postpartum hemorrhage	10,036 TXA group vs. 9,985 placebo group	1 g iv	Death due to bleeding within 42 days of giving birth was significantly lower in the TXA group (1.5%) than in the placebo group (1.9%)

(continued)

Table 1 (continued)

Trial	Target disease	Analysis objects	TXA dose	Results of primary outcomes
HALT-IT trial	Acute gastrointestinal bleeding	5,956 TXA group vs. 5,981 placebo group	1 g/10 min + 3 g/24 h	No significant difference in death due to bleeding within 5 days between the two groups (3.7% vs. 3.8%)

ATACAS, aspirin and tranexamic acid for coronary artery surgery; CRASH-2, clinical randomization of an antifibrinolytic in significant hemorrhage; CRASH-3, clinical randomization of an antifibrinolytic in significant head injury; HALT-IT, effects of a high-dose 24-h infusion of tranexamic acid on death and thromboembolic events in patients with acute gastrointestinal bleeding; TICH-2, tranexamic acid for hyperacute primary intracerebral hemorrhage; TXA, tranexamic acid; ULTRA, ultra-early tranexamic acid after subarachnoid hemorrhage; WOMAN, effect of early tranexamic acid administration on mortality, hysterectomy, and other morbidities in women with post-partum hemorrhage

Key Facts About Fibrinogen

- As far back as the fifth to fourth century BC, physicians recorded the presence of fibers in circulating blood (Costa-Filho et al. 2016).
- At the end of the seventeenth century, Malpighi used a single lens microscope to note that whole blood clots have a fibrous and corpuscular component (Douglas 1999).
- In 1847, the term "fibrinogen'" was coined by Virchow, who stated "should one want to give it a name, it could be called fibrinogen" (Virchow 1847), but Virchow had not discovered fibrinogen (Beck 1975).
- In 1859, Denis was the first to recognize that plasma contained a clottable substance that differed from fibrin and subsequently attempted to purify and characterize this protein.
- In 1879, Hammarsten was the first to isolate fibrinogen (Rosenfeld 1982).

Key Facts About D-dimer

- In 1973, Gaffney reported the finding of a unique fragment derived from human fibrin by the hydrolytic action of plasmin (Gaffney 1973).
- It has been shown that fragments, which are cross-linked dimers of fibrinogen fragment D, are released during plasmin digestion of fibrin clots cross-linked with γ-γ chains.
- Subsequently, this fragment was given the name of D-dimer (Gaffney and Brasher 1973).

Mini-Dictionary of Terms

- Fibrinogen: A glycoprotein coagulation factor generated in the liver. The normal range of plasma fibrinogen is 2.0–4.0 g/L.
- FDP: Abbreviation for fibrinogen/fibrin degradation products.
- D-dimer: A degradation product of stabilized (cross-linked and insoluble) fibrin.
- Primary fibrino(geno)lysis: A fibrinogen degradation pathway independent of the activation of coagulation cascade.
- Secondary fibrinolysis: A fibrin degradation pathway that is part of the activated coagulation cascade.

Summary Points

- This chapter focuses on fibrinogen, fibrinogen/fibrin degradation products (FDP), and D-dimer, which are important factors in patients with neurological injury.
- Fibrinogen is not present in the healthy brain and spinal cord, being blocked by the blood–brain barrier (BBB).
- When the BBB is disrupted by trauma, stroke, degenerative diseases, infections, and other diseases, it becomes permeable to fibrinogen, resulting in the deposition of fibrinogen and fibrin in the central nervous system (CNS).
- As fibrinogen is the only precursor of fibrin, which is indispensable for coagulation, it plays an important role in the coagulation-fibrinolysis cascade.
- Fibrinogen also directly affects inflammation, degeneration, and regeneration processes in the injured CNS by acting on all cellular components of the neurovascular unit and binding to receptors on glial cells.
- FDP includes both products from fibrinogen degraded by plasmin, and from fibrin degradation by plasmin, and reflects primary fibrino(geno)lysis and secondary fibrinolysis.
- D-dimer is a degradation product of stabilized fibrin and reflects only secondary fibrinolysis.
- Fibrinolytic markers are somewhat increased in cerebrovascular diseases, but it is in traumatic brain injury (TBI) that they are greatly elevated, with an associated hypercoagulable state caused by the production of large amounts of thrombin by tissue factor (TF) released from the injured brain, subsequent consumptive coagulopathy, and a secondary hyperfibrinolytic state caused by the release of tissue-type plasminogen activator (tPA) released from the injured brain and blood vessels.
- Among several randomized controlled trials, tranexamic acid was found to be effective in improving mortality in TBI because trauma, unlike cerebrovascular disease, is associated with hyperfibrinolytic disseminated intravascular coagulation (DIC) with abnormally high FDP and D-dimer levels.

Cross-References

► Viscoelastic Hemostatic Tests and Fibrinogen Concentrations in Trauma

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Nerve Injury and Biomarkers

4

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Contents

Abstract

The nervous system has enormous scale to its production. From the large-scale ganglia to the microscopic neuron, there is tremendous communication that serves a synergistic role. Our ability to identify deficits, or absences, in such communication is critical toward detecting and treating pathological conditions and states of injury, as well as optimizing human performance. Research to date has focused on very large-scale observations of the human nervous system, providing insight through computed tomography, positron emission tomography, and magnetic resonance imaging of the brain. More recent developments have allowed us to make significant advances in our ability to monitor neurological health in finer neuronal structures of the peripheral nervous system and identifying pathology through molecular and genetic markers. As we proceed from the more granular to fine-grained observations, our ability to detect pathology grows, but so does the level of noise. Techniques such as machine learning and artificial

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intelligence will help reduce this noise and provide a more accurate picture of a healthy neuron and clinical decision points for treatment.

Keywords

Biomarkers · Nerve injury · MRI · MRS · Brain injury · mTBI · Concussion · Machine learning · Artificial intelligence · Behavior · Affect

Abbreviations

AI	Artificial intelligence
CHO	Choline
EEG	Electroencephalography
fMRI	Functional magnetic resonance imaging
ML	Machine learning
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
mTBI	Mild traumatic brain injury
NAA	N-acetyl aspartate
PET	Positron emission tomography
TBI	Traumatic brain injury

Summary Points

- -Nerve injuries can be appreciated at multiple levels throughout the nervous system from behavior to cell.
- -Neuroimaging can act as an isolated and complementary technique that can provide information on nerve injury, neurodegeneration, and behavioral correlates.
- -Molecular methods of identifying nerve injury and secondary elements of nerve injury are becoming more common.
- -Standardized methods of interpreting nerve injury data are required to understand and classify healthy from pathological cases.
- -Machine learning and artificial intelligence will become central players in the identification of biomarkers of nerve injury.

Introduction

The ability to identify the presence of a nerve injury has evolved over the decades as technology has provided more insight and higher resolution for evaluating the central and peripheral nervous system as well as the statistical techniques for connecting findings with behavioral performance. In early decades, and in many places of the world today, insight may be limited to psychological observations and

identifying changes in behavior. This approach focuses on the behavioral outcome of the nervous system, its coordinated use of different pathways to ultimately produce a goal-directed, or reflexive, behavior. We, as a scientific community, have shown that as you strip down the layers of the nervous system, relying on behavior to determine changes in neuronal function has inherent limitations that supersede individual variability. The human nervous system is tremendously good at adapting and using alternative circuits, pathways, and networks when there are deficiencies in any previous one. This ability of the brain to work in series, as well as parallel, provides biological organisms an unparalleled ability to learn and adapt, but also represents the challenges in identifying a neurological injury. In this chapter, we walk through biomarkers of nerve injury from the level of psychological and behavioral, to brain imaging, and using molecular genetic approaches and conclude with a discussion on the use of higher-level analytical approaches including artificial intelligence to identify signal from noise.

Psychological and Cognitive and Behavioral Testing

The principle underlying identification of nerve injury using behavioral and psychological testing relies on an inferring *deviation* in behavior. It implies some level of either structural or functional deviation to neuronal integrity when understood in terms of pathology and has pioneered modern approaches in brain imaging toward understanding the relationship between brain and behavior. Similar to the presence of behavioral deviations in the classical case of Phineas Gage which were marked through heightened levels of aggressive behaviors from a disinhibited – damaged – frontal lobe, more recent attempts attempt to identify specific behaviors that can accurately identify a particular brain circuit.

In the context of brain injury, behavioral testing has long been applied to understand the behavioral manifestation of a potential underlying nerve injury. Disinhibited responses are common in persons with brain injury, and testing of traits such as aggression (Cusimano et al. 2014) is regularly performed to understand underlying pathology (Juengst et al. 2020). The identification of "clinically relevant" pathology in terms of concussion is a great case in point as, even today, it is difficult to resolve any quantifiable pathology; however, there is significant behavioral evidence for abnormal neurobehavioral performance. There are multiple tests available for identification of neurobehavioral differences after a suspected mTBI including the SCAT5 (Echemendia et al. 2017), post-concussion symptom scale (Riegler et al. 2019), and others. All of these effectively target symptoms including standard orienting questions (e.g., time and place), attention and memory function, as well as headaches and affective symptoms (e.g., depression or anxiety) can also occur (McCrory et al. 2017). More targeted approaches exist for specific populations including brain trauma from cancer (Zarrella et al. 2021), stroke (Pasotti et al. 2020), and multiple sclerosis (Wojcik et al. 2019) to ascertain the precise nature of a neurobehavioral insult.

More recently, researchers have attempted to understand how behavior connects with changes in the brain through online evaluation of neurofunctional characteristics during a task of interest. The focus in these investigations is to understand not only the behavioral elements but to evaluate changes in blood flow or oxygenation or electrical activity (EEG) that goes into task performance. This altered approach integrates two important perspectives: (1) does the task require more or less of the same mechanisms as would be seen in healthy controls; (2) does the task integrate brain regions that are not usually seen to support this task. Performance of such tasks can help understand that even when performance is equivalent between a patient cohort and a healthy control cohort that the underlying neurofunctional performance is different. This was demonstrated by Holmes and colleagues (2018) during a spatial navigation task, showing that pediatric persons with symptom levels comparable to healthy controls used unique regions of their brains to complete a given task (S. A. Holmes et al. 2019), a finding that could underlie residual fatigue and difficulties with attention in more chronic settings. Similar work has been completed with other neurocognitive tasks using different modalities such as the n-back task (R. S. Saluja et al. 2015), the flanker task (D. R. Sullivan et al. 2018), and visual tracking eye movements tests (Ventura et al. 2016). For a review of this work, see Lunkova et al. (2021).

Peripheral Neurography of Nerve Fiber Integrity

The peripheral nervous system is often overlooked during neuroimaging investigation, partly in response to the difficulty in isolating and evaluating nerve fibers and partly in response to the immense complexity of different functional subdivisions which include but are not restricted to the sympathetic and parasympathetic. Despite this, there is extensive opportunity to evaluate the range of peripheral nerve injuries (see Table 1) that can occur which can lead to chronic pain conditions. Currently, different methods exist for evaluating nerve fiber integrity in the peripheral nervous system that probe specific aspects of nerve structure or function. In the following sections, we outline several methods that are cable of providing insight into the peripheral nervous system and its subdivisions. The peripheral nervous system is composed of nerve fiber tracts which originate from or project to the spinal cord. Imaging of the peripheral nervous system is quite difficult based on the non-uniform

Table 1 Peripheral nerve injury grading system (R. Sullivan et al. 2016)

Grade	Relevant findings
Grade 1	Possible demyelination; neuropraxia
Grade 2	Disruption of axon; axonotmesis
Grade 3	Disruption of the axonal and endoneurial process
Grade 4	Disruption of the axonal, endoneurial and perineural process
Grade 5	Irreversible damage to the axon, myelin sheath, and stroma; neurotmesis

Peripheral nerve injury grades and characteristics

spatial distribution of nerve fiber bundles – whereas in the brain, there is relative consistency between where certain nerve fiber tracts are enabling the co-registration of images across participants and with a standardized atlas. As such, more manual work is required to delineate a nerve fiber from the surrounding tissue.

Diffusion tensor imaging (DTI): White matter in the central and peripheral nervous system is quite difficult to appreciate and is often evaluated based on its property of restricting water flow along axonal pathways. In doing so, markers sensitive to the axial, radial, and mean diffusion of water can be calculated with alterations highlighting potential interruptions in water flow or providing more movement than in a healthy situation. There appears to be significant variability present in nerve fibers of the peripheral nervous system. This has been observed when evaluating a person's lower legs where dominance confers a degree of lateralization in nerve fiber characteristics. In particular, it appears that the more dominant leg (taken relative to dominant hand) shows differences in the tibial and sciatic nerve fiber distributions suggesting less restricted water flow in the contralateral limb (Holmes et al. 2021a; b). The same group has also shown that in persons with neuropathic pain from an ankle sprain injury, there is quantifiable damage to peripheral nerve fiber structures that appears to be more pronounced as the nerve fiber gets more distal from the spinal cord. In this study, there were differences in axial, mean, and radial diffusivity in persons with a neuropathic pain injury suggesting a less confined environment relative to healthy controls, alluding to the presence of edema and inflammation from nerve injury (Holmes et al. 2021a, 2021b). These findings underscore the value of performing peripheral neurography to understand peripheral nerve fiber characteristics in clinical and non-clinical settings.

Central Markers of Nerve Fiber Integrity

We are only able to ascertain a coarse understanding of nerve fiber integrity from central nervous system imaging. There are roughly 100 billion neurons in the human brain (Herculano-Houzel 2012), and the size of a standard voxel resolution in MRI is roughly 0.8 mm. To combat this, averages are produced within known anatomically, or functional defined regions to assist in understanding gross neurological structure. Alternatively, post-mortem or biopsy-related investigations can be performed that offer more in-depth analyses but are only available during specific scenarios that warrant post-mortem investigation (e.g., Alzheimer's diagnosis) or neurosurgical biopsies (e.g., tumor resection). The following section outlines some techniques for understanding neurological health in different contexts using techniques that require varying levels of intervention.

The ability to resolve changes in neuronal tissue in the brain can be targeted toward both gray and white matter regions. For example, a progressive decrease in gray matter volume is suggestive of neurodegeneration. Traumatic brain injury, mostly the more severe forms but more recently evidence is suggestive of even in mild forms, can result in neurodegeneration and loss of brain tissue volume which

can be appreciated through a technique called voxel-based morphometry (Cole et al., 2018). This impact on neuronal tissue can be diffused in the context of a brain injury, limiting the sensitivity of the technique; however, there has been extensive research suggesting that certain brain regions may be more sensitive to the trauma of a TBI such as the hippocampus and thalamic nuclei (Sandry and Dobryakova 2021), suggesting that the technique may be better suited toward some neuroanatomical regions than others.

Techniques aimed at identifying changes in white matter pathology are currently best viewed, in an in vivo model, using diffusion tensor imaging (DTI). Researchers have noted that DTI is a strong predictor of neurodegeneration after moderate and severe traumatic brain injury (N. S. N. Graham et al. 2020). The specific ability of diffusion techniques (R. Saluja et al. 2018) provides a compelling opportunity to understand changes in axonal integrity, as well as the local environment (e.g., myelin sheaths, localized edema). Others have used more advanced versions of diffusion imaging such as a network diffusion model to again predict the presence of neurodegeneration in traumatic brain injury in more severe forms of brain injury (moderate to severe) (Poudel et al. 2020). The sensitivity of the technique for identifying and predicting long-term symptoms in less severe forms of brain injury is less reliable and a matter of current research.

Magnetic resonance spectroscopy is a technique that can provide insight into the molecular environment in a noninvasive way, ideal for special populations and informing clinical care. Available in different forms (e.g., proton and phosphorus-based), it can yield insight into the presence and extent of certain molecules that can inform neurological health. In the context of nerve injury biomarkers, one of the most prominent compounds is N-acytyl aspartate (NAA) that reflects neuronal health as a marker of CNS-based metabolism, connected to fatty acid production, myelin synthesis, and supporting neuronal mitochondria (Moffett et al. 2007). In persons with severe traumatic brain injury, biomarkers that showed elevated levels compared to controls were IL-4, IL-6, Il-8, IL-10, TNFa, sFas, BDNF, and cortisol. Of these, five were associated with unfavorable outcome: IL-6, IL-8, IL-10, TNFa, and cortisol (Hvingelby et al. 2022). In the context of brain injury cohorts, NAA has been shown to be significantly reduced in the majority of studies whereas choline signal (CHO) is usually found to be elevated, signaling membrane turnover (Croall et al., 2015). More support for the role of NAA and CR in the context of identifying brain injury shows an association for NAA/CR to Tau deposition in cognitively unimpaired older adults (Kara et al. 2022). In the pediatric setting, H-MRS has the potential for monitoring the metabolic arena of the central nervous system in a noninvasive way (Aida 2022; Menshchikov et al. 2020). Together, MRS has provided a powerful tool to understand changes in the molecular environment of persons with central nerve injuries, in a noninvasive way.

Use of Molecular Genetics to Understand Nerve Injury

Forces required to produce a traumatic brain injury or nerve injury are usually sufficient to produce a secondary injury, often implicating a diffuse perfusion event and inflammatory cascade. As technologies improve, our ability to resolve differences in the molecular genetics of a patient are improving. Either in terms of their base DNA, or in how the DNA becomes expressed, there are more findings supporting the use, and application, of molecular genetic approaches to understand aberrant nerve functioning and structural pathology. A major advantage to this approach is the resolution it offers, where other, less sensitive techniques such as magnetic resonance imaging may not be able to resolve. In the following paragraphs, we go into examples of how molecular genetics is contributing to our understanding of nerve fiber pathology.

As shown by Holmes and colleagues (2020), a peripheral nerve injury has the capacity to drive central brain changes. The authors demonstrated that an ankle sprain injury that leads to clinically confirmed neuropathic pain is associated with cortical atrophy of the primary motor and sensory cortex. The neurological basis for this could result from multiple aspects including: (1) neurological adaptation, where existing synapses are degraded and new ones formed to adapt to the injury; (2) atrophy resulting from associated disability; and (3) the impact of chronic pain on central brain processes. At a molecular level, the authors performed a transcriptome analysis and found that there were distinct elevations of neuroinflammatory markers in persons with clinically confirmed neuropathic pain. Markers such as GRIN2B, IL-1B, LINGO1, and 30 other mRNA molecules were either elevated or suppressed relative to an age- and sex-matched cohorts. Notably, integration of the molecular findings provides complementary evidence to the neuroimaging work, supporting the reason for their elevation or suppression to be due to central nervous system processes and informing neuro-adaptive efforts and chronic pain vulnerability.

Position emission tomography (PET) is a neuroimaging technique that uses radiolabeled compounds to identify the amount of a particular receptor or process in the brain. In the context of nerve injury, there are different PET tracers including 11C-Pittsburgh compound-B to identify fibrillar amyloid beta pathology that is routinely applied in the context of both brain injury and Alzheimer's disease populations. Another compound, 18F-AV1451, 18F-T807 shows specific non-displaceable binding to tau neurofibrillary tangles in post-mortem Alzheimer's disease brain tissue, and there is potential for the identification of phosphorylated version of Tau using 18F-FDDNP. The application of PET can be used to understand secondary events associated with neurodegeneration using 11C-PBR28 to identify activated microglia that may co-locate with amyloid and tau pathology (N. S. Graham and Sharp 2019). The limitations associated with PET ligands reflect principally the ability to match a ligand with a compound of interest and the facilities to generate the ligand in sufficient quantities to introduce it into the subject prior to the compounds half-life dissipating beyond detectable quantities.

There are other notable methods of identifying either trauma to neurons or the secondary events after neurotrauma. For example, post-mortem staining of brain

tissue can identify multiple processes that include protein biomarkers for neuronal cell body injury (UCH-L1, NSE), astroglial injury (GFAP, S100B), neuronal cell death (asll-spectrin breakdown products; e.g., cell body injury UCH-L1; necrosis SBDP150; apoptosis SBDP120), axonal injury (NF proteins), and white matter injury (myelin basic protein) (Wang et al. 2018). Alternatively, oxidized phospholipids, products of oxidative stress within the CNS, have been shown to be mediators of neurodegeneration in multiple disease models (frontotemporal lobe dimension, spinal cord injury, multiple sclerosis) (Dong and Yong 2022). More recently, work on SARS-CoV-2 in post-mortem samples has shown upregulation of inflammatory markers in neuronal tissues to include major histocompatibility complex-1, necrotizing myopathy (Suh et al. 2021). Please see Wilcox et al. (2021) for a review of the molecular profile of human neurons associated with nerve repair.

Artificial Intelligence

The acceleration of hardware and software to support machine learning and artificial intelligence pursuits is immediately appreciated in the research and clinical context of identifying nerve injuries. For a while now, there have been attempts to use AI-based algorithms to identify, from input images of CT or MRI scans, a person who has sustained a traumatic brain injury, or other neurological insult from a healthy person. Primitive attempts at this were moderately successful, especially with lower order (e.g., two dimensional data). The continued pursuit of higher-order magnetic fields to improve image resolution for MRI, as well as the integration of behavioral data, only reinforce the growing need for artificial intelligence to be a mainstay in the field of neuroscience and neurological injuries.

Deep learning is a field of artificial intelligence that uses multiple hidden layers of algorithms that help distinguish unique features of an input data space to classify a given output. These hidden layers provide a "black box" like approach, each responsible for assigning a weighting factor to a particular thronch of data; however, only the output layer provides actual output for the classifier. Deep learning has been used in the diagnosis of different forms of neuropathy such as diabetic neuropathy. In a study by William, Borroni, and colleagues (2019), the authors used a deep learning algorithm alongside confocal microscopy to distinguish persons with corneal neuropathic pain from healthy controls. Their algorithms developed a specificity of 0.87 and sensitivity of 0.68 for patient classification. A similar analysis was performed by Koseoglu et al. (2018) who used an AI-based approach on corneal neuropathic pain to successfully identify patients from healthy controls.

In the context of traumatic brain injury, machine learning and AI have been applied using multiple modalities. One of the most performed imaging scans clinically for TBI is the CT scan, used typically to rule out major brain hemorrhages after an injury. In a review by Brossard et al. (2021), the authors outline how AI approaches using CT have provided moderate success, with the largest gain provided in terms of automation, and the identification of patterns that are not perceivable by a

human. These algorithms (e.g., convolutional neural network) are capable with good levels of sensitivity and specificity to use an input image of a CT on persons ranging from mild to severe TBI to perform both the segmentation and quantification of lesions from a TBI (Monteiro et al. 2020). Their application has also been demonstrated in the context of prenatal and neonatal care, identifying patterns of brain injury from early life stressors in the womb, correlating with inflammation identified in the placenta after birth (Liao 2022). Together, there is significant potential for the growing capacity of machine learning and artificial intelligence algorithms to help inform nerve injury and improve clinical care.

Remaining Questions

Despite the progress that has been made in terms of the behavioral, imaging, and molecular sides of nerve fiber damage, there remain important questions to fully engage the field on a clinical level.

1. How do we determine deviance? This is a particularly important question when attempting to evaluate neuroimaging of nerve fiber integrity and emphasize the importance of including healthy controls, and if possible, within participant longitudinal analyses. As these are often not available, attempting to understand more distal divisions of nerve fiber bundles is currently very difficult as they can demonstrate significant spatial and temporal variability and therefore are harder to contextualize without a reference standard.
2. What is a clinically significant change? Nerve fiber pathology and the presence of pain/symptom presence are two very different elements. It is possible to have significant pathology to a neuron (centrally or peripherally) within any over behavioral symptoms. At this point, if there are no clinical symptoms, is the information clinically relevant? The answer to this question is very much a subject of current research efforts that attempt to understand the connection between nerve fiber changes and affective symptoms presentation, and the long-term implications of sub-clinical pathology (see, e.g., central sensitization (Borsook et al. 2018)).

Conclusions

As techniques improve, we are developing greater pictures for when nerve fiber damage occurs both in the central and peripheral nervous system. However, one of the major limitations that we have to address is the level of inter-subject variability in neural architecture and gene expression. This limitation presents a significant barrier from translational efforts. To help combat this, there is a growing use of techniques that integrate much larger data sets that include machine learning and artificial intelligence and more precisely, supervised and unsupervised approaches toward classification of tissue that can be deemed injured and tissue that is termed “normal.”

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Blunt Abdomen Trauma and Biomarkers

5

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Contents

Abstract

Blunt abdomen trauma (BAT) consists of one of the major emergencies that can result in a wide range of severity from mild simple stable to potentially life-threatening conditions. Recognition of the injury of the particular organ and the grading of the injury as early as possible is one of the keys to management of the blunt abdomen trauma. The recognition of biomarkers in diagnosis and management of BAT is trending slowly. The use of biomarkers in blunt abdomen trauma is still in its early stage, and no standard practice has been established till date. Several biomarkers are reported for specific organ injury in BAT and also several biomarkers are reported for overall prediction of the prognosis of the patients with severe BAT. In this chapter, we discuss the developments in various biomarkers for BAT and its fundamental application that can help the modern physician to provide supplementary arsenal in diagnosis and management of BAT.

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Keywords

Abdomen · Application · Biomarker · Blunt · Hollow · Kidney · Liver · Pancreas · Prognosis · Trauma · Viscus

Abbreviations

ALT	Alanine transaminase
AST	Aspartate transaminase
ATLS	Advanced Trauma Life Support
BAT	Blunt abdomen trauma
CRP	C-reactive protein
CT	Computed tomography
Cys-C	Cystatin-C
HMGB-1	High mobility group box-1
HVI	Hollow viscus injury
I-FABP	Intestinal fatty acid-binding protein
IL-18	Interleukin 18
KIM-1	Kidney injury molecule-1
LDH	Lactate dehydrogenase
NGAL	Neutrophil gelatinase-associated lipocalin
NLRP3	NACHT domain-, leucine-rich repeat-, and PYD-containing protein 3
PCT	Procalcitonin
WHO	World Health Organization

Introduction

Blunt abdomen trauma (BAT) consists of one of the major emergencies that require urgent care and specialized consultation. They can result in a wide range of severity from mild simple stable to potentially life-threatening conditions. It presents a major challenge to the attending emergency physicians and surgeons (Smyth et al. 2022). The basic mechanism of blunt abdomen trauma can have a spectrum of effect from particular organ to multiple organ injury including musculoskeletal system. Recognition of the injury of the particular organ and the grading of the injury as early as possible is one of the keys to management of the blunt abdomen trauma. With the development of ATLS (Advanced Trauma Life Support), the advancement in the management of trauma has taken a giant leap. With the introduction of several protocols and imaging investigations, the management of blunt abdomen trauma has improved within short duration. The recognition of biomarkers in diagnosis and management of BAT is trending slowly.

The World Health Organization (WHO), in coordination with the United Nations and the International Labor Organization, has defined a biomarker as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" (Nations et al. 2014). The use biomarkers in blunt abdomen trauma are still in its early stage and no standard practice has been

Table 1 List of all the possible organ specific biomarkers for diagnosis in BAT

Organs	Biomarkers
Liver	ALT, AST, LDH
Kidney	NGAL, KIM-1, Renalase, IL-18, Cys-C
Hollow viscus organs	I-FABP, NLRP3, HMGB-1
Pancreas	Amylase, lipase

ALT alanine transaminase, *AST* aspartate transaminase, *CRP* C-reactive protein, *Cys-C* cystatin-C, *HMGB-1* high mobility group box-1, *IL-18* interleukin 18, *I-FABP* intestinal fatty acid-binding protein, *KIM-1* kidney injury molecule-1, *LDH* lactate dehydrogenase, *NGAL* neutrophil gelatinase-associated lipocalin, *NLRP3* NACHT domain, leucine-rich repeat-, and PYD-containing protein 3, *PCT* procalcitonin

established till date. In this chapter we will discuss about application of biomarkers in BAT (Table 1).

Biomarkers for Liver Injury

Aspartate Transaminase (AST) and Alanine Transaminase (ALT) Transaminases are mitochondrial and cytoplasmic enzymes. AST and ALT are present in hepatocytes in high concentration, and following BAT, they leak into blood circulation. Their main function is to catabolize amino acids, permitting them to enter the citric acid cycle. AST is typically found in the liver only but ALT is also found in the heart skeletal muscle, kidney, brain, and RBC. Recently AST and ALT both have been studied extensively for its application to diagnose liver injury in BAT. Where computed tomography (CT) scan is available, it is considered the gold standard for the diagnosis of liver injury in BAT (Fig. 1) (Iacobellis et al. 2019). In the remote and periphery centers where CT scan is not available, AST and ALT may provide valuable guidance to the emergency physician to suspect liver trauma. Patients will be greatly benefitted from on timely referral of the patient to the tertiary trauma center (Shrestha et al. 2021).

Application to BAT: Koyoma et al. reported the optimal cut-off values of AST and ALT were 109 U/l and 97 U/l, respectively, for the patients with liver injury in blunt abdominal trauma. They suggested the optimal cut-off value as a predictor and also screening tool for CT scans for the presence of liver injury (Koyama et al. 2016). Recently various studies have shown that AST and ALT are some of the valuable biomarkers for liver injury in blunt abdominal trauma (Shrestha et al. 2021; Chang et al. 2017). These studies not only report that AST and ALT are important biomarkers for diagnosis of liver injury in BAT, they also suggested that the level of AST and ALT also helps to predict the severity of the liver injury. The higher the level of AST and ALT, the grade of liver injury increased accordingly (Shrestha et al. 2021). The cut-off value of various researches has been given in Table 2.

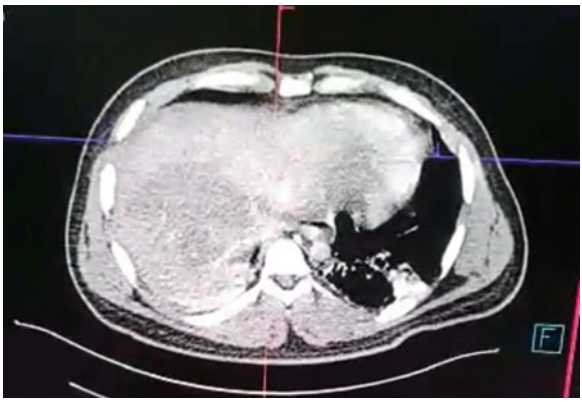

Fig. 1 CT scan showing injury of the right lobe of liver due to BAT (Courtesy of Dr. Yuvraj Raut, Chitwan Medical College Teaching Hospital, Department of Radiology, Chitwan, Nepal)

Table 2 List of cut-off value of AST and ALT by different authors for liver injury in BAT

Authors with publication year	Cut-off level for AST (U/L)	Cut-off level for ALT (U/L)
Tan et al. (2009)	82	126
Lee et al. (2010)	60	58
Tian et al. (2012)	113	57
Koyama et al. (2016)	109	97
Chang et al. (2017)	200	125
Shrestha et al. (2021)	106	80

ALT alanine transaminase, *AST* aspartate transaminase

Lactate Dehydrogenase (LDH) LDH is a cytoplasmic enzyme that is expressed in almost all major organ systems. It is released into the peripheral blood following cell death caused by ischemia or injury, for example. Because of its ubiquitous expression, the total serum LDH level is a highly sensitive, but nonspecific test.

Application to BAT: In the study done by Bilgic et al. the LDH level on admission was correlated with mortality and the severity of liver injury. It was also independently associated with mortality in multivariate analysis. High LDH levels may reflect the number of affected organs and the severity of injury (Bilgic et al. 2014). Similarly the study done by Tan et al. reported that patients with normal ALT, AST, and LDH are unlikely to have major liver injuries (Tan et al. 2009). It is advised that LDH can be used together with AST and ALT as a complementary test for liver injury in patients with blunt abdominal trauma. It cannot be used as diagnostic test since CT scan is the gold standard for the diagnosis of liver injury in blunt abdominal trauma (Iacobellis et al. 2019).

Biomarkers for Kidney Injury

Renal injuries are suspected frequently among patients who sustain blunt abdominal trauma. About 8 to 10 percent of the total patients presenting with blunt abdominal trauma was diagnosed with kidney injury (Alonso et al. 2009; Harris et al. 2001). Children are especially prone to blunt renal injuries because of the small proportion of retroperitoneal and abdominal fat tissue (Brown et al. 1998). The kidney size, the weak expression of muscles, the elastic ribs, and their anatomical position are the factors which add additional risk for blunt renal injury (Hirsch et al. 2017). Ultrasonography is the diagnostic method of choice for the examination of traumatic renal injury for children, and in case of severe injury, CT scan could be considered (Amerstorfer et al. 2015). For adults, CT is the technique of choice for evaluating the renal trauma as it can give accurate information about the status of the renal parenchyma, blood vessels, and collecting system (Bonatti et al. 2015; Park et al. 2012). Recently some of the new biomarkers have been reported for kidney injury in BAT.

Neutrophil Gelatinase-Associated Lipocalin (NGAL) NGAL is a 25 kDa protein belonging to the lipocalin family (Singer et al. 2013). The role of NGAL has been well established in patients with burn as a biomarker for acute kidney injury (Hong et al. 2013; Sen et al. 2015).

Application to BAT: Even though study in human population has not been done, promising reports on rat models have been done. In the experimental rat model study conducted by Bakal et al. they found that both serum and urinary level of NGAL were significantly increased immediately after the trauma. They concluded that the NGAL was an important noninvasive biomarker in the early recognition of blunt renal trauma (Bakal et al. 2019).

Kidney Injury Molecule-1 (KIM-1) KIM-1 is a type 1 trans-membrane protein, with an immunoglobulin and mucin domain, whose expression is markedly upregulated in the proximal tubule in the post-ischemic rat kidney. It was first reported by Ichimura et al. that KIM-1 was not present at a detectable level in normal rat kidneys, whereas it was increased in the kidneys of rats after acute renal ischemia (Ichimura et al. 1998). Similarly, another study indicated that urinary KIM-I might be preferable to conventional biomarkers in human studies as a noninvasive, rapid, sensitive, reproducible, and potentially high-throughput method to detect early kidney injury (Vaidya et al. 2006).

Application to BAT: No human studies have been done for the diagnostic utility of KIM-1 as biomarker for detecting blunt renal trauma. In the same experimental study conducted by Bakel et al. in rat model for NGAL, they reported there was a significant difference in the KIM-1 values after blunt renal trauma (Bakal et al. 2019). KIM-1 has shown positive evidence for the future research as a biomarker for blunt renal trauma.

Interleukin 18 (IL-18) Interleukin-18 (IL-18) was first described as "IFNg-inducing factor" in 1989 as a pro-inflammatory cytokine that is structurally and functionally related to the IL-1 family (Dinarello et al. 2013). A role for IL-18 has been implicated in several diseases, including renal injury (Leslie and Meldrum 2008).

Application to BAT: IL-18 is also one of the promising potential noninvasive biomarkers for blunt renal trauma. Like NGAL and KIM-1, this biomarker has not been studied in human population. In the study conducted by Bakel et al. in rat model, they reported that urine IL-18 levels of the blunt renal trauma group were significantly higher than those of the sham and control groups. However, serum IL-18 levels were significantly higher in both the sham and trauma groups compared with the control group (Bakal et al. 2019). Therefore, elevated serum levels of IL-18 do not seem to be associated with blunt renal trauma, and detailed clinical and experimental studies are needed to determine the potential utility of these markers in routine care.

Cystatin-C (Cys-C) Cystatin C (CysC) is a 13-kDa endogenous cysteine proteinase inhibitor produced at a constant rate by all nucleated cells and eliminated by glomerular filtration (Herget-Rosenthal et al. 2000). Hence, serum CysC is an early biomarker of AKI that can reflect the early changes in renal function and the decline of GFR (Liu et al. 2016). In traumatic hemorrhagic shock, CysC is significantly increased in the early stage of the shock and CysC can be used as a marker to predict AKI (Chen et al. 2015).

Application to BAT: Recently CysC has also been studied as a possible biomarker for blunt renal trauma. In the study done by Bakel et al. in the trauma group, serum and urine Cys-C levels reached their highest level within 60–72 h. They reported that although Cys-C levels do not seem to be beneficial in the early detection of trauma, they could be useful during the monitoring of the patient (Bakal et al. 2019). The limitation of this biomarker needs to be further studied in order to be used as a biomarker for kidney injury in BAT.

Renalase In 2005, the identification of renalase was reported. The human kidney releases this protein into the bloodstream to regulate blood pressure and breaks down catecholamines like adrenaline and noradrenaline in the blood circulation (Xu et al. 2005).

Application to BAT: Its role as a supplement in diagnosing of renal injury in blunt abdomen trauma is still in preliminary phase. Recently, the levels of renalase for the diagnosis of renal injury in rats with experimentally induced blunt renal trauma were investigated (Saraç et al. 2021). They reported that the level of renalase increased significantly in the rats grouped with blunt renal trauma (Saraç et al. 2021). Further studies are needed to be done for this biomarker.

Biomarkers for Pancreatic Injury Due to its retroperitoneal position, the injury to the pancreas in BTA is often difficult to diagnose. CT scan is the gold standard for diagnosing pancreatic injury in BTA. Serum amylase and serum lipase have been used as a diagnostic tool to diagnose acute pancreatitis. The use of serum lipase and amylase in the diagnosis of blunt pancreatic injury can be quiet challenging as the level of these enzymes may significantly increase in several other non-pancreatic-related conditions.

Serum Amylase and Lipase Amylase, a digestive enzyme, is predominantly secreted by the pancreas and salivary glands. Amylases' main function is to hydrolyze the glycosidic bonds in starch molecules, converting complex carbohydrates to simple sugars (Peyrot des Gachons and Breslin 2016). Serum amylase is elevated in a variety of conditions, including pancreatic disease, salivary disease, decreased metabolic clearance, intestinal disease, and macroamylasemia. It is one of the important criteria for diagnosing acute pancreatitis (Banks et al. n.d.). Lipase is an enzyme that is present in pancreatic secretions which breaks down triglycerides into free fatty acids and glycerol. They play a vital role in fat digestion. The increase in serum lipase can be seen in various pathologies like acute pancreatitis, chronic pancreatitis, peritonitis, small intestine manipulation, etc.

Application to BAT: Recently various studies have been reported as their role in blunt pancreatic injury. In the recent study reported from the Republic of Iran, they reported comparison of laboratory findings of amylase enzymes in patients with internal organ and pancreatic damage were higher than in patients without internal organ injury (Hosseininejad et al. 2020). Out of 384 patients, the patients diagnosed with pancreatic injury by focused assessment with sonography for trauma (FAST) scan, CT scan, and laparotomy had mean serum amylase level of 157.96, 83.47, and 93.42, respectively. There was statistical difference in mean amylase level between the non-pancreatic injury and pancreatic injury group. Similarly, serum lipase level was also higher in patients with blunt pancreatic trauma. The mean serum lipase level was 118.89 in patients with pancreatic injury. This suggests that measuring these enzymes could support the clinical suspicion of pancreatic damage as a reliable and cost-effective screening method in countries with limited resources where CT scan is not available. This may help the surgeons and emergency physicians to take proper measures in management of these patients. Similarly, a study done in India has also reported similar findings with some extra guidelines. Mahajan et al. have reported that combined serum amylase and lipase showed 100% specificity and 85% sensitivity in predicting pancreatic injury (Mahajan et al. 2014). The mean values of serum amylase in patients with low-grade injuries were 733 compared to 1323 in the high-grade group. For serum lipase, the corresponding figures were 618 and 1051, respectively. Elevated vs. normal serum amylase and lipase levels showed sole statistically significant association with time elapse since injury to admission, with a cut-off of 3 hours. They also pointed the time frame that persistently elevated or rising combined estimation of serum amylase and lipase levels are reliable indicators of pancreatic injury and is time dependent, non-diagnostic within 6 h or less after trauma.

Biomarker for Hollow Viscus Injury (HVI) CT has been shown to be accurate for the diagnosis of bowel and mesenteric injuries and is the diagnostic test of choice in the evaluation of BAT in hemodynamically stable patients. Sometimes injury to the hollow viscus is only found during laparotomy (Fig. 2).

Intestinal Fatty Acid-Binding Protein (I-FABP) The biomarkers for the HVI are the least explored one. The increase in WBC count has been studied as a biomarker for HVI in the first 24 hours. But the delay in diagnosis of HVI even for more than 8 hours has a very poor prognosis. Usually CT scan are considered gold standard for HVI. Recently I-FABP have been the point of interest by many researchers as a biomarker for intestinal diseases. I-FABP is a small (14–15 kDa), cytosolic, water-soluble protein that comprises up to 2% of the cytoplasmic protein content of the mature enterocyte and is abundant in bowel mucosa. If the intestinal mucosal tissue is injured, I-FABP is rapidly released into the bloodstream (Gajda and Storch 2015). I-FABP is elevated in several types of bowel disease, such as small bowel obstruction, mesenteric ischemia, acute enterocolitis, Crohn's disease, ulcerative colitis, and necrotizing enterocolitis (Cronk et al. 2006; Heida et al. 2015; Lieberman et al. 1997; Sarikaya et al. 2015; Wiercinska-Drapalo et al. 2008).

Application to BAT: Till now only one study has reported I-FABP as a potential biomarker for HVI in BAT (Matsumoto et al. 2017). The mean I-FABP was 9.92 ng/ml in patients with HVI and in patients with non-HVI was 3.97 ng/ml. The sensitivity of I-FABP was 76.9% and specificity 70.0%. Because the accuracy of I-FABP alone was insufficient, they combined the I-FABP with peritonitis sign to improve the sensitivity. None of the patients with negative I-FABP and a negative peritonitis sign developed HVI for which sensitivity was 100% and negative predictive value was 100%. Similarly, to improve specificity, I-FABP was combined with extra-luminal air findings on CT. The patients had both a positive I-FABP and extra-luminal air on CT, all of those patients developed HVI with specificity of 100%

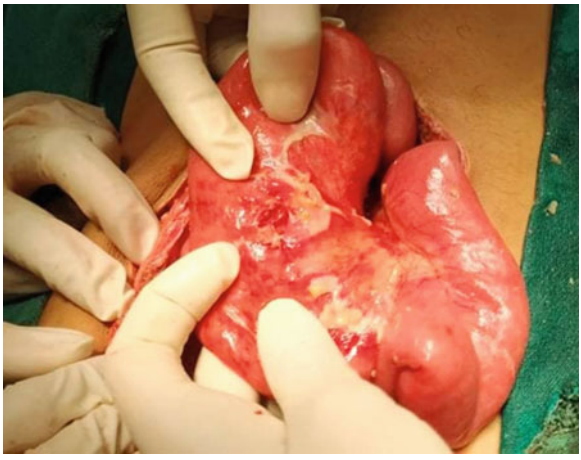

Fig. 2 Jejunal perforation due to BAT (courtesy of Dr. Dilip Baral, Department of Surgery, Pokhara Military Hospital, Pokhara, Nepal)

and positive predictive value of 100%. They suggested that for patients with negative I-FABP and negative peritonitis signs, HVI can be ruled out at the time of admission and patients with both a positive I-FABP and extra-luminal air on CT, diagnosis of HVI should be strongly considered. We conclude that the combination of I-FABP and physical examination may be able to rule out HVI. Further studies should be done in high-volume centers.

Biomarkers for Prognosis of the Patients with Severe BAT The diagnosis of blunt abdomen trauma is not only important; the proper protocol for the management of BAT is very crucial. During management of the BAT, determining prognosis of the patients with BAT is also very important.

NLRP3 and High Mobility Group Box-1 (HMGB-1) HMGB-1, a member of the alarmin group of cellular messaging proteins, is a pro-inflammatory cytokine that has been proven to be associated with post-traumatic inflammation (Yang et al. 2015). Studies have demonstrated that NACHT domain-, leucine-rich repeat-, and PYD-containing protein 3 (NLRP3) plays an important role in HMGB-1-mediated inflammation in many diseases and bioprocesses. NLRP3 can facilitate in vivo HMGB-1 release, and HMGB-1 can induce an increase in the level of IL-1b by activating the NLRP3 inflammasome.

Application to prognosis of BAT: In the study done by Sun and Xia et al. they demonstrated that the serum levels of NLRP3 and HMGB-1 were significantly higher in all BAT patients than in the healthy controls, and the serum levels of NLRP3 and HMGB-1 were significantly higher in the severe BAT group than in the mild/moderate BAT group (Sun and Xia 2019). They also reported that serum levels of NLRP3 and HMGB-1 were significantly higher in the deceased patients than in the living patients, suggesting that NLRP3 and HMGB-1 levels might be associated with death in severe BAT patients. They also reported that serum levels of NLRP3 and HMGB-1 were correlated with 6-month mortality in severe BAT patients. We strongly suggest that NLRP3 and HMGB-1 can play a significant role in determining the prognosis of the patients with severe BAT and multi-institutional study is required for its validity.

C-Reactive Protein (CRP) CRP is an acute-phase reactant protein synthesized by the liver, whose level rises in response to inflammation (Du Clos 2000). It is primarily induced by the IL-6 action on the gene responsible for the transcription of CRP during the acute phase of an inflammatory/infectious process (Sproston and Ashworth 2018). The alteration of baseline CRP levels are affected by many factors including age, gender, smoking status, weight, lipid levels, and blood pressure (Hage and Szalai 2007). The average levels of serum CRP in a healthy adult is around 0.8 mg/L. There is growing evidence of usefulness of CRP to predict prognosis in septic shock patients in ICU. The patients with dropping CRP level after admission had better prognosis than the patients whose CRP level didn't drop. The cut-off value of CRP for diagnostic accuracy of severe sepsis in critical patients was found to be 61 mg/L (Anush et al. 2019; Pradhan et al. 2016).

Application to prognosis of BAT: The role of CRP in prognosis of patients with trauma has been studied especially in pediatric population (Brunengraber et al. 2009). They reported that monitoring of the early CRP would correlate with clinical morbidity and outcome measures in pediatric trauma patients and also in estimating injury severity early in hospitalization. One study stated a controversial report that CRP is not valuable in adult population with BAT (Giannoudis et al. 2009). Still reliable study is needed to done for CRP to mark as a biomarker for predicting prognosis of patient with BAT.

Procalcitonin (PCT) Serum PCT a protein is the peptide precursor of calcitonin, a hormone that is synthesized by the parafollicular C cells of the thyroid and involved in calcium homeostasis. Although PCT is usually produced in the thyroid, during bacterial infections it is released by the neuro-endocrine cells of the lung and intestine and as an acute-phase reactant (Davies 2015). Studies have shown that, in ICU patients with sepsis, higher PCT levels are associated with a greater risk of progression to severe sepsis and septic shock, worsening the survival prognosis (Gregoriano et al. 2020; Rajkumari et al. 2013; Vijayan et al. 2017).

Application to prognosis of BAT: Recently PCT has recently become of interest as a possible marker for the prognosis of patients with severe trauma including abdomen trauma (Castelli et al. 2009; Maier et al. 2009; Sakran et al. 2012; Wanner et al. 2000). PCT has also been used as an independent prognostic biomarkers in children with severe trauma (Weber et al. 2021). Initial elevation of PCT is transient and after 48 hours they tend to decrease. During this time frame, higher serum PCT levels appear to indicate a poorer prognosis in patients (Koutroulis et al. 2014). In the recent review done by Alrawahi et al. they suggested that early rise of serum PCT may be used as an early predictor of severe injury, development of sepsis and MOD, and mortality in trauma population. They also indicated that patients with high level of PCT are warranted for aggressive management to prevent high morbidity and mortality (Alrawahi et al. 2019). However all these studies are not specific to blunt abdomen trauma. Multicenter prospective trials are needed to investigate the impact of PCT-guided decision-making on the clinical outcomes in the BAT setting.

Unsolved Mysteries

We all know that splenic injury is the most common organ injured in BAT but still no study has been done about the possible biomarkers for splenic injury. Also biomarkers for the injuries to the diaphragm and biliary system in BAT are also needed to in the future. To recognize these injuries, still we are fully dependent on imaging investigations (Gupta et al. 2004). They are also sometimes recognized during laparotomies (Figs. 3 and 4).

Fig. 3 Avulsed spleen following BAT (courtesy of Dr. Dilip Baral, Department of Surgery, Pokhara Military Hospital, Pokhara, Nepal)

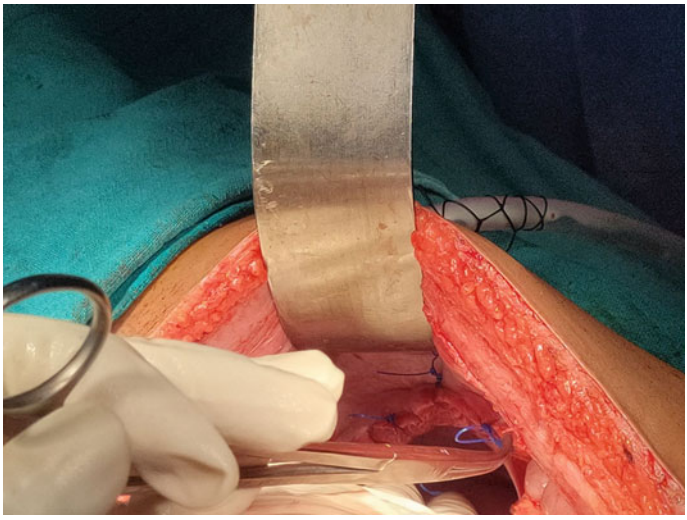

Fig. 4 Repaired diaphragm injury which was due to BAT (courtesy of Dr. Anup Shrestha, Department of Surgery, Indira Gandhi Memorial Hospital, Male, Maldives)

Summary

- The fundamental application of biomarkers can help the modern physician to provide supplementary arsenal in diagnosis and management of BAT.
- AST and ALT may provide valuable aid to the emergency physician to diagnose liver injury and also predict its grade of injury in BAT.
- LDH can be used together with AST and ALT as a complementary test for liver injury in patients with blunt abdominal trauma.
- The biomarkers for kidney injury in BAT are NGAL, KIM-1, IL-18, Cys-C, and renalase. They need to be studied in human population.
- Elevated vs. normal serum amylase and lipase levels showed sole statistically significant association with time elapse since injury to admission, with a cut-off of 3 hours.
- The combination of I-FABP and physical examination may be complementary to each other to rule out hollow viscus injury.
- The serum levels of NLRP3 and HMGB-1 correlates with 6-month mortality in severe BAT patients.
- Reliable studies are needed to be done for CRP to mark as a biomarker for predicting prognosis of patient with BAT.
- High levels of PCT are warranted for aggressive management of BAT to prevent high morbidity and mortality.
- The biomarker for splenic injury, biliary system, and rupture of diaphragm injury due to BAT remains an untouched subject.

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Therapeutic Strategies in Patients with Postoperative Elevation of Cardiac Biomarkers

6

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Abstract

Patients with preexisting cardiac conditions are at increased risk of perioperative complications, especially cardiac complications, during and after noncardiac surgery. The sensitivity and specificity of cardiac biomarkers has been steadily optimized in recent years, allowing improved assessment of the risk of cardiac complications perioperatively. In the nonsurgical setting, cardiac biomarkers have a high value in the diagnosis of acute cardiac events, and evidence-based treatment recommendations exist, but prospectively evaluated guidelines and recommendations for the management of perioperative myocardial injury and infarction are lacking and should be the subject of future clinical research. In this chapter, potential treatment strategies for patients with postoperative elevation of cardiac biomarkers and perioperative myocardial injury and infarction are discussed.

Keywords

Perioperative myocardial infarction · Perioperative myocardial injury · Postoperative elevation of cardiac biomarkers · Perioperative cardiac risk assessment · Therapeutic strategies · Treatment algorithm · Cardiac biomarkers · Troponin · Prevention · Perioperative optimization

Abbreviations

ANP	atrial natriuretic peptide
ASS	acetylsalicylic acid
BNP	brain natriuretic peptide
ECG	electrocardiography
HFpEF	heart failure with preserved ejection fraction
MACE	major adverse cardiac events
MET	metabolic equivalents
MINS	myocardial injury after noncardiac surgery
MR-proANP	midregional-proANP
NSTEMI	non ST-segment myocardial infarction
NT-proBNP	N-Terminal proBNP
PBM	patient blood management
PCI	percutaneous coronary intervention
PMI	perioperative myocardial injury or infarction
Pre-proAVP	pre-provasopressin
RAAS	renin angiotensin aldosterone system
RCRI	revised cardiac risk index
SGAR-SSAR	Swiss Society for Anesthesia and Resuscitation
STEMI	ST-segment myocardial infarction
URL	upper reference limit

Introduction

Peri- and postoperative mortality is among the most common reasons of death in Europe. The mortality rates are found to be 3% after elective and 5–10% after urgent or emergency surgery (Pearse et al. 2012; Nepogodiev et al. 2019). Diseases associated with increased perioperative mortality include preexisting cardiac conditions such as coronary artery disease or heart failure (Pearse et al. 2012). An analysis of over 600,000 patients showed a 90-day mortality of more than 10% in patients undergoing complex surgery and suffering from heart failure (Lerman et al. 2019).

With the widespread adoption of cardiac biomarker determination in the diagnosis of myocardial infarction, there is mounting scientific interest in the use of cardiac biomarkers for guidance of perioperative clinical decision-making in patients at cardiovascular risk.

Association Between Postoperative Elevation of Biomarkers and Outcome

The ground-breaking results of the VISION trials confirmed the findings of previous smaller studies (Filipovic et al. 2003; Levy et al. 2011) and established the association between postoperative troponin release and adverse outcomes. These data have transformed the paradigm of perioperative risk stratification in major (noncardiac) surgery, forming the basis of novel management strategies (Devereaux et al. 2012, 2017; Botto 2014). A further important finding is that prognostically relevant postoperative troponin release is often not associated with development of clinical symptoms (e.g., chest pain) or electrocardiographic (ECG) changes. Hence, prognostically relevant myocardial injury cannot be detected without systematic perioperative screening (Devereaux 2011; Smilowitz et al. 2019). In another investigation, perioperative myocardial injury was present in 16% of patients undergoing noncardiac surgery, of whom 9% died within 30 days, compared to a 30-day mortality of 2% in patients without postoperative troponin dynamics (Puelacher et al. 2018).

Systematic postoperative troponin screening has been recommended in selected patients at cardiovascular risk (Duceppe et al. 2017); however, to date there is a lack of consensus pertaining to treatment strategies for patients with signs of perioperative myocardial injury or infarction. In this chapter, we discuss potential treatment strategies for patients suffering from perioperative myocardial injury or infarction (PMI).

Pathophysiologic Background of Myocardial Injury and Infarction

According to the "Fourth Universal Definition of Myocardial Infarction," myocardial infarction is defined as the simultaneous presence of troponin elevation above the 99th percentile upper reference limit (URL) and newly developing ischemic

features such as chest pain, dynamic ST-segment changes, new wall motion disorders, or detection of coronary artery thrombus in coronary angiography or autopsy (Thygesen et al. 2018). Myocardial infarction can further be subdivided into type I and type II and can result in myocardial cell death due to prolonged ischemia. The distinction between myocardial infarction type I and type II is based on the etiology of myocardial ischemia. In contrast, myocardial injury is defined as any elevation of cardiac troponin above the 99th percentile URL without signs of ischemic features and consequently defined as **acute** myocardial injury if dynamic changes in troponin levels (rise and/or fall) are present.

In type I myocardial infarction, oxygen supply is absolutely impeded due to an acute plaque disruption and thrombotic occlusion of an atherothrombotic plaque. Erosion of the culprit lesion can cause mobilization of thrombotic material, which cuts off distal coronary supply, and plaque rupture may further lead to intraluminal thrombosis and/or coronary hemorrhage (López-Cuenca et al. 2016; Thygesen et al. 2018; Sabatine 2021). Integrating ECG findings in the clinical presentation, type I myocardial infarction can be subdivided into ST-segment myocardial infarction (STEMI) and non-ST-segment myocardial infarction (NSTEMI). The simultaneous presence of clinical symptoms suggestive of myocardial ischemia and ST-segment elevation in two adjacent leads indicate STEMI and reflect coronary occlusion. NSTEMI is present in patients presenting with clinical symptoms but nonpersistent signs of ST-segment elevation, though ECG findings may include ST-segment depression, T-wave inversion, or an unremarkable ECG. Current treatment recommendations for STEMI and NSTEMI will be discussed further.

Myocardial infarction – when caused by an imbalance between oxygen supply and demand – is designated as type II myocardial infarction and is commonly based on multiple concomitant mechanisms. Though type II myocardial infarction can develop regardless of coronary artery pathology, most events are associated with chronic coronary disease. A sudden trigger may provoke a disproportional increase in myocardial oxygen demand (e.g., tachyarrhythmias, arterial hypertension) and/or disturb myocardial oxygen supply (e.g., arterial hypotension, anemia, coronary-embolism, −dissection or -spasm). The extent of ischemia depends on the individual level of tolerance to the acute mismatch in oxygen demand and supply (Sandoval and Jaffe 2019; Horiuchi et al. 2020). Long-term survival is directly affected in the presence of a condition provoking type II myocardial infarction and can further contribute to higher mortality due to noncardiovascular events (Raphael et al. 2020) especially in patients traditionally not considered at risk of cardiac events (Singh et al. 2020). A recently published analysis of patients with suspected acute coronary syndrome identified similar risk factors for type I and type II myocardial infarctions and highlighted a prior history of type II myocardial infarction as the strongest predictor for type II events (Wereski et al. 2021).

Although cardiac troponin always originates from the myocardium, an increase in troponin cannot automatically be equated with myocardial ischemia or myocardial infarction. Causes of non-ischemic troponin release or myocardial injury are triggers such as thoracic trauma, severe and untreated sepsis, pulmonary embolism, or decompensated heart failure (Korff 2006), whereby the prognosis of patients with

non-ischemic troponin increase can be as poor as the one of patients with myocardial ischemia (Puelacher et al. 2018).

Evidence-Based Therapeutic Strategies in Nonoperative Myocardial Injury and Infarction

Therapeutic options for the treatment of STEMI have been steadily optimized and extensively studied over the years (Anderson 2017). A key principle represents the rapid restoration of perfusion of the myocardium as one of the most important goals of treatment. ECG (evidence level C) and determination of troponin (evidence level A) permit the differentiated determination and stratification of an acute event. Relevant medical treatment includes the administration of antiplatelet agents, statins, angiotensin-converting enzyme inhibitors, or angiotensin receptor antagonists and beta blockers, in the absence of cardiogenic shock (Amsterdam et al. 2014). Subsequent restoration of coronary perfusion is achieved by percutaneous coronary intervention (PCI) or intravenous fibrinolytic therapy and depends, among other factors, on the availability or time feasibility of PCI (O'Gara et al. 2013; Ibanez et al. 2018).

In the case of NSTEMI, an early invasive strategy might be superior to a drug-only approach, but angiography can be performed after 12/24 to 72 hours if there are no symptoms and no signs of circulatory instability (Collet et al. 2021). However, PCI is urgently indicated in cases of compromised/instable hemodynamic situations (Mehta et al. 2009; Amsterdam et al. 2014; Roffi et al. 2016). With regard to antithrombotic therapy, in addition to aspirin (evidence level A), the additional administration of a P2Y12 inhibitor in the case of a high-risk situation or before planned PCI is recommended and needs to be continued for up to 1 year after the coronary intervention (evidence level A) (Amsterdam et al. 2014). In addition, the administration of an anticoagulant is indicated until revascularization is achieved (evidence level A) (O'Gara et al. 2013; Amsterdam et al. 2014).

The current options for prevention and therapy existing for type I myocardial infarction and coronary artery disease have not been evaluated for the management of type II myocardial infarction or myocardial injury. A randomized control study comparing early coronary angiography with a conservative approach in patients with type II myocardial infarction or acute or chronic myocardial injury is currently underway (Lambrakis et al. 2019).

Derivation of Therapeutic Strategies in Perioperative Myocardial Injury and Infarction

To date, there is no consensus or evidence-based guidance regarding the treatment of patients sustaining PMI. The current data clearly indicate higher mortality in the presence of PMI and provide a rationale for the development of a unified guideline. Based on the existing evidence-based treatment options for myocardial infarction in the nonoperative setting, an analogue treatment strategy for PMI could be proposed

taking similarities and differences in etiology and pathophysiology into consideration.

In the nonoperative setting, a type I myocardial infarction is treated by rapid revascularization and dual platelet inhibition. In the perioperative setting however, dual antiplatelet treatment is problematic due to the increased risk of bleeding. Evidence and guidelines on this topic are urgently needed.

In type II myocardial infarction, the pathophysiological goal of rebalancing oxygen consumption and supply is independent of the operative or nonoperative setting and does not primarily ask for anticoagulation. Although guidelines for the peracute management of type II myocardial infarction in the nonoperative setting are lacking as well, we recommend a cardiological work up encompassing the evaluation of a coronary artery disease, treatment of the triggering factors, and optimization of long-term therapy.

This approach might also be of value as a treatment strategy of perioperative myocardial injury and myocardial injury after noncardiac surgery (MINS), as the potential causes of mismatch of coronary oxygen supply and demand are equivalent to the triggers of type II myocardial infarction.

As one of the first studies to evaluate a potential treatment strategy for postoperative myocardial injuries, the MANAGE trial investigated the application of dabigatran in patients with MINS. Patients suffering from MINS within the first 35 days after surgery were randomized to either receive either dabigatran or placebo for a maximum of 2 years. The primary endpoint was the development of major vascular complications, including vascular mortality, non-fatal myocardial infarction, non-hemorrhagic stroke, peripheral arterial thrombosis, amputation, and symptomatic venous thromboembolism, and further the occurrence of major bleeding as safety endpoint. The study found a reduction of major vascular complications (11% vs. 15%; hazard ratio 0.72, 95% CI 0.55–0.93). Remarkably, patients randomized early (<5 days) after occurrence of MINS seemed to profit more than those randomized late. There was no difference in the primary safety endpoint (Devereaux et al. 2018). However, it must be underlined that the risk of bleeding must always be taken into account before any anticoagulation is initiated. It is mandatory to weigh the potential benefits of anticoagulation against the potential bleeding risk, particularly with regard to the site of surgical intervention and hemostasis options. Therefore, an interdisciplinary approach is essential.

A retrospective case-control study evaluated the long-term impact of early cardiovascular therapy intensification in patients undergoing major vascular surgery sustaining myocardial injury and found an increase in survival when cardiac medication in patients with perioperative troponin elevation was optimized compared to patients not receiving cardiac medication or no improvement of present cardiac medication was conducted (Foucrier et al. 2014). We must point out that these findings have not been confirmed by further randomized control trials and consequently need to be regarded with caution.

However, we regard individual case assessment and optimization and/or initiation of cardiac medication including antiplatelet agents, statins, beta-blockers, and angiotensin-converting enzyme inhibitors – therapies recommended by the

American College of Cardiology/American Heart Association and the European Society of Cardiology – as important therapeutic principles (Roffi et al. 2016; Ibanez et al. 2018; Collet et al. 2021).

Proposed Algorithm for Therapeutic Strategies of Perioperative Myocardial Injury and Infarction (Fig. 1)

The management of PMI contains several steps: (1) identification of an elevation and dynamic change in troponin, (2) identification of features of myocardial ischemia, and (3) evaluation of a potential disruption of an arteriosclerotic coronary plaque or determination of further causes of changes in troponin and interprofessional discussion of the most appropriate treatment strategy.

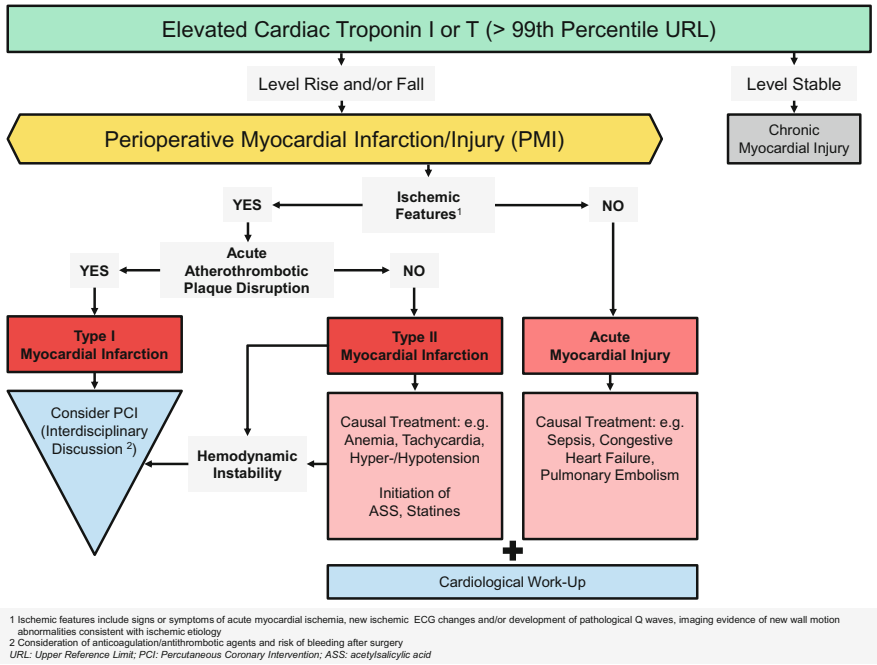

Fig. 1 Proposed treatment algorithm for patients suffering from perioperative myocardial injury/infarction. (Modified from Yurttas T, Hidvegi R, Filipovic M. Biomarker-Based Preoperative Risk Stratification for Patients Undergoing Non-Cardiac Surgery. Journal of Clinical Medicine. 2020; 9(2):351. https://doi.org/10.3390/jcm9020351, from the publishers MDPI and the authors with permission). 1 Ischemic features include signs or symptoms of acute myocardial ischemia, new ischemic ECG changes and/or development of pathological Q waves, and imaging evidence of new wall motion abnormalities consistent with ischemic etiology. 2 Consideration of risk of bleeding after surgery. *URL* upper reference limit; *PCI* percutaneous coronary intervention; *ASS* acetylsalicylic acid

1) A first step is a systematic perioperative troponin surveillance in patients at cardiovascular risk. A systematic troponin measurement (in contrast to a case by case approach) is essential since ischemic features (e.g., ECG changes or chest pain) are often missing in patients with prognostically relevant troponin elevations (Smilowitz et al. 2019). To recognize dynamic changes and to differentiate acute from chronic troponin elevations, a preoperative baseline value has to be obtained.

   Elevated troponin baseline values without subsequent dynamic changes are regarded as chronic myocardial injury, frequently associated with structural cardiac alterations as left ventricular hypertrophy or dysfunction or noncardiac causes commonly observed in patients suffering from diabetes mellitus or chronic kidney disease. Earlier investigations showed an association of chronic troponin elevation with an adverse postoperative outcome (Weber et al. 2013; Puelacher et al. 2018). There is no clear treatment strategy or recommendation on further preoperative measures that should be taken. However, postoperative surveillance (e.g., postoperative monitoring on the post-anesthesia care unit) is recommended, and cardiac reassessment needs to be taken into consideration if the patient's condition deteriorates. Such situations are becoming increasingly common since improved health care enables performing high-risk surgery in a broader population, especially in the elderly or in patients with multiple comorbidities (Park et al. 2017; DeFilippis et al. 2019).

   If preoperative troponin determination is not possible, e.g., in emergent surgery, relevant postoperative troponin dynamics can also aid in establishing the diagnosis of PMI (Puelacher et al. 2018).
2) A significant perioperative increase (or decrease) in troponin is defined as PMI. The first logical step is to consider the likely etiology of troponin dynamics. Clinical examination and search for ischemic features including signs and symptoms of acute myocardial ischemia, ECG changes (i.e., ST-segment alterations and/or new pathological Q-waves, new signs of left bundle branch block), and echocardiographic imaging evidence of presumed new wall motion abnormalities consistent with an ischemic etiology can provide clues as to whether troponin dynamic is based on an ischemic or non-ischemic cause. In the presence of new ischemic features, a distinction should be made based on the various pathophysiological properties described and the resulting treatment strategies.
3) Though uncommon, slightest indications of type I myocardial infarction urge for an early identification and the emergent need for treatment due to the risk of loss of myocardial viability (Sheth et al. 2018). Revascularization is indicated and should be carried out as promptly as possible (Ibanez et al. 2018). Suspicion should be raised in particular, if history of recent PCI and induced antiplatelet therapy, which might have been halted for the planned surgical intervention, is present (Ho et al. 2008). Similar to type II myocardial infarctions, induction or optimization of cardiac medication (antiplatelet agents, statins, beta-blockers, and angiotensin-converting enzyme inhibitors respectively) is recommended (Foucrier et al. 2014). However, timing and balanced dosage of induction of anticoagulation needs to be discussed in an interdisciplinary approach as risk of

perioperative bleeding is increased with respect to the type and/or location of the surgical intervention as well as the management and/or timing of removal of indwelling neuraxial catheters.

Type II myocardial infarction is triggered by factors such as tachycardia, hypertension, hypotonia, and bleeding anemia, which are frequently present in the perioperative setting, but may result in a pertinent imbalance of oxygen supply of the myocardium and consequently resulting in ischemia. Patients with type II infarction benefit from rapid correction of the triggering factor compromising oxygen supply or increasing oxygen consumption. Optimization of perioperative conditions (ensuring adequate oxygenation, avoiding hypotension, adjusting minimal hemoglobin concentration threshold) and elimination of triggering factors are the interventions of choice for prevention and treatment of perioperative myocardial supply/demand mismatch (Neumann et al. 2017; Sandoval and Jaffe 2019). Hemodynamic instability requires serial reevaluation and consultant involvement, since differentiation between type I and type II myocardial infarction might not be as clear as described and initially stable conditions might deteriorate and call for cardiological intervention in case of type I myocardial infarction. Apart from the acute situation of type II myocardial infarction, a subsequent cardiac workup to determine a formerly unknown cardiovascular condition, which may have predisposed to the event, has to be carried out prior to discharge, and initiation of preventive therapy like antiplatelet agents/statins needs to be discussed (Thygesen et al. 2018; Chuang et al. 2021).

Acute myocardial injury is present if there is evidence of troponin dynamics without signs of concurrent ischemic features. Identification, treatment, and postoperative cardiological follow-up of the eliciting mechanisms triggering an acute myocardial injury (commonly acute heart failure, cardiac trauma, severe sepsis, or pulmonary embolism or a multifactorial genesis, respectively (Landesberg et al. 2009)) are crucial, since these conditions are associated with a high mortality in the perioperative setting, as well as an ischemic cause (Puelacher et al. 2020).

Basic and Advanced Risk Stratifications and Strategies to Prevent Postoperative Myocardial Injury and Infarction

Depending on the risk of the respective surgical procedure, the European and American Societies for Anesthesiology and Cardiology recommend a preoperative cardiac risk assessment. Stratification process starts with the physical examination and the exclusion of an active or unstable cardiac condition (Fleisher et al. 2014; De Hert et al. 2018). Depending on the complexity of the planned intervention, a clarification of the physical performance (defined and evaluated as metabolic equivalents (MET)) and/or an additional clarification of cardiac risk factors, summarized in the latest version of the revised cardiac risk index (RCRI), is indicated (Lee et al. 1999; Gupta et al. 2011; Kristensen et al. 2014; Grant et al. 2015; Mauermann et al. 2017; Bartoszko et al. 2019).

If the patient's history and the procedure carry a correspondingly high risk, cardiac evaluation should be carried out, although this management is time-consuming and resource-intensive, and so far, no clear evidence of an improvement in perioperative mortality has been demonstrated.

Perioperative Optimization Strategies

Focusing on the physical and cardiac examination and patient's history, it is primarily advised to perform noninvasive investigations, e.g., ergometry, in case of symptoms of ischemic heart disease. If further diagnostic findings are positive, invasive and/or medical therapy and optimization of existing treatment as part of an interdisciplinary approach are recommended. Patients with heart failure in particular benefit from perioperative continuation of their existing cardiac medication (Foucrier et al. 2014). Treatment optimization with newly prescribed drugs, which are recommended as part of a cardiological evaluation, should be started at least 1 week before the planned operation (Fleisher et al. 2014). A detailed treatment scheme for perioperative use of platelet aggregation inhibitors or anticoagulants and the recommended interruption period in advance to surgical interventions can be found in this overview (Yurttas et al. 2017). A key concept besides physical examination is the optimization of further preexisting, altering but non-acute or impeding conditions like anemia. Patient blood management (PBM) comprises strategies to prevent and reduce incidence of perioperative anemia and blood loss (Spahn et al. 2019).

Advanced Strategies for Biomarker-Based Perioperative Risk Stratification

Cardiac biomarkers such as troponin and brain natriuretic peptide (BNP) are increasingly utilized in the perioperative setting to assess risk before or also during an operation. The relationship between a postoperative rise in troponin and morbidity and mortality as well as the high negative predictive value of normal BNP is of central importance (van Vark et al. 2017; Yang et al. 2018), since increased perioperative values of BNP correlate with the incidence of postoperative cardiac events (Duceppe et al. 2020).

In order to advance the stratification process, the Canadian Cardiovascular Society proposed an algorithm based on the perioperative determination of the cardiac biomarkers BNP, N-Terminal proBNP (NT-proBNP), and troponin (Duceppe et al. 2017). The latest statistical and economical results of the implementation process have been published and indicated no significant differences in costs, length of stay, or complication rates between pre- and post-implementation phases (McIsaac et al. 2021). An algorithm recently published by the Swiss Society for Anesthesia and Resuscitation (SGAR-SSAR) for perioperative risk assessment and optimization of patients for noncardiac surgery includes preoperative biomarker

determination as well as serial troponin monitoring in the postoperative course (Filipovic et al. 2018). Despite the lack of guidelines or prospective validations for the subsequent management of perioperative myocardial injuries, recent recommendations for active screening of patients at risk and determination of cardiac biomarkers can help the attending clinicians to recognize and optimize perioperative factors associated with myocardial injury (Kristensen et al. 2014; Fleisher et al. 2014; Duceppe et al. 2017; De Hert et al. 2018).

Other Biomarkers Under Investigation

The determination of BNP or NT-proBNP has so far been regarded as the clinical gold standard for the diagnosis of acute decompensated heart failure in patients with dyspnea and represents a considerable marker to quantify hemodynamic stress (Yagmur et al. 2019). Atrial natriuretic peptide (ANP) has biological properties comparable to BNP, whereby BNP is released through the activation of the renin angiotensin aldosterone system (RAAS), which is in turn induced by an increase in ventricular pre- and afterload, as well as an increase in ventricular wall tension. ANP, on the other hand, is released by stretching the atrial cells and is therefore less sensitive to intraventricular pressure increases and hemodynamic stress. Both peptides lead to a counter-mechanism of the RAAS by inducing natriuresis, promoting diuresis, and vasodilation. Midregional-proANP (MR-proANP) is excreted in the same amount as ANP and is more stable than the N- or C-terminal ends of proANP and therefore easier to use in clinical practice (Katan et al. 2010). Studies showed an association between increased MR-proANP levels and the incidence of cardiovascular events. In particular, MR-proANP detection was associated with the development of cardiovascular events in patients with heart failure with preserved ejection fraction (HFpEF) (Jensen et al. 2020), and MR-proANP levels corroborate a similar diagnostic performance as NT-proBNP in patients with chronic heart failure (Gohar et al. 2019).

Copeptin, a 39-aminoacid glycopeptide, is the C-terminal end of the precursor protein pre-provasopressin (pre-proAVP). Even minor cardiac stress situations lead to the release of copeptin, which makes it a promising biomarker. Concentrations below 14 pmol/l reliably excluded an acute coronary syndrome in a nonsurgical setting (Lipinski et al. 2014). In the perioperative setting, values of $\geq$9.6 pmol/l were associated with significantly higher myocardial injury rates and improved risk stratification process in patients who were scheduled for noncardiac surgery with missing increase in preoperative troponin (Mauermann et al. 2016). Even short periods of cardiac stress or constant but intensified hemodynamic pressure on the myocardium at a low level can be detected by copeptin before troponin dynamics indicate myocardial injury. Copeptin measured postoperatively was significantly higher in patients with myocardial injury than in patients without myocardial injury and was able to be detected immediately after the end of the operation. Thus, immediate postoperative copeptin concentrations hold promise for identifying patients at risk of myocardial injury (Kamber et al. 2018).

Differentiation between type I and type II myocardial infarction was superior by an assessment of novel biomarkers including copeptin and MR-proANP in the early onset of symptom development compared to an assessment using troponin I alone. Furthermore, the study reported that a combination of multiple biomarkers (including troponin I, copeptin, MR-proANP, procalcitonin, C-terminal proendothelin-1, and midregional proadrenomedullin) was able to detect type II myocardial infarction more reliably. With regard to predicting mortality during 180 days after clinical presentation, all biomarkers were superior compared to troponin I, not influenced by the diagnosis of type I or type II myocardial infarction. MACE during 180 days was predicted by all biomarkers but copeptin and troponin I.

Troponin is essential for the diagnosis of myocardial infarction but cannot determine type and etiology. However, other cardiac biomarkers might have stronger prognostic capability, and their implementation to the clinical process may help clinicians to confirm a suspected diagnosis of type II myocardial infarction, which may present with inconsistent clinical features compared to type I myocardial infarction (Horiuchi et al. 2020).

Yet, a lately published investigation of multiple biomarkers could not identify a marker, which warrants higher sensitivity or specificity to distinguish between type I or II myocardial infarction or injury, or grant superior diagnostic performance compared to BNP, troponin T, and clinical parameters (Nestelberger et al. 2021).

Conclusion and Perspectives

Identification of patients at cardiovascular risk and prevention of PMI are central goals of perioperative management. In those patients sustaining perioperative cardiac ischemic complications, timely institution of the correct therapies is paramount, with the potential to improve patient-centered outcomes (Puelacher et al. 2021). An interdisciplinary approach involving early cardiological consultation can aid in establishing an optimal therapeutic approach in individual patients, e.g., starting platelet inhibitors, as well as help in balancing the risk of postoperative bleeding complications.

As perioperative medicine specialists, we must not forget that our role is to choose the safest therapeutic options in the context of our patient's perioperative course.

Applications to Perioperative Myocardial Infarction/Injury

This chapter addresses the risks of perioperative myocardial injury, which has a significant impact on the postoperative outcome of patients undergoing noncardiac surgery (Devereaux et al. 2012). Cardiac biomarkers along with diagnostic tests and clinical assessment allow to diagnose and categorize myocardial infarction and to determine the appropriate treatment strategy. The established treatment recommendations for myocardial infarction refer to patients from the nonsurgical field, but due

to similarities in the pathophysiology, these treatment recommendations are the bases for the management for surgical patients, too (Ibanez et al. 2018; Collet et al. 2021). However, a prospective evaluation of the concepts in the perioperative setting is lacking.

Mini-Dictionary of Terms

- **Myocardial Infarction** Simultaneous occurrence of elevated cardiac biomarkers and diagnostic or clinical signs of ischemic features, resulting from a decreased oxygen supply to the myocardial tissue and may be caused by occlusion of an epicardial coronary artery or an imbalance between oxygen demand and supply.
- **Dynamic Changes in Troponin** Increase or decrease in measured troponin levels, relevant in the range of more than 20%.
- **Myocardial Injury** Elevation of cardiac biomarkers without diagnostic or clinical signs of ischemic features.
- **Perioperative Myocardial Infarction/Injury** Myocardial infarction occurring in the temporal context of a surgical procedure.
- **Systemic Troponin Surveillance** Troponin determinations performed at regular intervals pre- and postoperatively.

Key Facts of Perioperative Myocardial Injury/Infarction

Perioperative myocardial injury/infarction can be present in around 15% of patients undergoing noncardiac surgery and contribute to higher mortality.

Diagnostic detection could be improved through the use of biomarkers.

Perioperative myocardial injury/infarction is classified based on the dynamics of biomarkers and the occurrence of additional clinical and diagnostic signs and ischemic features.

Depending on the particular classification, a therapeutic strategy can be discussed analogue to existing evidence-based options from the nonsurgical field.

In patients with an increased risk of bleeding, the postoperative bleeding complication due to indicated therapy of myocardial injury/infarction has to be considered.

Summary Points

- Perioperative myocardial injury/infarction has an impact on the postoperative outcome of patients.
- Based on the pathophysiological differences, myocardial infarctions are divided into different types.
- During surgery, clinical signs associated with myocardial infarction may be absent, making awareness of such an event difficult.

- Combination of biomarkers and clinical features can better detect perioperative myocardial damage.
- The therapy indicated for myocardial injury/infarction depends on the trigger of the event.
- Although there are no evidence-based recommendations for the treatment of myocardial injury/infarction in the perioperative setting, it is possible to consult the published guidelines of the nonoperative field.
- The risk of postoperative bleeding complications is increased depending on the surgical procedure and should always be included in the discussion of a possible treatment strategy for perioperative myocardial injury/infarction.
- The determination of cardiac biomarkers in combination with the clinical presentation and diagnostic tools can help the treating physicians to recognize a perioperative myocardial injury/infarction and to indicate a necessary therapy.

Cross-References

► Intraoperative Management and Its Influence on Postoperative Biomarker Release

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Prognostic Biomarkers to Predict Outcomes in Trauma

7

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Abstract

Traumatic injury accounts for significant morbidity and mortality across all age groups. Unlike other diseases, the onset of injury is known and therefore trauma is well poised as an ideal disease entity for the development and use of predictive biomarkers. In this chapter, we describe biomarkers that have been used for or show promise for prognostication following traumatic injury. This chapter begins with a brief report on prognostic biomarkers specific to traumatic brain injury and goes on to describe several classes of biomarkers in polytrauma including cytokines, genomics, endothelial damage markers, and damage-associated molecular pattern molecules. Throughout the chapter, we make the important distinction between correlative biomarkers, which inform hypotheses and guide prospective experiments, and true predictive biomarkers established through rigorous testing and modeling. We close the chapter with recent work utilizing multi-omic and machine learning strategies which show great promise in the identification and utilization of predictive biomarkers following traumatic injury.

Keywords

Trauma · Biomarkers · Multi-omics · Predictive · Prognostic · Cytokines · Transcriptomics · Gene polymorphisms · DAMPs · TBI · SNPs

Abbreviations

ACIT2	activation of coagulation and inflammation in trauma
AKI	acute kidney injury
Ang-1	angiopoietin-1
Ang-2	angiopoietin-2
AUC	area under the curve
AUROC	area under the receiver operative characteristic curve
CAR	CRP to albumin ratio
CRP	C-reactive protein
CST5	Cystatin D
DAMPs	damage-associated molecular patterns
GCS	Glasgow-Coma score
GCSF	granulocyte colony-stimulating factor
GFAP	glial fibrillary acidic protein
HMGB1	high-mobility group box 1
HSP70	heat-shock protein 70
ICU	intensive care unit
IFN	interferon
IL-10	interleukin-10
IL17A	interleukin-17A
IL-2	interleukin-2

IL-33	interleukin-33
IL-5	interleukin-5
IL-6	interleukin-6
IL-7	interleukin-7
IL-8	interleukin-8
ISS	injury severity score
LOS	length of stay
LRS	lipid reprogramming score
MCP-1	monocyte chemoattractant protein-1
MHCII	major histocompatibility complex
MIP-1a	macrophage inflammatory protein-1 alpha
MODS	multiple organ dysfunction syndrome
MOF	multiorgan failure
MPPED2	metallophosphoesterase domain-containing 2
mtDNA	mitochondrial DNA
NAA	N-acetylaspartate
NETs	neutrophil extracellular traps
OR	odds ratio
PAMPer	The Prehospital Air Medical Plasma trial
PCA	principal component analysis
PE	phosphatidylethanolamine
RAGE	receptor for advanced glycation end products
ROC	receiver operating characteristic
SIRS	systemic inflammatory response syndrome
SNPs	single nucleotide polymorphisms
SOFA	sequential organ failure assessment
SSI	surgical site infection
sST2	soluble suppressor of tumorigenesis-2
TBI	traumatic brain injury
Th17	T-helper 17
TLR	toll-like receptor
UCH-L1	ubiquitin C-terminal hydrolase
UCLH1	ubiquitin carboxy-terminal hydrolase L1
VEGF	vascular endothelial growth factor
WBC	white blood cell

Introduction

Traumatic injury is the worldwide leading cause of morbidity and mortality in people under the age of 55 years. Trauma as a disease entity is well poised to utilize predictive biomarkers, as it is one of few disease processes with a known, precise time of onset. There is an immediate activation of immune and stress responses that

likely impact short- and long-term clinical outcomes. Of note, it has been demonstrated that these responses can be detected as early as 45 minutes from the time of injury (Hazeldine et al. 2017). The well-defined starting point also allows delineation of the subsequent phases of illness. Lamparello et al. refer to these time phases as time "windows" and propose a scheme of five time intervals after traumatic injury. These time windows may serve as a guiding framework by which to appropriately time and study the measurement of predictive biomarkers (Lamparello et al. 2019). Understanding the biomarker response to trauma is crucial to prognosticating outcomes and represents an opportunity to improve morbidity, mortality, and resulting healthcare expenditure from this ubiquitous disease process.

As data mining becomes increasingly utilized in the medical field, the term *predictive* has often been misused as meaning "associated or correlated with." It is important to distinguish these correlations and associations from true predictive utility. Much of the data on biomarker molecules is correlative in nature, meaning that marker levels correlate with outcomes of interest. This data is not to be ignored, as it adds valuable information, but it cannot be used to predict outcomes. For data to be considered predictive, it must be derived from modeling methods. These methods are numerous and varied and aim to use known patient data to predict outcomes of interest using techniques such as machine learning and hypothesis testing (Bellazzi and Zupan 2008; Bellazzi et al. 2011). For a biomarker to be reliably considered predictive, rigorous comparison of experimental groups with and without the biomarker must be performed, and threshold values must be established before proceeding to external clinical validation (FDA-NIH Biomarker Working Group 2016). We add one additional criterion; any meaningful predictive biomarker should significantly outperform data that is already readily available and gathered as part of standard of care.

Ideal predictive biomarkers should be either binary in nature (positive or negative) or quantifiable in an assay that is clinically available with reasonable turnaround time and high sensitivity and specificity. In this chapter, we will review molecules with potential to be used as predictive biomarkers in trauma. While some molecules have been validated in predictive models, none of them fulfill the requirements noted above. Furthermore, the majority of the data presented is only correlative. We will remind the reader of this difference as we proceed. In the following sections, we will cover putative biomarkers that have shown promise in the realm of traumatic injury. These range from cytokines to single nucleotide polymorphisms, with varying degrees of strength of evidence. It is important to note the general lack of available clinical assays able to measure many of the molecules discussed in this chapter. Most of the data is centered on patient samples gathered for research purposes only and not from clinical data. Research and development of clinical assays that are widely available with rapid results will be vital in utilizing any of these molecules to direct patient care or stratify patients during clinical trials. After reviewing putative biomarkers, we discuss methods for predictive modeling on a larger scale (i.e., multi-omics) as well as some of the early data produced using these tools.

Prognostic Markers in Traumatic Brain Injury

Traumatic brain injury (TBI) is a major cause of morbidity and mortality across a broad range of age groups, with an estimated 1.5 million Americans affected annually and nearly 61,000 TBI-related deaths in 2019 (Centers for Disease Control and Prevention 2021a, b). Whether it is a blow to the head or a penetrating injury, outcomes from TBI vary between patients and are known to be associated with a range of different immune responses (Georges and M Das J 2021). The response to polytrauma with TBI as a disease process may vary from polytrauma without TBI, and prognostic biomarkers are likely to be different for patients with TBI. We provide a brief overview here, but direct the reader to dedicated chapters in this text.

Studies have shown that several pro- and anti-inflammatory mediators are at play in the first 24 hours after injury that potentially impact host responses and outcomes in TBI. Most of these studies test for markers in the circulating peripheral blood compartment of patients presenting to the emergency room after polytrauma with an associated TBI (Edwards et al. 2020). Characterizing how immunologic markers affect clinical course trajectory is crucial to understanding the ongoing pathophysiologic processes and prognosticating clinical outcome following TBI.

Over the past decade, numerous markers have been studied to assess for the presence and severity of brain injury. IL-6 has initially shown promising results. As a pro-inflammatory cytokine, IL-6 has been implicated in various immunological and inflammatory disease processes ranging from disorders in the central and peripheral nervous systems, traumatic injury, autoimmune disorders, and several cancers (Tanaka et al. 2014; Alzghoul et al. 2019; Woodcock and Morganti-Kossmann 2013). In an attempt to quantify IL-6 levels in trauma patients with different brain injuries, Antunes et al. measured IL-6 levels in trauma patient plasma in the first 6 hours and found that patients with more severe brain injury had higher levels of IL-6 (Antunes et al. 2010). Another group reported specific cutoff values for IL-6 measured in plasma to stratify TBI patients based on injury severity and presence of elevated intracranial pressure. However, elevations in IL-6 are not specific to brain injury, and increase is also observed in trauma patients with orthopedic injuries and no brain injury (Hergenroeder et al. 2010). As with most studies of this nature, these results are correlative implying associations between markers and outcomes and not causation.

Glial fibrillary acidic protein (GFAP), ubiquitin C-terminal hydrolase (UCH-L1), and N-acetylaspartate (NAA) have also been recently studied as potential prognostic biomarkers in patients with TBI. Several research teams have successfully stratified patients with and without TBI based on these markers, but they were not able to predict which patients will deteriorate and have worse outcomes (Huebschmann et al. 2020; Gruen et al. 2020; Mozaffari et al. 2021; Osier et al. 2019). In an attempt to differentiate between patients with mild and severe TBI, Hill et al. measured a wide array of proteins in patients with varying severity of TBI at multiple timepoints. Of the proteins they identified, cystatin D (CST5) levels correlated with severe TBI within the first hour post-injury and were used to differentiate patients with severe TBI from patients with mild or no brain injury (Hill et al. 2017). As mentioned

above, these studies identified correlations for potential use as post-injury prognostic markers. Using multi-omic analysis of the blood plasma, we recently showed that ubiquitin carboxy-terminal hydrolase L1 (UCLH1) levels on admission distinguished patients with TBI and a severe systemic response to polytrauma from severely injured patients without TBI and TBI patients with minimal systemic response (Wu et al. 2021c). Adding other markers such as syndecan and IL-17A only slightly improved the model. Thus, it will likely be feasible to identify patients with TBI using plasma biomarkers; however, prospective validation of these findings is required. It may be feasible to assess for the presence of TBI and systemic responses in the field as point-of-care diagnostics are developed.

Putative Biomarker Molecules in Trauma

The following sections review several different classes of circulating molecules and those specific molecules that have potential as predictive biomarkers. Key findings from the literature are highlighted; however, these sections are not intended to represent an exhaustive assessment of any single biomarker.

1. **Cytokines**

Cytokines and chemokines are small proteins that act as important regulators of the systemic response to tissue injury and shock following polytrauma. These proteins are secreted by numerous cell types, both immune and non-immune, and play a role in coordinating the immune response. Cytokines and chemokines are pleiotropic in their function and can have both beneficial and adverse effects with regard to potentiating or diminishing the inflammatory response. However, cytokines can be classified as being generally pro-inflammatory or anti-inflammatory in their effects. Circulating levels of cytokines and chemokines can provide some insight into the state of the immune response and have been a focus of study in understanding the response to trauma (Bonaroti et al. 2021; DeLong and Born 2004; Guisasola et al. 2018).

IL-6

While there are several cytokines that demonstrate correlations with outcomes following trauma, IL-6 has proven to be most promising in this arena with regard to predictive potential (Biffl et al. 1996). Recent work demonstrates that serum levels of IL-6 in the first 24 hours following traumatic injury are highly correlative with a complicated clinical course and multiple organ failure (Qiao et al. 2018). It has also been shown that male trauma patients with high circulating levels of IL-6 are more susceptible to sepsis (Mörs et al. 2016) and that post-trauma inhibition of IL-6 with monoclonal antibodies in mouse polytrauma reduces levels of inflammatory mediators such as IL-10, MCP-1, and MIP-1a with attenuation of lung and

liver injury (Zhang et al. 2014). Furthermore, Yamamoto et al. have demonstrated that measurement of circulating IL-6 at 72 hours following injury had significant discriminatory power with an area under the receiver operative characteristic curve (AUROC) of 0.766 for predicting 28-day mortality. Sequential Organ Failure Assessment (SOFA) score alone had an AUROC of 0.766, while the addition of the 72 h IL-6 measurement to the SOFA score improved the AUROC for predicting 28-day mortality to 0.844. While the study did not establish a cut off value for IL-6, their results suggest that patients with an IL-6 concentration less than 74 pg/dL at day 3 and SOFA score less than 7 were likely to survive to 28 days (Yamamoto et al. 2021). While IL-6 is not yet utilized as a clinical assay, this biomarker shows significant promise in predicting outcomes in traumatically injured patients.

CRP

C-reactive protein (CRP) is an acute phase protein secreted by the liver in response to cytokine signaling, and circulating levels rise in response to inflammation and tissue injury (Du Clos 2000). CRP is perhaps the next most promising immune-related biomarker in terms of predictive potential following traumatic injury, particularly in traumatic brain injury (TBI). Wang et al. demonstrated that an elevated CRP-to-albumin ratio (CAR) was not only associated with in-hospitality mortality but also was predictive of mortality with an AUC of 0.710. In this study, combining CAR with admission Glasgow-Coma score (GCS) and acute kidney injury (AKI) into a predictive model performed better than GCS alone with AUC 0.832 (Wang et al. 2020). Elevated baseline CRP levels in TBI patient have also been shown to be associated with persistent post-concussion syndrome, cognitive impairment, and psychological problems at 3 months post-injury and may be an independent predictor of these outcomes in mild TBI (Su et al. 2014). In polytrauma, elevated CRP at 72 h is associated with prolonged mechanical ventilation (Honarmand and Safavi 2008).

MCP-1

Recent work has identified monocyte chemoattractant protein-1 (MCP-1) (also called CCL2) as a potential predictive marker for the development of surgical site infection (SSI) following traumatic injury. Cohan et al. investigated early cytokine mediators including IL-6, IL-8, IL-10, IP-10, granulocyte colony-stimulating factor (GCSF), and MCP-1 and created ROC curves for each of these in patients that did or did not develop SSI following trauma. Only MCP-1 had significant predictive potential with an AUROC of 0.71 ($p = 0.03$). The critical threshold value was 490 pg/mL (Cohan et al. 2020). We identified MCP-1 as one of the cytokines elevated early after injury in patients that go onto to develop nosocomial infection (Namas et al. 2016b).

Correlative Cytokines

Beyond IL-6, CRP, and MCP-1, numerous cytokines have been identified as having clear correlative capacity in polytrauma patients. Cytokine patterns that appear early in the clinical course can be correlated with adverse outcomes such as multiple organ dysfunction syndrome (MODS), nosocomial infection, tolerance to shock, and mortality. These patterns are characterized by an early predominance of pro-inflammatory cytokines including IL-6, IL-8, IL-10, and MCP-1 (Abboud et al. 2016; Namas et al. 2016a, b; McKinley et al. 2021; Almahmoud et al. 2019). Additionally, a pathogenic Th17 response has been identified in trauma patients who experience delayed adverse outcomes, which is characterized by elevated levels of lymphocyte associated cytokines after 24 h. These cytokines include those typically produced by lymphocytes and include IL-2, IL-4, IL-5, IL-7, and IL-17 (Schimunek et al. 2018; Abboud et al. 2016; Namas et al. 2016b). IL-33 increase is associated with an early pro-inflammatory response, and delayed increase of its soluble receptor, sST2, is associated with adverse outcomes (Billiar et al. 2019). These observations suggest that measurement of cytokine levels could be used to predict outcomes in trauma patients; however, the timing of these measurements is certainly critical and complex.

In general, three groups of cytokines have been observed that seem to respond together as modules in response to trauma. These groups are categorized as (1) pro-inflammatory, (2) lymphocyte-associated, and (3) protective/reparative/regulatory (Table 1). It has been demonstrated that in response to severe injury, the cytokines in the pro-inflammatory module increase, while the cytokines in the reparative/protective module are suppressed at high ISS (Cai 2021; Bonaroti et al. 2021). Use of these cytokine modules in future work may be beneficial for predictive models in trauma. However, it should be pointed out that a comprehensive assessment of all known circulating immune mediators has not been reported. Therefore, a full classification of the circulating mediator landscape awaits further studies.

2. Genomics

Genomic testing is a broad category of assays that involves sampling patient genetic material in order to obtain prognostic information. This includes using techniques such as MicroArray, NanoString, and single-cell RNAseq to evaluate

Table 1 Cytokine modules

Module 1: Pro-inflammatory	Module 2: Lymphocyte-associated	Module 3: Reparative/protective/regulatory
IL-6	IL-2	IL-22
IL-8	IL-4	IL-33
IL-10	IL-5	IL-9
MCP-1	IL-7	IL-21
MIG	IL-17A/F	IL-23
		IL-17E

the transcriptome as well as focal genomic features such as single nucleotide polymorphisms (SNP). These technologies have made significant advances over the past 20 years and have become crucial in understanding the response to traumatic injury. A robust utilization of these techniques, as well as elegant computational methods, in the study of trauma came from the Glue Grant project. This program included 22 academic medical centers in the USA and enrolled >2800 patients. Clinical and genomic data was gathered from a subset of these patients as well as healthy volunteers (Tompkins 2015). In a landmark study from this program published in 2011, white blood cell (WBC) gene expression over a time course spanning 12 h to 28 days was evaluated from 167 patients using gene microarrays. All of the patients had hemorrhagic shock as demonstrated by base deficit, systolic blood pressures <90, and blood transfusion within 6 h of admission. Average ISS was 32 and the incidence of multiorgan failure (MOF) was 35%. In the 167 trauma patients, 80% of WBC genes had significant changes in expression levels over 28 days when compared to normal volunteers. This study coined the phrase "genomic storm" in describing the dramatic transcriptional changes seen in immune cells following trauma (Xiao et al. 2011). While the changes were correlative to trauma outcomes and not predictive or diagnostic, this study suggested that the response to trauma is characterized by notable changes in transcription and that these changes may represent an opportunity for prognostication or intervention. Findings from this work lead to the subsequent work discussed below using nanostring technology.

Single Nucleotide Polymorphisms

Single nucleotide polymorphisms (SNPs) are DNA sequence variations that are the most common form of variation within the human genome. SNPs can be located in protein-coding and non-coding RNA genes and can have functional effects or be neutral. Several SNPs have been identified as being correlated with outcomes in trauma patients. Shimunek et al. identified 7 SNPs that distinguished survivors from non-survivors in a cohort of 397 blunt trauma patients. Interestingly, the authors also identified that survivors with those 7 SNPs demonstrated a unique inflammatory response when compared to similarly injured survivors that did not have the SNPs, suggesting an interaction between the polymorphism, the inflammatory response to trauma, and outcomes (Schimunek et al. 2018). The same authors went on to identify that patients with the rs2065418 TT SNP in the metallophosphoesterase domain-containing 2 (MPPED2) gene had higher MOD scores compared to those without the SNP. In severely injured patients (ISS > 25), those with the SNP had higher MOD scores, longer hospital LOS, greater requirement for mechanical ventilation, and higher plasma creatinine than those severely injured patients without the SNP. Severely injured patients with the SNP also had different levels of nine circulating inflammatory markers (Schimunek et al. 2019). These findings, while not predictive in nature, are important correlations and again suggest an interplay between genomic variation and outcomes following traumatic injury. In the future, a patient's SNP status may be assessed upon arrival to a trauma center in assays the could be

completed with hours. SNP status in individuals could theoretically be known prior to injury and in the future might be part of a patient's past medical record.

Transcriptomics

In addition to the work described above from the Glue Grant, Cabrerra et al. used whole blood transcriptomics to correlate gene expression in circulating leukocytes with trauma patient outcomes, showing that changes as early as 2 h within injury correlated with the development of organ dysfunction (Cabrera et al. 2017). Cuenca et al. used the microarray derived gene expression data from the 167 patients in the Glue Grant cohort and assessed differences in expression between patients with different clinical trajectories. The group identified 63 genes whose expression within leukocytes differed between patients with complicated or uncomplicated clinical outcomes over 28 days. They then went on to utilize nanoString, a multiplexed approach with rapid quantification of mRNA abundance, to validate the original Microarray findings using only the 63 identified genes. They were able to reduce the gene expression data to a composite score with the ability to discriminate between patients with complicated or uncomplicated outcomes (AUROC 0.81, $p < 0.001$) (Cuenca et al. 2013). This transcriptomic score, abbreviated "S63," was validated in a prospective fashion in a cohort of 127 trauma patients and was able to predict patients that would require ICU care longer than 5 days with ongoing organ dysfunction (AUC 0.80). Furthermore, the metric outperformed clinical markers and plasma IL-6 when measured at 24 h after admission. The group applied the metric to the activation of coagulation and inflammation in trauma (ACIT2) database of 26 patients and achieved an AUC of 0.85 for predicting multiorgan failure (Raymond et al. 2020). This S63 metric demonstrates promise to be clinically useful in prognosticating outcomes for trauma patients. Notably, the platform used to assess the metric has a turn around of under 24 h.

Single-cell RNAseq technology allows evaluation of transcriptomic changes occurring at the *single-cell level*, and the advancement of this technology over the past 10 years has enabled a greater understanding of the role of cell heterogeneity in many disease processes. Chen et al. used single-cell RNAseq to describe the landscape of transcriptomic changes occurring in bone marrow and circulating mononuclear cells after trauma in mice and humans. The most notable transcriptomic changes occurred within circulating CD14+ monocytes, and the major changes in transcriptomic patterns in monocytes after trauma were resolved to 129 genes with differential expression that could be clustered into six gene "signatures." The Glue Grant cohort (Xiao et al. 2011) of 167 trauma patients was clustered based on their scores within each gene signature determined within the first 12 h after injury. This resulted in two subgroups of patients, termed SG1 and SG2 by the authors, with SG1 patients demonstrating higher expression in gene signatures associated with inflammation and lower expression of MHCII and IFN signaling genes. Notably, subtype SG1 patients had longer hospital LOS, worse multiorgan dysfunction, and increased rates of complications. Furthermore, SG1 patients had

slower 28-day recovery. On multivariate analysis, the SG1 transcriptomic signature was an independent risk factor for slower recovery (Chen et al. 2021). This data demonstrates correlations between detectable transcriptomic changes and clinical outcomes, with the potential for predictive capability as technologies continue to improve. Thus, assessing transcriptomic changes within whole blood leukocytes holds promise as a rapid assay to prognosticate for outcomes.

3. **Endothelial Damage Markers**

The vascular endothelium is comprised of a single layer of cells lining the vasculature throughout the body. The luminal surface of endothelial cells is covered by the antiadhesive and anticoagulant endothelial glycocalyx which serves as a protective barrier to maintain vascular barrier function (Nieuwdorp et al. 2005). The endothelium plays a crucial role in the inflammatory response and activation of the coagulation system and maintains homeostasis between circulating blood and surrounding tissues. Further, as the endothelium is in close contact with organs, changes in endothelial homeostasis have been implicated in organ dysfunction. Thus, the endothelium serves as mediator of multiple biologic responses and is intimately involved in regulating the relationship between local and systemic inflammation (Chignalia et al. 2016; Reinhart et al. 2002).

Traumatic injury causes systemic activation and damage of the endothelium from either direct tissue injury or the downstream effects of hypoperfusion, inflammation, and sympathoadrenal activation (Johansson et al. 2012). Endothelial activation and damage disrupt the glycocalyx, which results in conversion to a procoagulant surface layer, microvascular permeability, adhesion of signaling/chemoattractant molecules in the inflammatory and coagulation cascade, and catecholamine release. The physiologic changes to the endothelium following traumatic injury in concert with sympathoadrenal activation and subsequent massive catecholamine release have been implicated as drivers of trauma-induced coagulopathy which is associated with poor outcomes (Johansson et al. 2017).

Several soluble biomarkers associated with endothelial dysfunction and disruption of the glycocalyx have been previously characterized and are summarized in Table 2.

Table 2 Circulating biomarkers of endothelial dysfunction

Biomarker	Physiologic role/significance in endothelial dysfunction
Syndecan-1	Damage to endothelial glycocalyx
Thrombomodulin	Endothelial cell damage
E-selectin	Endothelial cell activation
Angiopoietin-1 (Ang-1)	Maintains quiescent resting state of endothelium
Angiopoietin-2 (Ang-2)	Destabilization of blood vessels, indicates vascular leakage, marker of endothelial activation and dysfunction
Vascular endothelial growth factor (VEGF)	Endothelial cell-specific growth factor, mediator of vascular permeability

Syndecan-1

Syndecan-1, a biomarker of glycocalyx damage, has been shown to predict poor outcome and mortality in traumatic injury. A prospective cohort study by Johansson et al. measured 17 markers at admission including biomarkers of glycocalyx degradation and endothelial damage to examine the association between endothelial dysfunction, inflammation, coagulopathy, and mortality following injury (Johansson et al. 2012). Patients were stratified into high or low endothelial glycocalyx degradation groups based on the median cohort syndecan-1 concentration. Patients with high glycocalyx degradation had higher concentrations of catecholamines and biomarkers in the inflammatory, coagulation, and fibrinolytic pathways. Patients with high glycocalyx degradation also demonstrated a three-fold increased 30-day mortality compared to the low glycocalyx degradation group. This association remained significant after adjusting for injury severity score and age. Based on these findings, the authors proposed that the downstream effects of injury cause degradation of the endothelial glycocalyx and loss of vascular integrity resulting in thrombin generation, protein C activation and depletion, hyperfibrinolysis, organ damage, and subsequent increased mortality.

A subsequent study by Johansson et al. focused on the association between sympathoadrenal activation, endothelial damage markers, and poor outcome in a cohort of 424 trauma patients (Johansson et al. 2017). They examined circulating markers of sympathoadrenal activation (adrenaline, noradrenaline) and endothelial dysfunction (syndecan-1, E-selectin, thrombomodulin) from admission plasma samples of traumatically injured patients. They identified elevated plasma adrenaline as the only independent predictor of elevated syndecan-1 in an adjusted linear regression model, indicating a positive association of sympathoadrenal activation and endothelial damage. Using an adjusted Cox proportional-hazard analysis, they demonstrated that higher circulating levels of syndecan-1 were an independent predictor of early (<24-h) and late (7- and 28-day) mortality. Increased circulating thrombomodulin concentration was also an independent predictor of 7- and 28-day mortality, while E-selectin did not predict mortality in their model. The findings from these two studies suggest that elevated admission syndecan-1 levels may prognosticate increased risk for mortality following traumatic injury; however, further prospective trials investigating the predictive potential of syndecan-1 are needed.

Syndecan-1 and Thrombomodulin in Relation to Acute Kidney Injury Following Trauma

Syndecan-1 and thrombomodulin, which are both markers of endothelial dysfunction, are also associated with incidence and severity of acute kidney injury after trauma. In a prospective cohort study, Hatton et al. evaluated admission plasma levels of syndecan-1 and thrombomodulin in trauma patients surviving >24 h following traumatic injury (Hatton et al. 2021). In 477 patients, 242 (51%) developed acute kidney injury and had significantly higher plasma levels of syndecan-1

and thrombomodulin compared to patients who did not develop acute kidney injury. Additionally, elevated admission levels of thrombomodulin and to a lesser extent syndecan-1 were associated with increased risk, duration, and stage of acute kidney injury on adjusted models. Interestingly, thrombomodulin and not syndecan-1 was significantly associated with mortality. These findings suggest that evidence of endothelial dysfunction is associated with acute kidney injury. Further, measurement of admission thrombomodulin and syndecan-1 may predict development and severity of acute kidney injury in traumatically injury patients.

Angiopoietin-2

In addition to syndecan-1 and thrombomodulin, the endothelial damage marker angiopoietin-2 (Ang-2) has also been associated with poor clinical outcome after major trauma. An investigation by Ganter et al. examined circulating levels of Ang-2 in 208 adult trauma patients on admission to a single-institution level 1 trauma center (Ganter et al. 2008). They reported increased levels of Ang-2 compared to healthy controls in traumatically injured patients. Further, Ang-2 positively correlated with injury severity score. The authors also demonstrated that Ang-2 levels correlated with known markers of endothelial activation (von Willebrand factor and thrombomodulin), hypoperfusion (base deficit >7.6), and coagulopathy (prothrombin time, partial thromboplastin time). Demonstrated by regression analysis, increased Ang-2 levels had significantly increased odds for mortality (OR 4.0), ventilator-free days ($\leq$26 days; OR 3.0), acute lung injury (OR 2.0), acute renal failure (OR 7.4), and transfusion requirements (>2 units packed red blood cells; OR 2.7). These findings suggest that high circulating levels of Ang-2 represent endothelial cell activation and may predict coagulation abnormalities, organ dysfunction, and mortality following traumatic injury.

Taken together, these studies identify significant associations between admission biomarkers of endothelial damage and dysfunction and poor clinical outcomes (i.e., organ failure/dysfunction, mortality, coagulopathy) following traumatic injury. While prospective validation of these biomarkers in trauma is required prior to clinical prediction and adoption of these biomarkers, they represent a potential easily accessible modality for prediction of endothelial dysfunction and poor clinical outcome in trauma patients.

4. Damage-Associated Molecular Patterns (DAMPs)

Patients who survive the initial insult of severe injury are often affected by inflammatory-mediated complications including sepsis and multiple organ failure (Wafaisade et al. 2011; Osuka et al. 2014). Damage-associated molecular pattern molecules (DAMPs) represent a group of endogenous danger signals that are released from damaged, dying, or stressed cells (i.e., hypoxia), resulting in a non-infectious activation of the innate immune system. Disruption of macro- and micro-barriers (i.e., skin and cell membranes) following severe injury causes

systemic injury-related release of DAMPs which results in an unbridled innate immune response. Innate antigen-presenting immune cells are activated by this massive release of DAMPs after traumatic injury, causing a sterile systemic inflammatory response syndrome and downstream organ damage and dysfunction (Huber-Lang et al. 2018; Tsukamoto et al. 2010). Our group recently published the results of a large-scale multi-omics study of the levels of thousands of proteins and metabolites present in the circulation after severe injury in humans (Wu et al. 2021a). We found that over 1000 cell and tissue constituents were released into the circulation early after injury and that the levels were much higher in patients that died early or experienced a complicated clinical course (study described in greater detail below). Most of these biomolecules had not been previously measured. This finding indicates that there are many DAMP-like molecules released into the circulation and most of these have not been studied. Given the correlation between DAMPs, inflammatory mediators, and outcomes, these molecules have a potential role as prognosticators following traumatic injury. In this section, we will describe several DAMPs with prognostic potential for outcomes in the traumatically injured patient (Table 3).

High-Mobility Group Box 1 (HMGB1)

High-mobility group box 1 (HMGB1), a DNA nuclear binding protein, is one of the most extensively studied DAMPs in the context of traumatic injury. HMGB1 is involved in the initiation of sterile inflammation by activating macrophages and endothelial cells to release cytokines (i.e., IL-6, TNF-a, IL-1) as well as activating several receptors including toll-like-receptor 4 (TLR4) and receptor for advanced glycation end products (RAGE)(Fink 2007). Experimental studies have confirmed that HMGB1 drives systemic inflammation and organ injury after hemorrhagic shock (Levy et al. 2007) and peripheral tissue trauma in the form of lower extremity fracture (Levy et al. 2006). Yang et al. first showed that HMGB1 levels are elevated early in human trauma and related to severity (Yang et al. 2006). An investigation by Cohen et al. described the release of HMGB1 into the circulation within 30 minutes after severe traumatic injury. Further, they characterized a correlation of HMGB1 levels with increasing injury severity score and base deficit,

Table 3 DAMPs

Biomarker	Physiologic role/significance
High-mobility group box 1 (HMGB1)	DNA nuclear binding proteins involved in initiation of sterile innate inflammation
Mitochondrial DNA (mtDNA)	Circulatory mtDNA activates neutrophils through TLR-9
Heat-shock protein 70 (HSP70)	Activation of APCs through TLR-2 and TLR-4 pathways

indicating that release of HMGB1 requires severe tissue injury and hypoperfusion. The authors also demonstrated an association of HMGB1 levels with coagulopathy, hyperfibrinolysis, systemic inflammatory response, and activation of complement. In terms of clinical outcomes, elevated plasma HMGB1 levels were correlated with increased likelihood of death, acute lung injury, and acute renal failure (Cohen et al. 2009).

HMGB1 was also found to correlate to poor outcomes in a study by Abboud et al., which utilized stringently matched cohorts of survivors and non-survivors of blunt trauma to investigate the dynamic networks of systemic inflammation differentiating mortality outcomes. Non-survivors had significantly elevated circulating concentrations of HMGB1. Dynamic network analysis over time comparing survivors and non-survivors identified predominantly lymphoid-associated cytokine networks that were established within 24 h and persisted over 7 days in survivors. In contrast, non-survivors were characterized by both innate and lymphoid mediator networks early on that evolved into innate-dominant networks by 72 h. The authors also identified a link between HMGB1 and the IL-23/Th17 immune axis in non-survivors. Taken together, this study suggests that factors beyond injury characteristics influence non-survivors toward a loss of lymphocyte regulation and self-sustaining inflammation that results in organ dysfunction and death. HMGB1 may represent an upstream biomarker in these inflammatory networks to identify patients at risk for organ dysfunction and death within 24 h of traumatic injury (Abboud et al. 2016).

A similar investigation by Namas et al. examining circulating biomarker patterns in patients with and without nosocomial infection also identified elevated HMGB1 levels in patients who developed nosocomial infection. In one of three patterns that differentiated patients without nosocomial infection from patients with nosocomial infection, MCP-1/CCL2, IL-6, HMGB1, and IL-1RA were significantly elevated on presentation in those with nosocomial infection. Thus, in addition to risk stratification for mortality and organ dysfunction, elevated HMGB1 at admission may predict later development of nosocomial infection in patients sustaining traumatic injury (Namas et al. 2016b).

HMGB1 has also been shown to be predictive of outcome in patients with traumatic brain injury (TBI). In a study analyzing correlation of HMGB1 with traumatic brain injury outcomes, admission HMGB1 level was identified as an independent predictor for 1-year mortality. A receiver operative characteristic (ROC) curve identified that a baseline plasma HMGB1 concentration > 10.8 ng/mL predicted 1-year unfavorable outcomes based on Glasgow Outcome Scale (i.e., death, persistent vegetative state, severe disability) with 81.2% sensitivity and 84.5% specificity (area under curve [AUC] $= 0.88$). Further, a baseline plasma HMGB1 concentration > 11.7 ng/mL predicted 1-year mortality with 90.3% sensitivity and 73.3% specificity (AUC $= 0.883$). Thus, admission HMGB1 in patients with traumatic brain injury may assist in prognostication of 1-year unfavorable outcomes (Wang et al. 2012).

Mitochondrial DNA (mtDNA)

Mitochondrial DNA (mtDNA) is another form of DAMP that is released into the circulation by injured or dead cells resulting in polymorphonuclear neutrophil activation. Elevated concentrations of circulating mtDNA has been described following traumatic injury (Yamanouchi et al. 2013; Lam et al. 2004; Zhang et al. 2010). A study examining mtDNA concentrations in patients with severe sepsis or trauma identified a peak concentration of mtDNA on day 1 of admission in trauma patients that was significantly higher than healthy controls. Further, they identified a positive correlation of mtDNA concentration with injury severity score. Plasma concentrations of mtDNA on day 1 of admission were also significantly higher in non-survivors compared to survivors. Interestingly, the association between mtDNA concentration and mortality was not observed in patients with sepsis (Yamanouchi et al. 2013).

Another study that examined the concentrations of four sequences of mtDNA in trauma patients also identified associations with the development of systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), and mortality. They identified a significantly increased concentration of mtDNA among patients that developed SIRS and MODS compared to patients that did not develop these syndromes. Additionally, patients with above-median mtDNA levels in their cohort had a significantly increased relative risk of mortality (Simmons et al. 2013). While these studies are correlative, the observational associations of mtDNA concentration and poor outcome following traumatic injury suggest that mtDNA may represent a predictive biomarker following traumatic injury (Simmons et al. 2013; Yamanouchi et al. 2013).

Heat Shock Protein 70 (HSP70)

Heat shock proteins, particularly heat shock protein 70 (HSP70), represent an additional class of DAMPs that have been associated with outcome following traumatic injury. HSP70 plays a role in activating antigen-presenting cells to trigger the innate immune response (Zügel & Kaufmann 1999; Osuka et al. 2014). HSP70 has been shown to be released into the circulation following traumatic injury (da Rocha et al. 2005; Ren et al. 2016). A study by Ren et al. examined the expression of HSP70 in trauma patients with and without infection during their hospitalization. They reported increased levels of HSP70 within 6 h of injury across all injury severity categories compared to healthy controls, with the magnitude of HSP70 elevation correlating to increased injury severity. Further, trauma patients with infection had significantly higher serum HSP70 levels at 60–90 h after traumatic injury than patients without infection. Thus, the authors described a time-dependent characterization of HSP70 related to risk of infection. Patients who developed infection had persistently elevated levels of HSP70 at 24–48 and 60–90 h time periods, whereas patients who did not develop infection had decreases in HSP70 from baseline at these timepoints (Ren et al. 2016).

Similar findings regarding HSP70 concentration and immunosuppression/risk for infection were reported in a study by Timmermans et al. They described an immediate release and peak plasma concentration of HSP70 at the scene following injury which remained persistently elevated compared to normal controls for 10 days. While they did not report a significant difference in HSP70 concentration at arrival to the emergency room between patients who developed infection and those that did not, they did report a significant inverse relationship between HSP70 and HLA-DRA concentration. The authors also demonstrated that low HLA-DR levels were associated with decreased production of pro-inflammatory cytokines and development of infections. They suggest a potential mechanism of early DAMP release resulting in an acute suppressed immune state which was associated with development of nosocomial infections. While these studies are associative and not causal/mechanistic, they suggest that HSP70 may be a useful early biomarker for prediction of infection following traumatic injury (Timmermans et al. 2016; Ren et al. 2016).

Serum HSP70 has also been shown to predict mortality following traumatic brain injury (TBI) in males. In a study examining HSP70 levels at various timepoints (ICU admission, 24 h, 7 days) in males with severe TBI by da Rocha et al., mean HSP70 levels were significantly elevated at all time points compared to a control group of healthy volunteers. Further, on admission to ICU, nonsurviving patients had significantly higher proportions of elevated HSP70 compared to survivors. In addition, on receiver operator curve analysis, they determined a cutoff of 62 ng/mL within 20 h of injury had 70% sensitivity and 80% specificity for in-hospital mortality. This study is one of the first to identify diagnostic performance of HSP70 for in-hospital mortality among male patients with TBI (da Rocha et al. 2005). Despite these advances, further analysis of the diagnostic performance of HSP70 is required in additional subgroups of traumatically injured patients and adverse outcomes prior to clinical adoption.

Histones

Histones are nuclear chaperone proteins that interact with DNA and undergo numerous post-translation modifications that impact transcription. A nucleosome particle consists of DNA wrapped 1.7 times around a core of histone proteins (H2A, H2B, H3, and H4) with additional linker histones. This configuration gives chromosomes a more compact shape and structural support. Histones function as DAMPs as they are actively or passively co-released with nuclear DNA from dying cells and exert their influence on circulating immune cells through pattern recognition receptors, especially TLR4. The effects of histones include acting as chemokines, inducing cytokine release, inducing apoptosis, and direct cytotoxic activity (Szatmary et al. 2018). Histones, along with free DNA and granular proteins, are also able to form neutrophil extracellular traps (NETs) which are pro-inflammatory mediators and have been implicated as players in numerous inflammatory processes. Abrams et al. prospectively measured circulating histone levels in a cohort of 52 patients with severe nonthoracic blunt trauma in an effort to understand mechanisms behind

acute lung injury complicating trauma. The authors identified a surge of histone release at 4 h with circulating levels significantly higher in trauma patients than in healthy controls. Patients that went on to develop respiratory failure had significantly higher levels of circulating histones than those that did not develop respiratory failure. A threshold level of histone protein of 50 ug/mL appeared to be a threshold above which patients were more likely to develop respiratory failure. Furthermore, linear correlation analysis demonstrated that circulating histone levels correlated with SOFA scores (Abrams et al. 2013). This data suggests that circulating histones may play a role in the development of acute lung injury after trauma and that circulating histones may prove to be a prognostic biomarker in trauma patients.

Multi-Omics: Emerging Technologies with Predictive Capability

The deviation from homeostasis following trauma is abrupt and extensive across many cellular processes. A new approach to understanding the underlying biology is through a multi-omic approach consisting of distinct types of data, such as genomic, transcriptomic, metabolomic, proteomic, as well as many others. Thousands of measured molecules can be assessed qualitatively, looking at fold changes and intensities, or, quantitatively, by measuring concentrations. Advanced techniques have emerged to accurately study different molecular parts of the multi-omic landscape producing large integrated datasets. This has revolutionized how researchers look at diseases, including trauma (Subramanian et al. 2020). This field, while young, represents an opportunity for evaluating a massive number of molecules across numerous body compartments and assessing their potential as predictive biomarkers.

New techniques have been developed with the advent of multi-omics. Notably, liquid chromatography-mass spectrometry is a platform that measures molecules by separating them based on mass and charge and has been optimized for such approaches. A DNA-aptamer-based platform is another novel method for measuring proteins that has added thousands of distinct proteins to the multi-omic field (Krassowski et al. 2020; Kim et al. 2018).

Multi-omic assays bring the challenges of analyzing and interpreting high-dimensional, integrated datasets. Data science and computational biology methods with unsupervised and supervised statistical approaches are necessary to interpret and analyze multi-omic datasets. Unsupervised exploratory analysis uses various clustering and network-based correlations to identify dimensionality reduced patterns in data with no pre-assigned labels. Heatmaps, K-means clustering, and principal component analysis (PCA) are examples of unsupervised methods. Supervised analysis, on the other hand, includes predetermined groupings, such as outcome, that assess differences in expression profiles (Krassowski et al. 2020). Both methods are used concurrently to glean insight into the multifaceted underlying biological process resulting from traumatic injury.

While application of multi-omic strategies in predictive modeling is a relatively new field, there have been promising studies published recently. Cyr et al. evaluated

changes in the circulating metabolome (i.e., carbohydrates, lipids, amino acids, nucleic acids) in response to traumatic injury (Cyr et al. 2021). Plasma samples were drawn from 86 blunt trauma survivors with an average ISS of 23.8. 1000 metabolites were identified, with 45% being lipid molecules. Among the top 50 metabolites showing statistically significant differences from controls, there was enrichment of sphingolipids, and species of sphingolipids demonstrated a time-dependent pattern following trauma. Patients were clustered into three groups based on sphingolipid levels: those with no change in sphingolipid levels (non-responders), those with enrichment of sphinganine and sphingosine molecules, and those with enrichment in complex glycosphingolipids. The non-responder group had longer mean LOS, more ventilator days, higher MOD scores, and higher levels of circulating pro-inflammatory cytokines when compared to the groups of patients with enhanced sphingolipid levels despite similar ISS. This data suggests a correlation between circulating sphingolipid signatures, the immune response to trauma, and clinical outcomes. Wu et al. expanded on these findings and went on to characterize lipid metabolism following trauma and develop a lipid reprogramming score (LRS). The LRS is comprised of eight phosphatidylethanolamine (PE) species that were higher in slowly resolving patients, and the authors validated a predictive score that is representative of an overall reprogramming of lipid metabolism. The LRS was also shown to correlate with inflammatory markers. Furthermore, non-resolving trauma patients were shown to have dramatically increased LRS at 24 and 72 h after trauma when compared to resolving patients (Wu et al. 2021a).

Wu et al. have added to this work by using a true multi-omic approach in order to better understand the systemic response to trauma. In their recent analysis, they use clinical, cytokine, endotheliopathy markers, lipidome, metabolome, and proteome data from trauma patients enrolled in the PAMPer trial [The Prehospital Air Medical Plasma, (Sperry et al. 2018)]. The findings were validated in a second cohort of 472 blunt trauma survivors. The authors identified what they termed a "systemic storm" in the hyper-acute phase of traumatic injury with release of 1061 molecules into circulation. There was a concurrent "massive consumption" that occurred, consisting of consumption of 892 molecules from circulation. Following this hyper-acute phase, they identified molecules that defined "resolution" or "non-resolution" signatures at 72 h, consisting of 56 and 172 molecules, respectively. Using this data, they defined two endotypes of response to injury, which correlated with clinical outcomes. This data is the first of its kind to define the human response to trauma on a multi-omic, systemic scale. This database can serve as a resource to study a much larger number of potential biomarkers from across many families of biomolecules (Wu et al. 2021b).

Conclusions

Traumatic injury causes significant morbidity and mortality across all age groups and geographical regions. The widespread prevalence of traumatic injury emphasizes the importance of early prognostication and identification of patients with predicted poor outcomes to potentially intervene early in their clinical course. Additionally, trauma

has a unique benefit in the realm of prognostic biomarkers as it is the only disease process where the initiation of disease can be discretely identified. Thus, recent work in the field of trauma has aimed to identify early markers correlating with, and eventually predicting, injury characteristics (i.e., traumatic brain injury, injury severity) and clinical outcomes (i.e., mortality, organ failure).

In this chapter, we summarize several classes of biomarkers that are associated with injury characteristics and clinical outcome. Many of the studies described in this chapter identified correlations and associations of biomarkers with variables of interest. While this is an important initial step in understanding the pathophysiologic response to trauma, further work with modeling and diagnostic performance of these biomarkers is needed to establish their true predictive potential and potentially adopt them for use in the clinical setting. Nevertheless, we outline this promising preliminary work of classes of biomarkers in trauma that can potentially be used to prognosticate outcomes including cytokines, genomics, endothelial damage markers, and DAMPs.

From this body of work, the intricate interplay between inflammation, coagulation, metabolism, and sympathoadrenal activation after traumatic injury is highlighted. Thus, integration of these separate systems into cohesive analysis is essential to understanding the complex pathophysiology and predictive biomarker response in trauma. Multi-omics is a novel approach to examining the relationship between multiple systems and classifications of data and has shown promise in the identification of biomarkers predicting outcome and injury characteristics. While work remains to integrate predictive biomarkers into clinical practice for trauma, we outline several potential molecules and techniques that are integral to moving closer to predictive biomarker clinical assays.

Applications to Other Diseases or Conditions

In this chapter, we describe biomarkers that have been used for or show promise for prognostication following traumatic injury. Importantly, many of the findings described are relevant to sepsis, and there is a significant degree of overlap between the immune-inflammatory response to trauma and sepsis. CRP and IL-6 are examples of mediators that have prognostic value in both disease processes (Song et al. 2019). In addition, multi-omics holds equal promise for prognostication in sepsis as it does in trauma. In fact, multi-omics has been a focus in the sepsis field for quite some time. Several studies have used multi-omics to better understand the pathophysiology of sepsis and identify potential biomarkers (Liu et al. 2014; Langley & Wong 2017).

Mini-Dictionary of Terms

- Traumatic brain injury: a direct injury to the head affecting the brain.
- Multi-omics: an integrated biological field of study encompassing multiple layers including genomics, transcriptomics, metabolomics, lipidomics, proteomics, cytokines, and clinical data.

- Biomarkers: measurable substances in the human body that can indicate specific disease states.
- Cytokines: substances that regulate inflammation released by cells of the immune system such as interleukins and interferons.

Summary Points

- Traumatic injury, as a disease entity, is well suited to utilize biomarkers for prognostication due to a precisely identifiable time of disease onset.
- Alterations in circulating biomarker molecules can be identified as early as 45 min following injury.
- There is an immediate activation of an immune-inflammatory response following traumatic injury, which is reflected in many of the most promising circulating biomarkers.
- The response to polytrauma with TBI may vary from polytrauma without TBI, and prognostic biomarkers are likely to be different for patients with TBI.
- Biomarkers with promising data fall into several different classes including cytokines, DAMPs, endothelial damage markers, gene polymorphisms, transcriptional profiles, and multi-omic profiles.
- Multi-omic strategies can be utilized to assess the landscape of thousands of molecules in circulation and provide opportunities to identify novel prognostic biomarkers.

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Part II

Biomarkers in Critical Care and Illness

The Role of C-Reactive Protein and Implications to the Neonatal Intensive Care Unit

8

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Abstract

As neonatal infections are difficult to diagnose with accuracy and have a high prevalence with serious long-term consequences, biomarkers of infection have been widely studied to improve the diagnostic accuracy and reduce antibiotic exposure in patients who are not infected. C-reactive protein (CRP) is a serum protein that increases in the blood in response to inflammatory or infective triggers. The function of CRP is important in the humoral response to bacterial infection during the acute phase, and it has been widely used as a marker of severe bacterial infection in both adults and children; however, as a result of its delayed synthesis, the sensitivity during the earliest phases of infection is poor, and it may rise in response to other non-infectious triggers. Its accuracy and negative predictive value to rule out infection increase over time with serial investigations. In this review, we discuss the kinetics of CRP, its role in the diagnosis of infection in the neonate, factors that may affect its measurement, and its usefulness in monitoring the response to treatment in the infected neonate.

Keywords

C-reactive protein · Neonatal sepsis · Biomarkers · Neonate · NICU · Preterm · Term

Abbreviations

CRP	C-reactive protein
ELBW	Extreme low birth weight
EONS	Early-onset neonatal sepsis
hsCRP	High-sensitivity C-reactive protein
IL-1	Interleukin-1
IL-6	Interleukin-6
LONS	Late-onset neonatal sepsis
nCD64	Neutrophil CD64 percentage
NICU	Neonatal intensive care unit
PCT	Procalcitonin
POC	Point of care
RCT	Randomized controlled trial
ROC	Receiver operator characteristic curve
SNAP	Score for neonatal acute physiology
VLBW	Very low birth weight

Introduction

C-reactive protein (CRP) is an acute phase reactant protein measurable in serum that rises in concentration during states associated with inflammation or tissue necrosis (Gewurz et al. 1982). It is widely adopted as a marker of infection especially in the context of neonatal sepsis, which is any infection that occurs in the newborn in the first 28 days of life. C-reactive protein serum levels are often measured in serial specimens taken hours to days apart to improve the diagnostic likelihood of infection. Serial measurements may also provide a guide to clinicians to assess the response to antibiotic treatments and the necessary duration of treatments.

Many studies have evaluated neonatal sepsis and associated markers of infection with varying results (Hedegaard et al. 2015; Iroh Tam and Bendel 2017). These include blood cell counts and neutrophil ratios, acute phase reactants, and several cytokines and chemokines. Of these, CRP has been among the most extensively studied and widely used in practice (Hofer et al. 2012).

The plasma protein was discovered in 1930 by Tillet and Francis at Rockefeller University (Tillett and Francis 1930). While investigating the sera of patients suffering from acute pneumococcal infection, they observed a precipitation reaction between sera from patients suffering from acute pneumococcal pneumonia and the extracted polysaccharide fraction C from the pneumococcal cell wall. This reaction was not observed in healthy controls nor in patients that had recovered from pneumococcal pneumonia (Tillett and Francis 1930). CRP was thus named for its reaction with the capsular (C)-polysaccharide of pneumococcus. During the 20 years that followed, CRP was detected in more than 70 disorders including acute bacterial, viral, and other infections and non-infectious diseases such as acute myocardial infarction, rheumatic disorders, and malignancies (Pepys 1981). Though disorders of varying etiology, their commonality lies in an underlying process of inflammation and/or tissue injury.

C-reactive protein is homopentameric, composed of five identical subunits arranged in a cyclic pentameter shape (Fig. 1). The whole protein has a diameter of 102 Å (1 Ångström = 0.1 nanometer) and a molecular weight of 118,000 Daltons (Volanakis 2001). The major physiological role of serum CRP is to bind to microbial polysaccharides and immune complexes and activate the classical complement cascade.

In infants exposed to infectious inflammatory stimuli, serum CRP levels may rise by more than 100-fold, declining with a half-life of about 18 to 24 hours when the stimulus ceases (Ehl et al. 1999). However, many non-infectious inflammatory stimuli including chemical or physical irritation may also cause serum CRP levels to rise in infants (Hofer et al. 2012). Microbiological culture of a potentially pathogenic organism remains the gold standard for diagnosing early- and late-onset infection. However, a blood culture sample may take 24 to 48 hours to flag as positive (Cantey et al. 2016). Hence, CRP has been used as a biomarker to make an immediate assessment of the overall likelihood that an infant is truly infected.

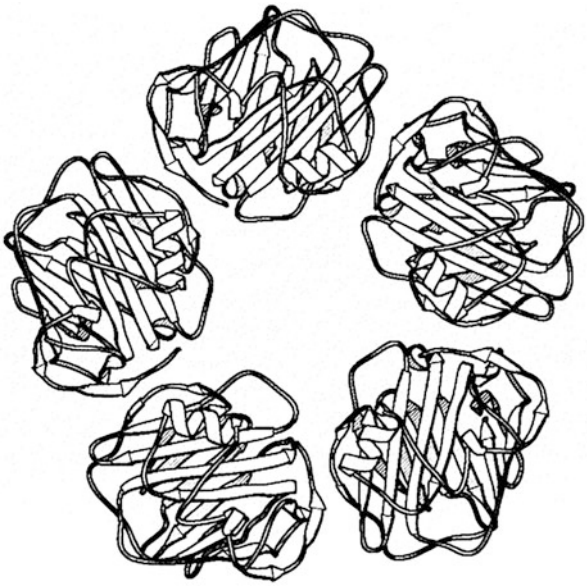

Fig. 1 Pentameric structure of CRP viewed down the fivefold symmetry axis. (From Volanakis (2001) with permission)

Neonatal Sepsis

The occurrence of sepsis in the neonate is a serious complication that is extremely common. Morbidity and mortality due to sepsis is high, particularly in preterm hospitalized infants. The incidence is estimated at three million cases annually worldwide live births with much higher incidences in developing countries reported (Fleischmann-Struzek et al. 2018). The reported mortality of neonatal sepsis accounts for up to 30% of infant deaths annually (Ershad et al. 2019).

The clinical diagnosis of neonatal sepsis is fraught with difficulties, some of which stem from the fact that neonates collectively are a heterogenous population with distinct subsets. They encompass the well neonate born at term with a normal birthweight who is discharged home within hours of birth and, on the other end of the spectrum, extremely preterm babies who have ineffective skin and mucosal barrier protection and limited humoral and immature cellular immune systems (Collins et al. 2018). The latter group are largely born with a very low birth weight (VLBW) of less than 1500 grams and remain in neonatal intensive care for several weeks to months. They have several risk factors which include invasive mechanical ventilation, nutritional, and feeding challenges. The presence of invasive indwelling vascular or other catheters and invasive procedures causes breaks in skin or mucosal integrity and places these patients at extreme risk of infection. A large study of extreme low birth weight (ELBW) infants born with birthweight below 1000 g found that 65% surviving infants had at least 1 infection during their hospital stay (Stoll et al. 2004).

Neonatal sepsis can be broadly categorized into early-onset neonatal sepsis (EONS) and late-onset sepsis and (LONS). Although definitions in the literature vary, EONS is typically described as infections that occur up to 72 hours of life and LONS is infections that occur after 72 hours of age up until the end of the neonatal period (Ershad et al. 2019; Walker et al. 2019). The distinction between EONS and LONS is useful for considering the different etiologies. Neonates with EONS are commonly infected by vertical transmission of pathogens from maternal sources, the commonest organisms being Group B *Streptococcus* and *Escherichia coli* (Stoll et al. 2011). Hospital-acquired infections account for the majority of LONS; predominant pathogens are coagulase-negative staphylococci, followed by Gram-negative bacilli and fungi (Dong and Speer 2015). Large multicenter studies in the United States have found that EONS occurs in 1.5 to 2% of VLBW infants and LONS in 21% of VLBW infants (Stoll et al. 2002; Stoll et al. 2011). Infants with LONS are significantly more likely to die than those who were uninfected, especially if they were infected with Gram-negative organisms or fungi (Stoll et al. 2002), and there is significant risk of long-term neurodevelopmental sequelae in survivors (Stoll et al. 2004).

Rational Antibiotic Use

Neonates who have infection may present insidiously with a constellation of non-specific symptoms, and prompt and reliable confirmation of infection remains challenging. Given the high risk of mortality of long-term morbidity in survivors, empirical antibiotic treatment is initiated on suspicion of infection. As the pathogens are variable and unknown, antibiotic therapy is generally broad spectrum; often unnecessary and frequently treatment is prolonged beyond what is needed (Dong and Speer 2015). A retrospective cohort study of more than 50,000 infants in 127 NICUs across a large US state demonstrated 40-fold variation of antibiotic usage, from 2.4% to 97.1% of patient days. At all levels of care, it was independent of proven infection, NEC, surgical volume, or mortality. Half of intermediate NICUs were in the upper quartile of antibiotic use despite most of the units reporting zero infections (Schulman et al. 2015), supporting the argument that antibiotics are overused. The serious and concerning impact of this is an increasing number of multidrug-resistant Gram-negative microorganisms in neonatal intensive care units (NICU) worldwide (Dong and Speer 2015). In addition, unnecessary antibiotic exposure may lead to an alteration in the preterm neonatal gut microbiome by diminishing microbial species alpha-diversity, reduced protective bacterial genera, and increased proportions of potentially pathogenic bacteria (Van Belkum et al. 2020). In the short term, there is concern that this dysbiosis will lead to gut illnesses like necrotizing enterocolitis in the preterm infant, but there are long-term concerns of immunologically mediated diseases like inflammatory bowel disease, wheezing, and eczema as well as obesity (Murgas Torrazza and Neu 2011; Turta and Rautava 2016) There is also emerging evidence of a microbiota-gut-brain axis in humans

during early life; exposure to gut-microbiome disruption may impact the neurodevelopment of infants (Hickey et al. 2021).

For the reason mentioned above, it would be desirable to limit the antibiotic exposure in these infants, using a structured antimicrobial stewardship program. This would include using local microbiological surveillance data to adapt empiric treatments that target the prevailing antimicrobial resistance patterns, to use narrow spectrum antibiotics wherever possible, and to only use antibiotics when significant infection is likely (Russell et al. 2012). However, the difficulty in diagnosing the infection or confirming with a reasonable certainty that infection is unlikely is where the challenge lies. Currently, diagnosis of suspected infection in the clinically ill neonate is confirmed by the isolation of the causative organism in cultures from blood, cerebrospinal fluid or other samples. In the absence of the confirmed bacteriological culture, the diagnosis of infection is often suspected and treated empirically by considering the clinical picture of the patient as well as the measurements of biomarkers of infection in patient blood samples. A host of biomarkers of infection have been identified and utilized in clinical practice, of which C-reactive protein is the most widely studied in the neonatal population (Iroh Tam and Bendel 2017).

Blood Cultures

Blood cultures are still considered the gold standard microbiological test in aiding the diagnosis of sepsis, although this method has several limitations, especially in relation to the neonate (Cantey and Baird 2017). The successful culture of a microorganism is dependent upon various factors including the number of blood cultures, volume of blood collected, technique, and antibiotic exposure.

In the neonate, the standard practice is for the collection of a single blood culture, often due to the small total blood volume of neonate, especially if in septic shock, the increased risk for the need of blood transfusions, and difficulty of venipuncture (Buttery 2002). A single blood culture is however considered of limited use; increasing the number of blood culture bottles from a single culture improves the diagnostic yield (Buttery 2002; Ntusi et al. 2010).

An adequate volume of blood is required to shorten the detection time by automated blood culture systems. At least 1–2 ml of blood per blood culture bottle is recommended (Schelonka et al. 1996). In the ELBW or infant with septic shock, these volumes might not be achievable. Contamination of the specimen due to poor technique complicates the patient management and prolong empiric antibiotic therapy (Cantey and Baird 2017). Arterial venipuncture is not considered superior to peripheral venous collection, although stringent skin preparation is recommended to reduce the risk of blood culture contamination (Buttery 2002). Prior antibiotic exposure via intrapartum antibiotic prophylaxis may reduce the bacterial concentrations, calculated as colony forming units, to below the detection limit of the automated systems. Ideally at such low bacterial concentrations, the neonate's innate immune system should be able to overcome the infection (Cantey and Baird 2017). Cantey et al. (2016) highlighted neonates with negative blood cultures, due to low

bacterial concentrations, and appropriate empirical antibiotic treatment of up to 48 hours did not require repeated antibiotic treatment. They questioned the relevance of low bacterial concentrations in the neonate with at least 48 hours of empiric antibiotic treatment (Cantey et al. 2016).

These various factors contribute to the low positivity rate of blood cultures, with only 30–50% of blood cultures collected from suspected septic patients reported as positive (Gupta et al. 2016). In many developing countries, blood culture negative sepsis accounts for most of the reported cases (Zea-Vera and Ochoa 2015). This sensitivity and the increased understanding of the systemic inflammatory response and the role of endo- and exotoxins in sepsis are indicative that bacteremia is not always present in patients with sepsis (Zrodlowski et al. 2020). With consideration for the prolonged period required to detect bacterial growth in automated systems, the diagnosis of sepsis cannot solely depend on a positive blood culture but requires the consideration of both clinical and additional laboratory biomarkers.(Zea-Vera and Ochoa 2015; Zrodlowski et al. 2020) Although newer molecular techniques could improve the sensitivity and specificity of microbiological testing for sepsis, these are more expensive and not readily available at most hospital laboratories. (Zea-Vera and Ochoa 2015).

CRP Kinetics and Clinical Utility

CRP production is triggered by inflammatory cytokine induction of CRP gene transcription. This predominantly occurs in the liver, but other tissues can also express the CRP gene. The strongest induction is through interleukin-6 (IL-6), a response often enhanced by interleukin-1 (IL-1), although IL-6 is not capable of triggering CRP gene expression on its own accord (Sproston and Ashworth 2018). Once released into circulation, CRP recognizes, binds, and aggregates various cellular structures. This includes plasma lipoproteins, phospholipids, damaged and apoptotic cells, as well as extrinsic components of various microorganisms (Pepys and Hirschfield 2003). The strongest binding affinity is towards phosphocholine, expressed on membranes of various microorganisms, as well as most eukaryotic cells. This partially explains the limited specificity of CRP in diagnostic testing (Sproston and Ashworth 2018). Once aggregated, CRP interacts with the complement pathway of the innate immune system. CRP is recognized by C1q, leading to formation of the terminal membrane attack complex and activation of the classical complement pathway. Binding to factor H through secondary binding sites, CRP also activates the alternative complement pathway with production of C5 convertases. The activated complement system is then able to facilitate opsonization and phagocytosis of microorganisms and partake in the proinflammatory response to infection (Pepys and Hirschfield 2003). Binding of CRP to the Fc receptors on IgG antibodies triggers the production of additional proinflammatory cytokines, and its interaction with neutrophils, natural killer cells, and platelets promotes antibody-dependent cellular cytotoxicity (Povoa 2002; Sproston and Ashworth 2018). Within 4 to 6 hours after cytokine stimulation, CRP will appear in circulation,

Table 1 Non-infectious conditions that have been associated with elevated C-reactive protein concentrations in infants categorized by occurrence in mother or infant

Non-infectious conditions that have been associated with elevated C-reactive protein concentrations in infants
Maternal and intrapartum:
Maternal intrapartum fever
Prolonged rupture of membranes
Prolonged labour
Fetal distress
Ventouse assisted delivery
Perinatal asphyxia
Infants:
Meconium aspiration syndrome
Surfactant administration for respiratory distress
Intraventricular hemorrhage
Pneumothorax
Tissue injury
Immunization

Adapted from (Hofer et al. 2012)

doubling every 8 hours and reaching a peak value in 36 to 50 hours. Termination of the cytokine stimulus will decrease the concentration of CRP, with an average half-life of 19 hours (Pereira et al. 2019).

The CRP concentration is independent of the causative pathology, and changes will only reflect interventions directed at reducing or removing the cytokine inflammatory stimulus that triggered the acute phase response. In essence, CRP value is only dependent on the degree of inflammation, with production rate and the concentration increasing with any inflammatory process, except when associated with hepatic failure (Povoa 2002). Marked CRP elevations are associated with most systemic bacterial and fungal infections, with only mild increases noted in acute viral infections, although some viral pathogens (adenovirus, measles/mumps, and influenza) can trigger high CRP concentrations during uncomplicated infections. When associated with systemic infections, cytomegalovirus and herpes simplex virus also cause severe increases in CRP (Povoa 2002). Non-infectious conditions associated with increases in CRP include malignancies, trauma, recent surgery, auto-immune diseases, and acute myocardial infarction (Table 1). It is widely accepted that CRP values are greater in infectious conditions than non-infectious conditions, and in adult patients with fever, a level of 87 mg/L or more is suggestive of infection. With severe infections, the value can be more than 1000 times the upper range of normal but will not correlate with the possible focus of the infection (Pereira et al. 2019).

Factors Affecting CRP Measurement

In healthy neonates, CRP increases physiologically over the first 24–48 hours, with concentrations affected (increased) by gestational age and birth weight, but not by gender (Chiesa et al. 2011a, b). Concentration peaks between 27–36 hours (to as

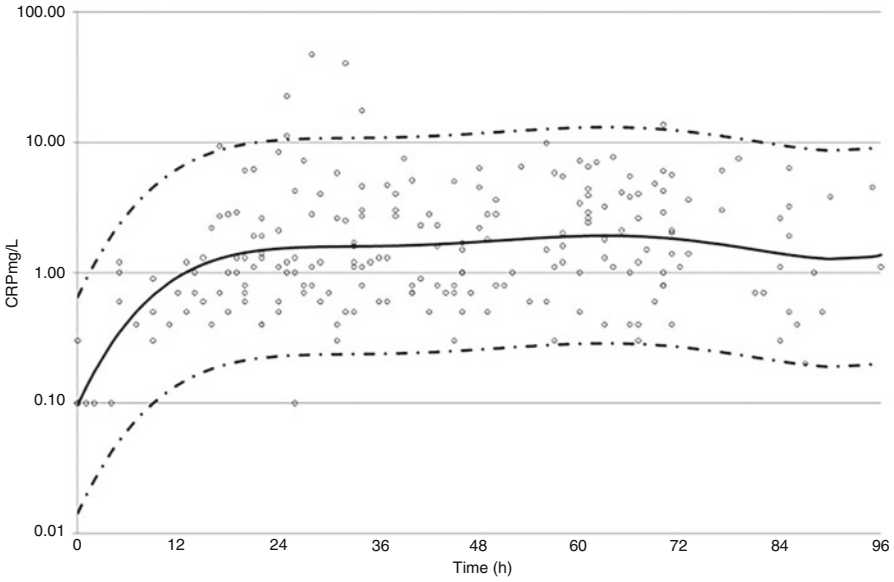

Fig. 2 Age-specific 95% reference intervals for C-reactive protein (CRP) in healthy-term neonates from birth to 96 h of life. The circles represent single values; the dotted lines represent lower and upper limits: the bold line represents the predicted geometric mean. Note the logarithmic scale of CRP. (From (Chiesa et al. 2011a, b) with permission)

high as 13 mg/L), declining by about 90 hours (to a maximum of 4.7 mg/L). Figure 2 shows age-specific 95% reference intervals for C-reactive protein (CRP) in healthy-term neonates from birth to 96 h of life (Chiesa et al. 2011a, b).

Very little CRP crosses the placenta, implying that any elevation represents endogenous synthesis (Hofer et al. 2012). Non-infective stimuli associated with CRP synthesis in the early neonatal period include shock, meconium aspiration pneumonitis, fetal distress, intraventricular hemorrhage, anoxic encephalopathy, respiratory distress syndrome, low 5 min APGAR, maternal fever, premature rupture of membranes, prolonged labor, pregnancy-induced hypertension, and vacuum extraction (Hofer et al. 2012). Early-onset sepsis may present with similar clinical signs to the conditions above, making the diagnosis of sepsis difficult to exclude or confirm using CRP. Furthermore, several studies have suggested that gestational age may play a role in CRP kinetics with lower baseline CRP values and lower sensitivities to infection in preterm newborns compared to term newborns (Hofer et al. 2012). In a study of 1010 episode of LONS confirmed by positive blood stream infections in 793 neonates, Lai et al. found patients with a low CRP ($\leq$ 10 mg/L) had a lower birth body weight and gestational age and an earlier onset of infection than patients with intermediate (11–100 mg/L) and high CRP (>100 mg/L) measurements (Lai et al. 2015).

Biological variation may be an additional consideration. In children, CRP has been shown to have a biological variation of 19.3% intra-individually and 125.4%

between individuals (Bailey et al. 2014). However, there does not appear to be a significant diurnal (Meier-Ewert et al. 2001) or seasonal rhythm (Sproston and Ashworth 2018).

In terms of preanalytical considerations of measurement, CRP is an ideal analyte as it is stable in serum. It is stable for 11 days at room temperature and 60 days in the fridge and remains unchanged for months to years at −70 degrees Celsius (Wilkins et al. 1998), adding to its practical utility. CRP levels are unaltered by enteral nutrition (Ledue and Rifai 2001) and display little interference by drugs that do not alter the inflammatory process. However, certain assays may be affected by lipemia (Knezevic et al. 2020) and the high-dose hook effect (antigen excess).

CRP is measured by immunoassay (competitive or sandwich), usually by immunoturbidimetry or nephelometry on automated analyzers. These methods are accurate, freely available, affordable, and rapid, with acceptable turnaround times, usually around 1–2 hours depending on the distance from the laboratory. There are many instrument platforms currently available for the measurement of C-reactive protein, demonstrating different performance characteristics (area under curve ROC, analytical sensitivity, measuring range, precision) and employing different methodologies for detection, antibodies, incubation periods, and wash steps. These differences result in variability in results obtained on different instruments, despite most being traceable to a single reference material (ERM-DA470) (Merlini et al. 2010, Päivi Ranta et al. 2017).

Point-of-care (POC) testing devices (usually employing lateral flow sandwich immunoassay methods) have been shown to be clinically viable in low-income settings where laboratory-based testing is not readily available or turnaround time is compromised (Prince et al. 2019); however, care must be exercised when employing published medical decision limits, as significant negative (Matheeussen et al. 2018) and positive biases may be present compared with automated laboratory-based methodologies. This variability is due to the different analytical methodologies employed, but also the different sample matrices being used. Point-of-care instruments use whole blood samples, which have been shown to demonstrate a negative proportional bias compared with serum samples, on which most medical decision limits have been derived (Roberts et al. 2001; Phommasone et al. 2016; Escadafal et al. 2020).

Clinical Applications of C-Reactive Protein in Sepsis

Features of infection, such as raised white cell counts and fever, are influenced by various factors and are of limited reliability as sepsis biomarkers (Povoa 2002; Hedegaard et al. 2015). The clinical applications of CRP have been studied extensively in both adult and neonatal patient populations for the diagnosis of infection, and the evidence favors the value of serial CRP measurements over a single reading, as CRP levels are only dependent on the rate of production (Povoa 2002).

Although several studies indicate that a single CRP measurement between 50 and 100 mg/L could be considered as a useful marker of sepsis, a true cut-off value for

sepsis is poorly defined and may differ in various types of infections and in various patient populations (Povoa 2002). Ugarte et al. reported that at a value of 50 mg/L, CRP had a sensitivity of 98.5% and specificity of 75% for the diagnosis of sepsis (Ugarte et al. 1999). Numerous studies agree that when serial CRP measurements show a steady increase in value over 48–72 hours, infection should always be considered. Limited data is available regarding the kinetics of CRP prior to the onset of sepsis, although an earlier report by Matson et al. showed that in critically ill patients, increases of 25% or more within 24 hours were a good indicator of sepsis (Matson et al. 1991).

In neonates, Benitz et al. (1998) found that the sensitivity of CRP to diagnose EONS increased from 39% at the initial sepsis workup to 84% by 24 hours to 89% for the higher of two levels obtained between 8 and 48 hours after initial workup. The corresponding specificities, however, declined from 90% at initial workup to 78% and 74%, respectively. They described the optimal cut-off value to be 10 mg/L (Benitz et al. 1998). Chiesa et al. (2003) found the cut-off value that maximized the sum of the sensitivity and specificity for CRP was 4 mg/L at birth, whereas at both 24 and 48 h of life, it was 10 mg/L in the diagnosis of culture-positive EONS (Chiesa et al. 2003). They found the sensitivity at those cut-off values to increase from 73% at birth to 91% at 24 and 48 hours at those cut-off values, while the sensitivity remained similar at 83%, 87%, and 84%, respectively. The low sensitivity at birth or initial sepsis workup in suspected EONS does not add value to clinical decision-making, as those patients who are ill or at risk of sepsis would not be spared from antibiotic exposure.

A recent systematic review and meta-analysis of 22 cohort studies with a total of 2255 infants included reported a pooled sensitivity of 62% (95% CI 50 to 72%) at a median specificity of 74% for CRP diagnosing LONS in newborn infants (Brown et al. 2020). The studies included mostly preterm or VLBW infants and used a prespecified CRP cut-off of 5–10 mg/L. Six studies calculated the CRP threshold level retrospectively by modeling the area under the ROC curve. In five of the studies, the threshold ranged from 2.2 mg/L to 18 mg/L, and in the sixth study, the threshold serum CRP level was 111 mg/L (Brown et al. 2020). Most studies used positive culture of a pathogenic organism from blood as a reference standard. The median prevalence sepsis rate in all the included studies was 40% (interquartile range 27–61%). If CRP determination was applied to a hypothetical cohort of 1000 newborn infants investigated for possible late-onset infection, the authors estimated that, if the prevalence of true infection was 40%, on average 152 cases of infection would be missed (false-negative) and 156 non-infected infants would be wrongly diagnosed (false-positive) (Brown et al. 2020). The review however was limited as it solely focused on the accuracy of CRP to determine the likelihood of infection in infants where there is a clinical suspicion of infection. Most of the studies in the meta-analysis were performed in high- or middle-income settings, limiting its generalizability.

The poor sensitivity and specificity of CRP to diagnose LONS in neonates from the meta-analysis by Brown et al. makes its utility questionable at best, especially in units with a high prevalence of infection. The result of the CRP would not

change the management of the patient with suspected infection when taken at initial presentation, as it would not prevent empirical antimicrobial treatment in an infant that appears unwell. Additionally, the positive predictive value worsens as the prevalence rate of LONS declines, leading to an increasing occurrence of treatment of "culture negative" sepsis (Cantey and Baird 2017; Cantey and Bultmann 2020).

Disease Severity and Outcome Prediction

Most agree that although serial measurements for trend analysis are of more clinical relevance than a single measurement, higher CRP levels correlate with more severe inflammatory responses and accordingly more serious or complicated infections (Chalmers et al. 2008). The CRP concentration may therefore reflect both the presence and severity of infection (Povoa 2002). Furthermore, CRP levels that fail to decrease or increase after initial decreases should raise the suspicion of the development of infection complications (Povoa 2002).

In newborn patients, CRP concentrations correlate with severity of illness as determined by the Score for Neonatal Acute Physiology (SNAP) and SNAP perinatal extension scores (Chiesa et al. 2003). The extent of response is further complicated by causative organisms. Much higher CRP concentrations are seen in neonates infected with Gram-negative organisms as well as *Staphylococcus aureus* and group B streptococci compared to other Gram-positive organisms (Hofer et al. 2012). Pourcyrous et al. reported normal CRP in patients with positive cultures with predominantly Gram-positive strains such as group D streptococci, *Streptococcus viridans*, and *Streptococcus epidermidis* who had uneventful clinical courses despite being inadequately or completely untreated, questioning the possibility of these organisms as contaminants rather than pathogens (Pourcyrous et al. 1993).

CRP kinetics has also been assessed as a prognostic marker, with several studies reporting on its value as a predictor of mortality, and is considered one of the more accurate inflammatory markers for the prediction of clinical outcomes in patients with sepsis (Povoa 2002; Pereira et al. 2019). In their meta-analysis, Zhang et al. (Zhang and Ni 2011) concluded that the mean difference between initial baseline CRP and measurements after 48 hours was lower in survivors when compared with non-survivors, suggesting serial CRP measurement over 48 hours can aid in the outcome prediction of patients with critical illness. Similarly, higher CRP levels on admission and higher peaks during the hospitalization have been reported in non-surviving patients than surviving patients presenting with infections (Povoa 2002). There is however sparse evidence in the literature regarding the utility of CRP to predict mortality in neonates. Singh et al. showed that infants with suspected serious bacterial infection and with a CRP raised above 40 mg/L showed a 4.1-fold increased risk of mortality in a low-income setting (Singh et al. 2018).

CRP Response to Guide Antibiotic Therapy

CRP kinetics have been described in terms of response to antibiotic treatment as "fast," "slow," "unresponsive," and "biphasic" patterns and associated with prognostic outcomes. Cases identified as either "fast" or "slow" responders had better clinical outcomes to antibiotic treatment in adult patients (Povoa et al. 2005). A single CRP value may not alter decisions about the initiation of antibiotic treatment; however, serial measurements can aid in identifying patients for safe discontinuation of empiric antibiotic treatment for neonatal sepsis, shortening the antibiotic exposure and hospital stay (Stocker et al. 2021). This makes CRP measurement an ideal tool in antibiotic stewardship programs with the aim of reducing antibiotic resistance and inappropriate antibiotic use (Povoa 2002).

In their study, Ehl et al. (1997) used a cut-off value of 10 mg/L to identify 99% of the neonates in the study group that did not have sepsis and could discontinue antibiotic treatment. Similarly, using a cut-off value of 10 mg/L, Philip and Mills (2000) were also able to discontinue treatment in almost 40% of neonates, with none of them representing within 30 days with features of recurrent or persistent infections.

A meta-analysis of studies assessing the use of CRP to tailor antibiotics found CRP-based algorithms reduced antibiotic treatment duration by −1.45 (95% CI -2.61 to 0.28) days in 2 RCTs and by −1.15 (95% CI -2.06 to −0.24) days with no differences in mortality or infection relapse (Petel et al. 2018). The authors caution about the relatively small sample sizes in the RCTs in the meta-analysis.

The timing of CRP testing to exclude infection has been looked at in several studies (Hofer et al. 2012). A repeat CRP taken 24 to 48 hours after initiation of therapy has been reported to have a 99% negative predictive value in ruling out EONS (Ehl et al. 1997). In a secondary analysis of the Neonatal Procalcitonin Intervention study, the authors found that normal serial CRP and procalcitonin measurements within 36 hours after the start of empiric antibiotic therapy can exclude the presence of EONS with a high probability. The negative predictive values of CRP and procalcitonin did not increase after 36 hours (Stocker et al. 2021).

CRP Combined with Other Biomarkers

CRP on its own is unreliable as a diagnostic marker of infection; however, several studies have investigated the utility of CRP combined with other biomarkers of infection to improve the accuracy of diagnosis (Deleon et al. 2015). Several combinations have been looked at, with an attempt to combine the earlier rise of some of the markers with CRP which rise more slowly but remain elevated for longer. The most extensively studied in combination with CRP are neutrophil CD64% (nCD64), PCT, IL-1β, IL-6, IL-8, and TNF-alpha.(Deleon et al. 2015) PCT may be a more sensitive marker of infection in adult and childhood infection; however, its utility in the NICU is reduced due to its physiological rise after birth (Chiesa et al. 2011a, b).

A meta-analysis of 28 studies found the pooled sensitivity and specificity increased from 71% (95% CI 63–78%) and 88% (95% CI 80–93%) for CRP alone to 91% (95% CI 84–95%) and 89% (95% CI 81–93%) when CRP was combined with PCT. There was however significant heterogeneity observed in the analysis (Ruan et al. 2018). The membrane glycoprotein nCD64, involved in the mediation of endocytosis, phagocytosis, and cytokine release, is expressed at low concentration on non-activated neutrophils but can be markedly upregulated at the onset of the sepsis process (Hashem et al. 2020). Song et al. (2019) found in their meta-analysis of 8 studies that the combined application of nCD64 and CRP produced a sensitivity of 95% (95%CI 86–98%) and specificity of 86% (95%CI 74–93%) (Song et al. 2019). However once again, the authors noted significant heterogeneity in the study analysis, due in part to different study designs and patient profiles so generalization of the findings was cautioned. Several studies have reported better diagnostic accuracy when combining IL-6 and CRP (Deleon et al. 2015). Combining multiple tests may greatly improve the sensitivity and negative predictive value of the test panel to 100% but reduce its specificity and its practical clinical application (Dilli et al. 2010). The discussion of the individual biomarkers' performance is beyond the scope of this chapter.

Conclusion

The literature on biomarkers and neonatal sepsis is challenged by the complexity and heterogeneity of the condition being studied. There is no consensus definition of sepsis and how it is diagnosed, with not all studies using positive cultures as the gold standard, and variability in what is considered pathogenic organisms. Even the timeframe after birth is variable for what is considered EONS or LONS (Shane et al. 2017). The ideal sepsis biomarker has been described as measurable with near-perfect sensitivity and specificity and would have a rapid turnaround time. Such a biomarker would facilitate a reasonable delay to the initiation of antibiotics for infants with a negative biomarker test (Cantey and Lee 2021). While rapid turnaround is possible in a cost-effective manner with existing POC tests, CRP lacks the sensitivity and specificity to reliably diagnose or exclude sepsis at the time of clinical assessment of the patient. At present, there is no single test for neonatal sepsis that meets the criteria of an ideal biomarker, and even when CRP is combined with other biomarkers, the accuracy of infection diagnosis is limited. Despite this, the use of CRP in the NICU continues to be widespread mostly with proponents of CRP citing its negative predictive value. There has been a recent shift in the literature away from CRP to rule out sepsis with some authors considering this negative predictive value only slightly better than flipping a fair coin in populations with a low prevalence of sepsis (Cantey and Bultmann 2020).

The accurate diagnosis of sepsis in patients with clinical suspicion of sepsis remains a challenge, and the investigation into diagnostic aids is widening to the fields of molecular diagnostics, proteomics, metabolomics, and gene expression offering a promise for potentially better diagnostic markers in the future (Iroh Tam and Bendel 2017). Currently, microbiological diagnosis for neonatal sepsis remains the cornerstone of diagnosis of infection. The overall approach to the management of neonatal sepsis in the NICU should start from before birth with adequate antenatal

and perinatal care to reduce the impact of maternal risk factors, followed by adherence to infection control practices in the NICU, gentle handling and meticulous skin care, early initiation of enteral feeding with human milk, limited use of invasive devices and catheters, and standardized bundles related to the care of catheters to reduce the burden of hospital-acquired LONS.

Applications to Other Diseases or Conditions

In this chapter, we reviewed the role of CRP in the diagnosis and management of neonatal sepsis. Studies suggest a single CRP test done at initial presentation has a low sensitivity to diagnose infection and would not effectively alter the decision to administer or withhold antibiotic therapy (Benitz et al. 1998; Brown et al. 2020; Stocker et al. 2021). Serial CRP testing did however improve diagnostic accuracy (Benitz et al. 1998; Stocker et al. 2021), and CRP-based algorithms can reduce the antibiotic treatment duration in newborn patients (Petel et al. 2018). There may however be a role for the use of point-of-care testing of CRP in adult outpatients. A meta-analysis of randomized controlled trials demonstrated reduced antibiotic treatment in adult patients who presented with acute fever or respiratory symptoms when a CRP-based algorithm for antibiotic initiation had been implemented (Petel et al. 2018), although the studies further demonstrated no differences in hospitalization or mortality when the algorithms were utilized. While CRP was used in these studies as a marker of infection, it is also elevated in states of inflammation and cardiac dysfunction (Pepys 1981). An association between high-sensitivity C-reactive protein (hsCRP) with risk for cardiovascular disease in adults has been well described. Patients with a higher baseline hsCRP have been shown to have an increased risk of cardiovascular and coronary events (Pepys and Hirschfield 2003). Baseline hsCRP greater than 3 mg/L can predict an approximately 50% increase in risk compared to levels below 1 mg/L. In addition, testing hsCRP has demonstrated value in predicting the risk of death or recurrent major cardiovascular events in patients with previous myocardial infarction when tested a month after initial recovery (Carrero et al. 2019). Since CRP may rise due to intercurrent pathologies, it is important to differentiate between true baseline values and temporarily elevated CRP with serial testing when being used to predict cardiovascular disease.

Mini-Dictionary of Terms

Acute Phase Reactant

Acute phase reactants are inflammation markers that exhibit significant changes in serum concentration during inflammation. Acute phase reactants can be classified as positive or negative, depending on their serum concentrations during inflammation. Positive acute phase reactants are upregulated, and their concentrations increase during inflammation. Negative acute phase reactants are downregulated, and their concentrations decrease during inflammation.

Antimicrobial Stewardship

Antimicrobial stewardship is a coordinated program that promotes the appropriate use of antimicrobials (including antibiotics), improves patient outcomes, reduces microbial resistance, and decreases the spread of infections caused by multidrug-resistant organisms.

Biomarker

A naturally occurring molecule, gene, or characteristic by which a particular pathological or physiological process, disease, etc. can be identified.

Immunoassay

Immunoassay is a bioanalytical method of measuring the presence of substances which range from small molecules to macromolecules in a solution by the use of an antibody or an antigen to recognize it.

Immunoturbidimetry

A method that measures the absorbance of light from a sample which is used for quantifying an amount of analyte based on the level of turbidity produced by the formation and precipitation of an immune complex containing the analyte.

Microbiome

A community of microorganisms (such as bacteria, fungi, and viruses) that inhabit a particular environment and especially the collection of microorganisms living in or on the human body.

Nephelometry

Technique used to determine levels of antibodies or antigens in a suspension based on its light-scattering properties.

Negative Predictive Value

The ratio of subjects truly diagnosed as negative to all those who had negative test results. The characteristic predicts how likely it is for a patient to truly be disease

free, in case of a negative test result. Negative predictive value = True negative/(true negative + false negative).

Point-of-Care Test

Point-of-care testing is defined as medical diagnostic testing at the time and place of patient care.

Positive Predictive Value

The ratio of patients truly diagnosed as positive to all those who had positive test results (including healthy subjects who were incorrectly diagnosed as positive). Positive predictive value = True positives/(true positives + false positives).

Sensitivity

Also known as true positive rate, refers to the proportion of those who received a positive result on this test out of those who have the condition when judged by the Gold Standard.

Specificity

Specificity or true negative rate refers to the proportion of those who received a negative result on this test out of those who do not actually have the condition when judged by the Gold Standard.

Vertical Transmission

A vertically transmitted infection is an infection caused by pathogens (such as bacteria and viruses) where transmission is directly from the mother to an embryo, fetus, or baby during pregnancy or childbirth.

Hospital-Acquired Infection

Healthcare-associated infections which are nosocomially acquired infections and are typically not present or might be incubating at the time of admission. These infections are usually acquired after hospitalization and manifest 48–72 hours after admission to the hospital.

Key Facts of Neonatal Sepsis

- Neonatal sepsis is a diagnosis made in infants less than 28 days of life.
- The clinical syndrome includes systemic signs of infection, circulatory shock, multisystem organ failure, and progress to death rapidly.
- An estimated 15% of all neonatal deaths globally are due to sepsis.
- Neonates are disproportionately affected in low-income and middle-income countries with a high prevalence rate of infectious disease and restricted access to care.
- In Sub-Saharan Africa, neonatal sepsis resulted in an estimated loss of 5.3–8.7 million disability-adjusted life-years and an estimated economic burden of up to US$469 billion (2014 data).
- Early-onset sepsis is disproportionally more prevalent in preterm infants.
- In countries with widespread use of intrapartum antibiotics and screening for Group B streptococcus infection in mothers, *Escherichia coli* is emerging as the predominant pathogen causing early-onset sepsis.

Summary Points

- C-reactive protein (CRP) is a nonspecific acute phase reactant protein that rises in states of inflammation and infection.
- CRP production is triggered by inflammatory cytokines, predominantly IL-6 and IL-1, whereafter it binds to various cellular structures to facilitate in opsonization and phagocytosis of microorganisms and assists in activation of complement system and the proinflammatory response to infection.
- The rise in CRP starts 4 to 6 hours after cytokine stimulation, doubling every 8 hours to reach a peak between 36 and 50 hours.
- The removal of the cytokine stimulus results in a decline of CRP concentration with an average half-life of 19 hours.
- In healthy newborns, serum CRP increases physiologically at birth reaching a peak as high as 13 mg/L between 27 and 36 hours after birth.
- Neonatal sepsis is a severe condition that presents with nonspecific clinical signs and has a high morbidity and mortality especially in developing countries.
- The diagnosis of neonatal sepsis is confirmed by positive microbiological cultures; however, these may only be reported positive 36 to 48 hours after the specimens have been taken.
- Current evidence suggests CRP, at a cut-off value of 5–10 mg/L, has a low sensitivity of 62% and median specificity of 74% for diagnosing neonatal sepsis.
- Serial normal CRP measurements taken 24 to 36 hours after initiation of empiric antibiotic can exclude the presence of early-onset infection with a high probability.
- Despite its limitations, CRP testing is still widely used in newborns to assist in the diagnosis of neonatal sepsis and in the safe discontinuation of antibiotics.

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Cell Cycle Arrest Biomarkers in the Intensive Care Unit

9

Zi-jun Zhou and Bo Yang

Contents

Abstract

AKI is a clinical syndrome mainly manifested as a sharp decline in renal function, which is a common complication in the ICU and is characterized by high mortality and poor prognosis, and early diagnosis and treatment of AKI is important to save the patient's life. When cells are stimulated by injury, the cell cycle cannot proceed normally and arrest at a certain stage. This phenomenon is called cell cycle arrest. Renal tubular epithelial cells of AKI patients usually have cell cycle arrest in G1 and/or G2 phases, which indicates that the expression

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changes of cell cycle arrest biomarkers can directly reflect the damage of renal tubular epithelial cells. Therefore, as a new standard for early prediction and diagnosis of AKI, the detection of cell cycle arrest biomarkers has become a current research hotspot.

Keywords

Cell cycle arrest · Biomarker · Acute kidney injury · Intensive care unit · [TIMP-2] × [IGFBP7]

Abbreviations

AKI	acute kidney injury
ALI	acute liver failure
ATM	activates the ataxia-telangiectasia mutated
ATR	ataxia telangiectasia and Rad3-related
CAK	CDK-activating kinase
CDKs	cyclin-dependent kinases
CHK1	checkpoint kinase 1
CKIs	Cdk inhibitors
CPB	cardiopulmonary bypass
CSA-AKI	cardiac surgery-associated AKI
ECMO	extracorporeal membrane oxygenation
GFR	glomerular filtration rate
ICU	intensive care unit
IGFBP7	insulin-like growth factor-binding protein 7
IL-18	interleukin-18
KIM-1	kidney injury molecule-1
L-FABP	L-type fatty acid-binding protein
NGAL	neutrophil gelatinase-associated lipocalin
Rb	Retinoblastoma protein
SA-AKI	sepsis-associated AKI
Scr	serum creatinine
TECs	renal tubular epithelial cells
TIMP-2	tissue inhibitor of metalloproteinase-2

Introduction

The research on cell cycle arrest biomarkers mainly focuses on the related fields of tumors and acute kidney injury (AKI). According to the admission criteria in the intensive care unit (ICU), the early diagnosis of tumors does not belong to the research scope in ICU, while AKI is characterized by high mortality and poor prognosis in ICU. Therefore, early diagnosis of AKI and provision of targeted treatment will be important to save the patient's life and improve the prognosis. With the important role of cell cycle arrest in the pathogenesis of AKI widely

recognized, tissue inhibitor of metalloproteinase-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7), biomarkers of cell cycle arrest, have become the current research hotspots for early prediction of AKI in the ICU. In this paper, we will focus on cell cycle and cell cycle arrest, cell cycle arrest in AKI, AKI in ICU, and early prediction of AKI by cell cycle arrest biomarkers.

Cell Cycle and Cell Cycle Arrest

Cell Cycle

Cell cycle refers to the whole process experienced by cells from the end of the last mitosis to the completion of the next mitosis. A complete cell cycle is divided into DNA synthesis phase (S phase) and mitosis phase (M phase), and the two intermediate preparation phases (G1, G2 phases) (Nurse 2000). G1 phase, also known as presynthetic phase, mainly synthesizes ribosomes and RNA and other substances to prepare for the replication of DNA; S phase is the DNA synthesis phase, which synthesizes histones and various other enzymes in addition to DNA; G2 phase is the anaphase of synthesis, which is mainly the material conditions required for mitotic preparation; M phase is the mitotic phase. In addition, cells can also be in a state of stopping proliferation and entering quiescence, which is called G0 phase at this time (Vermeulen et al. 2003).

The cell cycle progresses orderly under the common regulation of a variety of cellular proteins, the most critical regulatory proteins of which are cyclin-dependent kinases (CDKs), cyclins, and cyclin-dependent kinase inhibitors(CKIs), which bind to cyclins to form complexes and promote cell cycle progression, while CKIs prevent the transition of the cell cycle to the next stage by disrupting their binding (Lim and Kaldis 2013). So far, 21 CDKs and 5 CDK-like genes have been identified, which can be mainly divided into two categories: (1) subfamilies that directly or indirectly regulate the cell cycle and (2) subfamilies that regulate transcription (Whittaker et al. 2017). Among them, in the process of regulating the cell cycle, CDK1, CDK2, CDK4, and CDK6 play a major role, and they regulate different cell cycle stages by binding to specific cyclins (Zhang et al. 2021) (Fig. 1).

Cell Cycle Arrest

There is a special set of regulatory mechanisms in the cell cycle, known as cell cycle checkpoints, which can be divided into four checkpoints according to the cell cycle. When there are problems such as abnormal cell morphology, DNA damage, and abnormal spindle assembly, cell cycle checkpoints are activated to prevent the progression of the cell cycle to the next stage, so that cells have time to repair damage, correct abnormalities or exit the cell cycle (Justman 2017). This phenomenon that the cell cycle cannot proceed smoothly due to intracellular and

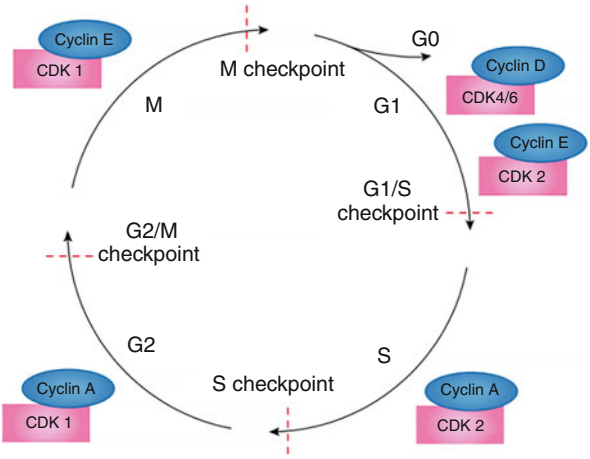

Fig. 1 CDK-cyclin complex regulates the cell cycle. This figure shows the role of different CDK-cyclin complexes at different stages of the cell cycle

extracellular stress stimuli and arrests at a certain stage is called cell cycle arrest (Pack et al. 2019).

Depending on the cell cycle checkpoint, the cell cycle can be arrested at different stages, and the signaling pathways involved are mainly divided into the following aspects:

(1) G1 arrest: In the classical cell cycle model, the G1 phase checkpoint is set at the end of G1 phase, through which cells will be allowed to enter S phase, and the cell cycle will be blocked in G1 phase when cells have factors such as lack of mitotic stimulation and/or DNA damage (Agami and Bernards 2002).

Normal mitotic stimulation can induce the expression of cyclin D. Cyclin D forms a complex with CDK4/6, and the cyclin-CDK complex enters the nucleus and is activated by CDK-activating kinase (CAK). The activated cyclin-CDK complex phosphorylates retinoblastoma protein (Rb); relieves the partial inhibition of E2F transcription factor family by Rb; promotes the expression of E2F target genes such as cyclin E, followed by the increase of cyclin E level; and activates CDK2. CyclE-CDK2 hyperphosphorylates Rb, which leads to increased release of E2F transcription factors, transcriptional initiation, and increased gene expression in S phase, and the cell cycle enters S phase from G1 phase (Goel et al. 2018; Ingham and Schwartz 2017; Yang et al. 2020). Cells are therefore unable to complete a normal G1/S phase transition when mitotic stimulation ceases or is lacking.

DNA damage caused by various endogenous or exogenous factors activates the ataxia-telangiectasia mutated (ATM) p53 signaling pathway; p53 acts as a transcription factor to activate the expression of the downstream protein p21, which, as a member of the CKI family, inhibits cyclin-CDK complex activity and prevents cells from entering S phase, thus providing time for DNA damage repair or induction of apoptosis (Georgakilas et al. 2017; Khoronenkova and Dianov 2015).

Thus, in the classical cell cycle model, hyperphosphorylation of Rb in late G1 becomes the molecular basis by which the cell cycle can enter S phase, so the G1 phase checkpoint is considered to be a limiting point located in late G1, after which cells will enter S phase. However, with the deepening of research, a new cell cycle model has been proposed in recent years, in which there are a total of three checkpoints to determine whether cells can enter S phase (Hume et al. 2020).

(2) S phase arrest: When cells have DNA replication stress factors such as abnormal replication origin excitation, DNA damage, or depletion of deoxyribonucleotide pools, the cell cycle cannot pass the S phase checkpoint and is arrested in S phase (Ciardo et al. 2019). In this process, endogenous or exogenous replication stress activates the ataxia telangiectasia and Rad3-related (ATR) and checkpoint kinase 1 (CHK1) signaling pathways, and ATR/CHK1-mediated phosphorylation events inhibit the activity of the CDK activator Cdc25, reduce CDK1/2 activity, and prevent the cell cycle from entering G2 phase (Giannattasio and Branzei 2017).

(3) G2 arrest: When there are checkpoint defects such as p53 gene mutation, cells with DNA damage may enter G2 phase normally through the first two checkpoints, so the G2 phase checkpoint is essential for maintaining the integrity of the genome, and cells with DNA damage will be arrested in G2 phase for repair or selected for apoptosis and cannot enter M phase (Kastan and Bartek 2004). Similar to S phase, ATR-mediated damage repair is activated during DNA damage, and ATR/CHK1 activates the mitotic repressors WEE1 and MYT1, while inhibiting the expression of Cdc25, which inhibits the activity of cyclin B-CDK1 through these three pathways and halts the cell cycle in G2 phase (Gorecki et al. 2021; Schmidt et al. 2017).

However, some cells can overcome G2 phase cell cycle arrest and carry damaged DNA into mitosis, a phenomenon known as G2/M checkpoint adaptation. Of these, most of the cells die during mitosis, and a small proportion may survive due to changing the original genome during repair and adaptation, which may be associated with inactivation of CHK1 (Kalsbeek and Golsteyn 2017).

(4) M phase arrest: When the spindle is not properly attached to the chromosome, the cell stops in M phase, so the M phase checkpoint is also called the spindle checkpoint (Musacchio 2015). After cells enter M phase, CDK1 releases APC/C from APC/C-CDH1 and phosphorylates APC/C, allowing it to disrupt the activity of cyclin B and securin, promote sister chromatid separation, and exit mitosis. When cells have abnormal chromosome segregation, however, the mitotic checkpoint complex will inhibit the activity of APC/C and maintain cells in M phase (Holder et al. 2019).

Recent studies have found that M phase may also have a DNA damage checkpoint due to the possible presence of persistent DNA replication defects, unrepaired pre-M phase DNA damage, and true M phase DNA damage, indicating that DNA

damage can prolong M phase, which may be associated with ATM and the ATR cascade (Thompson et al. 2019).

Cell Cycle Arrest in AKI

Normally, the vast majority of renal tubular epithelial cells (TECs) are considered to be in the quiescent G0 stage, and less than 1% of cells are proliferating to balance cell physiological death or accidental loss from the basement membrane. When renal injury events such as ischemia-reperfusion, oxidative stress, and toxic injury occur, they will lead to necrosis and apoptosis of TECs, at which time surviving G0 phase TECs will reenter the cell cycle and replace necrotic and apoptotic cells by migration, proliferation, and differentiation to restore renal function (Moonen et al. 2018). AKI often also accompanies DNA damage in TECs (Yan et al. 2016; Zhu et al. 2015). Thus, when AKI occurs, TECs that enter the cell cycle are arrested at a certain cycle by activated cell cycle checkpoints.

In AKI, TECs cell cycle arrest mainly occurs in two stages: G1 and G2, of which G1 phase is mainly mediated by p21 signaling pathway, and early transient G1 arrest of TECs will facilitate cell repair injury, while continuous stay in G1 phase will lead to apoptosis. G2 phase is mainly mediated by the ATR/CHK1 signaling pathway, and G2/M-arrested TECs present a pro-fibrotic phenotype, while activating c-jun NH_2 terminal kinase signaling pathway promotes the secretion of fibrotic factors, ultimately leading to the development of fibrosis and poor prognosis and even progression to chronic kidney disease (Andrade et al. 2018; Basile et al. 2015).

This seems to be a paradoxical phenomenon, and kidney injury stimulates cell proliferation and also leads to cell cycle arrest, but in fact, cell cycle arrest, as a protective mechanism, provides sufficient time for the repair of DNA damage to avoid genetic defects, cell necrosis, and other conditions that are not conducive to the recovery of kidney function during the proliferation and differentiation of TECs. Thus, AKI often undergoes cell cycle arrest in TECs in the early stage, and by detecting cell cycle arrest biomarkers, it is possible to detect whether TECs are damaged as early as possible, regardless of whether this damage is sufficient to lead to AKI, but this is the earliest detectable damage signal, so cell cycle arrest-related proteins are considered to be new biomarkers for predicting AKI.

Both TIMP-2 and IGFBP7 are closely associated with G1 arrest of TECs (Fig. 2) and can be detected in urine. When TECs are injured, both of them cause cell cycle arrest in TECs by autocrine and paracrine means, in which TIMP-2 stimulates p27 expression and IGFBP7 stimulates p21 and p53 expression, and these highly expressed p21, 27, and 53 inhibit the activity of cyclin D-CDK4 and cyclin E-CDK2 and arrest the cell cycle in G1 phase for repair (Kellum and Chawla 2015). Therefore, TIMP-2 and IGFBP7, as cell cycle arrest biomarkers, can indicate that TECs are damaged as early as possible and play an important role in the early prediction of AKI.

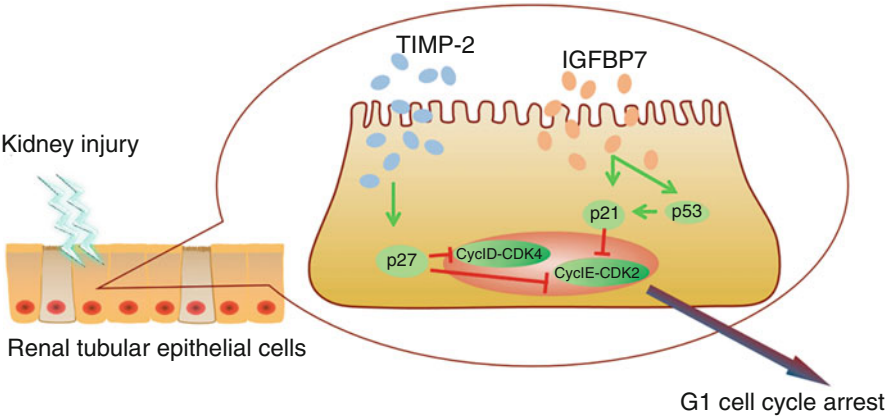

Fig. 2 TIMP-2 and IGFBP7 and G1 arrest. This figure shows the mechanism by which TIMP-2 and IGFBP7 lead to G1 arrest in tubular epithelial cells

AKI in ICU

AKI is a clinical syndrome characterized by a sharp decline in renal function and is a common complication in ICUs, and a multinational study of more than 1800 patients in 97 ICUs pointed out that about 57% of patients will experience varying degrees of AKI within 1 week of admission (Hoste et al. 2015). AKI in the ICU appears primarily as a complication of several of the following conditions (Griffin et al. 2020):

(1) **Sepsis:** Sepsis is the most important cause of AKI in the ICU, and sepsis-associated AKI (SA-AKI) occurs in 10%–20% of septic patients and 50%–70% of septic shock patients (Griffin et al. 2020), which accounts for 45%–70% of all AKI cases (Sun et al. 2019). The pathogenesis of SA-AKI has not been fully clarified. In the past, renal hypoperfusion and ischemia were considered to be the main causes of SA-AKI. However, recent studies have found that SA-AKI can also have normal or even increased renal blood flow. The classical renal hypoperfusion theory is not enough to explain the occurrence of SA-AKI. Therefore, a "unified theory" based on inflammation, oxidative stress, microvascular dysfunction, and endothelial dysfunction pointed out that tubular cells may adapt to these injurious and inflammatory danger signs caused by sepsis in a manner that sacrifices their own function, manifested as SA-AKI (Gomez et al. 2014; Poston and Koyner 2019).

(2) **Cardiac surgery:** In the ICU patient population, cardiac surgery-associated AKI (CSA-AKI) is the second most common type of AKI after SA-AKI, with an incidence ranging from 5% to 42% in patients after cardiac surgery (Griffin et al. 2020). CSA-AKI can be classified as type I cardiorenal syndrome, that is,

the dramatic deterioration of cardiac function leads to AKI, and its occurrence may involve several renal injury pathways such as renal hypoperfusion, ischemia-reperfusion injury, nephrotoxic drug stimulation, inflammation, and oxidative stress (Wang and Bellomo 2017).

(3) **Acute liver failure:** Approximately 70% of patients with acute liver failure (ALF) will develop AKI during hospitalization, with acetaminophen-induced ALF and ischemic ALF having the highest incidence of AKI (Tujios et al. 2015). AKI during ALF may be caused by renal hypoperfusion, endogenous injury (e.g., tubular toxicity of acetaminophen), and other complications of ALF (e.g., sepsis) (Leventhal and Liu 2015).

(4) **Application of extracorporeal membrane oxygenation:** Extracorporeal membrane oxygenation (ECMO) is widely used in the ICU, and the incidence of AKI is as high as 60%–70% in the ECMO patient population. ECMO can lead to the development of AKI through factors such as pulseless blood flow, coagulation disorders, and inflammatory response formed by cardiopulmonary bypass (Razo-Vazquez and Thornton 2016).

(5) **Other factors:** A series of complex clinical syndromes such as cardiorenal syndrome and hepatorenal syndrome also trigger AKI, and the mechanism is closely related to factors such as hemodynamics (Gonwa and Wadei 2013; House 2018); intra-abdominal hypertension may also cause decreased perfusion and trigger AKI through increased renal venous and renal parenchymal pressure (Mohmand and Goldfarb 2011).

In conclusion, AKI is not only one of the common complications in the ICU, but also AKI acts as an independent risk factor for mortality in the ICU, and its severity is directly closely related to mortality (Hoste et al. 2015). In addition to death, survivors of AKI often face the threat of poor prognosis and progressive or persistent renal injury, which may progress to chronic kidney disease or end-stage renal disease (Forni et al. 2017). Therefore, diagnosing AKI as early as possible, with targeted intervention, will be important to save patients' lives and improve prognosis.

Application of Cell Cycle Arrest Biomarkers in Predicting AKI

The diagnostic criteria for AKI have also not been uniform due to the lack of accepted definitions and grading criteria for AKI, and in this context, the Acute Dialysis Quality Initiative group proposed the RIFLE criteria in 2004 (Bellomo et al. 2004) AKI is divided into five stages: risk, injury, failure, loss, and end-stage renal disease, and is diagnosed by serum creatinine (Scr), glomerular filtration rate (GFR), and urine volume. Subsequently the AKIN improved the RIFLE criteria in 2007 (Mehta et al. 2007). GFR was canceled as the basis for the diagnosis of AKI, stages L and E were canceled, and AKI was classified into three grades based on the changes in Scr and urine volume. However, these diagnostic criteria still have some limitations, so in 2012 KDIGO synthesized the RIFLE criteria and AKIN criteria and released the first clinical practice guidelines (Kdigo.org.). KDIGO criteria followed

the changes in Scr and urine volume in AKIN criteria as the diagnostic basis, modified and improved the diagnostic criteria for AKI 3 stage in the original criteria, and became the most applied AKI diagnostic criteria at present.

However, these diagnostic criteria have some problems; GFR and Scr baseline in RIFLE criteria are difficult to determine, while it is unknown whether the recommended MDRD formula is accurate in assessing GFR baseline under pathological conditions. Although AKIN and KDIGO improved the baseline problem and improved the sensitivity of Scr in the diagnosis of AKI, since Scr and urine volume are the embodiment of renal function and cannot visually reflect the renal injury, the changes of Scr and abnormal urine volume can be detected only when the renal function has been significantly impaired, which seriously hinders the early diagnosis of AKI and has a huge adverse impact on the prognosis of patients (Kashani et al. 2017). Changes in Scr are more suitable as a criterion rather than a diagnostic criterion for defining AKI, and in this context, the use of biomarkers produced during kidney injury as a new criterion for predicting and diagnosing AKI has become a hot topic of current research.

At present, the research on biomarkers of kidney injury mainly focuses on neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), L-type fatty acid-binding protein (L-FABP), TIMP-2, and IGFBP7. This article mainly introduces TIMP-2 and IGFBP7.

[TIMP-2] × [IGFBP7] Findings

The earliest important studies on TIMP-2 and IGFBP7 for predicting AKI can be pursued until 2010–2013, in order to find more appropriate AKI biomarkers. Kashani et al. (2013), based on various hypotheses of AKI pathophysiology (inflammation, apoptosis, necrosis, cell cycle, etc.), identified a total of 340 AKI related proteins including NGAL, IL-18, KIM-1, TIMP-2, and IGFBP7 as AKI candidate biomarkers and analyzed these 340 candidate biomarkers in combination with blood and urine samples from 3 ICU clinical bases. TIMP-2 and IGFBP7 performed the best in this process. Within 12–36 hours, according to RIFLE criteria, the AUC for predicting phase I/F was 0.75 (95% CI 0.70–0.80) and 0.77 (95% CI 0.71–0.82), respectively, which was more advantageous than other biomarkers such as urine NGAL, urine IL-18, and urine KIM-1. Subsequently, Kianoush et al. studied the performance of TIMP-2 and IGFBP7 in predicting AKI in 728 critically ill patients (Sapphire study). In this study, they found that the product of TIMP-2 and IGFBP7 had higher value in predicting AKI. When subjects developed moderate to severe AKI within 12 hours (KDIGO2-3 stage), the AUC of [TIMP-2] × [IGFBP7] was 0.80 (95% CI 0.75–0.84), while the AUC of TIMP-2 and IGFBP7 alone was 0.79 and 0.76, respectively. Meanwhile, the AUC of [TIMP-2] × [IGFBP7] was significantly higher than that of all other existing biomarkers. These evidences suggested that [TIMP-2] × [IGFBP7] may have obvious advantages in early prediction of AKI in clinical practice. Meanwhile, this study also proposed the use of [TIMP-2] × [IGFBP7] for AKI risk classification. When [TIMP-2] × [IGFBP7] > 0.3 $(ng/mL)^2/1000$, the patient's

risk of AKI (KDIGO2-3 stage within 12 hours) and renal adverse events (death, dialysis, persistent renal insufficiency, etc.) increased dramatically; when [TIMP-2] × [IGFBP7] > 2 $(ng/mL)^2/1000$, the risk of AKI and renal adverse events increased to five times and two times, respectively.

However, the Sapphire study still has some limitations, and in order to better apply the findings to clinical practice, Bihorac et al. (2014) validation was performed against the two proposed AKI Risk Grading cutoffs in the Sapphire study (Topaz study). A total of 408 critically ill patients were enrolled in this study, which investigated the sensitivity and specificity of two cutoffs, [TIMP-2] × [IGFBP7] > 0.3 $(ng/mL)^2/1000$ and [TIMP-2] × [IGFBP7] > 2 $(ng/mL)^2/1000$, in predicting AKI, while clinical variables were introduced to investigate whether [TIMP-2] × [IGFBP7] remains a statistically significant biomarker for predicting AKI in a clinical model. In this study, the median value of [TIMP-2] × [IGFBP7] in critically ill patients diagnosed with AKI within 12 hours was 1.6 (0.7–2.8) $(ng/mL)^2/1000$, significantly higher than the median value of 0.3 (0.2–0.8) $(ng/mL)^2/1000$ in non-AKI critically ill patients, meanwhile, the AUC of [TIMP-2] × [IGFBP7] to predict AKI within 12 hours was 0.86 (95% CI 0.76–0.88) confirming the reliability and reproducibility of the AUC of [TIMP-2] × [IGFBP7] of 0.80 (95% CI 0.75–0.84) observed in the previous Sapphire study. The AUC of Scr determined in the same period was 0.63 (95% CI 0.56–0.70), indicating a significant advantage of [TIMP-2] × [IGFBP7] in early prediction of AKI. Compared with patients with [TIMP-2] × [IGFBP7] < 0.3 $(ng/mL)^2/1000$, patients with [TIMP-2] × [IGFBP7] 0.3–2 $(ng/mL)^2/1000$ had a fivefold increased risk of AKI (95% CI3–17), and patients with [TIMP-2] × [IGFBP7] > 2 $(ng/mL)^2/1000$ had a 17-fold increased risk of AKI (95% CI9–54). Notably, when clinical variables were introduced, the AUC for the combined [TIMP-2] × [IGFBP7] and clinical model was 0.86 (95% CI 0.80 to 0.90), while the AUC for the clinical model alone was 0.70 (95% CI 0.63 to 0.76), a significant difference, indicating that [TIMP-2] × [IGFBP7] remains a strong biomarker for predicting AKI. In a subsequent cutoff study, [TIMP-2] × [IGFBP7] had the highest sensitivity of 92% (95% CI85–98) and specificity of 46% (95% CI41–52) at a cutoff of 0.3 $(ng/mL)^2/1000$, and [TIMP-2] × [IGFBP7] had the highest specificity of 95% (95% CI93–97) and sensitivity of 37% (95% CI26–47) at a cutoff of 2 $(ng/mL)^2/1000$. This is consistent with the conclusion of Hoste et al. (2014) that [TIMP-2] × [IGFBP7] had the highest sensitivity of 89% and NPV of 97% at a cutoff of 0.3 $(ng/mL)^2/1000$ and [TIMP-2] × [IGFBP7] had the highest specificity of 95% and PPV of 49% at a cutoff of 2 $(ng/mL)^2/1000$ in the Opal study of Eric et al. This identified a cutoff value of 0.3 $(ng/mL)^2/1000$ for high sensitivity and 2 $(ng/mL)^2/1000$ for high specificity in moderate-severe AKI, which is clinically important for identifying patients with AKI.

So far, the US Food and Drug Administration (FDA) has approved [TIMP-2] × [IGFBP7] for marketing as a biomarker for predicting the risk of AKI, NephroCheck® (NC) test. By quantitatively measuring the value of [TIMP-2] × [IGFBP7] in the patient's urine, the test is compared with the clinical endpoint to provide a validated

AKI risk index in prospective clinical trials. Since then, the clinical application of [TIMP-2] × [IGFBP7] has become a current research hotspot.

[TIMP-2] × [IGFBP7] to Predict Different Types of AKI

As AKI in ICU is mainly closely related to sepsis and cardiac surgery, [TIMP-2] × [IGFBP7] predicted that AKI was mainly concentrated in CSA-AKI and SA-AKI, as shown in Table 1.

CSA-AKI: Meersch et al. (2014) found that [TIMP-2] × [IGFBP7] is the best predictor in the process of predicting the risk of developing AKI after cardiac surgery, with high specificity and sensitivity. They enrolled 50 patients with a high risk of AKI who underwent cardiac surgery under cardiopulmonary bypass (CPB). Among them, 26 patients (52%) developed AKI after operation. In these patients, the value of [TIMP-2] × [IGFBP7] was significantly increased compared with that before CPB, and [TIMP-2] × [IGFBP7] increased from an average of 0.49 (SE 0.24) preoperatively to 1.51 (SE 0.57) at 4 hours after CPB, while AKI diagnosis according to traditional criteria occurred 1–3 days after CPB; the AUC of [TIMP-2] × [IGFBP7] at 4 hours after operation was 0.81 (95% CI 0.68–0.93), while the AUC of NGAL was 0.68 (95% CI 0.53–0.84), indicating that [TIMP-2] × [IGFBP7] has more important value in predicting the early risk of AKI after cardiac surgery. In particular, the specificity was 0.83, and the sensitivity was 0.80 using a cutoff value of 0.3 $(ng/mL)^2/1000$, and the specificity was 0.81, and the sensitivity was 0.92 using a cutoff value of 0.5 $(ng/mL)^2/1000$, indicating that the highest concentration of [TIMP-2] × [IGFBP7] at 4 hours after surgery is a specific and sensitive indicator for predicting the risk of postoperative AKI. Wang et al. (2017) subsequently validated the ability of [TIMP-2] × [IGFBP7] to predict the risk of AKI within 4 hours after surgery in a study of 57 cardiac surgery patients, with an AUC of 0.80 (95% CI 0.68–0.91), and the value of [TIMP-2] × [IGFBP7] was significantly higher in patients who developed AKI compared to those who did not. Oezkur et al. (2017) investigated the relationship between [TIMP-2] × [IGFBP7] at different time points after surgery and the risk of AKI in a study of 150 cardiac surgery patients, and they indicated that immediate postoperative measurement of [TIMP-2] × [IGFBP7] was the best time to predict the risk of AKI. Gist et al. (2017) investigated the ability of [TIMP-2] × [IGFBP7] to predict the risk of AKI in infants and newborns after cardiac surgery. The study found that the risk of AKI in patients with [TIMP-2] × [IGFBP7] $\geq$ 0.78 $(ng/mL)^2/1000$ was three times higher than that in patients with [TIMP-2] × [IGFBP7] $<$ 0.78 $(ng/mL)^2/1000$. When combined with the clinical model, the AUC of [TIMP-2] × [IGFBP7] to predict AKI was 0.78 (95% CI 0.67–0.90). These studies showed that [TIMP-2] × [IGFBP7] also performed well in predicting the risk of AKI in infants, newborns, and children.

Postoperative AKI in other surgical procedures: In addition to cardiac surgery, other surgical procedures also have a higher risk of postoperative AKI. The study by Gocze et al. (2015) included 107 patients who underwent noncardiac surgery, and a total of 45 patients (42%) developed AKI within 48 hours after surgery, with an

Table 1 Researches of [TIMP-2] × [IGFBP7] in AKI. This table shows the clinical application of [TIMP-2] × [IGFBP7] in predicting AKI

Study	Patient	Sample size	AKI diagnostic criteria	AKI	[TIMP-2] × [IGFBP7] detection time	AUC	Cut off	Sensitivity	Specificity
Meersch et al. (2014)	Patients undergoing cardiac surgery with CPB	50	KIDGO	26	4 hours after CPB	0.81	0.3	0.8	0.83
						0.84	0.5	0.92	0.81
Wang et al. (2017)	Patients undergoing cardiac surgery	57	KIDGO	27	4 hours after CPB	0.8	0.3	0.75	0.7
							2	0.2	1
Oezkur et al. (2017)	Patients undergoing cardiac surgery with CPB	150	KIDGO	35	After postoperative ICU admission	0.81	0.3	0.6	0.88
Katja et al. (2017)	Infants undergoing cardiac surgery with CPB	94	KIDGO	31	2 hours after CPB	0.73	0.78	/	/
Gocze et al. (2015)	Patients at high risk for AKI (surgery and an additional risk factor for AKI)	107	KIDGO	45	After postoperative ICU admission	0.85	0.3	0.87	0.736

Gunnerson et al. (2016)	Patients at high risk for AKI (surgery and an additional risk factor for AKI)	375	KIDGO	35	At ICU admission	0.84	0.3	0.89	0.49
							2	0.4	0.94
Honore et al. (2016)	Patients with sepsis	232	KIDGO	40	At ICU admission	0.84	0.3	0.95	0.38
							1	0.78	0.75
							2	0.6	0.89
Cuartero et al. (2017)	ICU patients	98	AKIN	49	Within 12 hours of ICU admission	0.8	0.4	0.74	0.71
							0.8	0.72	0.78
Maizel et al. (2019)	Patients with septic shock and eligible for KDIGO1-2	112	KIDGO	45	6 hours after ICU admission	0.83	1.92	0.78	0.81
Ferrari et al. (2019)	Critically ill patients	442	KIDGO	188	12 hours after ICU admission	0.74	0.3	0.08	0.54

AUC of 0.85 (95% CI 0.78–0.93) for AKI 1 and 0.85 (95% CI 0.67–0.80) for AKI 2–3 according to the cutoff value of [TIMP-2] × [IGFBP7] > 0.3 $(ng/mL)^2/1000$. Simultaneous, the AUC of 0.3 (ng/mL) 2/1000 as the cutoff value for predicting early application of RRT in patients was 0.83 (95% CI 0.75–0.92), and the AUC to predict 28-day mortality was 0.77 (95% CI 0.67–0.80), indicating that [TIMP-2] × [IGFBP7] can be used not only to predict the risk of AKI but also predict the prognosis of AKI and guide early renoprotective treatment. Gunnerson et al. (2016) also noted [TIMP-2] × [IGFBP7] to be a strong predictor of risk of AKI in patients after surgery in their analysis of critically ill surgical patients in the Sapphire study and Topaz study, with an AUC of 0.84 (95% CI 0.76–0.90).

SA-AKI: Honore et al. (2016) studied the probability of AKI in sepsis and non-sepsis patients predicted by [TIMP-2] × [IGFBP7] and found that AKI prediction by [TIMP-2] × [IGFBP7] was not affected by sepsis and AUC was 0.85 (95% CI 0.76–0.94) in patients with SOFA score > 7 in addition to renal score, while AUC was 0.84 (95% CI 0.73–0.92) in patients with ≤7, indicating [TIMP-2] × [IGFBP7] can still accurately predict AKI in septic patients. Cuartero et al. (2017) also verified this in subsequent studies, [TIMP-2] × [IGFBP7] was not affected by sepsis when predicting AKI, and its AUC was 0.80 (95%CI0.71–0.89), and these studies showed that [TIMP-2] × [IGFBP7] could be an independent predictor of AKI independent of sepsis interference. Maizel et al. (2019) found that the AUC of [TIMP-2] × [IGFBP7] for predicting progression to KDIGO3 stage within 24 hours in patients with septic shock was 0.83 (95% CI 0.75–0.90), which was superior to other predictors such as serum creatinine. When [TIMP-2] × [IGFBP7] > 1.92 $(ng/mL)^2/1000$, the sensitivity and specificity for predicting AKI were 78% and 81%, respectively. In this study, [TIMP-2] × [IGFBP7] was only found to be clinically significant in predicting AKI in patients within 24 hours, but not 72 hours. When [TIMP-2] × [IGFBP7] > 2 $(ng/mL)^2/1000$, the risk of patients progressing to KDIGO3 stage within 24 hours will be increased by four times.

Other types of AKI: [TIMP-2] × [IGFBP7] predict the risk and prognosis of AKI in critically ill patients and is also widely used in general ICU wards. Fiorenza Ferrari et al. (2019) selected 442 ICU patients for observation. Among them, 188 patients (42.53%) developed AKI. The proportion of patients with [TIMP-2] × [IGFBP7] ≤ 0.3 $(ng/mL)^2/1000$ and [TIMP-2] × [IGFBP7] > 0.3 $(ng/mL)^2/1000$ was 31.9% and 68.1%, respectively. Meanwhile, the AUC of [TIMP-2] × [IGFBP7] for predicting AKI within 12 hours was 0.74 (95% CI 0.69–0.80), the AUC of AKI within 48 hours was 0.70 (95% CI 0.65–0.76), the AUC of AKI from 48 hours to 7 hours was 0.40 (95% CI 0.28–0.52), and [TIMP-2] × [IGFBP7] had the best prediction for severe AKI within 12 hours, with an AUC of 0.82 (95% CI 0.70–0.88). In addition, the risk of AKI increased by 2% for every $0.1(ng/mL)^2/1000$ units of [TIMP-2] × [IGFBP7] in Logistic regression model.

Applications to Prognosis

Koyner et al. (2014) found [TIMP-2] × [IGFBP7] also had a certain correlation with poor prognosis of AKI in secondary analysis of data after 9-month follow-up in Sapphire study. This study evaluated the value of [TIMP-2] × [IGFBP7] in predicting poor prognosis of AKI using Cox proportional hazard model with all-cause mortality or the need for renal replacement therapy as the composite endpoint. A total of 382 of these patients (55.2%) developed AKI within 72 hours of registration, and 217 patients (31.4%) met the composite endpoint. In univariate analysis, [TIMP-2] × [IGFBP7] > 2 $(ng/mL)^2/1000$ was associated with an increased risk of the composite endpoint, with a hazard ratio of 2.11 (95% CI 1.3–3.23), while [TIMP-2] × [IGFBP7] at 0.3–2 $(ng/mL)^2/1000$ had a hazard ratio of 1.22 (95% CI 0.91–1.62), which was not significantly associated with an increased risk of the composite endpoint. In multivariable analysis adjusted for clinical model, [TIMP-2] × [IGFBP7] > 0.3 $(ng/mL)^2/1000$ was only associated with a composite endpoint in patients who developed AKI, with adjusted hazard ratios of 1.44 (95% CI 1.00–2.06) for [TIMP-2] × [IGFBP7] at 0.3–2 $(ng/mL)^2/1000$ and 2.16 (95% CI 1.32–3.53) for [TIMP-2] × [IGFBP7] > 2 $(ng/mL)^2/1000$. These evidences suggest that early detection of [TIMP-2] × [IGFBP7] in critically ill patients can not only predict the occurrence of AKI but also have some significance for the prognosis of patients.

[TIMP-2] × [IGFBP7] Disadvantage in Predicting AKI

However, with the deepening of research, many evidences question the value of [TIMP-2] × [IGFBP7] in predicting the risk and prognosis of AKI. Fiorenza Ferrari et al.'s (2019) study stated that the traditional cutoff point for severe AKI was not confirmed in their study, while [TIMP-2] × [IGFBP7] was only optimal in predicting AKI within 12 hours. Bojan et al. (2020) also observed that the use of [TIMP-2] × [IGFBP7] in predicting AKI risk in infants and neonates undergoing cardiac surgery was not significant at 1 hour or 1 to 3 hours after surgery. Zaouter et al.'s (2018) study failed to demonstrate that [TIMP-2] × [IGFBP7] predicted the risk of AKI within 24 hours, within 1 week after cardiac surgery, and only predicted the risk of AKI within 12 hours, with an AUC of 0.65 (95% CI 0.53–0.84). In addition, Titeca-Beauport et al. (2020) found that [TIMP-2] × [IGFBP7] could not be used to accurately distinguish sepsis-related transient and persistent AKI, while other clinical prediction models such as serum creatinine and urine volume had better prediction effect for persistent AKI. These evidences suggest that [TIMP-2] × [IGFBP7], although it is currently an excellent new biomarker for predicting AKI with high specificity and sensitivity, it still faces many challenges, and more clinical studies are needed to validate its ability to predict AKI risk.

Conclusion

As a common clinical syndrome in ICU, early detection, diagnosis, and provision of early renoprotective therapy are important for patients to sustain their lives and improve their prognosis. Cell cycle arrest plays an important role in the occurrence and development of AKI. Cell cycle arrest biomarkers TIMP-2 and IGFBP7 are expected to play a role in clinical practice as biomarkers for early prediction of AKI, but their clinical effects have not been confirmed. Therefore, to explore the clinical effects of [TIMP-2] × [IGFBP7] and study the application of [TIMP-2] × [IGFBP7] in different clinical settings, different pathogenic factors and different patient populations will provide great help for the clinical application of [TIMP-2] × [IGFBP7].

Mini-Dictionary of Terms

- **Acute kidney injury**. Acute kidney injury is defined as a clinical syndrome arising from a dramatic decrease in renal function caused by a variety of etiologies and is particularly common in the ICU.
- **Biomarker**. Biomarkers refer to the indicator substances reflecting disease changes or therapeutic effects through detecting the proteins, genes, and other substances contained in the human blood, urine, and other body fluids or tissues.
- **Cell cycle arrest**. When the cell is stimulated by injury, the cell cycle cannot proceed normally and is blocked at a certain stage, which is called cell cycle arrest.
- **ICU**. The concept of ICU is often considered to have originated in the polio epidemic in Copenhagen in 1952 and is a place to provide higher quality medical services for acutely ill patients by concentrating manpower, strengthening equipment allocation, and treating and managing them.
- **[TIMP-2] × [IGFBP7]**. Also known as NephroCheck® (NC) test, it was approved by FDA for marketing in 2014. Predict the risk index of AKI in patients by quantitatively measuring the value of [TIMP-2] × [IGFBP7] in urine of patients.

Key Facts of AKI

- AKI is a clinical syndrome characterized by a dramatic decrease in renal function.
- AKI is a common complication in ICU. About 57% patients will develop different degrees of AKI within 1 week after admission.
- Sepsis is the most important cause of AKI in the ICU, and SA-AKI accounts for 45%–70% of all AKI cases.
- Scr and urine volume are both embodiments of renal function and do not intuitively reflect renal injury and are not conducive to the early diagnosis of AKI.

- The related studies of [TIMP-2] × [IGFBP7] in predicting AKI in ICU mainly focused on CSA-AKI and SA-AKI.

Key Facts of Cell Cycle Arrest

- The cell cycle refers to the entire process that cells undergo from the end of the last mitosis to the completion of the next mitosis.
- The normal cell cycle is regulated by cell cycle checkpoints, and when stimulated by stress, the cell cycle arrests at a certain stage for repair.
- Transient cell cycle arrest facilitates damage repair, and sustained cell cycle arrest may lead to apoptosis and necrosis.
- When AKI occurs, the cell cycle of tubular epithelial cells is mainly arrested in G1 and/or G2 phases.
- Both TIMP-2 and IGFBP7, as cell cycle arrest biomarkers, are closely associated with G1 arrest of TECs and can be detected in urine.

Summary Points

- Cell cycle arrest biomarkers are mainly used to predict AKI early in the ICU; AKI is a common complication in the ICU, with high mortality and poor prognosis; and early intervention is beneficial to improve prognosis.
- Both TIMP-2 and IGFBP7 are associated with G1 arrest in renal tubular epithelial cells and can be detected in urine, and the product of TIMP-2 and IGFBP7 has a higher value in predicting AKI.
- The existing diagnostic criteria do not indicate the occurrence of AKI as early as possible, so the search for biomarkers during early renal injury as novel biomarkers to predict AKI has become a hot topic in current research.
- [TIMP-2] × [IGFBP7] can not only predict the risk of AKI but also predict the prognosis of AKI patients.
- Although [TIMP-2] × [IGFBP7] is an excellent AKI biomarker, there are still some problems that need to be verified by more clinical studies.

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Plasma Markers of Cholestasis in Critical Illness

10

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Contents

Abstract

Cholestasis occurs often in critically ill for multiple reasons, whereas different mechanisms are not completely understood and a definition not uniform. In this

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chapter we summarize available information on plasma markers of cholestasis and discuss differential diagnosis, pathophysiology, epidemiology, and management of critical illness-associated cholestasis.

Keywords

Cholestasis · Bile acids · Bilirubin · Liver enzymes · Alkaline phosphatase · Gamma-glutamyl transpeptidase

Abbreviations

ALP	alkaline phosphatase
ALT	alanine aminotransferase
APACHE	Acute Physiology And Chronic Health Evaluation
AST	aspartate aminotransferase
BA	bile acids
BSEP	bile salt export pump
CAR	constitutive androstane receptor
FGF	fibroblast growth factor
FXR	farnesoid X receptor
GGT	gamma-glutamyl transpeptidase
ICU	intensive care unit
IFALD	intestinal failure-associated liver disease
MDR	multidrug resistance protein
MODS	Multiple Organ Dysfunction Score
MRP	multidrug resistance-associated protein
NTCP	Na + −dependent bile acid transporter
OATP	organic anion transporting polypeptide
PNALD	Parenteral nutrition-associated liver disease
PXR	pregnane X receptor
RXR	retinoid X receptor
SAPS	Simplified Acute Physiology Score
SOFA	Sequential Organ Failure Assessment
ULN	upper limit of normal
VDR	vitamin D receptor

Introduction

The role of the liver producing pro- and anticoagulatory factors, acute-phase proteins, and clearing pathogens and toxins cannot be overestimated in critically ill patients. At the same time, monitoring of liver function in critical illness is not well established, cholestasis not well-defined, and interpretation of plasma markers reflecting liver function and damage not straightforward.

Bilirubin is used to identify liver dysfunction in the most used organ dysfunction score in the critically ill – Sequential Organ Dysfunction Assessment (SOFA) score

(Vincent et al. 1996). Other plasma markers of cholestasis are often measured in patients admitted to intensive care units, but their interpretation is varying. There is no uniform definition of cholestasis based on plasma markers (Jenniskens et al. 2016, Kiss et al. 2020). To define cholestasis or cholestatic liver disease in the critically ill, some authors have used elevation of serum/plasma levels of alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT) (de Tymowski et al. 2019, Kiss et al. 2020, Mesotten et al. 2009), whereas others used serum/plasma bilirubin levels (Kramer et al. 2007, Vincent et al. 1996). Also aspartate aminotransferase (AST) and alanine aminotransferase (ALT), usually used as markers of hepatocellular (hypoxic) damage, have been included in a complex assessment together with previously mentioned markers (Grau et al. 2007, Hwang and Chung 2019, Lifshitz et al. 2021, Pollock and Minuk 2017). This approach is justified by some overlap between cholestatic and (ischemic) hepatocellular liver injury with bidirectional causal relationship between these entities. Determination of circulating bile acids is of interest to define cholestasis, but not available in clinical routine (Horvatits et al. 2017, Jenniskens et al. 2016). Parenteral nutrition-associated liver disease (PNALD) and intestinal failure-associated liver disease (IFALD) are described as specific features in the literature, based on the same markers as cholestasis and similarly lacking specific diagnostic criteria (set of markers with clear cutoff values) and further complicating nomenclature.

To move toward a unified definition that is meaningful in clinical practice (Titcomb 2003), understanding of pathophysiology of cholestasis together with mechanisms influencing outcome in critical illness is warranted.

This chapter summarizes available information on pathophysiology and epidemiology of critical illness-associated cholestasis, currently used and potential plasma markers, as well as current and potential management strategies.

Etiology and Pathophysiological Mechanisms

Normal Hepatobiliary Transport

The liver produces and excretes bile, which is essential for intestinal lipid emulsification and absorption and for the excretion of excess or toxic compounds. The main organic component of bile are bile acids, which are synthesized from cholesterol in the hepatocytes. The rate-limiting enzymes in this process are CYP7A1, CYP8B1, and CYP27A1 (Halilbasic et al. 2013). Through hydroxylation and further modification of the side chain and ring structure of cholesterol, the two primary bile acids cholic acid and chenodeoxycholic acid are produced. These primary bile acids are further conjugated in the liver by adding glycine or, to a lesser extent, taurine, which makes the molecules more amphipathic and enhances their physiological function. Excreted bile acids are delivered to the small intestine through the bile ducts. Primary bile acids are converted by the bacterial gut flora and can be deconjugated by bile salt hydrolases and converted into the secondary bile acids deoxycholic acid and litocholic acid and their respective conjugates through 7alpha-dehydroxylation

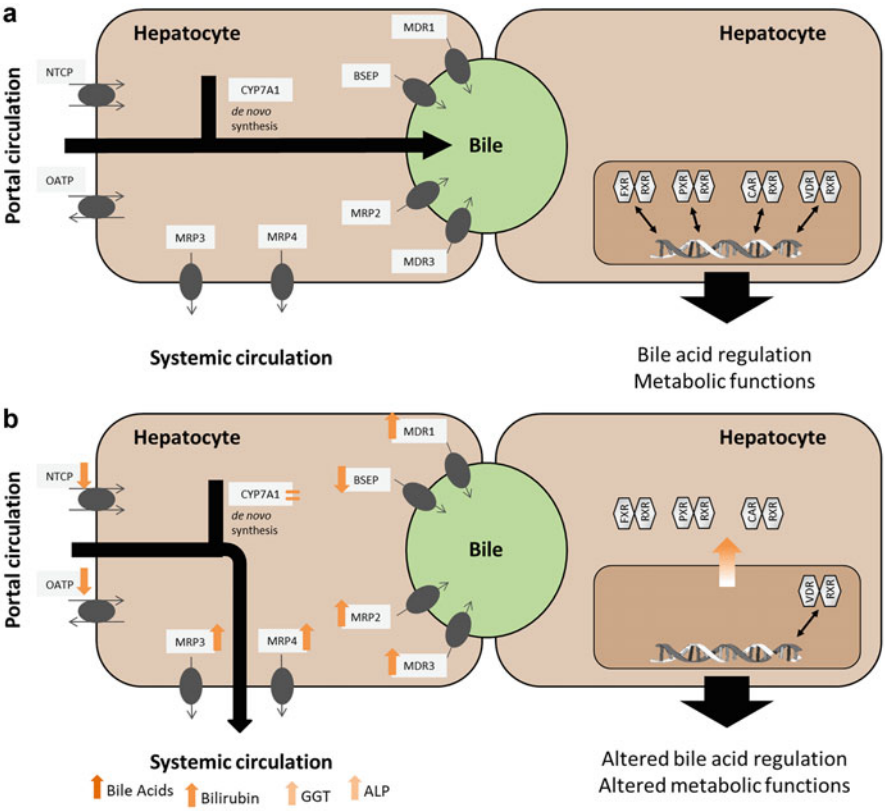

Fig. 1 Hepatobiliary transport system in normal conditions (**a**) and during critical illness (**b**). During the course of critical illness, the specific uptake transporter NTCP and less specific OATP transporters of bile acids are downregulated. Also the specific export pump BSEP is downregulated. MRP3 and MRP4, pumps mediating alternative export of bile acids to the systemic circulation, are upregulated. Simultaneously, nuclear receptors are no longer located in the nucleus of the hepatocyte and are unable to bind to their respective response elements. Plasma bile acids and bilirubin will increase and the cholestasis will also enhance synthesis and release of ALP and GGT to the circulation. Adapted with permission (Jenniskens et al. 2016)

(Long et al. 2017). This microbial-mediated biotransformation of the intestinal bile acid pool is indispensable for bile homeostasis, and perturbations of the gut microbiota can majorly affect bile acid signaling and homeostasis (Long et al. 2017). The majority of the bile acids are efficiently reabsorbed in the enterocytes of the small intestine and transported through the portal circulation back to the liver, known as the enterohepatic cycle.

The hepatocyte expresses specific hepatobiliary transporters for the excretion and uptake of biliary constituents (Fig. 1a). Apical excretion of bile acids into the bile ducts is primarily regulated by the bile salt export pump (BSEP) and by the less specific multidrug resistance protein (MDR) and multidrug resistance-associated

protein (MRP) family (Alrefai and Gill 2007, Kubitz et al. 2012). Apart from bile acids, MDR1 eliminates a wide array of toxins, MDR3 excretes phospholipids, and MRP2 transports endogenous and xenobiotic amphipathic organic anions (Alrefai and Gill 2007, Kubitz et al. 2012). Reuptake of bile acids from the blood into the hepatocyte is primarily regulated by the high affinity Na + −dependent bile acid transporter (NTCP) and the less specific organic anion transporting polypeptide (OATP). Hepatic excretion of bile acids to the canaliculi is the rate-limiting factor in the bile acid-dependent bile flow. A small fraction of bile acids is not exported via the bile but reenters the systemic circulation through basolateral membrane transporters MRP3 and MRP4 (Rius et al. 2006, Soroka et al. 2001).

The hepatic nuclear receptor farnesoid X receptor (FXR) is the main regulator of bile acid homeostasis and is activated upon bile acid binding (Fig. 1a). Other nuclear receptors with high affinity for bile acids are the pregnane X receptor (PXR), the vitamin D receptor (VDR), and the constitutive androstane receptor (CAR) (Halilbasic et al. 2013). Other biliary components such as bilirubin can also activate CAR (Halilbasic et al. 2013). These nuclear receptors exert their transcriptional activity by heterodimer formation with the retinoid X receptor (RXR). Ligand binding-induced activation of the bile-sensing nuclear receptors will lead to decreased synthesis and uptake and increased export of bile acids to limit intracellular bile acid levels, which will result in elimination of bile acids from the body (Panzitt and Wagner 2021). In the ileum, increased concentrations of bile acids will also activate FXR, which will increase expression of fibroblast growth factor 19 (FGF19), a strong suppressor of the rate-limiting enzyme in bile acid synthesis CYP7A1 (Holt et al. 2003, Zhang et al. 2013).

Of vital importance for the physiology and function of the liver is its blood supply. The liver has a unique dual afferent blood supply, receiving blood via the hepatic artery and via the portal vein draining the gastrointestinal tract. In normal physiology, the portal vein delivers approximately 75% of the blood supply, while the hepatic artery provides the remaining 25%. Hepatic arterioles and portal venules feed the liver sinusoids that supply blood to the hepatocytes. In contrast, the intra- and extrahepatic bile ducts only receive blood supply via the hepatic artery.

Hepatobiliary Transport During Critical Illness

Cholestasis is clinically defined as decreased or absent bile flow toward the small intestine, which is caused by impaired bile formation or the impaired bile secretion (Fig. 1b). Critical illness-induced cholestasis is related to inflammation-driven cellular alterations resulting in a transient accumulation of bile acids and bilirubin within the liver and in the systemic circulation. Histological postmortem abnormalities indeed point to frequent portal and lobular inflammation with large Kupffer cells and infiltrating inflammatory cells (Hirata et al. 2001, Koskinas et al. 2008).

ICU non-survivors demonstrated several-fold increased circulating levels of bilirubin and bile acids, whereas the hepatic protein expression of the rate-limiting enzyme for bile acid synthesis, CYP7A1, was not suppressed in postmortem liver

biopsies (Vanwijngaerden et al. 2011). This ongoing synthesis despite increased bile acid availability suggests that normal feedback inhibition is suppressed during critical illness. Such suppressed feedback is also supported by the observation that hepatocytic nuclear staining of the nuclear bile acid receptors FXR, PXR, CAR, and RXR was strongly suppressed in postmortem liver biopsies of critically ill patients and rodents (Jenniskens et al. 2018b, Vanwijngaerden et al. 2011). Inflammatory mediators can regulate such nuclear export through c-Jun N-terminal kinases (Kosters et al. 2009, Schneider Aguirre and Karpen 2013, Zimmerman et al. 2006).

Also hepatobiliary transport is affected during critical illness. Endotoxemic and septic animal studies and observations in critically ill patients demonstrated a clear suppression of the basolateral uptake pumps NTCP and OATP, again likely due to pro-inflammatory stimuli (Andrejko et al. 2008, Geier et al. 2003, Green et al. 1996, Kim et al. 2000, Vanwijngaerden et al. 2011). The export pumps BSEP displayed reduced hepatic expression in rodents and patients (Cherrington et al. 2004, Elferink et al. 2004, Vanwijngaerden et al. 2011). In contrast, export pump MRP2 and MDR1 and MDR3, involved in the export of toxic compounds to the bile canaliculi, were upregulated in postmortem liver biopsies of critically ill patients (Vanwijngaerden et al. 2011). The most remarkable observation in animal models and critically ill patients is the increased expression of the export pumps MRP3 and MRP4 (Cherrington et al. 2004, Donner et al. 2004, Jenniskens et al. 2018b, Vanwijngaerden et al. 2011). In normal healthy conditions, these basolateral export pumps are only expressed at very low levels, but their expression is increased in inflammation, sustained cholestasis, and critical illness, thereby potentially increasing export of bile acids back to the circulation in a compensatory reaction to cholestasis (Jenniskens et al. 2018c, Jenniskens et al. 2016). Indeed, in critically ill patients, hepatic MRP3 expression strongly correlated with histological signs of bilirubinostasis, circulating bilirubin and bile acids (Vanwijngaerden et al. 2014).

Bile acids play a vital role in intestinal integrity, and when bile flow is reduced, this may promote translocation of the gut microbiome with consequently increased concentrations of LPS in the portal circulation (Clements et al. 1996, Hegyi et al. 2018, Hofmann and Eckmann 2006). As this might result in sustained inflammatory signals, this could further enhance the already disturbed hepatobiliary transport (Donner et al. 2004, Elferink et al. 2004). On the other hand, intestinal malabsorption of bile acids could contribute to increasing circulating bile acids. Indeed, especially diarrhea, occurring in 14–21% of ICU patients (Dionne et al. 2019, Reintam Blaser et al. 2015, Tirlapur et al. 2016), can lead to decreased reuptake of bile acids, which would prevent negative feedback inhibition through FXR and FGF19, and support ongoing bile acid synthesis in the liver (Hegyi et al. 2018, Xiao et al. 2016). Changes in the composition of microbiome can also affect the biotransformation of the intestinal bile acid pool and, as a consequence, bile acid homeostasis (Long et al. 2017). In this regard, diarrhea and elevated markers of cholestasis often occur concomitantly in critically ill patients, although this association may be confounded by severity of illness (Kiss et al. 2020). On the other hand, lack of oral or enteral nutrition and also the administration of lipids through parenteral nutrition are

thought to contribute to the development of cholestasis during critical illness (Carter and Shulman 2007). Early use of artificial nutrition but also malnutrition was independently associated with liver dysfunction (Grau et al. 2007). Critically ill patients not receiving oral intake are more susceptible to develop biliary sludge (Kumpf 2006).

In selected patients, ischemia of the biliary system could add to the development of critical illness-induced cholestasis. Indeed, decreased blood supply via the hepatic artery can induce ischemic cholangiopathy, which can contribute to bile stasis and the formation of biliary casts, thereby promoting the development of clinical jaundice (Rady et al. 1998, Ruemmele et al. 2009). Ischemic cholangiopathy can arise from hepatic artery thrombosis, a frequent complication after liver transplantation that can also be triggered by a hypercoagulable state (Mourad et al. 2014, Piscaglia et al. 2007). Impaired microcirculation of the gallbladder wall, with secondary inflammation and necrosis, can lead to the potentially lethal condition of acalculous cholecystitis, associated with a complicated ICU stay and mortality (Ahvenjarvi et al. 2011, Kalliafas et al. 1998).

Diagnosis of Critical Illness-Induced Cholestasis

Critical illness-induced cholestasis, mainly caused by intrahepatic alterations in liver transport machinery and bile acid synthesis, results in elevated bilirubin and bile acid levels. This entity occurs more frequently than hypoxic hepatitis and presents with mild liver test abnormalities with predominantly cholestatic features (Fig. 2b). Cholestasis has been reported in 4–42% of ICU patients, depending on definition used, case mix, and assessment time point (Table 1). It can develop during the early stages of severe illness but also as a late event in sepsis and multiorgan failure (Kiss et al. 2020, Kramer et al. 2007, Mesotten et al. 2009, Thomson et al. 2009). Of note, one-third of patients suffering from hypoxic hepatitis or shock liver subsequently develops cholestasis, potentially triggered by (prolonged) inflammation associated with ischemia-reperfusion injury (Jager et al. 2012). Biliary sludge can develop because of a lack of bile flow and has a prevalence of 50–60% in patients requiring more than 5 days of intensive care (Mesotten et al. 2009).

Not only cholestatic liver injury or critical illness-induced cholestasis but also ischemic hepatocellular injury or hypoxic hepatitis (Fig. 2a) frequently occurs in critically ill patients and may lead to cholestasis (Horvatits et al. 2013, Jenniskens et al. 2018c). In hypoxic hepatitis or shock liver, impaired oxygen delivery and ischemia of the liver will lead to hepatocellular necrosis (Fig. 2a). As a consequence of this diffuse cellular damage, hepatic lysis enzymes ALT and AST and to a lower extent also ALP and GGT will be released into the blood stream. Typically, an early and steep increase in circulating ALT and/or AST is observed, with concentrations reaching more than 20× the upper limit of normality (Fuhrmann et al. 2011, Horvatits et al. 2013).

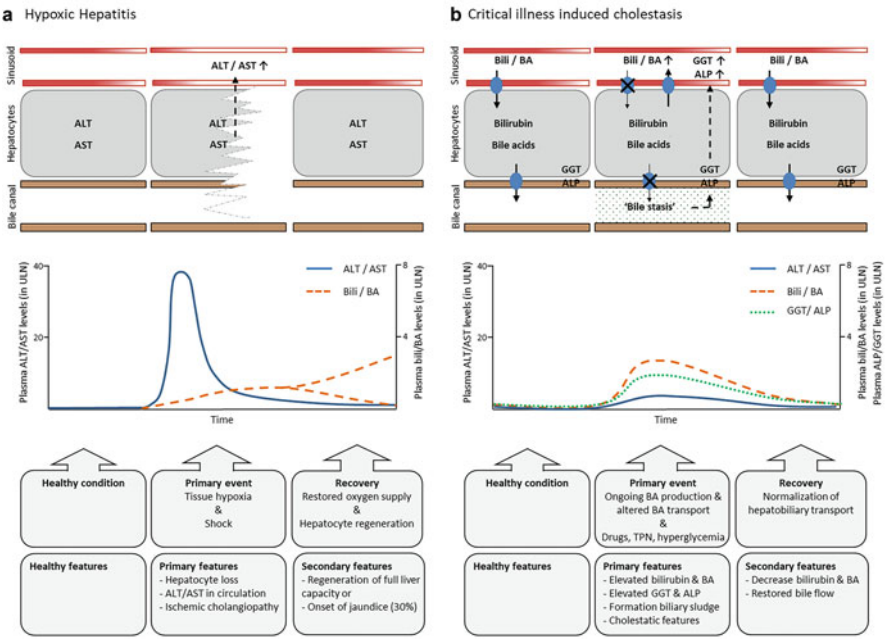

Fig. 2 Schematic overview of liver alterations and serum markers in hypoxic hepatitis and cholestasis induced by critical illness. Adapted with permission (Jenniskens et al. 2018c). Legend: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BA, bile acids; Bil, bilirubin; GGT, gamma-glutamyl transpeptidase; ULN, upper limit of normal (numbers presented in times ULN)

Plasma Markers of Cholestasis

Bilirubin and Bile Acids

Bilirubin is often used for the assessment of cholestasis and is incorporated in commonly used ICU prediction and organ assessment scores such as SOFA, SAPS, APACHE, and MODS (Jenniskens et al. 2018c, Kamath et al. 2001, Le Gall et al. 1993, Vincent et al. 1996, Zimmerman et al. 2006). In addition, increased circulating concentrations of the markers ALP and GGT can further strengthen the diagnosis of cholestasis (Kortgen et al. 2009, Thomson et al. 2009). These standard laboratory tests unfortunately lack specificity, challenging the diagnosis of cholestatic liver dysfunction (Thomson et al. 2009).

An increase in total bilirubin above 2 mg/dl is routinely used as a marker of hepatic excretory dysfunction (Dellinger et al. 2013). However, hemolysis and – to a lesser extent – prolonged fasting can also increase bilirubin levels, which may be a confounding factor (Bauer et al. 2008, Jenniskens et al. 2018c). Hemolysis will specifically increase concentrations of unconjugated (or indirect) bilirubin, due to increased conversion from hemoglobin before it is transported to the liver for further conjugation and excretion, whereas an increase in conjugated (or direct) bilirubin

Table 1 Studies reporting prevalence of cholestasis in critically ill

Author and year Number of patients	Study design and population	Definition cholestasis (cutoffs of markers)	Prevalence
Kiss et al. (2020) (n = 720)	Retrospective, single-center Mixed ICU patients who stayed for ≥3 days	ALP and GGT both 1.5× ULN	28% during ICU stay
de Tymowski et al. (2019) (n = 214)	Retrospective, single-center ICU patients with severe burns who stayed for ≥2 days	ALP 1.5× ULN and GGT 3× ULN Bilirubin 2× ULN	42% during ICU stay 20% during ICU stay
Jenniskens et al. (2018a) (n = 1231)	Prospective, multicenter (sub-analysis of an RCT) Pediatric (28 weeks – 17 y) ICU patients	Bilirubin 2× ULN	Between 3.8% and 4.9% during first 7 days in ICU
Patel et al. (2015) (n = 251)	Retrospective single-center study ICU patients with severe sepsis	Bilirubin 2× ULN	8% during first 72 h after ICU admission
Casaer et al. (2011) (n = 4640)	Prospective, multicenter (secondary endpoint of an RCT) Mixed ICU patients	Bilirubin 3× ULN GGT 1.5× ULN ALP 1.5× ULN	14% during ICU stay 35.5% during ICU stay 21% during ICU stay
Thomson et al. (2009) (n = 263)	Prospective, single-center Consecutive mixed ICU patients	Bilirubin 2× ULN[a] ALP 2× ULN	6% on admission 4% on admission
Mesotten et al. (2009) (n = 658)	Prospective, multicenter (sub-analysis of an RCT) Medical ICU who stayed for ≥5 days	Bilirubin 3× ULN Or ALP and GGT both 1.5× ULN	20% on ICU-day 10 (17% on admission) 34% on ICU-day 10 (13% on admission)
Kramer et al. (2007) (n = 38′036)	Prospective database analysis, multicenter Mixed ICU patients	Bilirubin 2× ULN	11% during the first 48 hrs after ICU admission
Brienza et al. (2006) (n = 141)	Prospective observational single-center study Mixed ICU patients who stayed for ≥2 days	Bilirubin 2× ULN for at least 48 h	31% during ICU stay

[a]Cholestasis was not defined a priori, prevalence of abnormality based on different cutoffs for each biomarker were presented

points to a hepatic origin. However, when (total and conjugated) bilirubin accumulates because of hampered bile flow, this is rather a consequence of retained bile acids, as they are the main osmotic drivers of bile excretion (Trauner and Boyer 2003, Trauner et al. 1998). This suggests that not bilirubin but circulating concentrations of bile acids might better reflect cholestasis in critically ill patients, with higher sensitivity and specificity (Jenniskens et al. 2018c, Kiss et al. 2020,

Recknagel et al. 2012). However, the quantification of bile acids is technically more challenging and is not part of routine clinical practice. A potential additional marker that could be used in the context of changes in bile acid homeostasis and more specifically to assess problems with intestinal malabsorption of bile acids could be plasma FGF19, although this requires further validation studies (Kiss et al. 2020).

Alkaline Phosphatase and Gamma-Glutamyl Transpeptidase

An increase in ALP $\geq 1.5\times$ the upper limit of normal (ULN) and in GGT $\geq 1.5\times$ (or $\geq 3\times$) ULN has been used to diagnose cholestasis, independently on concomitant increase in bilirubin (Table 1). In ICU patients, elevation of GGT above the normal appears to be more common compared to ALP (Thomson et al. 2009, Casaer et al. 2011, Kiss et al. 2020). With short-stayers excluded, cholestasis defined with ALP and GGT $\geq$1.5 ULN occurred in around 30% of ICU patients (Mesotten et al. 2009, Kiss et al. 2020). In approximately one-third of these cases, cholestasis was already present on admission to the ICU, whereas in remaining 2/3 it developed during the ICU stay. Of note, both ALP and GGT have a high sensitivity but low specificity, arguing against their use for the individual assessment of hepatic dysfunction (Giannini et al. 2005). Indeed, elevated GGT is also observed in non-cholestatic liver diseases such as nonalcoholic fatty liver disease, alcoholic hepatitis, and chronic hepatitis C but also in non-liver diseases such as chronic obstructive pulmonary disease, renal failure, and acute myocardial infraction. Based on the close link between cellular GGT and the metabolism of glutathione and glutathione conjugates, an increase in GGT is considered to be a marker of oxidative stress (Lee and Jacobs 2009). Also large quantities of alcohol intake and certain drugs (e.g., carbamazepine, phenytoin, barbiturates) can increase plasma GGT (Giannini et al. 2005, Lee and Jacobs 2009, Limdi and Hyde 2003). Non-cholestatic causes for elevated ALP include bone disease, pregnancy, and the use of certain drugs such as anabolic steroids (Giannini et al. 2005, Limdi and Hyde 2003).

Other Diagnostic Tools

Indocyanine Green Clearance

In contrast to the above described static markers of liver function, liver function can also be assessed by dynamic tests. Measuring the clearance rate of indocyanine green is a good indication of hepatic biotransformation and excretion (Recknagel et al. 2012). Indocyanine green is an organic dye, stable in blood, and almost exclusively taken up by the liver by and eliminated into the bile in an unconjugated state without undergoing metabolization (Sakka 2018). Transcutaneous systems for bedside assessments are available and were reported reliable in liver transplant patients (Faybik et al. 2004) and critically ill patients (Sakka et al. 2000). Plasma disappearance rate of indocyanine green correlated with mortality with comparable sensitivity and specificity as the SAPS and APACHE scores in critically ill patients (Sakka et al. 2002). However, the rate of dye clearance strongly depends on the perfusion of the liver, and impaired clearance rates may thus (partly) reflect liver hypoperfusion

rather than reduced excretory function (Uusaro et al. 1995). Also redistribution of the dye in extravascular tissues, protein binding, and complex pharmacokinetics can further complicate interpretation of this diagnostic tool, especially in the complex clinical context of critical illness (Stehr et al. 2005).

Abdominal Sonography

Mechanical obstruction of the extrahepatic bile duct can easily and reliable be diagnosed with abdominal ultrasonography. Such obstruction is however rarely the origin of deranged cholestatic liver parameters in critically ill patients (Boland et al. 2000, Murray et al. 1992). Abdominal ultrasonography can also be used to assess hepatic artery thrombosis (Mourad et al. 2014, Piscaglia et al. 2007) and acalculous cholecystitis (Mourad et al. 2014, Piscaglia et al. 2007, Thampy et al. 2019). The latter will present as a significantly thickened wall with edema and possible pericholecystic fluid on the abdominal ultrasonography (Thampy et al. 2019). Critically ill patients have increased risk to develop acalculous cholecystitis, which may be life-threatening through gallbladder perforation and necrosis (Ahvenjarvi et al. 2011, Kalliafas et al. 1998). Biliary sludge, which can develop because of a lack of bile flow, can also be diagnosed with ultrasonography (Mesotten et al. 2009). Critically ill patients not receiving oral intake are more susceptible to develop biliary sludge (Kumpf 2006), although withholding parenteral nutrition reduces the occurrence of biliary sludge (Vanwijngaerden et al. 2013). Importantly, ongoing cholestatic serum, even after clinical improvement, may indicate the development of secondary sclerosing cholangitis (Jaeger et al. 2006, Kulaksiz et al. 2008).

Other Laboratory Markers

Apart from diagnosing cholestasis, evaluation of other liver tests and liver function may assist in diagnosing etiology and severity of liver injury. Increases in ALT and AST are a consequence of cellular necrosis and are typically related to hypoxic liver damage (Fuhrmann et al. 2011, Horvatits et al. 2013). Mild elevations however can be observed in non-hypoxic conditions, as a consequence of inflammation-induced increase in cellular permeability (Jenniskens et al. 2018c). Moreover, ischemic or hypoxic liver injury may also lead to subsequent cholestasis.

Coagulation factors, assessed by measuring standardized prothrombin time (INR), are used to estimate the synthetic capacity of the liver in the Model for End-Stage Liver Disease score to prioritize end stage liver disease patients for liver transplant (Kamath et al. 2001). However, critical illness-related conditions, such as disseminated intravascular coagulation, the administration of coagulation factors, vitamin K deficiency or antagonists, blood transfusions, bleeding, or hemodilution can affect the INR in critically ill patients (Lescot et al. 2012). Albumin, a protein predominantly produced in the liver, is the most abundant circulating protein and accounts for up to 50% of hepatic protein synthesis. However, its circulating levels are also affected by breakdown and extravascular distribution, and low albumin concentrations can reflect blood loss, hemodilution, and capillary leakage, rather than reflect hepatic synthesis dysfunction (Lescot et al. 2012, Nicholson et al. 2000). An alternative diagnostic tool to estimate the synthetic capacity of the liver is the

quantification of circulating cholinesterase, although diagnostic cutoff values are not yet well-defined (Ba et al. 2014, Kamolz et al. 2002). Furthermore, healthy volunteers receiving endotoxins displayed increased as well as decreased cholinesterase activities (Ofek et al. 2007). Hyperammonemia is used as an indication of inadequate clearance by the liver but can also occur in ICU patients without liver failure (Sakusic et al. 2018).

Epidemiology of Cholestasis in Critical Illness

Pathologies Affecting Liver Function

Patients with sepsis or extrahepatic bacterial infections with inflammation often develop cholestasis (Geier et al. 2006). A prospective analysis showed that sepsis is the second leading cause of cholestasis in clinically jaundiced patients (Whitehead et al. 2001). Indeed, high circulating endotoxin levels and inflammatory cytokines are strong stimulators of the observed shift in bile acid metabolism and transport in critical illness-induced cholestasis (Jenniskens et al. 2018c, Jenniskens et al. 2016, Kosters et al. 2009, Schneider Aguirre and Karpen 2013, Zimmerman et al. 2006). Also presence of pneumonia, pyelonephritis, and endocarditis have been linked to jaundice in ICU patients, as well as presence of severe trauma or other severe shock states (Brienza et al. 2006, Trauner et al. 1999).

Preexisting presence of alcoholic, viral, or toxin-induced hepatitis might further augment liver test abnormalities during critical illness (Meersseman et al. 2018, Moreau et al. 2013). Similarly, patients suffering from primary sclerosing cholangitis or primary biliary cirrhosis have a cholestatic biochemical profile upon admission to the ICU (Chapman et al. 2010). Despite the even more disturbed liver tests, the ICU course and outcome of such acute-on-chronic liver failure patients is comparable to patients without chronic liver disease but with similar baseline severity of illness (Meersseman et al. 2018). Critically ill patients who require prolonged intensive care also have increased risk to develop secondary sclerosing cholangitis (Martins and Verdelho Machado 2020). Although rare, with an estimated incidence of 1/2000 ICU patients, this progressive cholestatic disease affecting the intra- and/or extrahepatic bile ducts can progress to biliary cirrhosis (Jaeger et al. 2006, Kulaksiz et al. 2008). Biliary tract infection probably plays a critical role in the pathogenesis of secondary sclerosing cholangitis, with episodes of cholangitis deteriorating the bile duct integrity (Voigtlander et al. 2015). More recently, an increased incidence of this severe complication was reported in COVID-19 patients (Butikofer et al. 2021, Meersseman et al. 2021). Critically ill patients with secondary sclerosing cholangitis have a worse ICU prognosis than patients suffering from primary sclerosing cholangitis (Kirstein et al. 2020).

Gastrointestinal disorders associated with malabsorption are associated with increased circulating cholestasis markers (Rubio-Tapia and Murray 2008). Indeed, cholestasis occurs more frequently in patients suffering from diarrhea, occurring often beyond the first week in the ICU (Kiss et al. 2020). Finally, cholestasis may be

caused by extrahepatic obstruction of bile flow, which includes obstruction by gallstones; pancreatic, biliary, or gastrointestinal malignancies; and intra-abdominal collections.

Interventions Affecting Liver Function

Patients with intestinal failure due to conditions such as necrotizing enterocolitis, gastroschisis, short bowel syndrome, and others, who need prolonged use (more than 2 weeks) of parenteral nutrition for survival or growth, can develop intestinal failure-associated liver disease (IFALD). This terminology is used for a spectrum of liver diseases, which encompasses cholestasis progressing to biliary cirrhosis, steatohepatitis, and gallbladder disease, where other causes besides the prolonged use of parenteral nutrition have been excluded (Lauriti et al. 2014, Lee et al. 2020). Other terminologies used to describe liver disease associated with the prolonged use of parenteral nutrition are parenteral nutrition-associated liver disease (PNALD) and parenteral nutrition-associated cholestasis (PNAC) (Khalaf and Sokol 2020). Pathogenic mechanisms are not completely understood, but an interaction of factors originating from the injured and hyperpermeable intestine with especially lipid components of the parenteral nutrition appears to activate hepatic inflammation which can progress to cholestasis (Carter and Shulman 2007, Khalaf and Sokol 2020). The intestinal failure strongly suppresses circulating levels of the feedback regulator FGF19 (Khalaf and Sokol 2020). Overlapping with the findings in critical illness-induced cholestasis are blunted signaling of the key regulator of bile acid homeostasis, FXR, and a shift in bile acid transporters, together increasing the hepatic bile acid pool (Khalaf and Sokol 2020).

During critical illness, not only the administration of lipids through parenteral nutrition but also lack of oral or enteral nutrition is thought to contribute to the development of cholestasis (Carter and Shulman 2007). Indeed, early use of artificial nutrition but also malnutrition was independently associated with liver dysfunction (Grau et al. 2007). Critically ill patients not receiving oral intake are more susceptible to develop biliary sludge (Kumpf 2006), although withholding parenteral nutrition reduces the occurrence of biliary sludge (Vanwijngaerden et al. 2013). Tight blood glucose control also lowered the occurrence of biliary sludge (Mesotten et al. 2009). Remarkably, delaying the initiation of parenteral nutrition for 7 days to supplement insufficient enteral nutrition also reduced biochemical markers of hepatocytic damage (maximum levels ALT) and of cholestasis (GGT, ALP) (Vanwijngaerden et al. 2013). In contrast, circulating bile acids were not affected by the nutritional therapy, whereas withholding parenteral nutrition increased plasma bilirubin, throughout the 7-day intervention window (Vanwijngaerden et al. 2013), which might have been induced by a normal fasting response (Whitmer and Gollan 1983). Indeed, circulating bilirubin levels have been shown to double during 24-h fast, of which the underlying mechanism is not clear (Meyer et al. 1995).

Drugs administered as part of intensive care can directly cause hepatocyte damage but also exert toxic accumulation of bile acids, bilirubin, and toxins by

inhibition of canalicular transporters and interference with nuclear receptors (Yang et al. 2013). Frequently used drugs in the ICU are indeed associated with the occurrence of cholestasis and can contribute to the development of jaundice (Gonnert et al. 2013, Lammert et al. 2010, Recknagel et al. 2012). Vice versa, the clinical impact of an altered hepatic transport on toxins, drugs, and other metabolic substances normally excreted by the hepatocyte is not clear. Selected antibiotics and other drugs with biliary excretion can easily accumulate in cholestatic conditions.

The occurrence of critical illness-induced cholestasis has also been independently related to the use of mechanical ventilation with high levels of PEEP, although one cannot exclude residual confounding by increased illness severity (Brienza et al. 2006).

Association of Critical Illness-Induced Cholestasis with Outcome

Only a few studies have addressed association of plasma markers of cholestasis with outcome of critical illness. A study addressing abnormal liver function tests (incl. Bilirubin, ALT, ALP, and GGT) in 263 adult ICU patients without an established hepatobiliary pathology observed abnormalities (any value above the reference level) in 61% of study patients (Thomson et al. 2009). In majority of them, the degree of abnormality was less than twice the upper limit of normal. However, the presence of an abnormal ALT, ALP, or GGT was associated with an increased risk of death within 30 days of admission in non-adjusted analyses, while not in adjusted analyses. Patients with extreme hyperbilirubinemia (>12 mg/dL) were shown to have very high mortality, increasing with the level of total bilirubin (Han et al. 2021). Less markedly elevated bilirubin levels were identified as independent risk factor for unfavorable outcome (Kramer et al. 2007, Mesotten et al. 2009, Patel et al. 2015) (Horvatits et al. 2017). Also total bile acids predicted 28-day mortality independently of sex, age, serum bilirubin, and severity of illness (Horvatits et al. 2017).

Outside the ICU context, a population-based sample (n = 14.950) revealed an association of elevated GGT with mortality from any cause, liver disease, cancer, and diabetes, while ALT was associated only with mortality from liver disease (Ruhl and Everhart 2009).

An independent association between serum cholestasis markers and outcome has been demonstrated in specific critically ill patient groups such as hypoxic liver injury with bilirubin levels >3 mg/dL (Jager et al. 2012) and liver-transplanted patients (Ben-Ari et al. 2004, Rhu et al. 2021). In one study bilirubin to albumin ratio was independently related to 28 days of mortality in patients without previous hepatobiliary disease (Choi et al. 2020). Bilateral associations between gut dysfunction and cholestasis, which could potentially influence outcome of critical illness, have been hypothesized but not yet proven (Kiss et al. 2020).

Management of Critical Illness-Induced Cholestasis

Current Management Strategies

To our knowledge, there are no specific management strategies that have been clearly proven to improve patient-relevant outcomes in nonobstructive cholestasis in critically ill (Horvatits et al. 2013). However, strict glucose control has been shown to reduce cholestasis and biliary sludge in one study (Mesotten et al. 2009).

The management is mainly based on assuring sufficient oxygen delivery to organs (including the liver) and elimination of all potential etiological factors, e.g., medications and source control of sepsis (Gilroy et al. 2003, Horvatits et al. 2013).

Nutrition strategy is likely to play a role as well, offering potential for adjustment, but specific evidence is scarce. Avoidance of overfeeding is important for all critically ill patients (Singer et al. 2019), whereas it may be especially relevant regarding cholestasis. Overfeeding with carbohydrates was shown to associate with PNALD in one small study (Lakananurak and Tienchai 2019), whereas the other authors have proposed reduction of lipid component in parenteral nutrition as a possible strategy for prevention of PNALD in neonates (Cober and Teitelbaum 2010). In a pediatric ICU population, incidence of overt cholestasis and hypoxic hepatitis was low and unrelated to the nutritional strategy, although withholding parenteral nutrition up to 1 week after ICU admission increased plasma bilirubin (Jenniskens et al. 2018a). Also in adult ICU patients, delaying the initiation of parenteral nutrition until the second week in ICU increased plasma bilirubin but reduced the occurrence of biliary sludge and reduced biochemical markers of hepatocytic damage and cholestasis, suggesting the increase in bilirubin is mediated by fasting rather than by increased cholestasis (Vanwijngaerden et al. 2013). Next to the amount of calories and each macronutrient, also composition of intravenous lipid emulsions has been suggested to play a role, mainly studied in patients with intestinal failure and long-time parenteral nutrition (Lee et al. 2020, Rochling 2021). However, respective evidence is scarce and recommendations not available (Hojsak et al. 2016).

Future Research

Future research should address clinical course along with dynamics of all cholestasis markers together with screening of possible pathophysiological mechanisms and outcome. Such research could assist in development of uniform definition of cholestasis and help toward recommendations for measurement of plasma markers of cholestasis in the ICUs. At the same time, experimental research on pathophysiological mechanisms should continue. Only if future research identifies the mechanisms that lead to cholestasis impairing outcome specific management strategies could be developed.

Mini-Dictionary of Terms

- Cholestasis: decreased or absent bile flow toward the small intestine, which is caused by impaired bile formation or the impaired bile secretion.
- Hypoxic hepatitis: hepatocellular injury caused by impaired oxygen delivery to the liver.
- IFALD or PNALD: spectrum of liver diseases, which encompasses cholestasis progressing to biliary cirrhosis, steatohepatitis, and gallbladder disease, where other causes besides the prolonged use of parenteral nutrition have been excluded.
- Jaundice: high circulating levels of bilirubin causing yellow pigmentation of the skin and the sclerae.

Key Facts of Plasma Markers of Cholestasis in Critical Illness

- Bilirubin is often used for the assessment of cholestasis and is incorporated in commonly used illness severity and organ assessment scores.
- Critical illness-induced cholestasis is mainly caused by intrahepatic alterations in liver transport machinery and bile acid synthesis, resulting in elevated bile acid and bilirubin levels.
- Bile acids are the intrahepatic synthesized drivers of bile flow and might better reflect cholestasis in critically ill patients than bilirubin, with higher sensitivity and specificity.
- The quantification of bile acids is technically more challenging and is not part of routine clinical practice.
- ALP and GGT are also often used for the assessment of cholestasis, with or without concomitant assessment of bilirubin.
- Both ALP and GGT have a high sensitivity but low specificity for cholestasis, arguing against use for the individual assessment of hepatic dysfunction.
- Elevated levels of plasma markers of cholestasis can indicate high secretion, intrahepatic dysfunction or posthepatic obstruction. Other laboratory markers including transaminases and other tools (e.g., ultrasound) are important in differential diagnosis.

Summary Points

- There is no consensus on the exact definition of critical illness-induced cholestasis including specific cutoffs of plasma markers.
- Bilirubin, ALP, and GGT remain widely used plasma markers of critical illness-induced cholestasis.
- The technically more challenging measurement of plasma bile acids could have a better diagnostic value. Other laboratory markers such as transaminases and other tools (including ultrasound) are important in differential diagnosis.

Cross-References

- A Synopsis of Routine Blood Biomarkers in Trauma, Injury Critical Care and Recovery: General Overview
- Blunt Abdomen Trauma and Biomarkers
- Intraoperative Management and Its Influence on Postoperative Biomarker Release
- Prognostic Biomarkers to Predict Outcomes in Trauma
- The Role of Vitamin D As a Biomarker in Trauma

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Biomarkers of Oxidative Stress in Neonatal Hypoxic-Ischemic Encephalopathy

11

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Abstract

Neonatal hypoxic-ischemic encephalopathy (HIE) results from the disruption of cerebral oxygen delivery and is a major cause of disability worldwide. The oxidative burst triggered by hypoxia-ischemia-reperfusion, glutamate excitotoxicity, and mitochondrial dysfunction plays a key role in the development of brain injury. Hence, multiple biomarkers of oxidative stress have been explored in asphyxiated infants and are reviewed in this chapter. While lipid and protein oxidation biomarkers, nonprotein-bound iron, and uric acid have long been studied, bringing encouraging predictive data on HIE severity and outcome, other biomarkers have been explored more recently and require further investigations. Oxidative biomarkers could be useful to identify infants at higher risk of moderate to severe HIE that would benefit from neuroprotective treatments. To date, however, they are not part of routine neonatal practice, mainly due to the high costs and complexity of the spectroscopic techniques required for their assessment.

Keywords

Hypoxic-ischemic encephalopathy · Perinatal asphyxia · Neonate · Infant · Oxidative stress · Free radicals · Biomarkers · Lipid peroxidation · DNA oxidation · Protein oxidation · Nitric oxide · Acid uric · Nonprotein bound iron · Bilirubin · Uric acid

Abbreviations

4-HNE	4-hydroxynonenal
8-OHdG	8-hydroxydeoxyguanosine
aEEG	Amplitude-integrated electroencephalography
AOPP	Advanced oxidation protein products
ATP	Adenosine triphosphate
CAT	Catalase
CNS	Central nervous system
Cr	Creatinine
CSF	Cerebrospinal fluid
EEG	Electroencephalography
ETC	Electron transport chain
Gal-3	Galectin-3
GC-MS/MS	Gas chromatography coupled to tandem mass spectrometry
GP	Glutathione peroxidase
HIE	Hypoxic-ischemic encephalopathy
LC-MS/MS	Liquid chromatography coupled to tandem mass spectrometry
MDA	Malondialdehyde
MRI	Magnetic resonance
MS	Mass spectrometry
NAD	Nicotinamide adenine dinucleotide

NADPH	Nicotinamide adenine dinucleotide phosphate
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NOS	Nitric oxide synthase
NPBI	Nonprotein bound iron
O_2	Molecular oxygen
PC	Protein carbonyls
PPPs	Prostaglandin-like peroxidation products
PUFAs	Polyunsaturated fatty acids
Quin	Quinolinic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
sTB	Serum total bilirubin
TH	Therapeutic hypothermia
UA	Uric acid
XD	Xanthine dehydrogenase
XO	Xanthine oxidase

Introduction

Oxidative Stress

Molecular oxygen (O_2) is key to guarantee aerobic metabolism via the mitochondrial oxidative phosphorylation. The electron transport chain in the inner mitochondrial membrane is the last component of aerobic respiration; at this level, more than 90% of the available O_2 is reduced directly to water by cytochrome oxidase through a series of redox reactions where electrons are passed rapidly from one component to the next, while less than 10% is reduced incompletely, leading to the formation reactive oxygen species (ROS) in aerobic conditions.

Superoxide anion (•O2-) results from O_2 reduction with one electron, and it is the most common oxidative free radical in human biology (Torres-Cuevas et al. 2017). O_2 reduction with two or three electrons forms hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH), respectively. Although H_2O_2 is not structurally considered a free radical, it is much more reactive than molecular oxygen and is therefore included in the ROS group. The production of hydroxyl and hydroxide radicals is also triggered by the Fenton reaction, which involves ferrous ion and H_2O_2. By combining with nitric oxide (NO), ROS can generate peroxynitrite and other reactive nitrogen species (RNS), which further enhance free radical production due to their highly unstable chemical behavior.

While, at low concentrations, ROS and RNS can exert beneficial effects on physiological functions, such as immune regulation, smooth muscle relaxation, modulation of gene expression, and programmed cell death, at higher concentrations they can react harmfully with nearby proteins, membrane lipids, nucleic acids, or

other cell components, leading to structural modifications that can alter their function (Valko et al. 2007). In order to protect biological structures from the ensuing oxidative damage by maintaining a redox homeostasis, the levels of ROS and RNS in biological tissues are regulated by specific antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GP). Reduced glutathione further contributes to reduce both ROS concentration, either via direct interaction or serving as a cofactor for ROS-detoxifying enzymes (Lushchak 2012).

Oxidative stress ensues from an imbalance between free radical production on one side and their physiological clearance by antioxidant enzymes on the other and leads to acute and chronic detrimental effects on vital organs and tissues.

Neonatal Hypoxic-Ischemic Encephalopathy: A Clinical Overview

Neonatal hypoxic-ischemic encephalopathy (HIE) is defined as a clinical syndrome of disturbed neurological function in the earliest days of life in the term infant, manifested by difficulty with initiating and maintaining respiration, depression of tone and reflexes, subnormal level of consciousness, and seizures resulting from an acute or subacute disruption of cerebral oxygen delivery (Nelson and Leviton 1991). Despite the advancements in perinatal care occurred over the past decades and the introduction of therapeutic hypothermia (TH) as a standard of care for neonatal HIE, this condition still represents a leading cause of neonatal mortality in low-income settings and of permanent neurologic disability in term neonates worldwide, with an estimated incidence that ranges from 1 to 8 per 1000 live births in high-income countries to as high as 26 per 1000 live births in low-income countries (Nelson and Leviton 1991; Lehtonen et al. 2017).

Antepartum risk factors for neonatal HIE include persistent occipital-posterior position, prolonged rupture of membranes, and maternal pyrexia. A sudden intrapartum event responsible for the acute decrease of fetal perfusion, such as placental abruption, uterine rupture, prolapse of the umbilical cord, or shoulder dystocia, is also often identified (Nelson et al. 2012; Douglas-Escobar and Weiss 2015). Repeated phases of fetal hypoxia due to reduced uterine perfusion during active labor contractions can further contribute to perinatal asphyxia. An additional although rarer condition associated with neonatal HIE is feto-maternal or feto-fetal hemorrhage, which determines a chronic hypoxic state due to the reduced levels of fetal hemoglobin.

A pH <6.8 or a base excess <-20 mEq/l on a blood sample obtained from the umbilical artery, an Apgar score ≤ 3 at 10 min, the loss of the physiological fetal heart rate variability during labor, seizures occurring within the first 24 h from the insult, and evidence of multi-organ injury (i.e., increased transaminase, creatinine, creatinine kinase MB, and troponin T levels on blood tests) are strong predictors of the development of brain damage after perinatal asphyxia (Tonni et al. 2014). The individual characteristics of the neonate (e.g., gestational age, individual sensitivity to oxidative stress, metabolic, and cardiovascular status) together with the features of

the hypoxic-ischemic insult (i.e., chronic vs. acute, intermittent vs. persistent) further contribute to determine the severity of HIE. In term neonates, chronic or mild to moderate hypoxia-ischemia is typically associated with evidence of brain injury in the parasagittal watershed zones between anterior/middle and middle/posterior cerebral arteries, while a global and acute reduction of cerebral hypoperfusion results in injury to metabolically active tissues such as the basal ganglia, hippocampus, sensorimotor cortex, corticospinal tracts, and also the brainstem (Bano et al. 2017).

Following a hypoxic-ischemic insult, it is fundamental to monitor electrical brain activity with electroencephalography (EEG) or with amplitude-integrated EEG (aEEG) in order to detect abnormal electrical patterns or to identify seizures. Based on the combination of EEG/aEEG abnormalities and of specific clinical signs such as abnormal level of consciousness, reduced reflexes, and hypotonia, the severity of HIE can be assessed using the Sarnat grading scale (Sarnat and Sarnat 1976), which classifies the affected neonates into three stages of increasing severity (i.e., stage I, mild; stage II, moderate; stage III, severe). This staging system also entails a prognostic value: the higher the stage, the more severe the HIE, the higher the probability of major neurological sequelae.

Brain magnetic resonance imaging (MRI), especially if associated with spectroscopy or diffusion studies, provides important information to assess the extent of HIE-related brain damage and to predict long-term neurodevelopmental outcomes. A lower diffusion coefficient in the deep grey matter within the first 7 days following the hypoxic-ischemic insult is a predictor for poor neurodevelopment. Evidence of an abnormal signal intensity in the posterior limb of the internal capsule and/or in the basal ganglia is associated with an increased risk of mortality and of cerebral palsy (Ferriero 2004).

The Role of Oxidative Stress in Neonatal HIE

The high oxygen requirements and the ample redox-active iron availability, together with the relatively low levels of antioxidants and the abundant contents of polyunsaturated fatty acids (PUFAs), make the infant brain particularly vulnerable to oxidative damage after a hypoxic-ischemic insult (Ferriero 2004). Cellular mechanisms underlying HIE are complex and involve a sequence of interconnected molecular events, such as oxygen deprivation, energy depletion, release of excitatory amino acids, and reperfusion that, as illustrated in Fig. 1, variously contribute to oxidative stress and to the ensuing cellular dysfunction and death.

In the first instance, acute cerebral hypoperfusion interrupts oxygen delivery to the brain, inhibiting oxidative phosphorylation in the mitochondria and shifting cellular metabolism from aerobic to anaerobic (Douglas-Escobar and Weiss 2015). Glucose utilization for anaerobic glycolysis is highly inefficient, and this, together with limited brain storages of glycogen, contributes to a rapid depletion of cerebral glucose, which represents the primary energy source for neural cells (Brekke et al. 2017). As a result of the decreased levels of adenosine triphosphate (ATP), ATP-dependent ion pumps are progressively inactivated, subsequently leading to

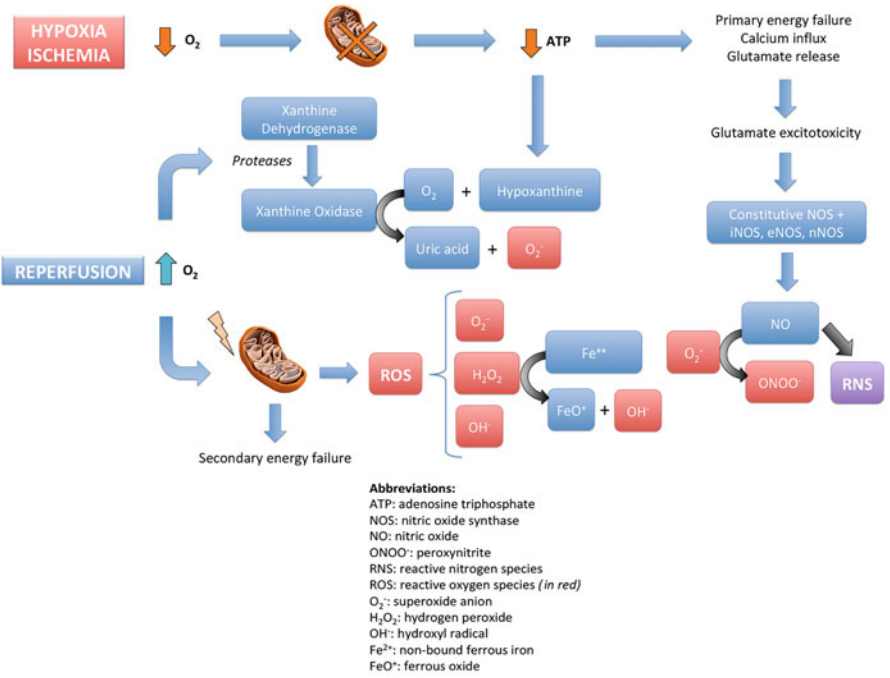

Fig. 1 Molecular mechanisms leading to oxidative stress generation following hypoxia-ischemia and reperfusion. From Martini et al. (2020) with permission

intracellular accumulation of sodium and water, cell swelling, and necrotic cell death. The sequence of neural hypoxia, ischemia, and energy depletion culminates into cell membrane depolarization and release of glutamate, an excitatory amino acid. This process, defined as glutamate excitotoxicity, triggers an intracellular calcium influx that contributes to cell damage not only by exerting necrotic effects but also activates apoptotic cascades via N-methyl-D-aspartate (NMDA) and glutamate receptors. In order to induce a compensatory increase in cerebral blood flow via NO-mediated vasodilation (Iadecola 1997), glutamate excitotoxicity upregulates constitutive and inducible nitric oxide synthase (NOS). The resulting increase in NO production, however, further contributes to brain injury by boosting RNS and ROS production (Ferriero 2004; Hsu et al. 2014). The role of oxidative and nitrosative stress is particularly important in the development of deep grey nuclei injury, which is the brain lesion most frequently observed in term neonates following an acute ischemic insult (Ferriero 2004). The reason underlying the enhanced susceptibility of this area includes the presence of NOS-expressing (NOS+) striatal neurons that, in the developing brain, are relatively resistant to the noxious effects of hypoxia-ischemia and glutamate excitotoxicity (Ferriero et al. 1990). Following calcium influx and the activation of NMDA receptors, NOS+ neurons produce excessive amounts of NO which, in turn, is converted to peroxynitrite, a potent mediator of free radical damage (Barkhuizen et al. 2017). Due to their proximity to

such NOS+ neurons, neural and glial cells located in the nearby striatal projections are exposed to a harmful bystander effect (McQuillen and Ferriero 2004). Evidence of reduced basal ganglia damage in association with selective NOS inhibition (Peeters-Scholte et al. 2002), after selective ablation of NOS+ neurons (Ferriero et al. 1995) or following targeted disruption of the NOS gene (Ferriero et al. 1996) in animal models of hypoxia-ischemia, further supports this mechanism of injury.

Depending upon the timing and the efficacy of cerebral blood flow restoration, an energetic recovery progressively occurs after the hypoxic-ischemic insult, as proved by proton magnetic resonance spectroscopy showing near normal levels of ATP (Hope et al. 1987). Although reperfusion and reoxygenation are key for survival, they also pave the way to the so-called reperfusion injury in the perfusion-deprived and oxygen-depleted tissues, first described four decades ago (Cerra et al. 1975). This process is characterized by a paradoxical overproduction of ROS by complex I and III secondary to the resumption of oxidative phosphorylation in disrupted mitochondrial ETC (McCord 1985) and ultimately leads to secondary ATP depletion and subsequent apoptotic brain damage (Wyatt et al. 1989). One of the most important mechanisms of ROS production following reperfusion is related to the proteolytic conversion of xanthine dehydrogenase (XD) to xanthine oxidase (XO), which is boosted by the intracellular influx of calcium occurring in energy-depleted cells (Amaya et al. 1990). While XD utilizes hypoxanthine or xanthine as a substrate and NAD as a cofactor to produce NADH, XO is a superoxide-producing enzyme and, using O_2 as a cofactor, produces $\bullet O_2^-$ and uric acid (UA) from the same substrates. This, together with the accumulation of hypoxanthine ensuing from ATP depletion during the hypoxic phase, remarkably enhances intracellular ROS production (Chung et al. 1997). Moreover, via the Fenton reaction, nonprotein bound iron (NPBI) and other transition metals available in the brain tissue further contribute to increase ROS levels exponentially (Valko et al. 2005).

The excessive ROS production that follows the hypoxia-ischemia-reperfusion cycle further contributes to worsen the mitochondrial damage begun by primary ATP depletion, calcium influx, and glutamate excitotoxicity. This latent disruption of oxidative phosphorylation starts with reperfusion and usually lasts up to 6 h, finally leading to secondary energy failure (Douglas-Escobar and Weiss 2015). For this reason, the first 6 h following the hypoxic-ischemic event represent a useful, though narrow, therapeutic window to start TH, which decreases cerebral metabolism by reducing the whole body temperature to 33.5 °C, and for other neuroprotective strategies that are currently being tested in clinical trials, such as inhaled xenon, melatonin, erythropoietin, and allopurinol (Martini et al. 2020).

The phase of secondary energy failure is characterized by a delayed and progressive failure of oxidative metabolism, despite normal oxygenation levels. This phase usually starts from the first 6–8 h and lasts up to 72 h after the insult, and its extent depends upon the severity of the hypoxic-ischemic insult. ROS and RNS overproduction plays a central role in determining mitochondrial collapse, secondary cytotoxic edema, and neuroinflammation and in triggering multiple apoptotic pathways. Even after the secondary phase has resolved, however, ongoing effects on the brain can persist for several weeks to years; these effects include persistent

inflammation, impaired oligodendrocyte maturation and myelination, altered synaptogenesis, and epigenetic alterations, with relevant implications on long-term neurodevelopment (Davidson et al. 2021).

Biomarkers of Oxidative Stress in Neonatal HIE

The main clinical challenge after perinatal asphyxia is to identify infants at higher risk of developing moderate to severe HIE, who would therefore benefit from prompt neuroprotective treatments. As previously mentioned, the assessment of asphyxiated infants mainly relies on clinical, neurophysiological, and neuroimaging abnormalities. Nevertheless, the neurological status and the aEEG/EEG patterns of cerebral electrical activity can be significantly altered by sedatives, by anticonvulsants, and by TH itself (Thoresen et al. 2010), thus hindering to evaluate not only HIE severity, but also the response to ongoing treatments. Characteristic changes on brain MRI, particularly with conventional imaging assessments, may take several days to become apparent; moreover, unstable neonates may not tolerate either transport to the MRI scanner or the scan duration itself (Douglas-Escobar and Weiss 2015). Hence, the validation of blood, urine, and cerebrospinal fluid (CSF) biomarkers could contribute to support the sensitivity and specificity of clinical, aEEG/EEG, and neuroimaging findings for HIE severity and outcome prediction.

Among the biochemical parameters that have been currently proposed for the assessment of perinatal hypoxic-ischemic brain damage in clinical and research settings, it is possible to distinguish between markers of tissue injury, resulting from neuronal necrosis (e.g., neuron-specific enolase, matrix metalloproteinase, S100B protein, ubiquitin carboxyl-terminal esterase L1, etc.), gliosis (e.g., glial fibrillary acidic protein) or inflammation (e.g., interleukins), and markers of free radical production and related oxidative activity; this chapter will selectively focus on the latter group.

The gold-standard methods to assess the redox status in biological fluids are gas chromatography and liquid chromatography coupled to tandem mass spectrometry (GC-MS/MS, LC-MS/MS) (Torres-Cuevas et al. 2017). Together with thiobarbituric acid assays, these techniques can be used to measure urinary products of lipid peroxidation (Kuligowski et al. 2014). Moreover, the development of high- or ultra-performance LC-MS/MS has recently allowed to determine the concentration of ROS, RNS, and their metabolites in very small amounts of biological fluids (Cháfer-Pericás et al. 2015), and this is particularly important to translate the assessment of oxidative biomarkers to the neonatal population.

An important limitation hindering the routine clinical use of GC-MS/MS or LC-MS/MS resides in their high cost, complexity, and the need for trained specialists; for these reasons, the availability of these techniques is mainly limited to academic settings with research facilities (Torres-Cuevas et al. 2017).

The following paragraph provides a detailed overview on the available preclinical and clinical evidence on the main oxidative stress biomarkers in the context of neonatal HIE. A summary of the available biomarkers is provided in Table 1.

Table 1 Summary of the main characteristics of the oxidative biomarkers investigated in the context of neonatal hypoxic-ischemic encephalopathy

Biomarker	Role in oxidative stress	Specimen			Proposed reference or cut-off values	Data during TH	Predictivity (HIE severity, outcomes)
		Cord blood, plasma/serum	Urine	CSF			
MDA	Lipid peroxidation marker	↑	↑	↑	Not available	Available	+
4-HNE	Lipid peroxidation marker	↑	Not available	Not available	Not available	Not available	/
Isoprostanes (IP) Neuroprostanes (NP)	Lipid peroxidation marker	↑8-IP	↑ NP	Not available	Normal cord IP values: <124.47 pg/mL	Not available	+ (8-IP)
PC	Protein oxidation markers	↑	Not available	Not available	Not available	Not available	+/−
AOPP	Protein oxidation markers	↑	Not available	Not available	Normal cord values: <80.39 μmol/dL	Available	+(early assessments)
8-OHdG	DNA peroxidation marker	↑	↑	↑	Not available	Available (plasma)	/
NPBI	Substrate for Fenton reaction	↑	Not available	↑	Normal cord values: <6.91 μmol/L	Available (plasma)	+
SOD GP CAT	Antioxidant enzymes	↑SOD ↑GP ↑CAT	Not available	↑SOD ↑GP ↑CAT	Not available	Available (plasma)	+ (SOD and CAT)
Uric acid	Catabolite of xanthine oxidase activity	Not available	↑	Not available	UA/creat ratio ≥ 2.3: Severe HIE and mortality	Not available	+
Nitric oxide	Substrate for nitrosative species production	↑	Not available	↑	Not available	Not available	+
Total bilirubin	ROS scavenger	↓	Not available	Not available	Not available	Available	+/−

Lipid Peroxidation Markers

Free radical species can insert oxygen molecules to lipids containing carbon-carbon double bonds, such as PUFAs or membrane phospholipids, to generate lipid peroxyl radicals and hydroperoxides (Ayala et al. 2014). Following hypoxia-ischemia, free radical overproduction can overcome the adaptive upregulation of antioxidants enzymes, resulting in noxious peroxidative modifications of neuronal membranes. Once this oxidative process is initiated, a propagation of chain reactions generates a wide variety of lipid peroxidation products that can serve not only as biomarkers of global lipid peroxidation but, given the rich lipid composition of the developing brain, may also provide useful information to estimate the oxidative brain damage in neonatal HIE.

Malondialdehyde

Malondialdehyde (MDA) is encountered among the most mutagenic products of the peroxidation of omega-3 and omega-6 fatty acids (Esterbauer et al. 1990) and has long been studied as a biomarker for this oxidative process (Ayala et al. 2014). Increased MDA levels in the cord blood of severely asphyxiated infants (Mondal et al. 2010; Mahmoud El Bana et al. 2016) and in the serum of newborns with HIE within the first 72 h of life (Singh et al. 1999; Thorat et al. 2004; Shouman et al. 2008; Kumar et al. 2008a; Mondal et al. 2010; Mutlu et al. 2015) have been consistently reported by currently available literature. Serum MDA concentration in the acute phase has also been shown to correlate with HIE severity (Thorat et al. 2004; Mutlu et al. 2015; Mahmoud El Bana et al. 2016); HIE infants who died or developed such complications as seizures, persistent neurological impairment, or evidence of brain lesions at neuroimaging showed the highest values of serum MDA (Yu et al. 2003; Shouman et al. 2008; Mondal et al. 2010).

MDA is water-soluble and, as such, is excreted in urines; hence, its excretion rate has also been investigated to evaluate lipid peroxidation extent in HIE. The ratio between urinary MDA and creatinine (uMDA/uCr) increases significantly over the first 24–48 h of life in asphyxiated neonates (Siciarz et al. 2001; Banupriya et al. 2008; Mahmoud El Bana et al. 2016), showing a positive correlation with Sarnat stage and a negative correlation with Apgar score (Banupriya et al. 2008; Mahmoud El Bana et al. 2016). Furthermore, a higher uMDA/uCr ratio was seen in HIE neonates who died compared to survivors; in this regard, a cut-off level of 3.495 μg/mg has been proposed to predict death following perinatal asphyxia with a sensitivity and specificity of 87.5% and 91.7%, respectively (Banupriya et al. 2008).

In the context of HIE, however, serum and urinary MDA are not specific for the lipid peroxidation processes within the brain. MDA concentration in CSF has the advantage of a higher specificity for the cerebral tissue compared to serum or urine and could therefore provide a better estimate of oxidative brain injury in neonatal HIE. Significantly higher MDA levels in CSF have been reported between 24 and 48 h of life in asphyxiated term infants who developed HIE compared to controls (Kumar et al. 2008b) and in HIE infants who expired or developed neurological

deficits compared to those with normal neurological status at hospital discharge (Shouman et al. 2008). Within each category, however, MDA levels in CSF were much lower than in plasma and, for increasing HIE stages, showed smaller surges, resulting in a paradoxically diminished ratio of CSF/plasma MDA in infants progressing to Sarnat stage III compared to controls and in fatal cases compared to survivors (Kumar et al. 2008b). Larger data are further required to validate CSF MDA as a possible marker for oxidative brain damage in neonatal HIE.

4-Hydroxynonenal (HNE)

4-hydroxynonenal (4-HNE) derives from peroxidation of omega-6 fatty acids and can either act as a signalling molecule modulating cell apoptosis or exert cytotoxic effects with long-lasting biological consequences (Esterbauer et al. 1990). Evidence on its role as a lipid peroxidation marker after perinatal asphyxia is limited to one study by Schmidt et al. (Schmidt et al. 1996), who analyzed 4-HNE levels in cord blood samples from preterm and term neonates. 4-HNE increased with increasing gestational age and rose significantly after the hypoxic insult, reflecting sensitively the extent of lipid peroxidation. However, while evidence on other lipid peroxidation products has progressively grown over the last two decades, no further data are so far available to add knowledge on 4-HNE as a biomarker of oxidative stress in HIE. This may be due to the unstable nature of 4-HNE that contributes to the technical complexity of its determination.

Prostaglandin-like Peroxidation Products (PPPs)

Prostaglandin-like compounds derived from free radical-catalyzed peroxidation of arachidonic and docosahexaenoic acid include isoprostanes, isofurans, neuroprostanes, and neurofurans. High brain levels of these compounds have been documented in animal models of global perinatal asphyxia (Calamandrei et al. 2004; Solberg et al. 2017) and in term, asphyxiated infants at the autoptic evaluation (Back et al. 2005).

Among PPPs, F2-isoprostanes have proved to reliably reflect the extent of oxidative processes after hypoxia-ischemia-reperfusion (Sakamoto et al. 2002), and the development of an ultra-performance LC-MS/MS, sensitive to very small amounts of serum, has allowed to investigate their role as lipid peroxidation biomarkers in the context of perinatal asphyxia (Cháfer-Pericás et al. 2015). Higher levels of 8-isoprostane and of total isoprostanes were detected in cord blood samples from acidotic and depressed infants compared to healthy neonates (Chafer-Pericas et al. 2016); in particular, 8-isoprostane positively correlated with the severity of perinatal asphyxia, defined according to cord gas pH, Apgar score, and neurological status at birth. However, the evaluation of serum F2-isoprostanes levels over the first 5 days in HIE infants treated with TH failed to demonstrate different concentrations in relation to the severity of HIE or of brain damage extent at neuroimaging (Negro et al. 2018).

Recently, a urinary panel including multiple PPPs has been tested to estimate oxidative processes during the first 5 days of life in term HIE infants undergoing TH (Cascant-Vilaplana et al. 2021). No difference in the urinary concentration of isoprostanes, isofurans, neurofurans, and neuroprostanes, except of 14(RS)-14-F_{4t}-neuroprostane, was detected over the study period, while significantly different levels of 14(RS)-14-F_{4t}-neuroprostane, total isoprostanes, 15(RS)-15-F_{2t}-isoprostane, and total dihomo-isoprostanes were observed in relation to specific MRI patterns of brain damage.

Given the heterogenic findings of currently available literature, further data are necessary to establish the feasibility of PPPs and, in particular, of F2-isoprostanes, as oxidative biomarkers in neonatal HIE.

Protein Oxidation Markers

The free radical overload generated by hypoxia-ischemia-reperfusion can lead to carbonylation, fragmentation, nitration, cross-linking, and loss of thiol groups in cell proteins. Among the ensuing oxidation products, protein carbonyls (PC) and advanced oxidation protein products (AOPP) have been proposed as possible biomarkers of protein oxidation processes in neonatal diseases, including HIE (Mondal et al. 2010; Perrone et al. 2012).

A rise in PC concentration on cord blood and in serum samples after 48 h from the hypoxic-ischemic insult has been reported in asphyxiated term neonates (Mondal et al. 2010). Although PC levels did not differ significantly in relation to Sarnat staging or developmental outcome at 9 months, HIE infants who developed seizures showed a higher concentration at 48 h compared to those who did not (Mondal et al. 2010).

As for AOPP, Buonocore et al. (2000) have analyzed the concentration of these markers in cord blood from normoxic and hypoxic preterm infants, observing increased levels in the latter group and a significant positive correlation with plasma hypoxanthine and total hydroperoxide levels. In a pre-TH study, increased AOPP levels were reported on cord blood and at 48 h of life in term HIE infants compared to controls. In term asphyxiated infants undergoing TH, however, AOPP levels were significantly higher only at 4–6 h of life in severe compared to mild-moderate HIE infants, whereas no difference between HIE stages was observed at later evaluations, ranging from 24 h to 5 days of life (Negro et al. 2018). Similarly, no significant difference in serum AOPP levels on day 1 (>6 h) and 5 was reported by Mutlu et al. (2015) between term HIE infants treated with TH and controls, although a trend toward increased levels in the HIE group was observed. Given the evidence of higher AOPP levels in untreated HIE infants compared to controls and, in studies performed in the TH era, prior (i.e., 4–6 h), but not during and after this treatment, a beneficial effect of TH in reducing protein oxidation processes may be hypothesized to explain these findings.

In their recent study, Cascant-Vilaplana et al. (2021) used a LC-MS-/MS-based urinary panel of oxidative biomarkers, inclusive of several compounds derived from

protein oxidation, to evaluate the extent of oxidative processes in HIE infants. The urinary concentration of protein oxidation biomarkers increased significantly throughout the first 5 days of life and showed significantly different levels in relation to specific MRI patterns of brain injury. A significant, independent association between serum AOPP levels during the first 5 days of life and the extent of hypoxic-ischemic brain injury, assessed using a validated MRI score, was also reported by Negro et al. (2018); this association was stronger in males infants, thus suggesting a possible gender-related susceptibility to oxidative neurological damage.

DNA Peroxidation Markers

The oxidized DNA nucleoside 8-hydroxydeoxyguanosine (8-OHdG) results from the harmful peroxidative changes to nucleic acids and, over the past decade, has been investigated as a potential biomarker for oxidative DNA damage in preterm and term neonates (Matsubasa et al. 2002; Fukuda et al. 2008; Gane et al. 2014; Bandyopadhyay et al. 2017; Cascant-Vilaplana et al. 2021). With regard to HIE, however, little data are available. In 2008, Fukuda et al. evaluated urine and CSF 8-OHdG concentration in a cohort of children with various types of brain damage, including a small subgroup of neonates with HIE, who showed significantly higher CSF and urinary 8-OHdG levels compared to control subjects (Fukuda et al. 2008). However, data on the timing of collection of CSF and urinary specimens in the HIE subgroup was not specified, and these, together with the small study sample, are potential limitations to these study results.

Of interest, Gane et al. (2014) examined the impact of TH on blood levels of 8-OHdG in treated vs. untreated HIE infants before this treatment became a standard of care worldwide. While pre-treatment levels were similar between the two study groups, untreated infants showed a significantly higher concentration of 8-OHdG compared to the treated group at 36 h of life and after the completion of the hypothermic treatment, thus supporting the effectiveness of TH in reducing the oxidative burden.

8-OHdG was also included among the urinary oxidative biomarkers recently evaluated in HIE neonates undergoing TH; according to this study results, the urine concentration of this biomarker increased significantly over the first 5 days of life despite the hypothermic treatment (Cascant-Vilaplana et al. 2021).

Antioxidant Enzymes

As previously specified, the main antioxidant enzymes involved in the regulation of the redox homeostasis and in the defense from oxidative damage include SOD, CAT, and GP. After the occurrence of a hypoxic-ischemic insult, the activity of these enzymes is significantly enhanced to counteract the overproduction of free radicals and their harmful effects. Consistently, significantly increased levels of SOD, GP

and CAT have been reported in the cord blood of term asphyxiated neonates who developed HIE compared with healthy controls (Singh et al. 1999; Kumar et al. 2008b; Bharti et al. 2009). SOD and CAT concentration in cord blood samples also showed a significant association with Sarnat stages (Kumar et al. 2008b), suggesting that the early upregulation of antioxidant enzymes may effectively reflect the severity of HIE, with potential prognostic implications.

At 24 h, blood concentration of SOD, but not of GP, was found to be significantly increased in infants with mild and moderate HIE compared to controls (Thorat et al. 2004; Mutlu et al. 2015). Furthermore, consistently with the progressive consumption of antioxidant capacities after the acute oxidative burst, a trend toward a decrease in plasmatic SOD levels from day 1 to 5 after perinatal asphyxia has also been reported; on day 5, however, SOD concentration in blood samples from HIE infants was still significantly higher compared to the control group (Mutlu et al. 2015).

With regard to antioxidant enzymatic activities in other biological fluids such as CSF, which may increase the sensibility toward the oxidative processes ongoing in the brain, current data are limited to Gulcan et al. (2005), who evaluated SOD, GP, and CAT activity in the CSF of full-term asphyxiated neonates over the first 72 h of life. According to their findings, SOD activity was significantly higher in HIE infants versus controls, whereas a significant increase of GP and CAT activity was observed only in neonates with severe HIE compared to mild HIE and to the control group. However, this study was performed in the pre-TH era; therefore these results require further confirmation on treated HIE cohorts.

Recently, a possible association between HIE sequelae and specific functional polymorphisms of manganese SOD2, GP1, and CAT genes has been investigated. No difference in SOD2, GP1, and CAT genotype distribution between HIE infants developing epilepsy and controls was observed (Esih et al. 2017), whereas CAT rs1001179 polymorphisms resulted significantly associated with the development of cerebral palsy (Esih et al. 2016), thus hypothesizing a possible role for this polymorphism in identifying highly susceptible asphyxiated infants.

Uric Acid

The restoration of adequate O_2 supplies following a hypoxic-ischemic insult enhances the conversion of XD to XO, which uses hypoxanthine or xanthine as substrates and O_2 as a cofactor to generate $\bullet O_2^-$ and UA. Hence, by serving as a proxy for XO activity, the concentration of UA in biological fluids may reflect the extent of the ensuing ROS production and has therefore been proposed as an economical and easily accessible oxidative biomarker. Being water-soluble, UA is excreted by the kidney; hence, urinary levels of UA have been largely evaluated as noninvasive biomarkers for free radical production following perinatal asphyxia.

Current evidence is consistent in reporting an increased ratio between urinary UA and urinary creatinine (uUA/uCr) in both term and preterm asphyxiated

newborns compared to controls within the first 48–72 h of life (Chen et al. 2000; Banupriya et al. 2008; Basu et al. 2008; Bhongir et al. 2015; Mahmoud El Bana et al. 2016; Patel et al. 2017). This ratio has shown a significant association with both Apgar score (Banupriya et al. 2008; Basu et al. 2008; Bhongir et al. 2015) and Sarnat stage (Akisü and Kültürsay 1998; Banupriya et al. 2008): the lower the Apgar score, the more severe the HIE, the higher the urinary excretion of UA. Cut-off levels of uUA/uCr $\geq$ 2.3 have been proposed to be reliably diagnostic of HIE and to predict the related mortality with good sensitivity and specificity in term asphyxiated infants born in a low-resource setting (Banupriya et al. 2008; Patel et al. 2017). However, the cohorts on which this cutoff was determined were not treated with TH; therefore this data needs to be confirmed on cooled infants to ascertained possible effects of the hypothermic treatment on uUA/uCr ratio.

Nonprotein-Bound Iron

The hypoxia-ischemia-reperfusion cycle and the ensuing inflammation triggers the release of NPBI from erythrocyte hemoglobin. In turn, through the Fenton reaction, NPBI interacts with $\bullet O_2$- and H_2O_2 to form highly reactive •OH, which enhances protein (Marzocchi et al. 2005) and lipid oxidation (Signorini et al. 2008). The first report on NPBI as an OS biomarker in perinatal asphyxia dates 20 years back, when Dorrepaal et al. (1996) observed an increased prevalence of detectable plasma NPBI with increasing HIE severity in asphyxiated infants >34 weeks' gestation. In a recent study on HIE infants undergoing TH, increased levels of plasma NPBI were observed at 4–6 h, but not later, in neonates with severe compared to mild-moderate HIE (Negro et al. 2018), suggesting a potential role of TH in dampening down the related oxidative stress.

A potential predictive role for NBPI levels on HIE neurodevelopmental outcomes has also been proposed. Dorrepaal et al. noticed that three out of four severely asphyxiated neonates with a normal neurological outcome at 1 year of age had no detectable NPBI plasma levels during the first 8 h after birth (Dorrepaal et al. 1996). In a similar fashion, higher levels of plasma (Yu et al. 2003; Shouman et al. 2008) and CSF NPBI (Shouman et al. 2008) within the first 72 h of life were observed in neonates with moderate or severe HIE who died or developed an abnormal neurological status compared to those who were neurologically normal at discharge. However, these data were collected before TH introduction and thus require further validation in treated HIE cohorts.

Of note, while a cut-off value of NPBI to identify HIE infants has not been defined yet, reference intervals for cord blood NPBI have been recently investigated, and a cut-off value of 6.91 μmol/L has been proposed as the upper normal threshold in healthy, non-asphyxiated term infants (Longini et al. 2017).

Nitric Oxide

The upregulation of NOS expression triggered by hypoxia-ischemia enhances the production of NO, which acts as a free radical and reacts with other substrates to form peroxynitrite and other RNS. As such, NO concentration and the nitrates/nitrites ratio, which serves as a proxy for NO levels, have been investigated in the context of neonatal HIE. In several studies performed in pre-hypothermia years, increased plasma levels of NO in plasma (Shi et al. 2000; Thorat et al. 2004) and CSF samples (Gunes et al. 2007), as well as higher plasma nitrates/nitrites ratio (Kumar et al. 2008a), have been detected within the first 24 h of life in neonates with HIE compared to controls. A positive correlation between the plasmatic and CSF concentration of NO and HIE severity, expressed by increasing Sarnat staging, was observed (Shi et al. 2000; Thorat et al. 2004; Gunes et al. 2007). Moreover, plasma NO was much higher in HIE infants with neuroradiological evidence of brain damage compared to those with no abnormalities at brain MRI (Shi et al. 2000). Although these data are consistently supportive of the role of NO as a biomarker for OS and clinical severity in HIE, the possible effect of TH still needs to be evaluated.

Bilirubin

Despite its long-established toxicity on the cerebral tissue at high concentrations, unconjugated bilirubin is also endowed with antioxidant properties (Stocker et al. 1987). In particular, it can act as a highly efficient ROS scavenger in conditions of oxidative stress and may serve as a reducing substrate for peroxidases in the presence of H_2O_2 or other organic hydroperoxides (Vitek and Ostrow 2009). Hence, also considering the low costs and wide availability of its assessment, serum total bilirubin (sTB) levels in neonatal HIE have been investigated. Preliminary data from a retrospective study on HIE cohorts have shown an inverse correlation between lactate concentration, which reflect the extent of perinatal hypoxia-ischemia (Shah et al. 2004), and sTB (Haga et al. 2020). Consistently, lower peak and mean sTB levels were observed in HIE infants with moderate to severe HIE compared to controls, independently of ongoing TH, throughout the first 5 days after birth (Bin-Nun et al. 2018; Dani et al. 2018). These results are consistent with the scavenging role of bilirubin, which can be therefore consumed following perinatal asphyxia, and the degree of sTB consumption is likely associated with the severity of HIE.

Galectin-3 and Quinolinic Acid

Galectin-3 (Gal-3) is a β-galactoside-binding lectin produced by activated tissue macrophages and microglial cells (Liu et al. 1995) that, among its functions, enhances ROS production by activating NADPH oxidase in primed inflammatory cells (Karlsson et al. 1998). Microglia and macrophages also produce quinolinic acid

(Quin), a neurotoxic metabolite of L-tryptophan that, acting as a NMDA receptor agonist, triggers not only excitotoxic brain damage (Schwarcz et al. 1983) but also the oxidative burst (Santamaría et al. 2001).

In 2013, Savman et al. investigated CSF levels of Gal-3 and Quin in relation to perinatal asphyxia, observing increased levels of both biomarkers in asphyxiated infants compared to controls (Sävman et al. 2013). In addition, Gal-3 was significantly higher in HIE infants who died or developed neurological sequelae compared to those with normal outcome. In order to evaluate their specificity, the authors measured Quin and Gal-3 levels also in a cohort of non-asphyxiated septic infants, showing no difference with healthy controls. This encouraging preliminary evidence paves the way to a wider evaluation of Gal-3 and Quin validity as potential OS biomarkers in neonatal HIE.

Diagnostic and Prognostic Applications of Oxidative Biomarkers in Neonatal HIE

This chapter has reviewed the available evidence on the oxidative and nitrosative biomarkers that have been investigated to assess the extent of brain injury following a hypoxic-ischemic insult in the neonatal population.

While data on oxidative biomarkers that have more recently come into the spotlight (e.g., PPPs, gal-3, Quin) are very preliminary, other biomarkers, such as MDA, NPBI, UA, and NO, have been long investigated in the context of perinatal asphyxia, providing encouraging evidence for the identification of asphyxiated neonates at higher risk for brain injury, especially if evaluated in early phases (e.g., cord blood or 4–6 h of life). Since TH needs to be undertaken within the first 6 h after the hypoxic-ischemic insult to guarantee its therapeutic potential, the development of oxidative biomarker panels on easily available samples (i.e., urine or blood) may aid to identify infants at higher risk of brain injury or of HIE progression that would benefit from treatment. Although CSF biomarkers may better reflect the extent of oxidative brain injury compared to other biological fluids, the need to perform an invasive maneuver such as a lumbar puncture reduces their clinical potential in asphyxiated neonates.

The translation of oxidative biomarkers to routine neonatal practice, however, has not been achieved yet, due to the following important limitations. First, despite the development of ultra GC-MS/MS or LC-MS/MS has allowed to determine several oxidative biomarkers on very small biological samples, the high costs and the complexity of these techniques limit their availability and have likely contributed to the small study samples on which these biomarkers have been tested. Hence, further data on larger cohorts are required to validate the biomarkers reviewed in this chapter and to try to establish possible reference intervals. Moreover, the introduction of TH as a standard of care has split the available literature into pre- and post-TH epochs. By decreasing cerebral metabolism, TH contributes to reduce the oxidative burst that follows a hypoxic-ischemic hit and, as such, may influence the levels of oxidative biomarkers. Therefore, data obtained from untreated infants after the first

6 h need to be confirmed during TH, to rule out a reduction of the diagnostic and prognostic biomarker potentials while this treatment is ongoing. Finally, it has been shown that several conditions, either antenatal (sepsis, maternal preeclampsia, maternal tobacco, etc.) or postnatal (sepsis, meconium aspiration, etc.), can alter the neonatal redox homeostasis and should be thus taken into account when the oxidative status of asphyxiated neonates is investigated.

Mini-Dictionary of Terms

- **Hypoxic-ischemic encephalopathy (HIE):** clinical syndrome of disturbed neurological function resulting from an acute or subacute disruption of cerebral oxygen delivery.
- **Reactive oxygen species (ROS):** highly reactive chemical compounds derived by incomplete O_2 reduction or by redox reactions.
- **Oxidative stress:** condition characterized by an enhanced ROS production that overcomes the antioxidant capacities of the organism and causes detrimental effects on biological structures.
- **Sarnat stage:** staging system for HIE severity classification (stage I, mild; stage II, moderate; stage III, severe) based upon specific clinical and electroencephalographic features.
- **Therapeutic hypothermia (TH):** standard treatment for neonatal HIE, characterized by whole body cooling to 33.5 °C starting within the first 6 h following the hypoxic-ischemic insult and continuing for 72 h.

Key Facts of Oxidative Biomarkers in Neonatal HIE

- **Lipid peroxidation biomarkers:** this group of biomarkers has been most extensively investigated in neonatal HIE, providing consistent evidence on their diagnostic and prognostic value; data on CSF samples are also available.
- **Protein oxidation biomarkers:** limited, although promising evidence for early assessments in neonatal HIE; possibly influenced by TH. Normal cord blood ranges available.
- **Nonprotein-bound iron:** increased in HIE infants; the predictive value on HIE severity needs to be validated during TH. Normal cord blood ranges available.
- **Uric acid (UA):** urine UA concentration is easily and noninvasively available and may represent a promising oxidative biomarker in neonatal HIE. A urinary UA/creatinine ratio $\geq$ 2.3 may predict severe HIE and the related mortality.
- **Antioxidant enzymes:** correlation between cord blood and HIE severity; more heterogeneous evidence on blood levels over the first 5 days of life. Limited data on CSF specimens.

Summary Points

- Given the key role of oxidative stress in the development of brain injury following a hypoxic-ischemic insult, multiple oxidative biomarkers have been explored in the context of neonatal HIE, with the aim to implement available diagnostic and prognostic tools.
- Serum biomarkers may have a low specificity to effectively estimate the extent of oxidative processes underlying brain injury, as they may rather reflect systemic oxidative stress.
- CSF specimens may increase the diagnostic value of oxidative biomarkers toward the development of neonatal HIE; however, their collection require invasive maneuvers, and the available evidence on CSF biomarkers is still limited.
- Data on oxidative biomarkers obtained before the introduction of TH as a standard treatment for neonatal HIE may not be applicable to cooled infants, since this treatment contributes to reduce the oxidative burden during the secondary energy depletion.
- Validating these biomarkers in relation to the available neuroprotective treatments and, in particular to TH, may add useful information also to monitor the efficacy of these treatments in dampening down oxidative stress.
- The high costs, the technical complexity, and the need for trained personnel associated with GC-MS/MS or LC-MS/MS have limited their availability; hence, despite encouraging evidence, oxidative biomarkers are not routinely used in neonatal clinical settings yet.

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Measures of Classical and Alternative Complement Function in Serum as Markers in Critical Care

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William Bain, Shekina Gonzalez-Ferrer, S. Mehdi Nouraie, and Janet S. Lee

Contents

Abstract

The human complement system is a crucial component of the host response to pathogen invaders, cellular stress, and injury – all of which are common causes and manifestations of critical illness. The complement cascade can be activated by alternative, classical, and lectin pathways that function as immune recognition pathways. Yet, the capacity of the complement system to respond to infection and injury depends not only on the ability to activate but also on effective regulation to prevent complement factor exhaustion, limit inflammation, and preserve

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complement function to respond to threats. Therefore, measurements of complement pathway function and levels of specific complement proteins may serve as useful biomarkers during critical illness. Complement activity or function is widely variable during critical illness caused by sepsis and other infections, acute respiratory failure including pneumonia, and during the massive injury of trauma. Preserved complement function has been associated with improved outcomes across numerous investigations. However, whether preservation of complement function is a causal mechanism or the consequence of decreased burden of pathogen or injury is unclear and worthy of future research. Herein, we present key biology of the complement system, review assays of complement function, and describe key findings of the existing literature evaluating complement function during critical illness. We further present potential topics for future research on the complement system during critical illness.

Keywords

Complement · Alternative pathway · Classical pathway · Lectin pathway · Complement function · Hemolytic assays · Critical illness · Sepsis · Pneumonia · Acute respiratory failure · Trauma

Abbreviations

AH50	Hemolytic assay utilized to evaluate alternative complement pathway function
ARDS	Acute respiratory distress syndrome
CH50	Hemolytic assay utilized to evaluate classical complement pathway function
COVID-19	Coronavirus disease 2019, the human disease caused by the SARS-CoV-2 virus
EDTA	Ethylenediaminetetraacetic acid, a protease inhibitor that chelates calcium, magnesium, and other ions
EGTA	Ethylene-bis(oxyethylenenitrilo)tetraacetic acid, a chelating agent related to EDTA but with lower affinity for magnesium
ELISA	Enzyme-linked immunosorbent assay
ICU	Intensive care unit
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LPS	Lipopolysaccharide
MAC	Membrane attack complex
MASP	MBL-associated serine protease
MBL	Mannose-binding lectin
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
sC5b-C9	Soluble form of protein complex known as membrane attack complex that is terminal protein complex of complement action
SNP	Single nucleotide polymorphism

Introduction

The complement system is an evolutionarily ancient component of the vertebrate immune system (Elvington et al. 2016). The complement system has long-recognized roles in providing host defense against pathogen invaders, activating as well as regulating inflammatory pathways during infection and injury. There is also emerging recognition of complement functions at the cellular-molecular level, including intracellular "complosome" pathways. Complement function appears to be crucial to the host because genetic deficiencies of complement components are rare and when present, increase risk for infection and inflammatory diseases (Ram et al. 2010; Harris et al. 2012). Furthermore, complement proteins are among the most abundant proteins in the circulation (Walport 2001). Therefore, the complement system appears to be a vital component of host resilience, particularly during biological stress caused by infection and injury. In this chapter, we will review the complement cascade and markers of complement system function during critical illness. We will begin by reviewing activation of the complement system.

Review of Classical, Lectin, and Alternative Pathways of Complement Activation

There are a wide variety of biological stressors that can activate the human complement system including infection, (Ecker et al. 1946; McCabe 1973; Fearon et al. 1975; Whaley et al. 1980) ischemia, (Heideman et al. 1988) trauma, (Fosse et al. 1998; Ganter et al. 2007) burns, (Dobke et al. 1984) and surgery (Kvarnström et al. 2012; Rønholm et al. 1994). Complement activation is initiated by one or more of the three well-characterized pathways of complement proteolysis – the alternative, classical, and lectin pathways (Fig. 1). Each pathway is initiated via unique factors, but all converge upon formation of complexes called C3 convertase that cleave complement component C3 to yield the anaphylatoxin C3a and C3b. The C3b protein can function as an opsonin by coating and targeting pathogens for phagocytosis. In addition, C3b cleaves additional C3 molecules through alternative pathway auto-amplification or can serve as a component of the C5 convertase that cleaves complement component C5 to initiate assembly of a membrane attack complex composed of five sub-unit proteins (C5b-C9) that contribute to lysis of a targeted cell.

The alternative pathway is the oldest complement pathway with homologues identified in invertebrates such as sea urchins and sea anemones (Elvington et al. 2016; Poole et al. 2016; Al-Sharif et al. 1998). In humans, the alternative pathway is unique from other pathways in two important ways. First, the alternative pathway is spontaneously and constitutively active and therefore is tightly regulated by negative regulatory proteins and protective host-cell surface membrane inhibitory proteins. Second, the alternative pathway amplifies all complement pathways including self-feedback that can amplify its own activity (Blatt et al. 2016). Alternative pathway activation is initiated by spontaneous "tickover" hydrolysis of C3 to form C3(H_2O),

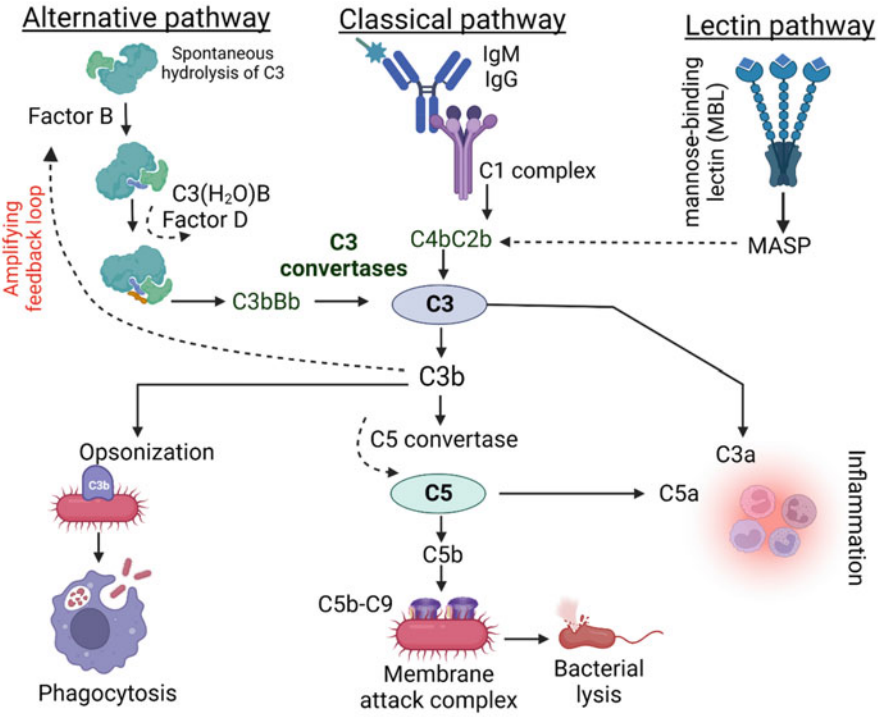

Fig. 1 Overview of complement pathway activation and complement function. There are three well-characterized pathways of complement activation: alternative, classical, and lectin. All pathways of activation converge to form C3 convertase that cleaves C3 into C3b and C3a. C3b can function as an opsonin or form a protein complex, C5 convertase, to cleave C5 as the first step in formation of the membrane attack complex. Created with BioRender.com

which in the absence of inhibitory proteins changes the conformation of C3 protein and enables binding of Factor B (Thurman and Holers 2006). The C3(H_2O)-bound factor B can then be cleaved by factor D to yield the C3(H_2O)-Bb complex, which is a C3 convertase that can cleave C3, thereby producing C3b. This new C3b can bind with the C3bBb complex to form the C5 convertase (C3bBbC3b or C3b_2Bb) and initiate the final common pathway of membrane attack complex formation. Alternatively, C3b can complex with factor B to cleave additional C3 proteins to form additional C3b, which can thereby self-amplify alternative pathway activation. Properdin is a key positive regulator of the alternative pathway by stabilizing surface-bound C3bBb complexes (Blatt et al. 2016). In fact, the alternative pathway was previously called the properdin pathway after the first description of properdin by Pillemer and colleagues in 1954 (Blatt et al. 2016).

In contrast, the classical pathway of activation was first described more than 50 years earlier by Ehrlich, Bordet, and others (Kaufmann 2008) in the context of antibody-mediated cytotoxicity as the classical pathway is activated by antigen-

immunoglobulin complexes. Given its functional role in concert with the adaptive immune system, the classical pathway is thought to be evolutionarily younger and restricted to jawed vertebrates (Dunkelberger and Song 2010). Immunoglobulins such as IgM and IgG can bind at their Fc region to the C1 complex composed of C1q, C1r, and C1s. The Fc-bound C1 complex can then cleave complement components C4 and C2 to yield C4b and C2b that can bind to function as a C3 convertase, cleaving C3 into C3a and C3b. The C4bC2b complex can then complex with C3b to form another C5 convertase, C4bC2bC3b. Therefore, the classical pathway is distinct from the spontaneously and constitutively active alternative pathway because the classical pathway is only activated upon antibody-mediated recognition of a target.

The third recognized pathway, the lectin pathway, is similar to the classical pathway because it is activated upon recognition of a target: specific carbohydrate sequences on micro-organisms that are bound by host pattern recognition proteins such as mannose-binding lectin (MBL) (Dunkelberger and Song 2010). Upon oligomerization of MBL, MBL-associated serine proteases (MASP) 1, 2, or 3 can then cleave C4 and C2 in a similar fashion to the classical pathway to form the C4bC2b complex that can cleave C3. MBL can recognize pathogen-associated molecular sequences on bacteria, fungi, and some viruses. Despite its more recent recognition, the lectin pathway is likely evolutionarily older than the classical pathway and may have been present in early invertebrates (Elvington et al. 2016).

The three pathways of complement activation are all pertinent to patients with critical illness as they contribute to inflammation, opsonization and recognition of pathogens, and initiation of the terminal pathway of complement with formation of the membrane attack complex. However, complement activation is only one aspect of complement function. The functional capacity of complement depends not just on initiation of activation in response to threats but also on the ability to maintain action to clear pathogens and cellular debris. In addition, complement activity provides effective regulation to restrain excessive inflammation and activation that can lead to consumption of complement factors. Therefore, complement regulation is a key aspect of complement function that is of importance during the extensive biological stress of critical illness.

Key Principles of Complement Pathway Regulation

Both prior to and upon activation, complement is tightly regulated to prevent maladaptive inflammation and excessive consumption of complement factors (Ram et al. 2010; Ekdahl et al. 2018; Joiner et al. 1983). To perform the critical functions of complement regulation, there are both circulating regulators such as C1 inhibitor and C4 binding protein that regulate the classical pathway, factors H and I that regulate the alternative pathway, and membrane-bound regulators such as CD59, CD55 (decay accelerating factor), CD46 (membrane cofactor protein), and

complement receptor 1 (Ram et al. 2010; Ferreira et al. 2010; Noris and Remuzzi 2013). Notably, properdin is a circulating positive regulator of the alternative pathway by stabilizing and increasing the half-life of C3 and C5 convertases (Blatt et al. 2016; Kemper et al. 2010). It is important to note that complement activation is not the same as complement function, as the latter includes the capacity not only to initiate activation in response to threats but also the ability to provide effective regulation to prevent excessive inflammation.

In contrast to the target-based activation of the classical and lectin pathways, the alternative pathway is spontaneously and constitutively active. Therefore, alternative pathway regulation may be crucial to prevent or limit inappropriate activation (Fig. 2). Furthermore, the alternative pathway is able to amplify all complement activity through a feedback loop that amplifies its own activation, which can lead to exhaustive consumption of complement factors (Blatt et al. 2016). Notably, several human diseases including recurrent infection during genetic deficiency (Reis et al. 2006) and kidney diseases such as membranoproliferative glomerulonephritis and atypical hemolytic uremic syndrome (de Córdoba and de Jorge 2008; Tortajada et al. 2009; Westra et al. 2017) are defined by impaired regulation of complement activation leading to over-consumption of complement factors.

Taken together, complement regulation may be crucial to maintain appropriate complement functional balance, which can be perturbed during the life-threatening.

biological stress of critical illness (DeCoux et al. 2015). Therefore, complement function may be an important biomarker for development to improve the practice of critical care.

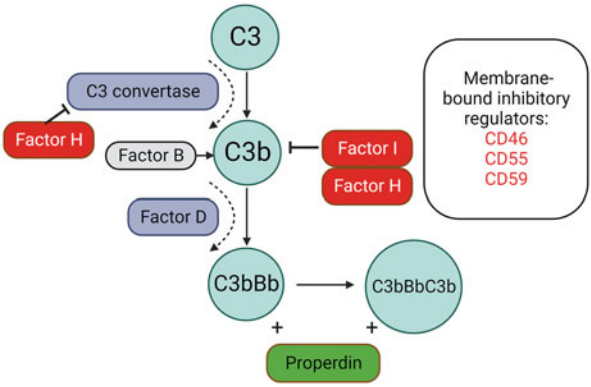

Fig. 2 Schematic of alternative pathway regulators. The alternative pathway of complement activation is unique because it is spontaneously and constitutively active and it can amplify all complement activity, including its own activity. The key positive regulator of the alternative pathway is properdin, which stabilizes C3 convertases (C3bBb) and C5 convertases (C3bBbC3b). The key negative regulator of the alternative pathway is factor H, which inhibits C3 convertase formation and is a cofactor for factor I-mediated cleavage and inactivation of C3b. There are several cell membrane-bound negative regulators including CD46, CD55, and CD59. Created with BioRender.com

Evaluation of Complement Pathway Function

When evaluating complement as a biomarker, it is important to note the potential for artifact with improper specimen collection, processing, and/or handling (Yang et al. 2015; Prohászka et al. 2016; Vercauteren et al. 2019; Mollnes et al. 1988; Stöve et al. 1995; Mollnes et al. 2007). Functional assays of the complement system are generally not compatible with additives such as ethylenediaminetetraacetic acid (EDTA) that chelate magnesium and calcium, which inhibits proteolytic activity (Prohászka et al. 2016). Therefore, assays of complement function in blood utilize serum, which maintains the capacity for proteolytic action necessary for complement function. However, complement activity can continue without proper handling of serum, potentially leading to artifactual elevation in markers of complement activation and artifactually low complement function due to exhaustion of complement factors (Prohászka et al. 2016). Therefore, accurate measurement of complement function requires proper techniques for collection, processing, storage, and handling to ensure validity of results.

Complement function has historically been measured by hemolytic assays (Kirschfink and Mollnes 2003) that incubate serum with a target erythrocyte and measure the amount of hemolysis that occurs across a range of dilutions to quantify function (Fig. 3). Hemolytic assays are commonly used to identify deficiencies in complement function, which typically occurs through deficiency in one or more complement factors (Mollnes et al. 2007). Total classical complement pathway function is measured by the CH50 hemolytic assay, which utilizes antibody-coated sheep erythrocytes as the target for complement action (Mollnes et al. 2007). The reported quantity is the reciprocal of the amount of serum at which 50% of the erythrocytes are lysed, adjusted to units of hemolysis per milliliter of serum (Kirschfink and Mollnes 2003; Costabile 2010). The CH50 assay is specific for classical pathway function by including Ca^{2+} ions and by diluting serum (e.g., 100–fold) (Bain et al. 2020) to limit alternative pathway function. The total function of the alternative complement pathway is measured by a similar hemolytic assay, AH50. Rather than antibody-sensitized sheep erythrocytes, the AH50 is measured by hemolysis of uncoated rabbit erythrocytes (Mollnes et al. 2007; Kirschfink and Mollnes 2003). To isolate the alternative pathway, the assay is performed in buffer with ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) to chelate Ca^{2+} and with addition of an optimal concentration of Mg^{2+} (Joiner et al. 1983; Mollnes et al. 2007). Similar to CH50, the AH50 uses serial dilution of serum to calculate the amount required to lyse 50% of the available red blood cells. Hemolytic assays of complement function have been used for decades to identify complement deficiencies in both clinical and research settings but have also been used to compare complement function between critically ill subjects in research settings (Bain et al. 2020).

More recently, several enzyme-linked immunosorbent assays (ELISAs) that measure complement function have been developed (Roos and Wieslander 2014; Zwirner et al. 1989; Fredrikson et al. 1993). These functional assays can be pathway-specific by utilizing a combination of specific activating substrates – e.g., IgM for classical pathway – and specific pathway inhibitors, e.g., EGTA, can be used to

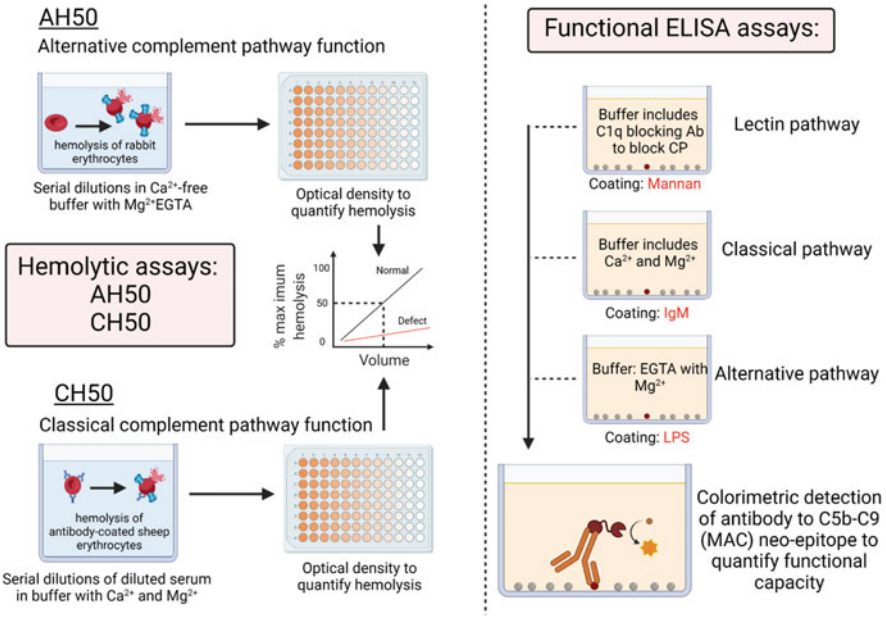

Fig. 3 Complement functional assays. Complement function can be measured by hemolytic assays or by functional enzyme-linked immunosorbent assay (ELISA). Hemolytic assays entail the incubation of serial dilutions of serum with erythrocytes – uncoated rabbit erythrocytes to assess the alternative pathway of activation and IgM-coated sheep erythrocytes to measure the classical pathway of complement activation. Upon incubation, hemolysis of erythrocytes is measured by optical density and plotted as linear regression across dilutions of serum to determine the volume of serum required to hemolyze 50% of erythrocytes. Functional ELISAs utilize pathway-specific well coatings – mannan for lectin, IgM for classical, and lipopolysaccharide (LPS) for alternative pathways, respectively. Each pathway is isolated by a specialty buffer – for example, to isolate the lectin pathway, the buffer incudes a blocking antibody against C1q to block the classical pathway. Upon pathway activation, the membrane attack complex (MAC) is formed upon the well coating and an antibody specific for the MAC is used to determine the "function" of the specific pathway. Created with BioRender.com

block classical and lectin pathway activation to measure alternative pathway function (Roos and Wieslander 2014). Importantly, functional ELISAs enable specific evaluation of the lectin pathway of complement activation that was not previously possible with hemolytic assays (Roos and Wieslander 2014; Fredrikson et al. 1993; Roos et al. 2003; Petersen et al. 2001; Seelen et al. 2005). There is a commercially available functional ELISA (Weislab) that provides semi-quantitative measurement of the function of alternative, classical, and lectin pathways on a single plate (Seelen et al. 2005). These functional ELISAs have been used in the study of some disease processes (O'Brien et al. 2020) but have not been widely utilized in critical care research to date (Li et al. 2019). Regardless of the method used to measure complement function, there is accumulating evidence that complement function may be a useful biomarker during critical illness caused by infection, respiratory failure, and trauma.

Complement Pathway Biomarkers During Critical Illness

Sepsis and Other Infections

Although complement function during infection has been studied for more than a century, two of the earliest robust assessments in patient with acute illness were published during the 1940s (Ecker et al. 1946; Rutstein and Walker 1942). Both reports demonstrate a narrow distribution of complement function in serum from normal healthy controls that widened, and frequently was below normal, in serum from patients during acute illness. In a series of 71 patients during hospitalization for acute illness with pneumonia, approximately 17% of patients demonstrated low levels of complement function and low levels were frequently noted in patients with fatal outcome (Rutstein and Walker 1942). In a series of 248 patients hospitalized for a variety of acute infectious diseases, there was a broader range of complement function with 31% of patients with complement titers below normal and 13% of patients with supra-normal complement titers (Ecker et al. 1946). Patients with very low complement function were more likely to suffer fatal outcomes demonstrating 40% mortality with lowest complement function compared to an overall 5% mortality rate for the entire cohort (Ecker et al. 1946).

These early studies suggested wide variation in complement function during acute illness that was subsequently validated in other reports (Bain et al. 2020; Coonrod and Rylko-Bauer 1977; Kalter et al. 1985). For example, AH50 but not CH50 was low early during the course of hospitalization in patients with pneumococcal pneumonia and AH50 remained below normal during recovery 1–3 weeks following acute illness (Coonrod and Rylko-Bauer 1977). Patients with pneumonia and bacteremia demonstrated decreased levels of key alternative pathway factors factor B, properdin, and C3 compared to patients with pneumonia alone, (Coonrod and Rylko-Bauer 1977) which is consistent with other reports demonstrating decreased alternative complement pathway factors during sepsis with shock (McCabe 1973; Fearon et al. 1975). Furthermore, a progressive decrease in AH50 and CH50 was demonstrated in a cohort of 45 critically ill patients with bacteremia as patients with shock or fatal shock had significantly decreased AH50 and C50 compared to patients with uncomplicated bacteremia (Kalter et al. 1985). In a series of surgical intensive care unit patients enriched for abdominal sepsis, CH50 was significantly lower in non-survivors compared to survivors and healthy controls (Schreiber et al. 2006). Similar results noting decreased CH50 in sepsis non-survivors compared to survivors and healthy controls were published in a separate report that also demonstrated increased markers of complement activation C3a and C5a, suggesting complement activation and consumption during septic shock (Unnewehr et al. 2013). However, it should be noted that C3a and C5a levels were compared between septic shock patients and healthy controls and were not described between septic shock survivors and non-survivors (Schreiber et al. 2006; Unnewehr et al. 2013). This discrepancy is mitigated by several other reports demonstrating increased C3a levels in septic shock with fatal outcome (Hack et al. 1989; Gardinali et al. 1992; Stöve et al. 1996; Wolbink et al. 1998). Together, these

studies suggest consumption of complement factors can occur during critical illness with sepsis and other infections. However, it is unclear whether complement activation is due to increased pathogen load, impairment in host complement regulation, or both pathogen and host factors.

Pathogen load likely contributes to complement activation. For example, levels of *Neisseria meningitidis* lipooligosaccharide strongly correlate with C3 activation products in patients with systemic *N. meningitidis* infection (Brandtzaeg et al. 1989). Notably, lipooligosaccharide levels peaked approximately 7 h after initiation of antibiotic treatment, which may correspond to peak lysis of bacteria (Brandtzaeg et al. 1989). Additionally, in a study of complement activation in neonates with infection, antibiotic administration led to decreased levels of the C3 convertase, C3bBbP, suggesting that decreasing pathogen load may limit complement activation (Zilow et al. 1993). Furthermore, intravenous administration of 2.5 mg/kg of *Escherichia coli* lipopolysaccharide infusion in a baboon model caused consumption of factors and deficiency of alternative pathway function (Joiner et al. 1983). However, a 106-fold lower dose (2 ng/kg) did not activate complement in experimental intravenous endotoxin challenge in healthy humans (van Deventer et al. 1990).

In addition to pathogen factors, it is likely that host factors contribute to complement derangement during critical illness with infection. There are two potential human models to assess the role of host factors in complement functional derangements during critical infection: genetic studies and cirrhosis. First, the importance of complement genotype in resilience during infection is highlighted by a unique history of Dutch immigrants who emigrated to Surinam in 1845 and subsequently faced new pressure from typhoid and yellow fever, which led to 60% mortality among colonists (de Vries et al. 1979). Comparing the genotypes of Dutch descendants in Surinam with a random Dutch cohort revealed enrichment for a pro-activation functional variant in the C3 gene (Heurich et al. 2011) in the Surinam cohort, suggesting that genotype played a role in survival against the selective pressure of infection (Harris et al. 2012; de Vries et al. 1979). Further evidence of the potential importance of complement genetics comes from a cohort of critically ill pediatric patients in the United Kingdom in which a pro-activation genetic variant in the factor H gene (Y402H, rs1061170) was associated with decreased risk of systemic inflammatory response syndrome or sepsis early in the ICU course (Agbeko et al. 2010). Others have demonstrated that genetic variants of key alternative pathway proteins can modulate complement function in vitro although the functional consequences in vivo during critical illness remain unclear (Heurich et al. 2011). Furthermore, although a "complotype" that dictates susceptibility to infection and inflammation has been proposed, the role of functional variants in complement genes has not been widely studied during critical illness (Harris et al. 2012; van den Broek et al. 2020). Therefore, the role of functional variants in complement genes in determining outcome during critical illness is an important subject area for future research.

Beyond genetic data, cirrhosis provides another model for probing the role of complement function during critical illness. Because many complement factors are synthesized in the liver, cirrhosis can be associated with acquired complement

deficiency. Prior work has demonstrated that patients with decompensated cirrhosis were more likely to demonstrate impairments in AH50 compared to healthy controls (Homann et al. 1997). Furthermore, impairments in AH50 and CH50 were associated with significantly increased risk of infection and mortality in patients with cirrhosis, although only C3 deficiency remained significant for increased risk of mortality and infection after multivariate analysis (Homann et al. 1997). Despite the potential role of cirrhosis in acquired complement deficiency during critical illness, it should be noted that several studies have demonstrated the importance of complement function during critical illness independent of cirrhosis (Bain et al. 2020; Coonrod and Rylko-Bauer 1977). Therefore, rather than driving the observed association between complement function and worse outcomes during sepsis, cirrhosis instead provides a conceptual framework for understanding the role of acquired complement deficiency. It is likely that acquired complement deficiency increases the risk of infection, which is supported by a rat model of cirrhosis that demonstrated increased disseminated pneumonia and decreased CH50 compared to non-cirrhotic controls. Furthermore, the risk of spontaneous bacterial peritonitis, which is a major cause of morbidity and mortality during cirrhosis, is associated with decreased complement function in ascites fluid (Runyon 1988; Runyon et al. 1985). Additionally, there is a generally increased risk of infections including spontaneous bacterial peritonitis, bacteremia, and pneumonia in hospitalized patients with cirrhosis (Caly and Strauss 1993; Di Pasquale et al. 2013; Falguera et al. 2009). Taken together, using cirrhosis as a model, acquired complement deficiency may increase the risk of infection and worse outcomes during infection. This concept is further supported by the protection provided by complement function, which was demonstrated in a glycoproteomic comparison of survivors and non-survivors of sepsis enrolled in a clinical trial that revealed increased alternative and classical complement pathway activation in sepsis survivors (DeCoux et al. 2015).

In summary, preserved complement pathway function during critical illness with sepsis, which can be modeled by AH50 and CH50, is associated with improved clinical outcomes. However, significant further work is required to understand host-pathogen interactions that influence complement function and clinical outcomes. We will next consider patients that are critically ill with acute respiratory failure.

Acute Respiratory Failure

The complement system has primarily been studied during acute respiratory failure in the context of whether complement activation is associated with development of the acute respiratory distress syndrome (ARDS). A pivotal early study of complement activation as measured by plasma C3a levels in 40 patients with extra-pulmonary sepsis (suspected or confirmed) at a single academic center in the United States did not demonstrate a significant increase in C3a levels in those patients with progression to lung injury (Weinberg et al. 1984). Despite the absence of statistical significance, median levels of C3a were higher in patients with lung injury and in those with progression to lung injury (Weinberg et al. 1984). However,

the relationship between the development of acute respiratory failure and complement activation may be due to the severity of the injury or underlying risk factor for complement activation rather than complement activation causing respiratory failure. For example, in a study of 50 patients in a surgical ICU in the Netherlands with heterogeneous risk factors for ARDS that used both healthy control and patients with minor abdominal surgery as controls, complement activation as measured by C3d/C3 ratio was elevated in both patients with minor surgery and to a greater extent in those with risk factors for ARDS (Duchateau et al. 1984). Notably, C3d/C3 ratios inversely correlated with CH50 levels suggesting complement activation was associated with consumption of complement factors although C3 levels were preserved (Duchateau et al. 1984). Furthermore, in a study of 87 patients admitted to the ICU of a single academic center with either sepsis alone or sepsis with ARDS, there was no difference in serum CH50 or plasma C3a levels between patients with sepsis alone or sepsis with ARDS (Langlois et al. 1989). However, patients with ARDS did have significantly higher levels of plasma terminal complement complex (sC5b-C9) compared to patients with sepsis alone although the authors did not differentiate between sepsis due to pneumonia or extra-pulmonary sepsis so the significance of this finding remains uncertain (Langlois et al. 1989). Yet, it is clear from these early studies that the complement system is not a biomarker for development of acute respiratory failure. However, more recent data have demonstrated that complement function may be of importance during the course of acute respiratory failure.

The largest study of complement function during acute respiratory failure included 321 patients with acute respiratory failure from heterogeneous etiologies, nearly all of whom were mechanically ventilated, at two affiliated academic medical centers in the United States (Bain et al. 2020). We demonstrated a wide distribution of serum AH50 and CH50 values and that patients with AH50 $>$ median were significantly more likely to survive to 1 year. In contrast, there was no relationship between CH50 and survival (Bain et al. 2020). We also noted that levels of key alternative pathway regulators – factor B, properdin, and factor H – were positively correlated with alternative pathway function as measured by AH50 (Bain et al. 2020). Furthermore, re-analysis of publicly available whole blood transcriptomic data from independent cohorts demonstrated that survivors had significantly higher transcriptional expression of factor D, factor B, and properdin in whole blood compared to non-survivors (Bain et al. 2020). Although complement activation was not assessed, we showed that impairments in alternative pathway function were associated with impairments in host defense using in vitro bacterial killing assays and mouse models of disseminated pneumonia (Bain et al. 2020). Therefore, AH50 may be an important biomarker during critical illness with acute respiratory failure and impairments in alternative pathway function may be associated with impairments in host defense. In addition, there may be pathogen-specific consequences of impairments in complement function during pneumonia and respiratory failure (Coonrod and Yoneda 1982).

One notable pathogen that has conferred significant attention on the complement system during respiratory failure is the SARS-CoV-2 virus that caused the COVID-19 pandemic as early reports suggested that complement activation contributed to disease severity (Bosmann 2021; Afzali et al. 2022). One report from two academic medical centers in the United States identified increased alternative complement

pathway activation in critically ill patients noting higher levels of activated factor B as measured by Ba levels (Ma 2021). Additionally, higher plasma C5a levels were noted in patients with COVID-19 that required mechanical ventilation compared to those who did not, and there were significantly higher plasma levels of sC5b-C9 in mechanically ventilated patients with COVID-19 compared to mechanically ventilated patients in a cohort evaluating sepsis and pneumonia caused by pathogens other than SARS-CoV-2 (Ma 2021). The investigators reported no difference in AH50 between patients with COVID-19 that required ICU level care and those who did not (Ma 2021). In contrast, others have noted significantly decreased function of the alternative pathway, but not classical or lectin pathways, by functional ELISA in critically ill patients in Hungary (Sinkovits et al. 2021). Similar to the other report, there were significantly increased levels of C3a and sC5b-C9 with increasing disease severity and increased C3a/C3 ratio in non-survivors compared to hospitalized but not critically ill patients (Sinkovits et al. 2021). Furthermore, the authors demonstrated high predictive value of C3a/C3 ratio for prediction of in-hospital mortality during COVID-19 (Sinkovits et al. 2021). Therefore, complement activation leading to exhaustion of factors appears to be associated with disease severity during COVID-19 although it is unclear whether complement activation reflects viral load or dysregulated complement regulation or both processes. Given that the lung is the primary site of severe infection and injury leading to morbidity and mortality during acute respiratory failure during COVID-19, there is developing evidence that lung epithelium deploys intracellular complement pathways in response to SARS-CoV-2 infection (Yan et al. 2021). Bulk RNA-sequencing of lung from patients with COVID-19 demonstrates a transcriptional signature of complement activation, which was confirmed using lung epithelial cells in vitro (Yan et al. 2021). During in vitro studies, higher viral titers led to higher expression of C3 in lung epithelial cells, which highlights the concept that the lung epithelium may use complement proteins to defend against stress (Kulkarni et al. 2019). Notably, the role of intracellular complement pathways in the lung and other tissues including immune cells is an emerging area of study that may be of significance during critical illness and respiratory failure (Kulkarni et al. 2019; Arbore et al. 2017; Liszewski et al. 2013). Regarding complement activation during COVID-19, inhibition of JAK/STAT pathways or intracellular inhibition of complement factor B normalized C3 generation in respiratory epithelial cells (Yan et al. 2021). Although this finding is of uncertain significance, it is intriguing as there may be clinical benefit to inhibition of the JAK-STAT pathway during COVID-19 pneumonia in reducing the risk of respiratory failure and death (Guimarães et al. 2021; Ely et al. 2022). However, no specific complement inhibitors have thus far demonstrated clinical benefit during COVID-19 disease, and there have been reports of increased risk of infection, including fatal infection, with pharmacologic inhibition of complement pathways (Afzali et al. 2022). Therefore, the global burden of acute respiratory failure during the COVID-19 pandemic has highlighted the importance of the complement system and identifies both intracellular complement pathways and complement function in the alveolar space during pneumonia and acute respiratory failure as crucial areas for future research (Robbins et al. 1987). We next discuss complement function during major injury not caused by pathogens, but instead by major trauma.

Trauma

Major trauma causes massive tissue injury that can lead to complement activation, which can result in consumption of complement factors and subsequent impairment in complement function. For those patients that survive their initial injury and are stabilized in a hospital intensive care unit, infection and multi-organ system failure including ARDS are major causes of morbidity and mortality (Baker et al. 1980). Therefore, complement factor exhaustion may contribute to worse outcomes after trauma.

One of the earliest studies of complement activation and function after polytrauma was published in the early 1990s (Zilow et al. 1992). The authors demonstrated a marked decrement in AH50 during the first 24 hours of hospitalization that slowly returned to near normal function by days 4–14 after injury. CH50 values were within normal limits in the first 48 hours after injury and rose to supra-normal levels during days 4–14 after injury (Zilow et al. 1992). Contemporaneous with AH50 decline, there was a decrease in C3, factor H, and factor I levels during the first 48 hours of hospitalization suggesting increased complement activation (Zilow et al. 1992). The authors did calculate C3a/C3 ratios, which were higher in plasma and broncho-alveolar lavage fluid of patients with ARDS compared to patients without ARDS in the first 24 hours of hospitalization, but they did not provide comparison to healthy controls or normal assay ranges to allow confirmation of activation (Zilow et al. 1992). Yet, subsequent studies have confirmed complement activation during massive trauma.

In a study of 56 polytrauma patients hospitalized in the surgical ICU of three different European hospitals, the authors noted an approximately twofold elevation in C3a levels and more than threefold increase in C3a/C3 ratio in non-survivors compared to survivors on the first day post-injury (Roumen et al. 1995). Furthermore, patients who subsequently developed multi-organ system failure demonstrated higher C3a levels compared to patients without multi-organ system failure. Others have demonstrated that complement activation was present at hospital admission as measured by C3b levels in EDTA plasma and was associated with injury severity score (Fosse et al. 1998). C3b levels at admission and peak C3b levels were higher in non-survivors compared to survivors (Fosse et al. 1998). Similarly, in a study of 208 polytrauma patients admitted to the emergency department at a urban trauma center in the United States, there was evidence of complement activation within 30 minutes of injury (Ganter et al. 2007). Bb levels, which are a marker of factor B cleavage and alternative pathway activation, as well as soluble C5b-C9, which is a marker of terminal complement complex activation, were highest in patients with the highest injury severity score and in non-survivors (Ganter et al. 2007). Notably, the authors also demonstrate correlation between C3a levels and markers of activation of both the alternative (Bb levels) and classical (C4d levels) pathway (Ganter et al. 2007). Data from a unique investigation of pre-hospital trauma patients in Germany indicate massive complement activation may occur at the time of injury (Burk et al. 2012). The investigators collected serum from 40 polytrauma patients in the field at the site of injury, in the emergency department, and in the hospital after admission

(Burk et al. 2012). They found significant decrease in CH50 pre-hospital at the injury scene, in the emergency department, and nadir approximately 4 hours after trauma with a slow return to normal levels by day 5 post-injury with supra-normal levels by day 10 compared to healthy volunteers (Burk et al. 2012). Levels of C4 binding protein and factor I were similarly decreased upon injury compared to healthy controls and remained low until day 5 post-injury with supra-normal levels at day 10 post-injury. They further note increased levels of C3a and C5a in serum in trauma patients at the scene of injury compared to healthy controls and found higher C5a levels at the scene and higher C3a levels in the emergency department when comparing non-survivors to survivors (Burk et al. 2012). However, the findings of increased complement activation should be interpreted with some caution as they were conducted using serum, which may increase risk of artifactual elevation in markers of complement activation (Prohászka et al. 2016). It should also be noted that the authors did not measure alternative pathway function. The importance of the classical pathway of complement during trauma is further supported by a manuscript from 2019 that enrolled trauma patients at a US Army Level 1 trauma center in Texas (Li et al. 2019). The investigators demonstrated a significant decrease in classical complement pathway function using a functional ELISA at time of emergency department presentation that persisted through day 5 and 7 post-injury compared to healthy controls (Li et al. 2019). In contrast, the functional ELISA demonstrated a modest decrease in alternative pathway function at emergency department presentation but otherwise no significant difference in either alternative pathway activity or lectin pathway activity compared to healthy controls (Li et al. 2019). Taken together, these studies suggest massive activation of complement during major trauma that is associated with decreased complement function. Some have described this phenomenon as a "complementopathy" early after trauma that may be analogous to coagulopathy that can occur with consumption of clotting factors after trauma (Burk et al. 2012). However, whether supplementation of specific complement factors would be of clinical benefit is unclear (Sperry et al. 2018).

Although the functional consequences of complement activation and consumption during polytrauma are unclear, it is notable that complement genetics may play a role in the risk for infection post-trauma. Investigators in Norway studied the genotype of 219 polytrauma patients, most of whom required ICU level care, with 51% developing infections, 36% developing sepsis, and 17% experiencing septic shock during their hospitalization (Bronkhorst et al. 2013). Notably, carrying a DD genotype in mannose-binding lectin-associated serine protease 2 (MASP2 Y371D, SNP rs12711521) or AS genotype in the ficolin-2 gene (FCN2 A258S, SNP rs7851696) were both associated with a significantly increased risk of developing septic shock post-trauma. Genetic variation at the MASP2 371 allele may affect binding of C4 and has been associated with increased risk of bacterial infection after liver transplantation (de Rooij et al. 2010). The functional consequences of the AS genotype in ficolin-2 are not clear, but it has been associated with decreased serum levels of ficolin and with increased risk of infection in cystic fibrosis (Bronkhorst et al. 2013). Therefore, complement function maybe an important component of host defense after trauma, which supports the concept that complement consumption

during the massive tissue injury of trauma may increase the risk for worse clinical outcomes due to infection.

Applications to Prognosis

In this chapter, we have summarized research on complement function and key biomarkers of complement regulation during critical illness in humans. The bulk of this research has been conducted in adults primarily in North America and Europe. Therefore, the applicability of this data to critically ill pediatric populations and populations enriched for geographic ancestry from regions other than North American and Europe is undetermined.

Conclusion

We have cataloged a number of studies spanning 70 years demonstrating wide variation in complement function during critical illness caused by sepsis and other infections, acute respiratory failure including pneumonia, and major trauma. We note improved outcomes associated with preserved complement function as measured by AH50 and CH50 assays of the alternative and classical pathways, respectively. Notably, there is significant inter-study variability in evaluation of AH50 and CH50, but impairments of both pathways have been noted in sepsis and trauma. In contrast, the AH50 may be more important for clinical outcomes during acute respiratory failure compared to the CH50 based on results of the largest study of complement function during critical illness to date. Impairments in complement function appear to be associated with complement activation in several studies, suggesting that consumption of complement factors may lead to complement exhaustion. Yet, complement activation appears to correlate with burden of pathogen during infection and burden of injury during trauma. Therefore, it is unclear whether impairments in complement regulation can also contribute to over-exuberant activation. It is notable that genetic deficiency of complement factors, acquired complement deficiency (e.g., in cirrhosis), and genetic variation in complement regulation may all contribute to increased risk of infection. Further research is necessary to determine whether the complement system can be modulated for clinical benefit during critical illness.

Mini-Dictionary of Terms

1. **Acute respiratory distress syndrome:** a clinical syndrome of fulminant acute hypoxemic respiratory failure marked by non-cardiogenic pulmonary edema and bilateral lung involvement that develops within 1 week of an injurious risk factor (e.g., pneumonia).

2. **C3:** a key complement protein upon which all known pathways of complement activation converge resulting in proteolytic cleavage of the C3 protein by C3 convertases to yield C3b and C3a.
3. **C3 convertase:** a complex of proteins formed by activation of complement, which is composed of C3bBb or C3(H_2O)Bb when formed by alternative pathway activation or C4bC2b when formed by classical or lectin pathway activation. C3 convertase proteolytically cleaves C3 into C3b and C3a. N.B.: C2b was originally named C2a so the C3 convertase of the classical and lectin pathways may also be known as C4bC2a.
4. **Membrane attack complex:** a collection of proteins that embed in the membrane of a target cell to form a lytic pore. C5b, C6, C7, C8, and C9 are the proteins that collect to form the membrane attack complex.
5. **Sepsis:** a clinical syndrome during infection, frequently bloodstream infection, which involves disarray of multiple organ systems and can progress to organ failure and death.

Key Facts of "Measures of Classical and Alternative Complement Function in Serum as Markers in Critical Care"

- There is wide variation in complement function during critical illness caused by sepsis and other infections, acute respiratory failure including pneumonia, and major trauma, which has been associated with worse clinical outcomes.
- In numerous studies, decreased complement function is associated with increased complement activation suggesting consumption and exhaustion of complement factors. It remains unclear whether complement activation is due to increased pathogen load, impairment in host complement regulation, or both pathogen and host factors.
- Impairments in alternative pathway function, whether genetic and/or acquired, may impair host defense during acute respiratory failure.
- The global burden of acute respiratory failure during the COVID-19 pandemic has highlighted the importance of the complement system during acute respiratory failure and identifies both intracellular complement pathways and complement function in the alveolar space during pneumonia and acute respiratory failure as crucial areas for future research.
- Although a "complotype" that dictates susceptibility to infection and inflammation has been proposed, the role of functional variants in complement genes has not been widely studied during critical illness.
- Massive activation of complement during major trauma that is associated with decreased complement function may be considered an early "complementopathy" that may be analogous to trauma coagulopathy that can occur with consumption of clotting factors and may increase risk for worse outcomes in those that survive their initial injury.

Summary Points

- The complement system is a vital component of host resilience, particularly during biological stress caused by infection and injury.
- There are a wide variety of biological stressors that can activate the human complement system including infection, trauma, injury, ischemia, burns, and surgery.
- Complement function requires effective regulation to maintain complement homeostasis as well as to manage its target and intensity of action.
- Function of the alternative and classical pathways can be measured by hemolytic assays, which are termed the AH50 and CH50, respectively. Functional ELISAs can be used to measure function of both the alternative and classical, as well as the lectin, pathways.
- Several studies note wide variation in complement function during acute illness, which is associated with worse clinical outcomes.
- Complement activation is common during sepsis and there is increased activation with increased severity of disease, which may reflect higher burden of pathogen and tissue injury and/or result from impaired complement regulation.
- Preserved alternative pathway function as measured by AH50 is associated with improved outcomes during acute respiratory failure including COVID-19.
- Major trauma is notable for marked complement activation that correlates with severity of injury and is associated with decreased complement function that may lead to worse clinical outcomes.

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Serum Aromatic Microbial Metabolites as Biological Markers in Intensive Care

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Natalia V. Beloborodova

Contents

Abstract

Thanks to modern technical solutions and advances in the field of metabolomic research, it has become possible to search for new biomarkers among low-molecular compounds, including microbial origin. In intensive care, the most urgent and unresolved problem is the syndrome of systemic inflammatory reaction, one way or another associated with bacteria, for example, complications of artificial lung ventilation, postoperative complications, attachment of multiple organ failure (sepsis), etc. Therefore, among a wide range of small molecules, metabolites of aromatic amino acid (tyrosine, phenylalanine) are of the greatest

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interest, since the metabolic pathways leading to their accumulation or disposal are closely interrelated in the human body with the human microbiota.

Over the past two decades, in clinical studies, results of the search for metabolites of microbial origin using GC-MS were obtained in various groups of critical patients with pneumonia, abdominal sepsis, septic shock, infection complication in cardio surgery, neuro surgery, etc. The data indicate a high diagnostic and prognostic significance of some aromatic microbial metabolites (AMM), such as ***4-hydroxyphenyl lactic acid*** (HPhLA), ***4-hydroxyphenyl acetic acid*** (HPhAA), ***phenyllactic acid*** (PhAA). Serum AMM level in sepsis was not inferior and even had advantages over such well-known biomarkers as PCT or lactate, and was comparable in importance to multi-parametric severity scales such as SOFA. The use of AMM to predict the outcome of patients admitted to the intensive care unit is no less reliable than the widespread multi-parametric APACHE II scale.

In vitro experimental studies have confirmed the ability of human microbiota bacteria to produce and consume the abovementioned, clinically significant aromatic metabolites; a decrease in the biodiversity of the microbiota and its metabolic function leads to an excess of AMM in the blood.

In intensive care, AMM can be used to assess the severity of patients' condition and the risk of death, to predict complications in big surgery (abdominal, cardiac, neurosurgery, etc.), as well as to monitor the effect of treatment.

Today, the serum level of AMM is determined using GC-MS, HPLC-MS, etc. For widespread implementation in intensive care units, an accessible express method is needed, which will help improve results in patients with life-threatening diseases and sepsis.

Keywords

Sepsis · Shock · Mortality · Microbiota · Systemic Inflammation · Biomarker · Aromatic microbial metabolites · Phenylcarboxylic acids · Phenyllactic acid · p-Hydroxyphenylacetic acid · p-Hydroxyphenyllactic acid · Gas chromatography - Mass spectrometry · Prognosis in intensive care

Abbreviations

AMM	Aromatic microbial metabolites
BA	Benzoic acid
CSF	Cerebrospinal fluid
GC-MS	Gas chromatography - mass spectrometry
HMDB	Database of the human metabolome
HPhAA, p-HPhAA, HPAA	4-hydroxyphenyl acetic acid
HPhLA, p- HPhLA, HPLA	4-hydroxyphenyl lactic acid
HPLC -MS	High-performance liquid chromatography - mass spectrometry
HVA	Homovanillic (3-methoxy-4-hydroxyphenylacetic) acid

L-DOPA	L-3,4-dihydroxyphenylalanine
DOPAC	3,4-dihydroxyphenylacetic acid
DHMA	3,4-Dihydroxymandelic acid
MOF	Multiple organ failure
NMR	Nuclear magnetic resonance
NT-proBNP	Pro-brain natriuretic peptide
PhAA	Phenylacetic acid
PhCAs	Phenylcarboxylic acids
PhLA, PLA	Phenyllactic acid
PhPA, PPA	Phenypropionic acid, phenylpropanoic acid
SIRS	Systemic inflammatory reaction syndrome
SOFA	Sequential organ failure assessment

Summary Points

1. Aromatic metabolites are fundamentally different from other biomarkers, since the metabolic pathways leading to their production or biodegradation in the human body are closely related to bacteria.
2. The concentration of aromatic microbial metabolites (AMM) in the biological fluids of a healthy body, it remains at a constant level, since aromatic metabolites have their own biological activity; the intestinal microbiota plays an important role in ensuring homeostasis. Serum levels of AMM may increase in patients with local bacterial infection, but reaching maximum values in sepsis.
3. Serum levels of 4-hydroxyphenyllactic acid (HPhLA), 4-hydroxyphenylacetic acid (HPhAA), phenyllactic acid (PhAA), and/or their sum are an integral indicator of serious disorders in the interaction of the metabolome and microbiome, therefore these metabolites can be called universal biomarkers for patients in the intensive care. Studies in the ICUs have shown that these biomarkers are superior to other laboratory measures, such as lactate or PCT, and that they are comparable to multivariate severity scales such as SOFA.
4. In severe diseases, the phenylcarboxylic acids profile in blood serum is associated with the bacterial infections, reflects the degree of organs dysfunction. In patients with sepsis, the levels of phenylcarboxylic acids in the blood increase tenfold, the highest concentrations are associated with the development of multiple organ failure, septic shock, and death.
5. In intensive care, AMM can be used to assess the severity of patients' condition and the risk of death, to predict postoperative complications (for example, in large abdominal operations, cardiac surgery, neurosurgery, etc.), as well as to monitor the effect of treatment.
6. An significant increase in the serum level of AMM above the threshold value indicates the development of metabolic dysfunction of the microbiota, which may coincide with the moment of ***transition from the systemic inflammatory reaction syndrome (SIRS) to sepsis***.

7. To improve results in patients with life-threatening diseases in the intensive care units, it would be useful to develop and implement an affordable method for measuring serum AMM in a clinical laboratory, regardless of the availability of expensive equipment (GC-MS).

Introduction

By the beginning of the twenty-first century, medical science in developed countries demonstrates great achievements in various fields, which positively affects the quality and life expectancy of people. Despite the general progress in medicine, the urgency of the problem of infection does not decrease in intensive care units. Infectious complications after injuries or complex reconstructive surgery, the development of multiple organ dysfunction, sepsis, and septic shock come out on top in the structure of hospital mortality (Dellinger et al. 2008; Kumar et al. 2009; Hotchkiss et al. 2016; Rudd et al. 2020). The relevance of the search and implementation of new biomarkers and diagnostic technologies for early detection of sepsis risk and monitoring of critical conditions does not decrease.

The World Health Organization defines a biomarker as any substance, structure or process that can be quantified in the body or produced (outside of it), capable of predicting the outcome of a disease and/or establishing a diagnosis (WHO 1993; Strimbu and Tavel 2010) A biomarker can be called a compound that has the following three signs:

1. If its content can be an indicator of normal, pathogenic processes, or a response of a living system to pharmacological effects.
2. If its dynamics correspond to the clinical picture (how the patient feels, survives, dies).
3. Substance content level may be more informative than clinical diagnosis.

Over time, biomarkers have been used in clinical practice and for broader purposes. For example, to monitor the severity or the stage of the disease, to compare the effectiveness of treatment with different drugs, to assess the effect of certain medical procedures, and so on. In modern critical medicine, biomarkers occupy a special place if they meet the requirements of urgency, helping to make the right decision regarding the patient, including in life-threatening situations. For example, procalcitonin, originally created as a biomarker of sepsis, was subsequently widely used to determine indications for prescribing antibiotics and to evaluate the effectiveness of antibiotic therapy, since the use of adequate antibiotic therapy in ICU reduces the risk of death (Kollef et al. 1999; Kumar et al. 2009).

In 1971 Nobel laureate Linus Pauling published an article with the results of the quantitative determination of low molecular weight volatile organic compounds in human biological samples. Now that research is deservedly called fundamental for the beginning of the era of metabolomics studies in biology and medicine, to discover new diagnostic approaches and search for new biomarkers (Pauling et al. 1971).

Thanks to the development and improvement of technologies of gas and liquid chromatography, mass spectrometry (GC-MS, HPLC), nuclear magnetic resonance (NMR) spectroscopy, etc., by the beginning of the twenty-first century, it became possible to conduct research of the qualitative and quantitative composition of low-molecular compounds, in cells, tissues, organs, and biological fluids. The data obtained during the research accumulated and amounted to hundreds intermediate and final metabolites found in blood, urine, exhaled air condensate, cerebrospinal fluid (CSF), etc., which could be useful for assessing the functional state of the body. This contributed to the creation of a freely accessible electronic database of the human metabolome (HMDB), which contains detailed information about low-molecular metabolites found in the human body and regularly updated (Wishart et al. 2007, 2013, 2022).

It was found that among the low-molecular substance in human blood there are different classes of compounds with not produced by mammalian eukaryotic cells. Late in metabolomic studies it was confirmed that many compounds found in human blood have exclusively microbial origin, because they are contained in the blood of ordinary mammalian animals, but are absent in the blood of germ-free (gnotobiotic) animals. For example, many phenolic metabolites in free form or in the form of conjugates, such as phenyl sulfate, p-cresol sulfate, conjugates of phenylpropionic and cinnamic acids (respectively phenylpropionylglycine and cinnamoylglycine) are found in the blood only in ordinary animals with microbiota, and never in sterile ones (Wikoff et al. 2009). Other phenolic compounds in the blood of ordinary animals are found in higher concentrations compared to germ-free, for example, for conjugates of benzoic or phenylacetic acid with glycine (respectively, hippuric acid or phenylacetylglycine), the reliability of differences reaches values of $p < 10^{-8}$–10^{-9} (Wikoff et al. 2009).

In a clinical metabolomic study by Beloborodova and Osipov (2000), it was shown that microbial components are contained in the blood of both group – healthy people and critically ill patients with sepsis, peritonitis, endocarditis, postoperative complications, etc., – but differ significantly. Several groups of microbial molecules (some fatty acids, hydroxy acids, alcohols, aromatic, cyclic, branched compounds, etc.) were measured in blood samples using GC-MS; the greatest differences between healthy and seriously ill patients were obtained in phenyl carboxylic acids. The results obtained allowed the authors to formulate the **Concept of homeostasis of small molecules originating from microbes** (**SMOM**) and suggested for the first time that a severe disturbance of SMOM homeostasis serve as an inducer of a systemic inflammatory response, septic shock, and multiple organ failure (Beloborodova and Osipov 2000).

In Search for Promising Metabolites

Clinical and experimental researches of low molecular weight compounds was described in review of Chernevskaya and Beloborodova (2018), six groups were divided into according to their chemical structure: (1) amino acids and their

derivatives; (2) polyols and their derivatives; (3) fatty acids and their derivatives; (4) hydroxy acids and their derivatives; (5) amines, nitrogen-containing heterocyclic compounds and their derivatives; (6) nitrogen-containing bases of nucleic acids, nucleosides, and their derivatives. Summarizing the results of the analysis, the authors concluded that in human body fluids, metabolites of aromatic amino acids – phenylalanine, tyrosine, and tryptophan – are of particular importance, among other small molecules may be the most promising biomarkers for clinical use. The hypothesis formulated earlier about the integration of human metabolism and its microbiome can serve as a fundamental basis for explaining the results obtained (Beloborodova 2012).

In recent years, there has been a rapidly growing number of publications that attach importance to metabolic research in critical care (Beloborodova 2017), including metabolic markers of oxidative stress, steroid hormone, nutritional markers, and amino acid pathways (Zurfluh et al. 2018). Some authors suggest not to limit the measurement of individual biomarkers in the blood, but to use several death-related metabolic pathways (DRMPs) including amino acids, mitochondrial metabolism, eicosanoids, and lysophospholipids (Wang et al. 2020). Developing the hypothesis of metabolic interactions, the authors are inclined to the need to integrate several methods to search for new biomarkers or therapy strategies (Araújo et al. 2022). In one of the last big reviews on this topic, metabolomic studies were grouped into five issues, including the application of metabolomics for (1) sepsis diagnosis, (2) septic shock diagnosis, (3) prognostication in sepsis, (4) prognostication in septic shock, and (5) monitoring treatment response in sepsis and septic shock (Trongtrakul et al. 2022). Extensive factual material is presented in detail, but without specific recommendations for practical application, as a justification for continuing numerous metabolomic studies to search for optimal biomarkers.

Metabolites of Aromatic Amino Acids

Among the metabolites of bacteria, of particular interest is the study of aromatic microbial metabolites (AMM) – products of biotransformation of three essential aromatic amino acids – tryptophan, tyrosine, phenylalanine.

The aromatic amino acid tryptophan is actively involved in the intersection of the metabolic pathways of bacteria and the host organism, which are summarized and described in detail in the chapter of the Book Metabolomics (Beloborodova et al. 2019a, b). In the gut, the three main pathways of tryptophan metabolism are under the direct or indirect control of the microbiota. Tryptophan biotransformation products such as serotonin (5-hydroxytryptamine), kynurenine, and a number of indole derivatives (indole-3-acetic acid, 5-hydroxyindole-3-acetic acid, indole-3-propionic acid) they play a certain role in pathology of the human body, including diseases of the intestines, kidneys, central nervous system, cardiovascular, etc. (Agus et al. 2018; Pautova et al. 2020a, b, c; Beloborodova et al. 2020a, b) (Fig. 1). Tryptophan metabolites have been little studied in intensive care and have not yet been used as biomarkers.

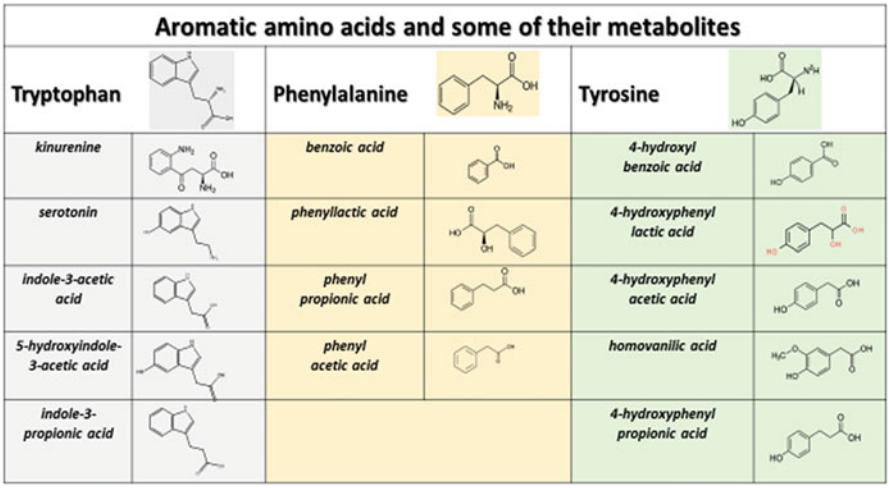

Fig. 1 Clinically significant metabolites of three aromatic amino acids and their chemical structure

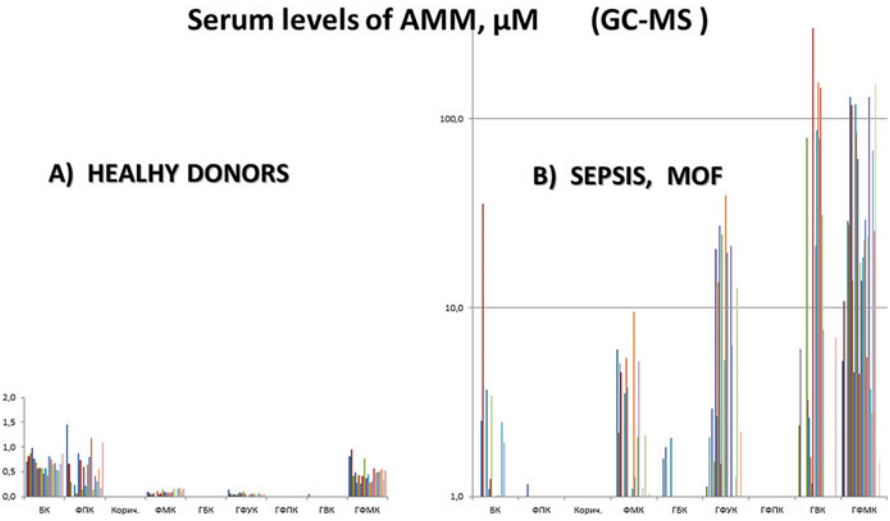

Fig. 2 Comparison of the profile of nine aromatic microbial metabolites (AMM) in the blood of healthy and septic patients, microns (GC-MS method). Individual concentrations of metabolites in blood serum are shown (**A**) in 42 healthy donors, (**B**) in 36 patients with sepsis/shock/MOF

Studies of the last two decades show that metabolites of tyrosine and phenylalanine in the form of phenylcarboxylic acids are the most promising biomarkers for ICU.

Critically ill patients are characterized by the totally different AMM profile than in healthy people, particularly by the absence of PhPA; but dominated by p-HPhAA and p-HPhLA, as clearly shown in the Fig. 2.

The Origin of Aromatic Metabolites in Human Blood

According to current ideas, the accumulation of phenyl carboxylic acids as the main representatives of aromatic microbial metabolites (AMM) in the human body can occur in several ways:

1. If the endogenous metabolism of aromatic amino acids is disrupted.
2. As a result of the intake of microbial metabolites or from the source of infection or from the gut (Fig. 3)
3. Or accumulate in violation of the mechanisms of binding and excretion (renal-hepatic insufficiency).

Normally, in a healthy person, AMM in the form of phenylcarboxylic acids (PhCAs) always detected in the systemic bloodstream, but in very low concentrations, about 1-3 μM. Depending on the type of disease, the profile and the total number of PhCAs may change in the patient's body. The Fig. 4 allows to visually asses the differences in the PhCAs profile depending on the disease and shows a significant predominance of some metabolites (HPhAA, HPhLA, PhLA) in patients with sepsis (Fig. 4A).

The sensitivity and specificity of this three metabolites in sepsis were high (Fig. 4B).

The first clinical study to assess the clinical significance of AMM in intensive care was described by Beloborodova et al. (2011): 175 patients after cardiac surgery, including those with serious complications (pneumonia, sepsis) and 25 healthy volunteers (control). Mortality rate in patients was 27% (47/175). The following graph (Fig. 5) shows a real gap between the study groups: patients with sepsis had

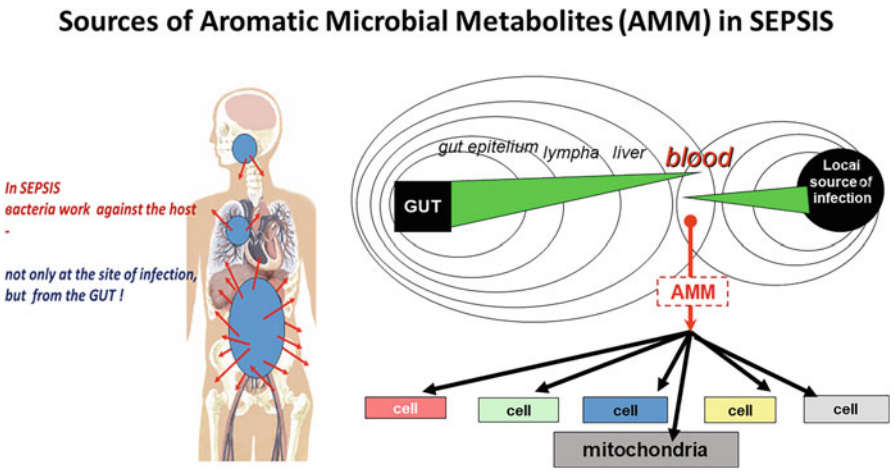

Fig. 3 The aromatic microbial metabolites (AMM) enter the bloodstream from two sources where do bacteria realize their metabolic activity: the infectious focus and the gut

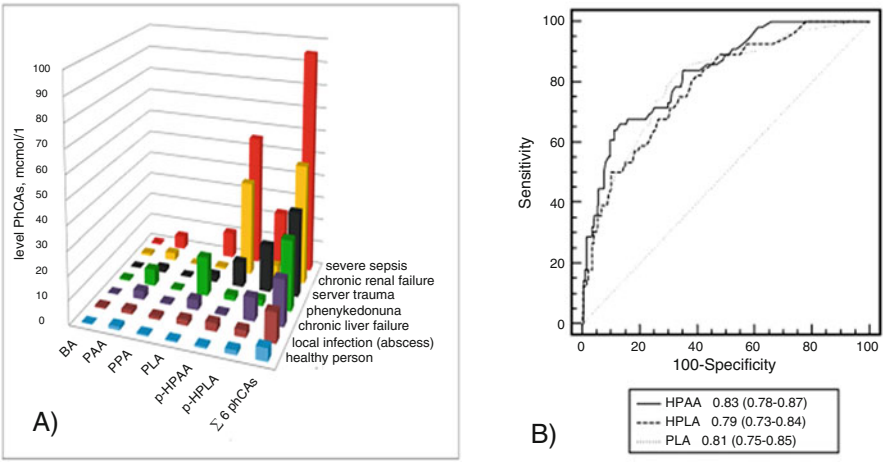

Fig. 4 The significance of the serum level of aromatic microbial metabolites (AMM) in sepsis (**A**) compared with other acute/chronic diseases or pathological conditions; (**B**) sensitivity and specificity of three AMM (HPhLA, HPhAA, and PhLA) in patients with sepsis

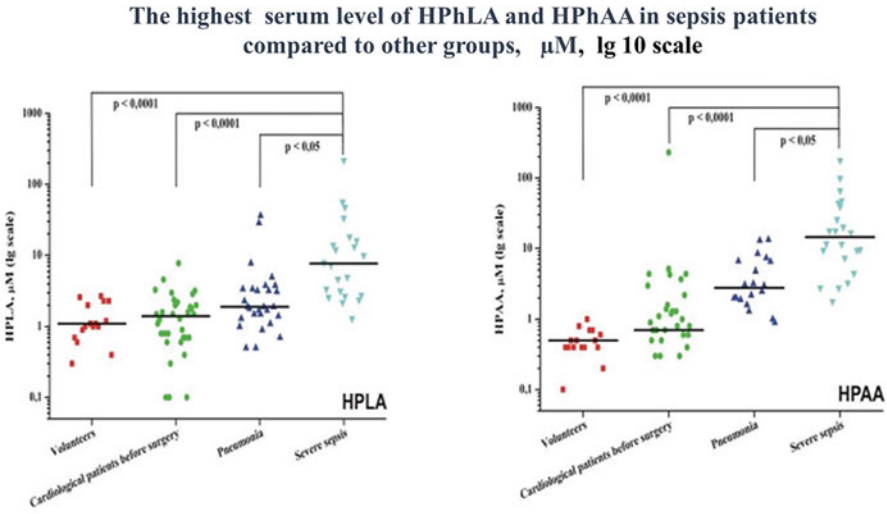

Fig. 5 The result of comparing four different groups by serum levels of aromatic microbial metabolites: in sepsis, the level of HPhLA and HPhAA is many times higher than in healthy volunteers ($p < 0.0001$), or than in patients before cardiac surgery ($p < 0.0001$), or than in pneumonia after cardiac surgery ($p < 0.05$)

significantly higher serum levels of HPhAA and HPhLA compared to healthy (control) or cardiac surgery patients without sepsis.

In this study, mortality in patients was 27% (47/175). AMMs reflected the severity of sepsis; PhLA and HPhLA were significantly higher in patients who died of sepsis compared to surviving sepsis patients (Fig. 6).

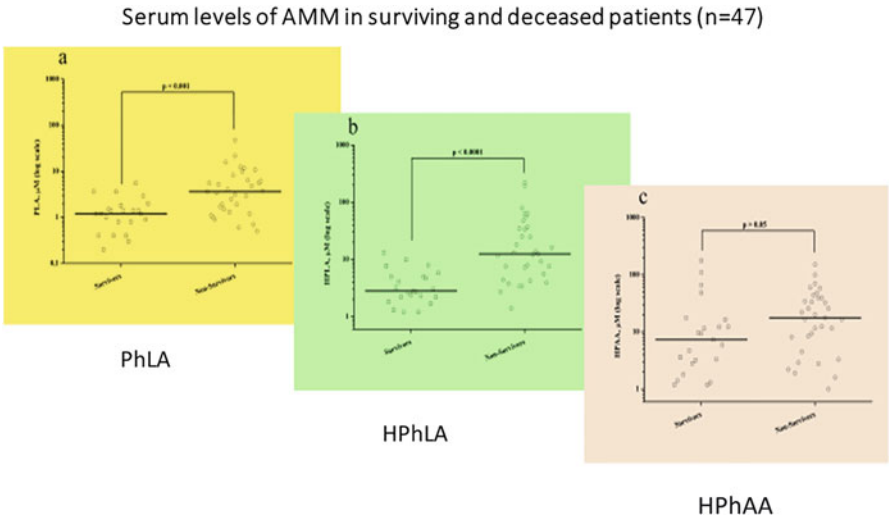

Fig. 6 The differences of serum levels in surviving and non-surviving patients were statistically significant for PhLA and HPhLA

Moreover, the results of this study showed for the first time the existence of a direct correlation between HPhAA, HPhLA, and PhLA (Spearman $r = 0.71–0.84$, $p < 0.05$), and even then an interesting conclusion was made that any separately or the sum of the three PCAs can be used as a marker of sepsis (Beloborodova et al. 2011).

A natural question arises: how to explain the high information content of these AMMs in sepsis? Aromatic metabolites in sepsis may have a higher value compared to other known biomarkers, since, unlike other biomarkers, the metabolic pathways leading to the formation or utilization of AMM in the human body are closely related to bacteria. In other words, AMMs can be called "common metabolites" for human and his microbiota (Beloborodova et al. 2018a, b).

Serious disorders of the microbiota naturally develop and accompany critical conditions, often aggravated by massive antibiotic therapy. So, sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al. 2016). The microbiota is also an organ made up of trillions of microbial cells. The severity of the metabolic dysfunction of the microbiota in sepsis is manifested by a change in the profile of aromatic metabolites in the blood (Beloborodova 2017). Consequently, the level of aromatic metabolites in the blood serum, such as HPhAA, HPhLA, PhLA, serves as a biological marker of sepsis. This issue will be discussed in more detail below.

Bacteria Producing AMM

Ten years ago, in 2012, data were published comparing the ability of different bacteria to produce in vitro aromatic metabolites – potential biomarkers for intensive care (Beloborodova 2012). The study included the main representatives of the

human microbiota: (A) obligate anaerobic bacteria, mainly from the list of healthy human microbiota, and (B) aerobic and facultative anaerobic bacteria, among which pathogens of bacterial infections and sepsis are often found. During in vitro cultivation, standard multicomponent nutrient media containing all the necessary components for the growth and reproduction of bacteria were used, that is, the internal environment of the human body was simulated for bacteria. The results are shown in Fig. 7.

The maximum production of PhLA and p-HPhLA in vitro was found in gram-negative bacteria of the *Enterobacteriaceae* family and *Staphylococcus aureus*, which, by the way, are known as the most frequent pathogens of bacterial infections in the ICUs.

The dominant source for the accumulation of AMM in the blood of patients is the intake of microbial metabolites from the intestine. This conclusion based on the following established facts: most of the clinically significant microorganisms studied are capable of producing the identified AMM. It is known that in septic patients, *Bifidobacteria* spp. and *Lactobacilli* practically disappear from the microbiota, which is usually explained by pronounced metabolic disorders in the patient's body, which does not correspond to the habitat of these "capricious" bacteria. Therefore, despite the ability of *Bifidobacteria* spp. and *Lactobacilli* spp. to produce AMM in vitro, they cannot show their metabolic abilities in the body of a seriously ill person, and they cannot be attributed to bacteria that are involved in increasing the circulating AMM in sepsis.

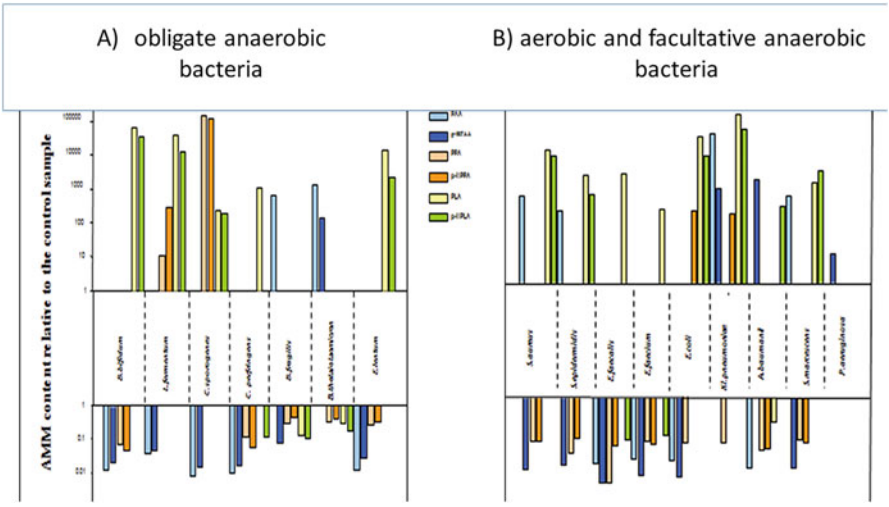

Fig. 7 Evaluation of the ability to produce and consume AMM of pure bacterial cultures in an experiment.The cultivation 16 species of bacteria in vitro: (**A**) obligate anaerobic bacteria in anaerobic conditions, (**B**) aerobic and facultative anaerobic bacteria in aerobic conditions. For each studied type of bacteria, the multiplicity of increase (or decrease) in the culture fluid of certain aromatic metabolites (by 10–100 or more times) in relation to the control sample of the nutrient medium is shown

In real conditions, microbial metabolism in the gut is carried out quite difficult, according to the principle of "a kind of relay race", when the biodegradation of a compound can be transmitted from one type of bacteria to another like "from hand to hand".

Therefore, in vitro study with pure bacterial cultures can only approximately reflect the real process.

Why AMMs Are Biomarkers in Intensive Care?

The main secret – why aromatic metabolites are so informative in critical ill patients – is that bacteria of human microbiota are involved in the metabolic pathways of aromatic amino acids (phenylalanine, tyrosine, tryptophan).

The microbiota compensates for the absence of a shikimate metabolic pathway in the healthy human body, the biodiversity of bacteria in gut ensures the formation of phenylcarboxylic acids by sequential biodegradation of the initial compounds - aromatic amino acids and polyphenols.

Today, modern studies have proved that in critical conditions, deep violations of both the taxonomic composition and the metabolic function of the microbiota occur (Zaborin et al. 2014; Beloborodova et al. 2014; Ojima et al. 2016; Lankelma et al. 2017; McDonald et al. 2016; Chernevskaya and Beloborodova 2018; Prevel et al. 2022), what can be called the phenomenon of total suppression of normal metabolic activity of the microbiota (Beloborodova et al. 2019a, b).

Evidence of microbial origin of phenylcarboxylic acids in the human body has been shown previously (Beloborodova et al. 2009). Anaerobic bacteria of the gut microbiota take part in the metabolism of AAAs phenylalanine and tyrosine (Fig. 8A).

Normal microbiota metabolized PhCAs to the end products – benzoic (BA), PhPA. PhAA – regardless of human or microbial origin, since phenyl carboxylic acids formation from phenylalanine or tyrosine by both endogenous and microbial metabolic pathways.

As previously described by Beloborodova et al. 2014, the anaerobic microbiota has the ability to reduce the number of clinically significant aromatic metabolites (Fig. 8B).

Anaerobic bacteria of the intestinal microbiota take part in the metabolism of phenylcarbonic acids, including both pathways of endogenous and microbial origin, to the final products such as BA, PhPA, PhAA (Fig. 8A). Suppression of normal gut anaerobes and their metabolic activity (as result of antimicrobial therapy) leads to the accumulation of metabolic intermediates. Figure 8B show that inhibition of

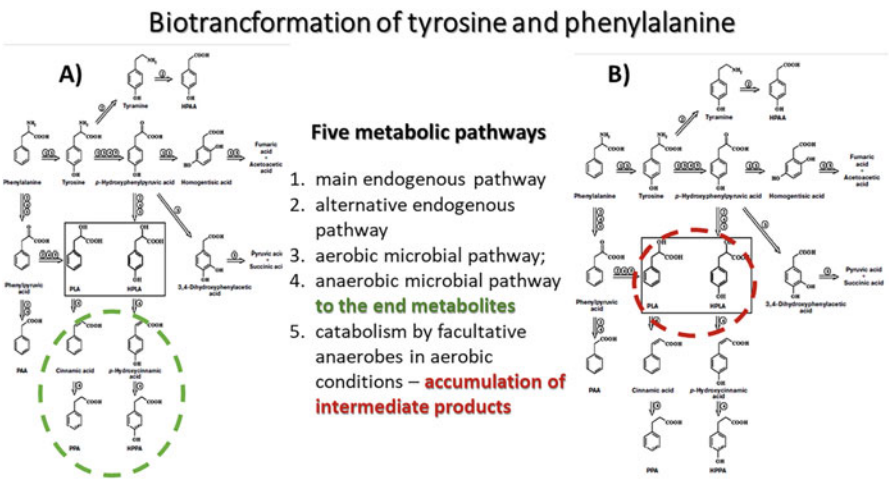

Fig. 8 Biotransformation of aromatic amino acids phenylalanine and tyrosine in the human gut: (**A**) to final metabolites (PhAA, PhPA, BA) by normal metabolic pathways; (**B**) to metabolic intermediates (PhLA, HPhLA) under conditions of suppression of gut anaerobes and their metabolic activity in sepsis

microbial pathways of phenylalanine and tyrosine biotransformation will lead to an excess of intermediates, namely, PhLA and HPhLA.

The review by Chernevskaya and Beloborodova (2018) analyzes the causes of violations of the taxonomic composition of the microbial community in the intestine and, accordingly, the AMM profile in the blood. The authors point out the importance of changing the internal environment of a seriously ill human body, which is also the habitat of the microbiota; the negative role of aggressive antibiotic therapy, which damages the species diversity of the intestinal microbiota, is emphasized. A large clinical study by Prevel et al. (2022) shows even more convincing that among critical ill patients, the diversity of the intestinal microbiota in non-survivors is significantly reduced compared to survivors, and is independently associated with mortality on day 28.

Association of AMM with Mortality

The strongest reason for the recognition of AMM as biomarkers for ICU is the close association of these metabolites with mortality.

In clinico-metabolomic study by a large group of authors (Langley et al. 2013), examined different profile of proteins and metabolites in patients with sepsis upon their arrival at hospital emergency departments and 24 h later. The discovery set of 150 patients have five groups, 332 metabolites were detected both t_0 and t_{24}. Seventy

six metabolites differed between sepsis survivor and death groups at $t_{0,}$ and 128 metabolites at $t_{24.}$ The authors point to a high interindividual variability of the values of some metabolites. Reliability of differences between survivors and non-survivors was confirmed by the discovery that many members of biochemical families had the same direction of changes, among which amino acid catabolites predominated. The usefulness of predictive models has been tested additionally by clinical measures and targeted quantitative analyses of 11 metabolites, including aromatic metabolites 4-hydroxyphenyl lactate (HPLA). The results obtained by the level of HPhLA in the comparison groups are shown in one of the graphs of this article: the maximum concentrations of HPhLA were found in patients who died of sepsis in the first 7 days after admission, although the authors unfortunately do not focus on this fact in the conclusions.

In another big study by Roger et al. (2014), blood samples from critically ill patients ($n = 90$) were purposefully studied dozens metabolites for association with 28-day mortality. Among the identified lipids, amino acids or breakdown products of amino acids, carbohydrates and nucleotides using gas and liquid chromatography and mass spectroscopy, a link with death was found in 7 metabolites, one of which is aromatic metabolites 4-hydroxyphenyl lactate (HPhLA), $p < 0.001$.

As another example from Beloborodova et al. (2018a, 2020b), the association of AMM level with mortality was also shown in a clinical study on a group of very severe of patients, where the 28-day mortality rate was 40%. On admission to ICU the increased level of ∑3AMM was detected in 76% (26/34) of patients, while elevated PCT levels were found only in 59% (20/34) of patients.

How significantly the dynamics of the serum level of AMM differed when comparing the groups of survivors and non-survivors by the end of the first day of treatment in the intensive care unit is shown in Fig. 9. It is important to emphasize that the cause of death was septic shock in 82% of those who did not survive, and this issue requires special discussion.

AMM and Septic Shock

Septic shock in patients in ICUs develops as sudden arterial hypotension, refractory to therapy and is characterized by high mortality. Septic shock was first postulated about 40 years ago. The mechanisms of its development are being intensively studied, the diagnostic criteria are being revised, but despite major advances in research a mortality rate of up to 50%. The reasons leading to septic shock are still poorly understood. Septic shock is one of the most common causes of mortality in intensive care units (ICUs) worldwide (Mickiewicz et al. 2015; Singer et al. 2016). It is extremely urgent to search for early biomarkers capable of predicting the development of life-threatening arterial hypotension in patients at risk with bacterial infection and sepsis.

In a clinical study by Beloborodova et al. (2018b) reported a correlation between aromatic microbial metabolites (AMM) with conventional clinical and laboratory parameters in a population of 41 patients with severe community-acquired

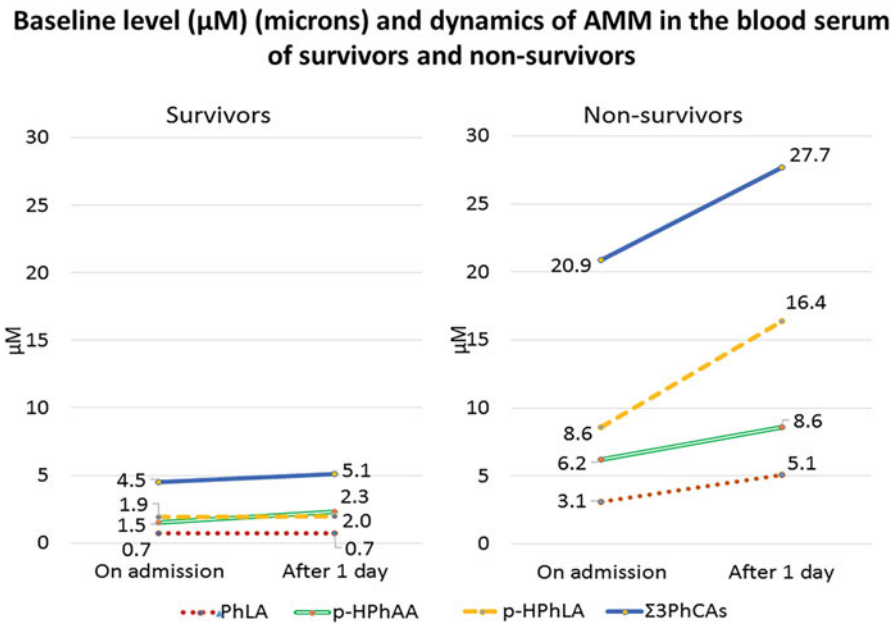

Fig. 9 Dynamics of serum level of aromatic microbial metabolites AMM concentration in critically ill, survivors and non-survivors, by the end of the first day of treatment in ICU

pneumonia. The authors controlled their parameters on the admission and by the end of the first day of treatment in the intensive care unit; then two groups of patients were compared – with and without septic shock. The correlation between sum of 3 AMM and presence of shock, levels of lactate, HVA, and NT-proBNP on admission was 0.44, 0.67, 0.57, and 0.38, respectively, but the correlation on the next day was 0.59, 0.73, 0.76, and 0.6, respectively ($p < 0.01$).

The results obtained can be explained if we assume that some AMMS have the ability to inhibit tyrosine hydroxylase, thereby limiting the synthesis of endogenous catecholamines. Patients with septic shock were found to have high concentrations of not only p-HPhLA (as usual in sepsis), but also p-HPhAA, in parallel with HVA. This allowed the authors to postulate that an excess of p-HPhAA and its microbial biodegradation products, by analogy with HVA, promotes the inhibition of tyrosine hydroxylase, which reduces the production of catecholamines and creates the risk of septic shock.

This allowed to postulate that an excess of p-HPhAA and its microbial biodegradation products, by analogy with HVA, has an inhibitory effect on tyrosine hydroxylase, what disrupts the metabolic pathway of catecholamine synthesis, increasing the risk of septic shock (Beloborodova et al. 2018a, b) (Fig. 10).

It should be noted that the clinical data presented above were carefully compared with the results of experiments conducted by other researchers in previous years, (Shen 1984; Laschinski et al. 1986), which allowed us to formulate an idea.

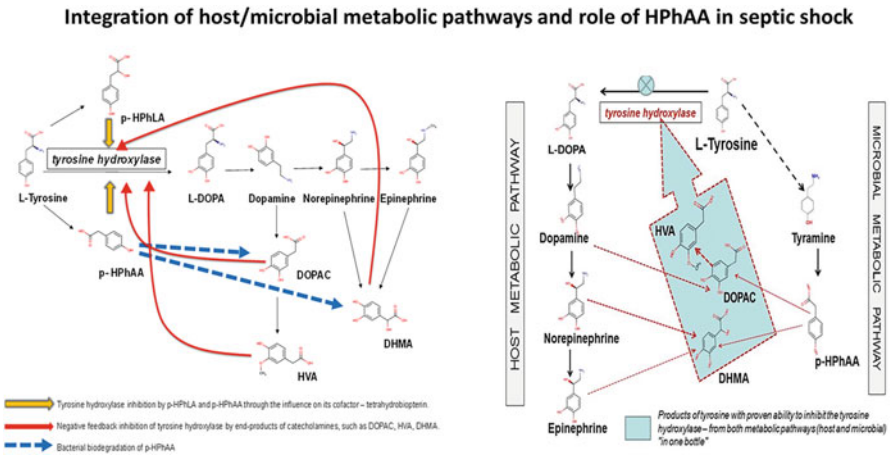

Fig. 10 Illustration to the postulate about the participation of AMM in the development of septic shock by inhibiting tyrosine hydroxylase and disruption of the metabolic pathway of catecholamine synthesis

According to Morrison and Scalea (2018), although these findings are compelling, it is unknown whether AMMs are a marker or a mediator of septic shock and whether they provide for a therapeutic target.

Aromatic metabolites, especially p-HPhAA and its biodegradation products, are involved in the pathogenesis of septic shock, which opens up the prospect of studying the possibilities of measuring AMM for predicting and monitoring this life-threatening condition.

Applications to Prognosis

The possibility of using AMM biomarkers for prediction has been shown in a number previous studies, including for assessing the outcome of severe pneumonia or abdominal surgery infections (Beloborodova et al. 2015a, b, 2020a, b).

This is also indirectly evidenced by the analysis of 90 critically ill patients in ICUs, where one of aromatic microbial metabolites HPhLA was associated with a fatal outcome (Roger et al. 2014).

In a study of patients ($n = 58$) with acute surgical diseases and bacterial complications, it was shown that the levels of PhLA, p-HPhAA, p-HPhLA and the total concentrations of the three PhCAs were in direct correlation with the APACHE II score (r_s: 0.624; 0.757; 0.763, and 0.804, respectively); $p < 0.001$; areas under the ROC (AUC) curves were within the range of 0.800–0.900 ($p < 0.001$). Moreover,

the dynamics of the lactate level as a prognostic criterion was inferior in accuracy to the dynamics of the total PhCA concentration: lactate AUCC, % 0.667 ($p = 0.071$) versus AUCC 3PhCAs, % 0.862 ($p < 0.001$). The authors showed that the change in the concentration of PhCA in the blood serum reflects the dynamics of the patient's condition and can be used for objective monitoring of treatment (Moroz et al. 2016).

In one of the recent studies (Pautova et al. 2022a) a multivariate prognostic model of aromatic metabolites detected by gas chromatographic mass spectrometry has been developed to predict the outcome of critically ill patients. The analysis showed that univariate model based on *p*-HPhLA is able to classify correctly more survivors (less false-positive samples) than multivariate models. The authors concluded that aromatic metabolites (one or a number of them as profile of AMM) may be useful in clinical practice to reliably predict the outcome of critically ill patients on the admission to intensive care units, and this is no less reliable than the widespread multi-parametric scales.

The most widely used APACHE scale is generally recognize, it has been known for several decades (Knaus et al. 1985), at the same time, the prospect of predicting the outcome on the day of admission to the ICU using only one laboratory indicator, optimal p-HPhLA, is quite attractive.

Application to Meningitis

In intensive care units of neurosurgical clinics, the development of postoperative (hospital) meningitis is an urgent problem (van de Beek et al. 2010; Hussein et al. 2017; Zlokovic et al. 2021), therefore, the search for early laboratory markers of bacterial infection of the central nervous system is extremely relevant to improve the outcome of patients after brain surgery.

In a study by Pautova et al. (2022b), in post-neuro-surgical patients ($n = 82$), the levels of aromatic metabolites and some biomarkers in samples of cerebrospinal fluid (CSF) were determined to assess the risk of post-neuro-surgical meningitis. It was revealed that *p*-HPhLA, an aromatic metabolite of microbial origin, can be considered as a specific marker of the post-neuro-surgical meningitis. ROC-analysis for *p*-HPhLA resulted in 0.734 values of the area under the ROC-curve, the sensitivity 66.67% and specificity 82.69%. If the concentration of *p*-HPhLA in CSF was more or equal to 0.9 μmol/l the risk of bacterial complications was 9.6 times higher. For comparison, the data obtained for the nonspecific biomarker of inflammation IL-6, the sensitivity was 96.30, but the specificity was only 54.17, that is, significantly lower than that of HPhLA. The authors concluded that *p*-HPhLA is a promising marker for the prognosis of the post-neurosurgical meningitis; studies involving a larger group of patients may subsequently prove its diagnostic significance HPhLA for bacterial infections of the central nervous system.

Short About Methods

Metabolic product determination in biological fluids is mostly performed using two groups of methods: NMR spectroscopy and different combinations of gas or liquid chromatography, particularly gas or liquid chromatography – mass spectrometry (Tomiko 2007; Nicholson and Lindon 2008; Lawton et al. 2008; Wishart 2011; Dunn et al. 2011). Researchers also use approaches based on metabolic profiling, reflecting the qualitative and quantitative composition of certain substances in the test sample. Studies in the clinic have shown that an imbalance in the profile of aromatic metabolites in the blood of patients indicates serious metabolic disorders, which can be used for the purpose of diagnosis and monitoring in intensive care.

For practical use in the clinic, methods of sample preparation and measurement on GC-MS of the level of several clinically significant AMM in blood serum (Pautova et al. 2018) or liquor samples have been developed and described in detail (Pautova et al. 2022a, b). After a short small sample preparation (about half our) and derivatization with BSTFA, the sample is analyzed on the GC-MS equipment (Fig. 11).

In previous years, normal concentrations of AMM in the blood of healthy people have been studied and published, which will be useful for novice researchers in this field (Beloborodova et al. 2015a, b).

For widespread implementation in intensive care units, an accessible express method is needed, which will help improve results in patients with life-threatening diseases.

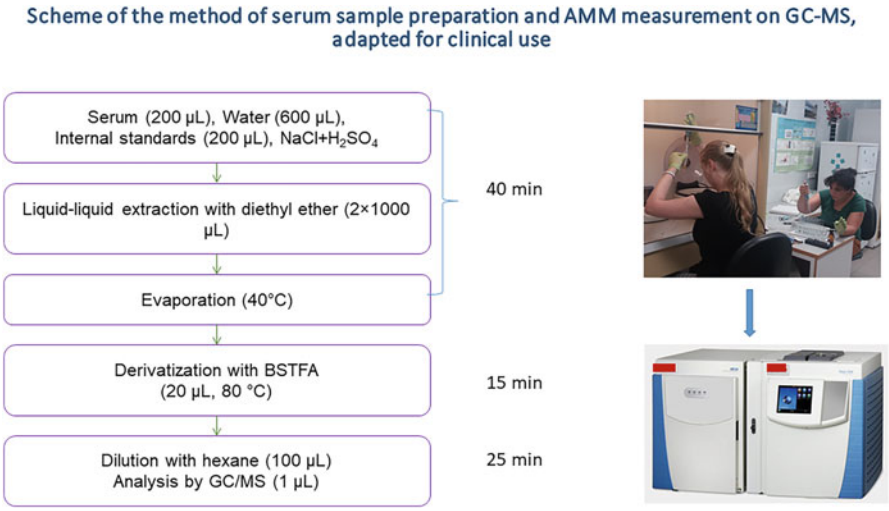

Fig. 11 Scheme of the method of serum sample preparation and AMM measurement on GC-MS, adapted for clinical use

Conclusion

The data presented in this chapter give grounds to consider aromatic microbial metabolites as biological markers in intensive care.

> Serum AMM is an integral biomarker that characterizes the state of the interaction between the critically ill patient's body and the microbial community inside it.

An increase in serum concentrations of such metabolites as PhLA, HPhLA, HPhAA, or their sum above 5 μM in critically ill patients indicates a high bacterial load and serious microbiota dysfunction, which poses a threat to the development of multiple organ dysfunctions. Thus, AMM as a biomarker can serve for early detection of the risk of MOF, sepsis, and septic shock, as well as for predicting the outcome.

> The accumulated data are the basis for a very important **postulate**: in a seriously ill patient, an increase in the serum level of AMM above the threshold value reflects the fact of the development of metabolic dysfunction of the microbiota, followed by the addition of multiple organ dysfunctions; in other words, the **moment of transition from systemic inflammatory response syndrome (SIRS) to sepsis**.

For wider application and study in intensive care units, an accessible express method is needed that allows measuring the level of AMM in serum or cerebrospinal fluid in a clinical laboratory, regardless of the availability of complex and expensive equipment such as GC-MS.

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Part III

Biomarkers in Trauma

Viscoelastic Hemostatic Tests and Fibrinogen Concentrations in Trauma

14

Henry T. Peng and Andrew Beckett

Contents

Abstract

Thrombelastography (TEG) and rotational thromboelastometry (ROTEM) are used to diagnose trauma-induced coagulopathy, fibrinogen deficiency, and guide fibrinogen transfusion in trauma, as well as to study the hemostatic effect of fibrinogen supplementation. We reviewed the clinical applications of TEG and ROTEM focusing on two functional fibrinogen (FF) tests, TEG FF and ROTEM FIBTEM, for assessing and guiding fibrinogen replacement in trauma patients. ROTEM FIBTEM, the standard FF test, measures clot amplitude. In contrast,

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while TEG FF, which is considered the standard FF test, also measures clot amplitude, other TEG tests, e.g., kaolin and rapid TEG, measure several coagulation parameters (maximum amplitude, K value, and angle α) to assess FF. Some confounding factors (e.g., hematocrit, factor XIII, and resuscitation fluids) need to be considered when interpreting the hemostatic effect of fibrinogen replacement measured by TEG and ROTEM. Different cutoff values for TEG and ROTEM parameters, particularly for maximum clot firmness (MCF) in FIBTEM, have been used for fibrinogen replacement. The dosage of fibrinogen replacement can be calculated based on the desired increment in the FIBTEM MCF or plasma fibrinogen level. In addition, we compared the clinical performance of the two FF test systems; the results were correlated but not interchangeable.

Keywords

Coagulopathy · Hemorrhage · Hypofibrinogenemia · Fibrinogen · Rotational thromboelastometry · ROTEM · Thrombelastography · TEG · Trauma · Viscoelastic tests · Conventional coagulation tests

Abbreviations

ACT	Activated clotting time
CAs	Clot amplitudes
CCTs	Conventional coagulation tests
CFT	Clot formation time
CL30	Clot amplitude at 30 min after MA relative to MA
CT	Coagulation time
ELISA	Enzyme-linked immunosorbent assay
FC	Fibrinogen concentrate
FF	Functional fibrinogen
FFP	Fresh frozen plasma
INR	International normalized ratio
K	Kinetic time
LI30	Lysis index at 30 min after CT
LY30	Clot lysis at 30 min after MA
MA	Maximum amplitude
MCF	Maximum clot firmness
PT	Prothrombin time
PTT	Partial thromboplastin time
R	Reaction time
RBC	Red blood cells
ROTEM	Rotational thromboelastometry
SLT	Standard laboratory test
TEG	Thrombelastography
TIC	Trauma-inducted coagulopathy

Introduction

Hemorrhage is the leading cause of preventable death in combat trauma (Eastridge et al. 2012) and secondary cause of death in civilian trauma (U. S. Burden of Disease Collaborators 2013). Coagulopathic bleeding is frequently present (at least one quarter of civilian trauma patients and one third of military trauma patients present with a laboratory-defined coagulopathy) early after major trauma (Chang et al. 2016) and causes a three- to five-fold increase in mortality (Hess et al. 2008; Davenport and Brohi 2015). Nearly one third of severe trauma patients present with trauma-inducted coagulopathy (TIC) which carries a 50% mortality rate (Maegele et al. 2012; Simmons et al. 2014). The degree of TIC worsens as the injury severity increases (Cohen and West 2011; Frith et al. 2010). Therefore, early diagnosis, prevention, and treatment of TIC in the prehospital setting and at admission are of major interest in trauma patients.

Viscoelastic Hemostatic Tests

Thrombelastography (TEG; Haemonetics Corporation, Haemoscope Division, Nile, Illinois, USA) and rotational thromboelastometry (ROTEM; Tem Innovations GmbH, Munich, Germany succeeded by Instrumentation Laboratory, Bedford, Massachusetts, USA) are two point-of-care systems for hemostatic tests in whole blood (Whiting and DiNardo 2014). Both provide a global measure of hemostasis by quantitatively measuring the elasticity of blood from the beginning of coagulation to the ending with fibrinolysis. This includes the onset of clot formation, its progress, maximum clot strength, and clot stability, which provides important information about coagulation, fibrinolysis, and platelet function (Luddington 2005). TEG and ROTEM can also identify the relative contributions of clotting factors, such as fibrinogen and platelets, to the overall coagulation process (Whiting and DiNardo 2014).

TEG and ROTEM have been increasingly used in various clinical settings involving bleeding patients to diagnose and treat TIC (Hartmann et al. 2020a) including fibrinogen deficiency (Schlimp and Schöchl 2014), predict the risk of bleeding and mortality, and guide fibrinogen transfusion in trauma (Figueiredo et al. 2016), cardiac surgery (Görlinger et al. 2013), liver transplantation (Goerlinger 2006), and postpartum bleeding (Ranucci et al. 2016). A randomized clinical trial has concluded that TEG-guided massive transfusion protocol for severe trauma improves survival compared with that guided by conventional coagulation tests (CCTs, e.g., prothrombin time [PT]/international normalized ratio [INR], fibrinogen, and D-dimer) and utilizes less plasma and platelet transfusion during the early phase of resuscitation (Gonzalez et al. 2016). However, the latest multicenter randomized controlled trial (iTACTIC; NCT02593877) involving nearly 400 patients comparing TEG- or ROTEM-guided transfusion therapy with CCT-guided transfusion showed that the 28-day mortality was strongly reduced by TEG- or ROTEM-guided transfusion in patients with major hemorrhage who had a severe traumatic brain injury;

however, there was no difference in the proportion of patients who were alive and did not require massive transfusion at 24 h after injury (Baksaas-Aasen et al. 2021). Although the evidence on the benefit of TEG and ROTEM over CCTs in trauma is limited at this time, substantial evidence from elective cardiac and liver transplant surgery studies provides further support for the use of TEG and ROTEM (Dias et al. 2019).

Fibrinogen

Fibrinogen plays a central role in both primary and secondary hemostasis (Levy et al. 2012) and TIC (Schlimp and Schöchl 2014). Upon major trauma, fibrinogen reaches levels critically below the physiological level of 2–4 g/L, earlier than those of other routine coagulation parameters and before patients meet the criteria for massive blood transfusion (Schlimp and Schochl 2014; Hayakawa et al. 2015). Low fibrinogen levels are associated with increased bleeding and coagulopathy and, as a result, poor clinical outcomes (Schlimp and Schochl 2014). The fibrinogen level is an independent predictor of mortality in major trauma patients and of the requirement for massive transfusion in patients with pelvic fractures (McQuilten et al. 2017a; Notani et al. 2020).

Different cutoff values of fibrinogen concentrations ranging from 1 to 1.8 g/L were used to define hypofibrinogenemia (Peng et al. 2019; Rourke et al. 2012). The current guidelines recommend fibrinogen supplementation (with fibrinogen concentrate (FC) or cryoprecipitate) in a bleeding patient with fibrinogen levels <1.5 g/L (Kozek-Langenecker et al. 2017; Rossaint et al. 2016) or equivalent by viscoelastic testing (Černý et al. 2022).

There is, however, a paucity of evidence to support the early replacement of fibrinogen in severely injured trauma patients. A systematic review and meta-analysis of the use of FC for trauma-related bleeding found no statistically significant difference in mortality between the groups, with 22% and 23.4% in the FC and comparator arms, respectively: risk ratio 1.00 [95% confidence internal 0.39–2.56], $p = 0.99$. Additionally, there was no statistical difference between FC and control in packed red blood cells (RBC), fresh frozen plasma (FFP), platelet transfusion requirements, and thromboembolic events (Stabler et al. 2020). On the other hand, another recent review of 21 major randomized controlled trials assessing FC use in perioperative settings found that approximately 60% of the studies in which FC was used to treat clinically relevant bleeding showed decreased bleeding tendency and decreased transfusion requirements versus comparative treatment (Cushing and Haas 2019).

Viscoelastic functional fibrinogen tests in particular ROTEM FIBTEM have been widely used for assessment of fibrinogen deficiency, prediction for transfusion requirement, and guided fibrinogen replacement, while studies focusing on TEG functional fibrinogen (FF) are limited (Peng and Nascimento 2018). A retrospective observational study showed that the incorporation of TEG FF into TEG-based

coagulation management and FC administration reduced the need for transfusion in patients undergoing liver transplantation, with no impact on survival (Kozek-Langenecker et al. 2017; Peng and Nascimento 2018).

The review is structured into four main sections. The first section describes the principles of the two systems and various commercially available tests employing them, with an emphasis on FF tests. The similarities and differences of the two systems along with new viscoelastic testing systems are discussed as well. The second section reviews the use of TEG and ROTEM for diagnosis of TIC including TEG FF and ROTEM FIBTEM for hypofibrinogenemia. The third section depicts the use of TEG and ROTEM to assess FF levels and the hemostatic effect of fibrinogen replacement. The fourth section discusses TEG- and ROTEM-guided fibrinogen replacement.

Principles of TEG and ROTEM FF Tests

Figure 1a and b show the testing principles of the two most commonly used systems for FF tests: the TEG 5000 Hemostasis Analyzer and the ROTEM delta system. Both systems measure the viscoelastic properties of blood as it clots under low shear stress, but there are primary hardware differences between the two, as detailed by Peng et al. (2018). Briefly, the hardware differences include the mechanisms for cup/pin rotation and the detection of the rotation, cup materials, and interior surface properties.

For both systems, measurement is graphically represented as a characteristic shape profile over time (Fig. 1c). From this graph, the following parameters can be derived for TEG: (1) the reaction time R, which is related to plasma clotting factors and circulating anticoagulants; (2) the kinetic time K, which is associated with the activities of the clotting factors, fibrinogen, and platelets; (3) the rate of clot polymerization, represented by the angle α, which is a main function of the platelets, fibrinogen, and plasma components residing on the platelet surface; (4) the maximum amplitude (MA) or maximum clot strength, which is a direct function of the maximum dynamic properties of fibrin and platelet number and functions; and (5) fibrinolysis at 30 min or the rate of amplitude reduction 30 min after MA, LY30/CL30, which is related to plasma levels and activities of tissue plasminogen activator and its inhibitors. For the TEG FF test, the FF level (FLEV) in mg/dL or g/L can be calculated from MA using analytical software (Agarwal et al. 2014). For rapid TEG, in which both intrinsic and extrinsic activators are used, the activated clotting time (ACT) is calculated from the R value using the TEG software and may provide a better measure of initial clot formation than R itself (Blaine and Steurer 2019).

Similar parameters, as shown in Fig. 1c (e.g., coagulation time (CT), clot formation time (CFT), angle α, MCF, and clot lysis index LI30), are measured by ROTEM. In addition, clot amplitudes (CAs) at 5 and 10 min after CT (i.e., CA5 and CA10) have been reported for ROTEM.

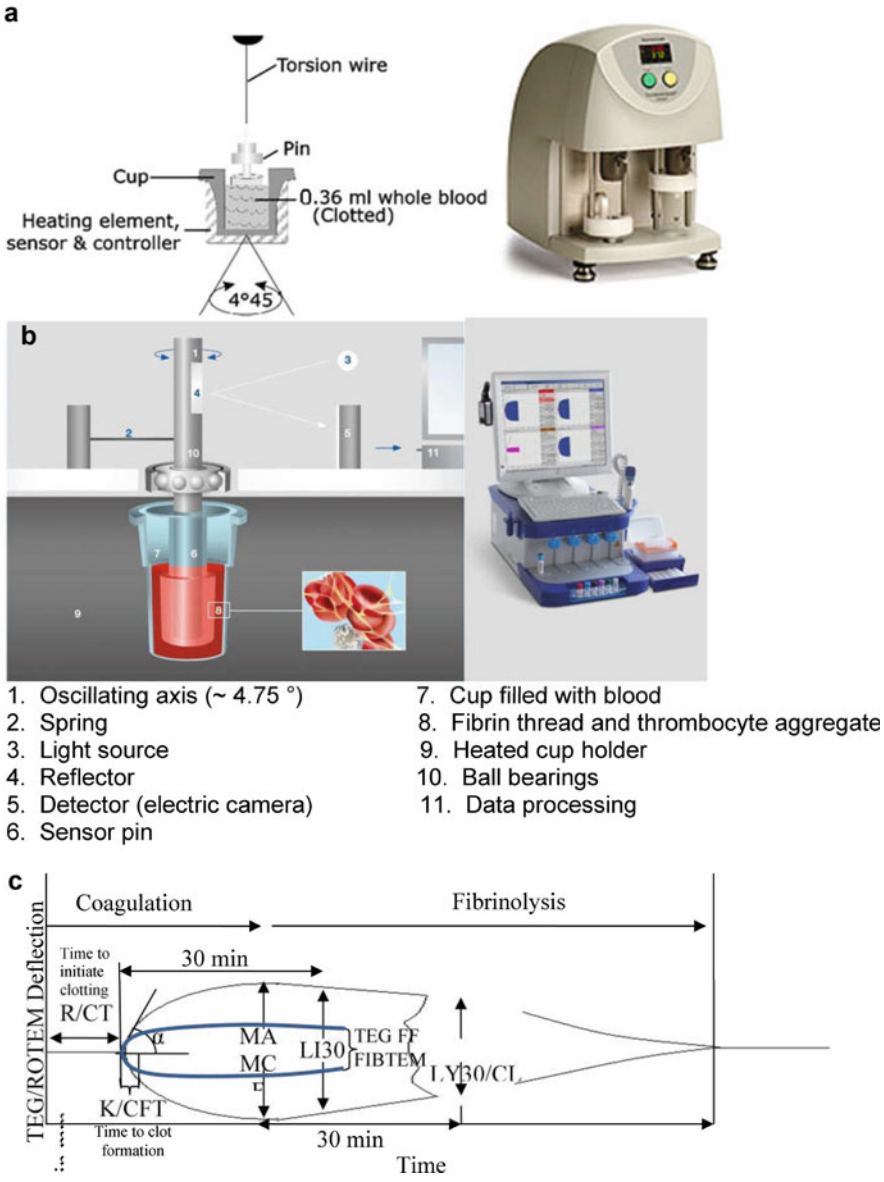

Fig. 1 Principles of and instruments for two viscoelastic testing systems. Schematic illustration of the mechanism and photograph of each instrument: (**a**) TEG 5000 (Haemonetics Corp., Niles, IL, USA), (**b**) ROTEM delta (Instrumentation Laboratory, Bedford, MA, USA), and representative TEG/ROTEM tracing showing the relationship between qualitative tracing and quantitative parameters (**c**). Panels A and B: courtesy of Haemonetics Corp. and TEM Systems Inc.

Comparison of TEG and ROTEM FF Tests

In addition to instrumental differences, the two abovementioned viscoelastometric systems use different reagents, as summarized in Table 1 (Whiting and DiNardo 2014; Schöchl et al. 2013a; Carroll et al. 2009; Blaine and Steurer 2019). Specifically, the FF reagent for TEG is composed of lyophilized tissue factor and a platelet inhibitor (abciximab) that binds to glycoprotein-IIb/glycoprotein-IIIa receptors to inhibit platelet aggregation and exclude platelet contribution to clot strength (Solomon et al. 2012). For the TEG FF test, 0.5 mL of citrated or native blood is activated with a mixture of tissue factor and a monoclonal glycoprotein IIb/IIIa receptor antagonist, and then 340 μL of the activated blood is added to a TEG cup preloaded with 20 μL of 0.2 M $CaCl_2$ (Ferrante et al. 2016). For the ROTEM FF test (FIBTEM), 20 μL of ex-TEM, 20 μL of fib-TEM solution, and 300 μL of citrated blood are mixed directly in a ROTEM cup (Solomon et al. 2012). The ex-TEM solution contains a combination of recombinant tissue factor and phospholipids that activates the extrinsic pathway of the coagulation system, whereas the fib-TEM solution contains $CaCl_2$ as a recalcification reagent and a platelet inhibitor (cytochalasin D) that inhibits the actin/myosin system. Studies comparing different TEG and ROTEM FF tests have shown that the platelet inhibitor abciximab in TEG FF is less effective in eliminating the platelet contribution to clot strength than cytochalasin D in ROTEM FIBTEM; this results in a larger MA in TEG FF than MCF in ROTEM FIBTEM, affecting their dependence on the fibrinogen level (Solomon et al. 2012; Schlimp et al. 2014). Moreover, the TEG FF reagent does not contain heparinase or polybrene and therefore cannot be used for heparinized patients, unless a heparinase TEG cup is used. In contrast, the ROTEM FIBTEM reagent ex-TEM contains the heparin inhibitor polybrene. A new reagent, fib-TEM PLUS, containing two platelet inhibitors, cytochalasin D and tirofiban, provided the most accurate assessment of clot strength ascribed to fibrinogen function (Solomon et al. 2013a).

TEG and ROTEM have yielded different results for diagnosing coagulopathy and guiding transfusion (Sankarankutty et al. 2012), and different transfusion algorithms have been developed for each system (Coakley et al. 2006; Enriquez and Shore-Lesserson 2009). For example, ROTEM-based algorithms tended to recommend the use of FC (Schöchl et al. 2010b) or cryoprecipitate (Tanaka et al. 2012), whereas TEG-based algorithms tended to recommend the use of plasma (Coakley et al. 2006); however, both systems recommended lower transfusion than standard laboratory measures of coagulation. These differences may be due to the tests rather than the instruments, as most ROTEM-guided transfusions involve FIBTEM, which is a specific test for fibrinogen level and function (Görlinger et al. 2012), while the TEG FF test is less involved in TEG-guided transfusions (Sawyer et al. 2012) and FC administration (Levy et al. 2014; Spahn et al. 2016).

Agreements between TEG and ROTEM recommendations to transfuse platelets were fair (kappa coefficient of agreement (κ) = 0.33 between ROTEM INTEM and native heparinase TEG, and κ = 0.28 between ROTEM INTEM and kaolin heparinase TEG) but were low in case of a low MA, suggesting the need for transfusion of either fibrinogen or platelets. There was a moderate agreement

Table 1 Summary of TEG and ROTEM tests, their corresponding activators and inhibitors, and their applications (Whiting and DiNardo 2014; Schöchl et al. 2013a; Carroll et al. 2009; Blaine and Steurer 2019)

Type of reagents	TEG			ROTEM		
	Constituents	Test	Applications	Constituents	Test	Applications
Calibration reagents	Lyophilized animal citrated plasma with stabilizers and buffers	Level 1 for normal control and level 2 for abnormal control	Quality control	Lyophilized human citrated plasma with stabilizers and buffers	ROTROL N for normal control and ROTROL P for abnormal control	Quality control
Recalcification reagent	0.2-M $CaCl_2$ aqueous solution	Native TEG	Not often used because of long runtime and high variability	Star-TEM: 0.2 M $CaCl_2$ and 0.1% NaN_3 in pH 7.4 buffer	NATEM	Not often used because of long runtime and high variability
Surface activator	Kaolin suspension in a buffered stabilizer and a mixture of phospholipids	Kaolin TEG	Information similar to that of aPTT; thrombin generation as indicated by R	In-TEM: ellagic acid and partial thromboplastin phospholipid and preservatives in buffer	INTEM	Information similar to that of aPTT for intrinsic coagulation pathway; thrombin generation as indicated by CT
Extrinsic activator	8% kaolin, human recombinant tissue factor, phospholipids, buffers, and stabilizers	Rapid TEG	Both intrinsic and extrinsic pathways are activated to more rapidly assess coagulation properties	Ex-TEM: a combination of recombinant tissue factor, polybrene, and phospholipids	EXTEM	Information similar to that of PT for extrinsic coagulation pathway, indication for FFP/PCC administration
Platelet inhibitor	Lyophilized tissue factor and abciximab	FF TEG	Used in conjunction with kaolin TEG can assess relative contribution of	Fib-TEM: a combination of a platelet inhibitor (cytochalasin D) and $CaCl_2$ and ex-TEM	FIBTEM	Measurement of fibrinogen and platelet contribution (in conjunction with EXTEM) to clot

			platelets and fibrin to overall clot strength			strength, indication for cryoprecipitate or fibrinogen and platelet administration
Heparin inhibitor	Lyophilized heparinase I from *Flavobacterium heparinum* at 2 IU in a TEG cup is sufficient to reverse 6 IU of heparin/mL of blood	HTEG	Compared with kaolin TEG to assess heparin effects	Hep-TEM: lyophilized heparinase I from flavobacteria, preservatives and buffer and Ca-containing diluent and start reagent with NaN_3 (< 0.1%) and preservatives and in-TEM	HEPTEM	Assessment of heparin effect in conjunction with INTEM
Platelet activator	AA ADP	Platelet mapping TEG	Assessment of coagulopathy, plate dysfunction, and hyperfibrinolysis and suggestion of interventions	ADP or thrombin receptor-activating peptide 6, buffers and stabilizers with ROTEM platelet module	ADPTEM TRAPTEM	Assessment of platelet function by activating either the ADP or the thrombin receptor pathway
Fibrinolysis inhibitor	Not available			Ap-TEM: aprotinin, 0.2 M $CaCl_2$, and 0.1% NaN_3 in a pH -7.4 buffer and ex- TEM	APTEM	Assessment of fibrinolysis in conjunction with EXTEM, indication for tranexamic acid administration

Abbreviations: *aPTT* activated partial thromboplastin time, *Ca* calcium, *$CaCl_2$* calcium chloride, *HTEG* kaolin TEG with heparinase, *NaN_3* sodium azide, *PT* prothrombin time, *FFP* fresh frozen plasma, *PCC* prothrombin complex concentrate, *TEG* thrombelastography, *ROTEM* rotational thromboelastometry, *CT* coagulation time, *FF* functional fibrinogen, *AA* arachidonic acid, *ADP* adenosine 5′-diphosphate

between ROTEM INTEM and prothrombin time ($\kappa = 0.42$) and a poor agreement between the recommendations of viscoelastic tests to administer FFP (Coakley et al. 2006). ROTEM FIBTEM has been used to assess and guide fibrinogen replacement. In contrast, TEG FF is less used for TEG-guided transfusion (Sawyer et al. 2012). There is a lack of studies directly comparing the utilities of ROTEM FIBTEM and TEG FF for the diagnosis of coagulopathies, including hypofibrinogenemia, and the guidance of transfusions, including fibrinogen replacement, although both have been reported to be useful (Carroll et al. 2009; Rugeri et al. 2007).

Meyer et al. (2014) compared different TEG and ROTEM tests, including TEG FF and FIBTEM, and the Clauss method to detect trauma-induced coagulopathy and goal-directed transfusion therapy. TEG FF and ROTEM FIBTEM early amplitudes (CA5, CA10) and MA/MCF had similar correlations with Clauss fibrinogen levels and could differentiate coagulopathic and transfused patients from non-coagulopathic and non-transfused patients. In a similar study, TEG and ROTEM were compared for FF tests in trauma patients (Meyer et al. 2015). TEG FF MA and ROTEM FIBTEM MCF correlated well with each other ($\rho = 0.71$, $p < 0.001$) and with the Clauss fibrinogen level ($\rho = 0.64$ for both, $p < 0.001$).

We compared the capabilities of the TEG and ROTEM FF tests to detect coagulation and fibrinolysis changes in response to hemostatic treatment and to predict acute traumatic coagulopathy and transfusion requirements in a randomized, controlled trial for fibrinogen in the initial resuscitation of severe trauma (Peng et al. 2018; Peng et al. 2019). Overall, we found significant differences in TEG FF MA and ROTEM MCF between placebo- and fibrinogen-treated groups over hospitalization time. ROTEM FIBTEM MCF seemed to be more consistent with the duration of the between-group difference, as indicated by fibrinogen levels, than TEG FF MA. There were significant correlations between corresponding parameters of TEG FF and ROTEM FIBTEM, with TEG FF MA and ROTEM FIBTEM MCF showing the strongest correlation ($\rho = 0.80$, $p < 0.001$); however, they were not interchangeable, and MA was larger than MCF. In addition, ROTEM CT and LI30 indicated the effect of fibrinogen administration on coagulation time and fibrinolysis. There were discrepancies between TEG and ROTEM in their detection of coagulation abnormalities, hypofibrinogenemia, and hyperfibrinolysis (Peng et al. 2018).

New Viscoelastic Hemostatic Testing Systems

New and fully automated (no pipetting) TEG and ROTEM systems (TEG 6 s [Haemonetics Corp.] and ROTEM sigma [Instrumentation Laboratory]) are now available. Both work with four-channel cartridges but are based on different mechanisms. TEG 6 s uses a new technology termed “coagulation resonance analysis” and microfluidic cartridges containing dried reagents (Gurbel et al. 2016). ROTEM sigma operates on the same pin and cup technology as ROTEM delta but uses cartridges containing lyophilized bead reagents instead of liquid reagents (Görlinger et al. 2016).

TEG 6 s reportedly is highly reliable, with results strongly correlating with those derived from TEG 5000 (linear correlation estimates >0.9) (Neal et al. 2020). ROTEM sigma also has a high precision, with results being strongly correlated with those derived from ROTEM delta (Pearson correlation coefficients ≥0.8) (Schenk et al. 2019). Furthermore, when compared for use in trauma patients, strong to very strong correlations (Spearman correlation coefficients >0.6) were observed between corresponding TEG 6 s and ROTEM sigma parameters, although there were significant differences in absolute values for most measurements (Ziegler et al. 2019).

Furthermore, other viscoelastic hemostatic testing systems are available and emerging (Hartmann et al. 2020b). Sonoclot is a legacy device developed by Sienco, Inc. The Sonoclot device differs from TEG and ROTEM in that it is not a rotational-based system but a linear motion system (Ganter and Hofer 2008). Quantra hemostasis analyzer is a relatively new product developed by HemoSonics based on a proprietary technology that uses ultrasound to measure clot time and clot stiffness from changes in viscoelastic properties of whole blood during coagulation (Ferrante et al. 2016). Multicenter evaluation of the Quantra system in adult patients undergoing major surgical procedures consisting primarily of cardiac and major orthopedic surgeries was conducted, showing that the correlation between ROTEM and Quantra was very strong with correlation coefficients ranging between 0.84 and 0.89. (Groves et al. 2020). Additional receiver operating characteristics analysis indicated sensitivities and specificities in the 80–90% range when Quantra parameters were used to discriminate ROTEM threshold values currently used in goal-directed treatment algorithms. Several emerging technologies are currently in development for point-of-care viscoelastic hemostatic testing, including microfluidics, fluorescent microscopy, electrochemical sensing, photoacoustic detection, and micro−/nano-electromechanical systems (MEMS/NEMS) (Mohammadi Aria et al. 2019).

Applications to Diagnosis of TIC and Hypofibrinogenemia

Historically, TIC is defined by CCTs, such as INR above a threshold of 1.2 (Frith et al. 2010; Meyer et al. 2014; Davenport et al. 2011; Hagemo et al. 2015), 1.3 (Tonglet et al. 2018; Kornblith et al. 2014; Cohen et al. 2013), 1.5 (Niles et al. 2008), and 1.6 (Rugeri et al. 2007), PTT ≥ 35 s (Cohen et al. 2013); by plasma fibrinogen levels, ranging from 1.0 to 2.0 g/L; and by platelet counts below 100×10^9/L (Rossaint et al. 2016). However, this is no sound evidence to support the usefulness of these tests in particular INR, PTT for diagnosis of coagulopathy, or to guide hemostatic therapy (Haas et al. 2015). There are several limiting factors with these assays, such as the time to obtaining results from multiple tests; sole measurement of contribution of plasma proteins to clot formation, without regard for the central role of platelets; and the inability to identify hyperfibrinolysis (Moore et al. 2021). The use of CCTs such as INR in trauma has been severely criticized due to the lack of association with bleeding and blood transfusion. It has been reported that INR

overestimated coagulopathy and should not be used to guide blood transfusion in stable trauma and surgical patients (McCully et al. 2013).

Consequently, viscoelastic hemostatic tests in particular TEG and ROTEM have been adopted for the diagnosis of TIC, owing to their assessment of whole blood clot formation and degradation in real time, and rapid availability of the comprehensive information. TEG has been shown to identify additional coagulopathies compared to CCT methods (Sumislawski et al. 2019). TEG and ROTEM can detect different fibrinolysis phenotypes in trauma (Stettler et al. 2019).

Table 2 summarizes the literature on the use of viscoelastic hemostatic tests for diagnosis of TIC according to test done (TEG or ROTEM) and the study design, method of blood sampling, activators used, and parameters studied along with main findings.

TIC was defined by celite- and kaolin-activated TEG, respectively, when $\geq$2 TEG parameters are abnormal (Kaufmann et al. 1997; Rizoli et al. 2011). TIC was also defined according to rapid TEG if any of the following variables were abnormal (Holcomb et al. 2012; Ostrowski et al. 2017): ACT>128 s, R time $>$ 66 s, K $>$ 150 s, Alpha<56°, MA $<$ 55 mm, and LY30 $>$ 3%. When defined by kaolin TEG, one or several of the TEG parameters could be abnormal to indicate coagulopathy in an algorithm with R $\geq$ 11 min, MA $\leq$ 50 mm, angle<52°, or LY30 $>$ 8% (Johansson et al. 2010). Rapid TEG is faster than kaolin TEG and CCTs for providing reliable information on coagulopathy in patients with multiple injuries (Jeger et al. 2009).

TEG and ROTEM could detect a hypercoagulable state, hyperfibrinolysis, and were better tests than PT or PTT (Branco et al. 2014; Park et al. 2009; Schreiber et al. 2005; Spasiano et al. 2022; Watters et al. 2010). However, single TEG R performed worse than INR at identifying vitamin K-dependent coagulation factor deficiency (Nascimento et al. 2012).

TIC was defined by hypocoagulable state on ROTEM which was determined by one of the principle parameters (CT, CFT, MCF, ML) outside the manufacturer's normal ranges by 20% (i.e., CT $\geq$ 94 s, CFT $\geq$ 190 s, MCF $\leq$ 40 mm, ML $\geq$ 12%) (Tonglet et al. 2018). EXTEM which includes the platelet contribution to the development of coagulation abnormalities would be more suitable to detect coagulopathy. EXTEM CA5 $\leq$ 35 mm could predict massive transfusion and was used to define TIC (Rourke et al. 2012). EXTEM CA5 $\leq$ 35 mm (Davenport et al. 2011) and $\leq$ 37 mm (Hagemo et al. 2015) threshold values for detection of TIC resulted in a detection rate of 77% and 66.3%, respectively, and FIBTEM CA5 $\leq$ 8 mm detected TIC in 67.5%, while fibrinogen concentration $\leq$ 1.6 g/L detected TIC in 73.6% (Hagemo et al. 2015). TIC defined by EXTEM MCF $<$ 40 mm was 39% in combat casualties (Woolley et al. 2013). ROTEM also detected more abnormal coagulation status than CCTs (PT and PTT) in a deployed military setting (Doran et al. 2010) as well as in-hospital emergency department (Spagnolello et al. 2021). EXTEM MCF showed 100% sensitivity and specificity for detection of hyperfibrinolysis defined as a euglobulin lysis time (ELT) $<$ 90 min (Levrat et al. 2008). Combined with INR $>$ 1.2, EXTEM A5 $\leq$ 35 mm and/or LI30 $<$ 97% on admission classified 15% more patients with TIC and predicted massive transfusion

Table 2 Viscoelastic hemostatic tests (TEG and ROTEM) for diagnosis of TIC

Study type	Tests and blood samples	Parameters and cutoff values	Definition of TIC/abnormal coagulation	Main findings	References
Prospective observational cohort study of 69 adult blunt trauma patients with a median ISS of 5 (range, 1–75)	Celite-activated TEG (model 3000) native blood	R 5–7 min K 1.5–3 min α angle 54–67° MA	PT/PTTs normal, 11.0–14.0 and 25–36	Forty five patients were hypercoagulable (mean ISS 13.1), and 7 were hypocoagulable (mean ISS 28.6). TEG is predictive of early blood transfusion with the first 24 h of admission	Kaufmann et al. (1997)
Prospective observational study of 65 patients with mean injury severity score was 23 ± 12	Kaolin TEG with native blood	R, α, MA	R < 3.7 min for hypercoagulability	Significant correlation only on day 1; between R and PTT, MA and platelets hypercoagulability in the first 24; women more hypercoagulable	Schreiber et al. (2005)
Prospective observational study of 20 patients with a median ISS of 29 (range 16–65)	Kaolin and rapid TEG with native blood	R, K, α, MA rapid TEG: >2 min, α < 66°, MA < 54 mm; for laolin TEG: R > 8 min, K > 4 min, α < 47°, MA <54 mm	INR > 1.25, aPTT >36 s, thrombin time > 15 s, platelet count <150 nL	Strong correlation between k, α, and MA in rapid TEG and TEG; moderate correlation between k/α/MA and platelet count and INR, no significant correlations were found between TEG parameters and TT or aPTT. Rapid TEG provides a fast and reliable indication of coagulation status in trauma patients	Jeger et al. (2009)

(continued)

Table 2 (continued)

Study type	Tests and blood samples	Parameters and cutoff values	Definition of TIC/abnormal coagulation	Main findings	References
Prospective observational study of nonburn trauma patients ($n = 33$) with an average ISS of 21.7, burned patients ($n = 25$) with an average of ISS of 18.1	Rapid TEG with native blood	R, α, MA,LY30	Healthy volunteers ($n = 20$)	α and MA were greater in patients than in controls; TEG was more sensitive than PT and PTT, to hypercoagulable state in post-injury, non-bleeding patients	Park et al. (2009)
Prospective observational study of 30 trauma patients with splenectomy	Kaolin TEG	R, K, α, MA, LY30	Splenic preservation group ($n = 50$)	MA significantly greater postsplenectomy; platelet and fibrinogen remained higher; persistent hypercoagulable state after splenectomy	Watters et al. (2010)
Prospective observational cohort study of 110 severely traumatized patients with an ISS $\geq$ 16	Kaolin TEG with citrated blood	R, K, α, MA $\geq$ 2 abnormal parameter values	Clotting factor (II, V, VII, VIII, IX, X, XI, and XII deficiency ($\geq$ 30% activity)	TEG had a sensitivity of 35.3% (14.2%, 61.7%), specificity of 88.8% (79.7%, 94.7%), PPV of 40.0% (16.3%, 67.7%), and NPV of 86.6% (77.3%, 93.1%) for critical clotting factor deficits	Rizoli et al. (2011)
Prospective observational cohort study of 219 patients with an ISS 26 $\pm$ 12	Kaolin TEG with citrated blood	R > 8 min	Vitamin K-dependent coagulation factor (II, VI, IX, and X) deficiency ($\geq$ 50% activity)	TEG had a sensitivity of 33% (95% CI, 16%– 55%), specificity of 95% (95% CI, 91%–98%), PPV of 47% (95% CI, 23%–72%), and NPV of 92% (95% CI, 87%–95%) in detecting coagulation factor deficiency	Nascimento et al. (2012)

a single-center, retrospective cohort study of 1974 trauma patients with a median ISS of 17 (9, 26)	Rapid TEG with citrated blood	ACT 86–118 s R 0–1 min K 1–2 min α angle 66–82° MA 54–72 mm LY30 0.0–7.5%	PT, aPTT, INR, platelet count, and fibrinogen concentration	Rapid TEG was clinically superior to CCTs for identifying patients with an increased risk of early RBC, plasma and platelet transfusions, and fibrinolysis	Holcomb et al. (2012)
Prospective observational study of 404 trauma patients with a median ISS of 17 (9–26)	Rapid TEG with citrated blood	ACT >128 s, R > 1.1 min, K > 2.5 min, α < 56°, MA < 55 mm, and LY30 > 3%	TG lag time ≥ 4.9 min, ETP < 1000 nM*min, peak <250 nM, time to peak ≥7 min, rate of TG ≤ 100 nM/min	One third (35%) of the patients had rapid TEG coagulopathy which was associated with sympathoadrenal activation, endotheliopathy, and increased mortality	Ostrowski et al. (2017)
Prospective observational study of 45 trauma patients with a median ISS of 24.0 (17.5–29.0)	TEG FF with citrated blood	MA 1–24 mm	INR ≥ 1.2 fibrinogen concentration < 1 g/L	TEG FF MA poorly detected coagulopathy as defined by INR ≥ 1.2 (AUC 0.557 (0.480–0.634) but well predicted hypofibrinogenemia (fibrinogen concentration < 1 g/L) (AUC 0.948 (0.886–1.000)	Peng et al. (2019)
Prospective observational 2 years cohort study on 83 severe trauma patients with a mean new ISS of 36 (±13)	Kaolin TEG	R 9–27 min MA 44–64 mm G 3.6–8.5 dyne/s ≥ 2 abnormal parameter values LY30 > 3%	INR > 1.5 LY30 > 3% hyperfibrinolysis	88.8% of the trauma population had coagulation abnormalities immediately after the trauma; with 71.3% procoagulant changes and 8.8% hyperfibrinolysis	Spasiano et al. (2022)

(continued)

Table 2 (continued)

Study type	Tests and blood samples	Parameters and cutoff values	Definition of TIC/abnormal coagulation	Main findings	References
ROTEM					
Prospective observational study of 88 patients with an ISS of 22 (12–34)	EXTEM, INTEM, FIBTEM	EXTEM CA15 < 32 mm	PT > 1.5 of control value	EXTEM CA15 had a sensitivity of 87 (72–87), specificity of 100 (99–100), PPV of 100 (83–100), NPV of 99 (98–99), AUC of 0.98 in predicting PT > 1.5 of control value	Rugeri et al. (2007)
		INTEM CFT 112 s	APTT >1.5 of control value	INTEM CFT has a sensitivity of 100 (84–100), specificity of 74 (73–74), PPV of 23 (19–23), NPV of 100 (98–100), and AUC of 0.94	
		INTEM CA15 < 46 mm	Platelet count $<50 \times 10^9$/L	INTEM CA15 has a sensitivity of 100 (71–100), specificity of 83 (82–83), PPV of 17 (12–17), NPV of 100 (98–100), and AUC of 0.92	
		FIBTEM CA10 < 5 mm	Fibrinogen <1 g/L	FIBTEM CA10 had a sensitivity of 91 (72–93), specificity of 85 (84–86), PPV of 55 (45–60), NPV of 99 (97–100), and AUC of 0.96	

Prospective observational study of 23 patients with a median ISS of 38 (24–75)	INTEM and EXTEM	CT, CFT, MCF, EXTEM CA10 $\leq$ 10 mm, CA15 $\leq$ 12 mm, MCF $\leq$ 18 mm; LI30 $\leq$ 71%, LI60 $\leq$ 1%; FIBTEM; APTEM	Hyperfibrinolysis was defined as a ELT $<$ 90 min	EXTEM MCF correlated better with ELT ($r^2 = 0.68$) than LI60 ($r^2 = 0.63$), CA10 ($r^2 = 0.53$) or LI30 ($r^2 = 0.15$). ROTEM accurately and rapidly diagnosed hyperfibrinolysis. At the threshold of 18 mm (EXTEM MCF), 71% (LI30) and 7% (increase of APTEM MCF), the sensitivity for detection of hyperfibrinolysis was 100%, 75%, and 80%, respectively, with a specificity of 100%	Levrat et al. (2008)
Prospective observational field study of 25 combat trauma patients	EXTEM INTEM FIBTEM	CT, CFT, MCF, ML $<$ 15%	PT $>$ 18 s, and PTT $>$ 60 s	Standard laboratory testing showed that 10% of all patients were coagulopathic on admission compared with 64% with an abnormal ROTEM trace ($p = 0.0005$)	Doran et al. (2010)
Prospective observational study of 300 patients with ISS of 12 (4–25)	EXTEM with citrated blood	CT $>$ 94 s, $\alpha < 65°$, CA5 $\leq$ 35 mm	PT ratio $>$ 1.2	CFT, α, CA5, MCF significantly different in group with coagulopathy; CA5 predicts transfusion better than PT and rapidly diagnoses coagulopathy and predicts transfusion	Davenport et al. (2011)

(continued)

Table 2 (continued)

Study type	Tests and blood samples	Parameters and cutoff values	Definition of TIC/abnormal coagulation	Main findings	References
48 severe trauma patients including 31 battlefield casualties with ISS of 34	EXTEM with citrated blood	CA5 < 32 mm and CA10 < 40 mm	MCF < 40 mm	CA5 and CA10 predicted hypocoagulation in individual samples with sensitivities/specificities [95% CI] of 0.96 [0.91–1.00]/0.58 [0.45–0.71] (CA5) and 1.00/0.70 [0.58–0.82] (CA10), respectively	Woolley et al. (2013)
Observational cohort study of 808 patients with an median ISS of 16	EXTEM and FIBTEM	EXTEM CA5 ≤ 37 mm and FIBTEM CA5 ≤ 8 mm	INR > 1.2	Sensitivity for diagnosis of TIC is 66.3% (55.1–76.3) and 67.5% (55.9–77.8), respectively; specificity 81.2% (15.9–21.9) and 100–79.3 (17.7–23.9); PPV 29.9 (23.4–37.1) and 26.9 (20.8–33.8); NPV 95.2 (93.2–96.8) and 95.6 (93.5–97.1); AUC 0.79 (0.76–0.81) and 0.80 (0.77–0.83)	Hagemo et al. (2015)
Prospective observational study of 45 trauma patients with a median ISS of 24.0 (17.5–29.0)	EXTEM and FIBTEM	MCF 52–70 mm and 7–24 mm	INR ≥ 1.2 fibrinogen concentration < 1 g/L	EXTEM and FIBTEM MCF poorly detected coagulopathy as defined by INR ≥ 1.2 (AUC 0.564 (0.488–0.640); 0.609 (0.535–0.683), but well predicted hypofibrinogenemia	Peng et al. (2019)

				(fibrinogen concentration < 1 g/L) (AUC 0.920 (0.833–1.000); 0.962 (0.900–1.000)	
Prospective observational study of 40 trauma patients with a median ISS of 22 (CI 14–27)	EXTEM citrated blood	EXTEM CA5 ≤ 35 mm EXTEM LI30 < 97%	INR >1.2	A total of 35% patients showed EXTEM abnormality within which 20% were coagulopathic as defined by INR. In contrast, 55% patients presented with INR >1.2	Cohen et al. (2019)
Fibrinogen concentration					
Observational cohort study of 808 patients with an median ISS of 16	Clauss method	≤1.61 g/L	INR > 1.2	Fibrinogen concentration had a sensitivity of 73.6 (63.0–82.4), specificity of 100–11.5 (9.2–14.1), PPV of 45.1 (36.7–53.6), NPV of 96.3 (94.5–97.7), and AUC 0.87 (0.84–0.89) in detecting TIC	Hagemo et al. (2015)

Data represent medians and interquartile range, unless specified. *ACT* activated clotting time, *aPTT* activated partial thromboplastin time, *AUC* area under the curve, *CI* confidence interval, *ELT* euglobulin lysis time, *ETP* endogenous thrombin potential, *GCS* Glasgow coma scale, *INR* international normalized ratio, *ISS* injury severity score, *NPV* negative predictive value, *NR* not reported, *PPV* positive predictive value, *PT* prothrombin time, *TBI* traumatic brain injury, *TG* thrombin generation

with higher sensitivity (86% vs. 64%) than INR > 1.2 alone in military trauma patients (Cohen et al. 2019).

High-quality studies are need for diagnosis of TIC using viscoelastic devices (Sakamoto et al. 2017). Additionally, a clinical scoring system for assessing TIC, which includes subclassifications for the anatomic location of injury and interventions required for bleeding control, has been proposed (Neal et al. 2015). European trauma experts recommend a grading system comprising three severity levels based on fibrinogen level, INR, and platelet count, to define TIC (Černý et al. 2022).

Table 3 summarizes the predictive accuracy of TEG FF and ROTEM FIBTEM for hypofibrinogenemia in trauma. MA and MCF are the main parameters used for the predictions of hypofibrinogenemia and blood transfusions. The prediction accuracy was evaluated by sensitivity, specificity, and area under the receiver operating characteristic curve (AUC) and variate regression analyses. Different cutoff values of fibrinogen concentrations ranging from 1 to 1.8 g/L were used to define hypofibrinogenemia. Traditionally, a plasma fibrinogen level of 1 g/L was established for fibrinogen replacement in patients with congenital fibrinogen deficiency, whereas the threshold varied from 0.8 to 2.0 g/L in patients with acquired fibrinogen deficiency (Levy et al. 2014). In contrast, a critical fibrinogen concentration of 2.29 g/L was identified in trauma below which a significant increase in mortality occurred (Hagemo et al. 2014). The discrepancy implies that the negative impact of fibrinogen deficiency in trauma may have been underestimated. It should also be noted that hypofibrinogenemia prevalence in major bleeding varies across clinical contexts (McQuilten et al. 2017b).

Most clinical studies of hypofibrinogenemia in trauma are prospective observational, while a few are retrospective and randomized controlled. Sample size ranged from 23 to 1077 patients. In contrast with ROTEM, TEG FF has been used less to detect hypofibrinogenemia and predict blood transfusion requirements. Among various clinical settings, ROTEM FIBTEM has been mostly used in trauma, cardiac surgery, and liver transplantation with best predictive power for hypofibrinogenemia (fibrinogen <1.5 g/L) (AUC = 0.99) in cardiac surgery (Bhardwaj et al. 2017). Furthermore, several studies have shown that TEG FF and ROTEM FIBTEM could predict bleeding and transfusion requirements in trauma (Johansson et al. 2013; Schöchl et al. 2011), with various accuracies. It appeared that ROTEM would have better predictive accuracy than TEG because it has greater specificity for some common coagulopathies in cardiac surgery, such as fibrinogen deficiency. The averaged likelihood ratio of TEG FF MA for diagnosis of hypofibrinogenemia is 4.71 ± 2.18 based on a number of studies (Gautam et al. 2017; Meyer et al. 2015; Peng et al. 2018), while the corresponding value of ROTEM FIBTEM MCF is 9.24 ± 2.64 calculated from the literature (Meyer et al. 2015; Peng et al. 2018; Jeong et al. 2015).

Only a few studies demonstrated ROTEM FIBTEM provided faster and better prediction than plasma fibrinogen concentration for massive transfusion (Schöchl et al. 2011) and bleeding (Dötsch et al. 2017), respectively. ROTEM FIBTEM provided early prediction of massive transfusion in trauma similar to the most predictive laboratory parameters (e.g., fibrinogen and hemoglobin concentrations)

Table 3 Clinical evaluation of TEG and ROTEM functional fibrinogen tests for diagnosis of hypofibrinogenemia in trauma

Study design and patients	Blood collection and analysis	Findings	Ref.
TEG FF			
Randomized controlled trial of trauma patients at risk of significant hemorrhage ($n = 45$, ISS = 18–29) receiving either 6 g fibrinogen concentrate (RiaSTAP™) or placebo (normal saline)	Citrated whole blood was collected from the randomized trauma patients at admission, 1-, 3-, 11-, 23-, and 47-h post-infusion time. Standard FF TEG was performed on a computerized TEG Hemostasis System 5000 (Haemonetics Corporation, Haemoscope Division, Niles, IL, USA) according to the manufacturer's protocol	FF TEG MA predicted hypofibrinogenemia (fibrinogen concentration < 1 g/L) and 24-h plasma transfusion with high accuracies (AUC = 0.95, $p = 0.002$ and AUC = 0.70, $p = 0.042$)	Peng et al. (2018)
A prospective study of 182 adult trauma patients with a median ISS of 17 (9–26)	Blood was sampled immediately upon arrival to trauma center and evaluated in tissue factor-activated and platelet-inhibited TEG (i.e., FF TEG) precisely 1 h after sampling by a hemostasis analyzer system (TEG 5000, Haemonetics Corp., Braintree, MA) according to the manufacturer's recommendations. All analyses were conducted at 37 °C	Sensitivity, specificity and AUC of TEG FF MA for detection of fibrinogen <1.5 g/L were 77%, 81% and 0.869, respectively. TEG FF MA was also a univariate predictors of massive transfusion (>10 units of RBCs) at 6 and 24 h with odd ratios of 0.79 and 0.82 and mortality at 28 days with a hazard ratio of 0.84	Johansson et al. (2013), Meyer et al. (2015)
ROTEM FIBTEM			
Randomized controlled trial of trauma patients at risk of significant hemorrhage ($n = 45$, ISS = 18–29) receiving either 6 g FC (RiaSTAP™) or placebo (normal saline)	Citrated whole blood was collected from the trauma patients at admission, 1-, 3-, 11-, 23-, and 47-h post-infusion time. Standard ROTEM FIBTEM was performed on a ROTEM delta system (tem innovations GmbH, Munich, Germany; succeeded by instrumentation	ROTEM FIBTEM MCF predicted hypofibrinogenemia (fibrinogen concentration < 1 g/L) and 24-h plasma transfusion with high accuracies (AUC = 0.96, $p < 0.001$) and AUC = 0.72, $p = 0.023$)	Peng et al. (2018)

(continued)

Table 3 (continued)

Study design and patients	Blood collection and analysis	Findings	Ref.
	laboratory, Bedford, MA, USA) according to the manufacturer's protocol		
A prospective observational study of 88 trauma patients an median ISS score of 22 (12–34)	Blood samples were collected immediately after the patient's arrival to the trauma room (H0) and at 6 h (H6), 12 h (H12), and 24 h (H24) after admission, representing a total of 270 samples. The ROTEM measurements and standard coagulation tests were performed within 2 h of collection of blood samples	Sensitivity, specificity and AUC of FIBTEM A10 for detection of fibrinogen <1 g/L were 91%, 85% and 0.96, respectively	Rugeri et al. (2007)
A retrospective analysis of data from 323 patients with an injury severity score (ISS) ≥16 (20–50)	Blood samples were taken immediately upon admission to ER. ROTEM analyses (EXTEM, INTEM, FIBTEM) were typically performed at the bedside within minutes of sample collection. Fibrinogen concentration was measured by the Clauss method (STA-fib assay (Roche diagnostics GmbH); optical read-out), using a STA compact machine (Roche diagnostics GmbH, Vienna, Austria)	Sensitivity, specificity and AUC of FIBTEM A10/MCF for prediction of massive transfusion (≥10 units RBC transfused in 24 h) 63.3/77.5%, 83.2/74.9%, 0.83/0.84 (95% CI 0.78–0.87/0.79–0.88), similar to fibrinogen concentration	Schöchl et al. (2011)
A prospective cohort study of 517 trauma patients with a median ISS of 14 (8–27)	Blood was drawn from either the femoral vein or antecubital fossa into a 2.7-mL citrated vacutainer within 20 min of arrival in the emergency department (ED). ROTEM tests were performed within 2 h of blood draw with a ROTEM delta instrument, at 37 °C	Sensitivity, specificity and AUC of FIBTEM A5 for detection of fibrinogen <1.5 g/L 87%, 70% and 0.8 (95% CI 0.7–0.9)	Rourke et al. (2012)

(continued)

Table 3 (continued)

Study design and patients	Blood collection and analysis	Findings	Ref.
A prospective, single-center, noninterventional, noncontrolled, open clinical study of 50 trauma patients with a median ISS of 13 (4–66)	Blood was collected at hospital admission, 3- and 24-h after admission and analyzed by ROTEM assays (EXTEM and FIBTEM). EXTEM was considered positive if one of the four principle parameters (CT, CFT, MCF, and maximum lysis) greater than 20% of the expected highest or lowest normal value of the manufacturer normal value ranges (CT $\geq$ 94, CFT $\geq$ 190, MCF $\leq$ 40, ML $\leq$ 12). FIBTEM was considered positive if MCF was at least 20% smaller than the expected mean normal value (MCF $\leq$ 7)	Sensitivity, specificity, and AUC of FIBTEM MCF $<$ 7 mm within normal EXTEM patients are 100%, 90.2%, and 0.951 and 0%, 87.5%, and 0.563 for predictions of coagulopathy (INR $\geq$ 1.3) and mortality at 30 days	Tonglet et al. (2018)
A prospective study of 182 adult trauma patients with a median ISS of 17 (9–26)	Blood was sampled immediately on hospital arrival. FIBTEM assays were performed with citrated blood precisely 1 h after sampling according to the manufacturer's recommendations. Fibrinogen level was determined by Clauss method	Sensitivity, specificity and AUC of FIBTEM MCF $<$ 10 mm were 80%, 89%, and 0.889 for detection of fibrinogen $<$1.5 g/L	Meyer et al. (2015)

AUC area under the receiver operating characteristic curve, *CI* confidence interval, *CPB* cardiopulmonary bypass, *ICU* intensive care unit, *ISS* injury severity score

(Schöchl et al. 2011). A separate study comparing standard fibrinogen measurement methods (i.e., Clauss method and thrombin clotting time) with ROTEM FIBTEM in patients with cirrhosis suggested FIBTEM as a promising alternative to standard plasma fibrinogen measurement in cirrhotic patients, especially in evaluating fibrin polymerization disorders in these patients (Vucelic et al. 2015).

There is insufficient evidence or low-quality evidence for the benefits of TEG and ROTEM for the prediction of bleeding and adverse outcomes beyond that achieved using routinely measured baseline factors or CCTs except for rapidity. ROTEM EXTEM and FIBTEM were no better than routine laboratory tests for detecting

differences between surviving and nonsurviving critically ill patients (Larsson et al. 2015). ROTEM FIBTEM was not a good test to predict the presence of acute coagulopathy of trauma defined as an INR $>$ 1.3 or a fibrinogen level $<$ 1.5 g/L unless combined with EXTEM, and either of the tests could predict the need for emergent blood product transfusions (defined as $\geq$5 units of RBC and $\geq$ 3 units of plasma within the first 24 h of care) (Tonglet et al. 2018).

Finally, if fibrinogen deficiency has a causal relationship with bleeding and adverse clinical outcomes, it is sensible to suggest that TEG and ROTEM FF tests that improve clinical prediction for fibrinogen-related bleeding may also have the potential to predict adverse clinical outcomes. However, randomized trials are needed to provide high-quality evidence for the role of TEG and ROTEM in diagnosis, management, and monitoring of fibrinogen function and replacement in bleeding patients.

Assessment of the FF Level and Hemostatic Effect of Fibrinogen Replacement

The Clauss test is considered a standard FF test for determining the plasma fibrinogen level, although other methods, such as the prothrombin time-derived method (Blasi et al. 2012) and enzyme-linked immunosorbent assay (ELISA) (Kalina et al. 2008), are also used. However, ELISA does not discriminate between functional and nonfunctional immunoreactive fibrinogen proteins or even some fibrinogen degradation products (Mackie et al. 2002).

The Clauss method is limited to low levels of heparin (which inactivate thrombin through antithrombin III), which is a serious limitation to its use in cardiac surgery. It may be affected by fibrin degradation products and polymerization inhibitors as well as inhibitors of fibrin formation (Koh et al. 1994). Its turnaround time is approximately 40 min (Asmis 2015). In comparison, the TEG and ROTEM FF tests can be completed in 15 min and provide rapid and accurate detection of hyperfibrinolysis (Schöchl et al. 2010a). Another advantage of TEG and ROTEM is that they can be used for fully heparinized patients (Solomon et al. 2012; Gertler et al. 2011).

TEG has been used to study in vitro effects of fibrinogen on coagulation of plasma deficient in coagulation factors and diluted by colloids (Nielsen et al. 2005; Nielsen 2005). It has been used to monitor the effect of a cardiopulmonary bypass system with biocompatible coating on fibrinogen levels (Fluger et al. 2011). ROTEM has been used to determine the usefulness of fibrinogen substitution to reverse dilutional coagulopathy in in vitro (Fries et al. 2006), animal (Fries et al. 2005), and ex vivo models (Fenger-Eriksen et al. 2005). In vitro study showed dose-dependent increase in ROTEM MCF with the amount (0–3 mg/mL) of FC (Haemocomplettan P, CSL Behring GmbH, Marburg, Germany) added to normal human plasma pool, fibrinogen-deficient plasma pool, and individual plasma samples from 17 patients with fibrinogen deficiency (Kalina et al. 2008). All these studies showed that to various extents, fibrinogen improved clot strength (MA or MCF), clot formation (R or CT), and clot propagation (α) as measured by TEG or ROTEM.

In addition, ex vivo ROTEM studies indicated that administration of 6 g FC to samples of coagulopathic trauma patients could correct FIBTEM CA5 and MCF to the level of patients with minor injury (Rourke et al. 2012). In contrast, the ex vivo addition of cryoprecipitate at a standard dose of cryoprecipitate (equivalent to 2.6 g fibrinogen) was unable to reverse the coagulopathy until a high dose (equivalent to 7.8 g).

As summarized in Table 4, a number of clinical studies on TEG and ROTEM tests, especially those on ROTEM FIBTEM, have assessed hemostatic effects of FC administration in major trauma (Peng et al. 2019; Rourke et al. 2012; Ponschab et al. 2015; Schlimp et al. 2013a; Innerhofer et al. 2013, 2017; Schöchl et al. 2010b; Ziegler et al. 2021), including early cryoprecipitate transfusion (Curry et al. 2015), cardiovascular surgery with cardiopulmonary bypass (Schlimp and Schöchl 2014; Gautam et al. 2017; Meyer et al. 2015), liver transplantation (Görlinger et al. 2013; Peng et al. 2018), and orthopedic surgery (Jeong et al. 2015). Unless specified otherwise, the TEG and ROTEM tests were performed using TEG 5000 and ROTEM delta and the reagents and procedures recommended by the respective manufacturers.

Most of these clinical studies were randomized and controlled (Peng et al. 2019; Innerhofer et al. 2017; Curry et al. 2015; Ziegler et al. 2021; Nascimento et al. 2016), whereas a few were prospective, observational, or retrospective (Ponschab et al. 2015; Schlimp et al. 2013a; Innerhofer et al. 2013; Schöchl et al. 2010b; Seebold et al. 2019). Fibrinogen replacement was conducted pre-emptively or was guided by ROTEM or TEG. ROTEM FIBTEM has been well used in trauma, showing a dose-dependent increase in MCF immediately after fibrinogen administration. The hemostatic effect could last from 4 to 48 h (Peng et al. 2019; Wikkelsø et al. 2015). Furthermore, several studies have shown that the TEG FF- and ROTEM FIBTEM-measured hemostatic effect mirrored plasma fibrinogen profiles in response to fibrinogen replacement (Peng et al. 2019; Curry et al. 2015).

Some of these studies also used ROTEM to guide FC administration (Ponschab et al. 2015; Schlimp et al. 2013a; Innerhofer et al. 2013, 2017; Schöchl et al. 2010b). Few studies on the effects of FC administration on TEG FF have been reported (Peng et al. 2019), although some have shown a correlation between TEG FF MA and the Clauss fibrinogen level (Kornblith et al. 2014; Harr et al. 2013). Alternatively, TEG FF has been used to measure the effect of fibrinogen levels on heparin resistance/thromboprophylactic treatment in trauma (Harr et al. 2014).

Fibrinogen is not the only contributor to TEG FF and ROTEM FIBTEM CAs, which may limit their utility for the assessment of the hemostatic effect of fibrinogen replacement. Activated factor XIII and hematocrit levels may affect clot firmness as well (Schlimp et al. 2013b; Solomon et al. 2013b; Nielsen et al. 2004; Ogawa et al. 2012; Thomas et al. 2016). In one study, postoperative factor XIII levels correlated with FIBTEM MCF more significantly than fibrinogen levels in patients undergoing major upper gastrointestinal surgery (Thomas et al. 2016). The same study showed a significant correlation between platelet count and ROTEM FIBTEM MCF ($r = 0.55$,

Table 4 Hemostatic effects of fibrinogen replacement as measured by TEG and ROTEM

Clinical setting	Study design	Fibrinogen replacement and TEG/ROTEM test	Results	References
Preemptive fibrinogen replacement				
Trauma	Single-center, randomized, controlled, double-blind feasibility trial of adult trauma patients requiring blood transfusion and randomly and preemptively treated with FC ($n = 21$) or normal saline (placebo, $n = 24$)	Within 1 h after hospital admission, 95% of patients received a single dose of 6 g FC (RiaSTAP, CSL Behring GmbH, king of Prussia, PA, USA). ROTEM FIBTEM and TEG FF were performed at hospital admission and 2, 4, 12, 24, and 48 h after admission	TEG FF MA and ROTEM FIBTEM MCF mirrored plasma fibrinogen profiles and reached a maximum difference between the two groups at 1–3 h after fibrinogen administration. TEG FF MA for placebo patients was significantly lower than that for FC patients at all time points ($p \leq 0.019$) during the 48-hr hospitalization, except at admission ($p = 0.11$). ROTEM FIBTEM CT and MCF showed between-group differences 2–24 h after admission ($p \leq 0.028$ for CT and $P \leq 0.002$ for MCF)	Nascimento et al. (2016), Peng et al. (2019)
	Randomized, placebo-controlled, double-blind trial of adult trauma patients treated with FC ($n = 28$) or placebo (25) before hospital admission	FC (Clottafact, LFB, Les Ulis, France) at a dosage of 50 mg/kg body weight or an equivalent amount of placebo was administered on site or during transportation to the study center. ROTEM FIBTEM at baseline (onsite, prior to study drug administration) and on ED admission and 3, 9, 24, and 48 h and 7 days after ED admission	Median FIBTEM MCF decreased in the placebo group between the baseline and ED admission, from 12.5 (interquartile range: 10.5–14) mm to 11 (9.5–13) mm, $P = 0.0226$ but increased in the FC group from 13 (11–15) mm to 15 (13.5–17) mm, $P = 0.0062$. The median between-group difference in FIBTEM MCF was 5 (3–7) mm, $P < 0.0001$	Ziegler et al. (2021)

(continued)

Table 4 (continued)

Clinical setting	Study design	Fibrinogen replacement and TEG/ROTEM test	Results	References
	Randomized, controlled trial of adult trauma patients with ISS $\geq$ 15 who received 10 U of cryoprecipitate and MHT or MHT alone	Eighty-five percent of patients received cryoprecipitate (CRYO) within 90 min after hospital admission. Blood samples were drawn for ROTEM tests immediately upon admission, during active bleeding (immediately after transfusion of 4, 8, and 12 U RBC), and 24 and 72 h after randomization	FIBTEM data mirrored changes in Clauss fibrinogen levels, with higher FIBTEM CA5 and MCF levels observed in the CRYO arm during active bleeding. A significant rise in CA5 and MCF values for both FIBTEM and EXTEM measurements was seen between 24 and 72 h in both study arms ($P < 0.0001$), with a greater increase observed in the CRYO group	Curry et al. (2015)
Guided fibrinogen replacement				
Trauma	Single-center, parallel-group, open-label, randomized study of patients with an ISS > 15, bleeding signs, and FIBTEM CA10 < 9 mm or EXTEM CT > 90 s, randomly treated with FFP ($n = 48$) or CFC (primarily FC, $n = 52$)	ROTEM analyses were conducted at the ED and ICU and at 24 and 48 h after admission. Patients were randomized to receive FC (CSL Behring, Marburg, Germany) at 50 mg/kg body weight or placebo when FIBTEM CA10 < 9 mm or EXTEM CT > 90 s	EXTEM CT was shorter in the CFC group. EXTEM α and EXTEM CA10 worsened after FFP treatment, whereas they normalized quickly in patients receiving CFC. FIBTEM CA10 increased insufficiently with FFP, whereas values well above the threshold for transfusion were achieved with CFC. Most of these differences persisted until 24 h after admission, except EXTEM α, which was comparable between the two groups at 24 h after admission	Innerhofer et al. (2017)

(continued)

Table 4 (continued)

Clinical setting	Study design	Fibrinogen replacement and TEG/ROTEM test	Results	References
	Retrospective, observational study of 96 trauma patients with a median ISS of 34.0 (25.0–44.5) treated with FC only (FC group), FC and PCC (FC + PCC group), or PCC only (PCC group)	Blood samples for ROTEM tests (EXTEM, FIBTEM, and INTEM) were collected as soon as possible following ED admission and during initial operative treatment and ICU stay. Patients with severe coagulopathy upon admission received immediate treatment with both FC (Haemocomplettan P, CSL Behring) (6–8 g) and PCC (20–30 lU/kg body weight). Additional fibrinogen treatment was administered for a FIBTEM CA10 < 7 mm (target FIBTEM CA10: 10–12 mm). If EXTEM CT remained prolonged (>80 s) following FC treatment, PCC (Baxter, Vienna, Austria) was administered	Administration of FC resulted in reductions in EXTEM and FIBTEM CT and an increase in FIBTEM CA10 but had no effect on INTEM CT and CA10 and EXTEM CA10. Combined administration of FC and PCC increased FIBTEM MCF and normalized EXTEM CT but did not change either INTEM or FIBTEM CT. PCC therapy normalized EXTEM and FIBTEM CT and decreased CA10 in EXTEM, INTEM, and	Ponschab et al. (2015)
	Retrospective study of 157 trauma patients with a median ISS of 29, treated with FC alone (FC group), FC and PCC (FC + PCC group), or FC with PCC and FFP (FC + PCC + FFP group)	Blood samples were drawn following ER and ICU admission and at 24 h after admission for EXTEM and FIBTEM tests. FC (Haemocomplettan P) was administered at 2–6 g (2–4 g if initial FIBTEM CA10 = 4–6 mm and 6 g if FIBTEM CA10 = 0–3 mm)	EXTEM CT and CFT in the FC + PCC + FFP group were prolonged upon ICU admission; low MCF and reduced α were also observed in the FC + PCC + FFP group at the same time point. Between-group differences in all EXTEM parameters reached statistical significance upon ER and ICU	Schlimp et al. (2013a)

(continued)

Table 4 (continued)

Clinical setting	Study design	Fibrinogen replacement and TEG/ROTEM test	Results	References
			admission but not at 24 h FIBTEM CA10 increased between ER and ICU admission in the FC + PCC group but not in either of the other groups. FIBTEM CA10 was lower in the FC + PCC + FFP group than in the other two groups at ICU admission. No between-group differences were observed in any of these parameters at 24 h; all were in the normal range	
	Prospective study of 144 patients with major blunt trauma (ISS > 15) who received FC and/or PCC alone (CF group, $n = 66$) and were compared with those additionally receiving FFP transfusions (CF + FFP group, $n = 78$)	ROTEM was conducted using blood samples collected at ED admission and 4, 6, and 24 h thereafter. FC (Haemocomplettan P) was administered at 25–50 mg/kg body weight when fibrinogen <1.5–2.0 g/L, which indicates FIBTEM MCF < 7 mm	CF + FFP patients showed increased FIBTEM MCF at 4, 6, and 24 h when compared with that at ER admission. This group also showed higher FIBTEM MCF at 4 and 6 h than in the CF group	Innerhofer et al. (2013)
	Retrospective study of 131 trauma patients with a mean ISS of 38 ± 15 who received ≥5 U of RBC concentrate within 24 h	Blood was drawn immediately after ER and ICU admission. ROTEM tests were performed according to the manufacturer's recommendations within 5 min of blood sampling. When FIBTEM MCF < 10 mm, 2–4 g of FC (Haemocomplettan P) was administered.	On ER admission, the mean EXTEM MCF was 50 mm, and the median FIBTEM MCF was 6 mm, which is lower than the normal range (9–25 mm). The median EXTEM CT was 78 s, which is within the normal range (35–80 s). On ICU admission, ROTEM parameter	Schöchl et al. (2010b)

(continued)

Table 4 (continued)

Clinical setting	Study design	Fibrinogen replacement and TEG/ROTEM test	Results	References
		Patients showing prolonged EXTEM CT (> 1.5 times normal) received an additional 1000–1500 U PCC	values were comparable with preoperative values. Mean plasma fibrinogen was 1.26 g/L on ER admission and 1.50 g/L on ICU admission. The mean fibrinogen level only reached below-normal values 24 h after ER admission (2.28 g/L, normal range – 4.5 g/L)	
	Retrospective, observational study of 36 adult trauma patients with an ISS $\geq$ 15	ROTEM analysis was conducted at ED admission, before and after FC transfusion, after a bleeding episode, and 24–48 h after admission. Median of 22 min (IQR, 17–30 min) from time of FIBTEM CA5 analysis to FC administration. If FIBTEM CA5 $\leq$ 6 mm, an initial dose of 4 g FC was transfused	FIBTEM CA5 and the Clauss fibrinogen level were correlated (correlation coefficients 0.7–0.8), and both were increased significantly ($p < 0.05$) at 24 and 48 h after admission. One gram of FC raised the FIBTEM CA by approximately 1 mm	Seebold et al. (2019)

Abbreviations: *CA* clot amplitude, *CA5* clot amplitude at 5 min after CT measurement, *CA10* clot amplitude at 10 min after CT measurement, *CFC* coagulation factor concentrates, *CPB* cardiopulmonary bypass, *ED* emergency department, *ER* emergency room, *FC* fibrinogen concentrate, *FFP* fresh frozen plasma, *IQR* interquartile range, *MCF* maximum clot firmness, *PC* platelet concentrate, *PC* prothrombin complex concentrate, *ICU* intensive care unit, *ISS* injury severity score, *PC* platelet concentrate, *RBC* red blood cells, *ROTEM* rotational thromboelastometry, *MCF* maximum clot firmness, *TEG* thromboelastography, *FF* functional fibrinogen, *CT* coagulation time, *MHT* major hemorrhage therapy, *PPH* postpartum hemorrhage, *CFT* clot formation time

$p < 0.01$), which implied that the test might be profoundly impaired by the incomplete inhibition of platelet contribution to clot strength. Factor XIII levels and platelet count might also affect TEG FF (Gautam et al. 2017; Nielsen et al. 2004). The correlation between FIBTEM CA10 and Clauss fibrinogen became weaker as the hemoglobin level increased, suggesting that the hemoglobin level could influence the measurement of fibrinogen by FIBTEM (Mace et al. 2016). The

correlation could also be weakened by fibrinogen replacement in trauma patients (David et al. 2016).

Hemostatic effects, as measured by TEG and ROTEM, can be affected by resuscitation fluids. Fenger-Eriksen et al. (2010) assessed fibrinogen levels in plasma diluted in vitro with different fluids (isotonic saline, hydroxyethyl starch, human albumin), using an antigen determination method, three photo-optical Clauss methods, one mechanical Clauss method, a prothrombin-derived method, and viscoelastic measurement through ROTEM. Fibrinogen levels were overestimated by the photo-optical Clauss methods due to dilution with hydroxyethyl starch. In contrast, ROTEM FIBTEM MCF was reduced by dilution with hydroxyethyl starch and, to a lesser extent, by dilution with human albumin; the former effect was ascribed to an unexplained interference with the optical source by hydroxyethyl starch, and the latter was due to impairment of fibrin polymerization induced by the fluids. Mittermayr et al. (2007) reported that the magnitude of clot firmness reduction was determined by the type of fluid used in major orthopedic surgery. FIBTEM MCF was most strongly affected by hydroxyethyl starch, followed by gelatin solution and Ringer's lactate solution.

In addition to MA, other TEG parameters, e.g., estimated FLEV, kinetic time K, and α, and kaolin TEG K and α can be used to assess fibrinogen levels (Kornblith et al. 2014; Harr et al. 2013). Kornblith et al. (2014) confirmed a significant correlation between TEG FF FLEV and Clauss fibrinogen test in trauma patients, similar to results by Harr et al. (2013). However, the correlations were affected by the fibrinogen level; they decreased at low and high FLEVs (Harr et al. 2013).

In contrast, the FLEV estimated using TEG FF was, on average, 1.0 g/L higher than that determined by the Clauss method in both surgical patients and healthy controls (Fries et al. 2006). This is consistent with other reports of higher TEG FF FLEVs than Clauss values in cardiac surgery (Fries et al. 2005) and obstetric patients (Fenger-Eriksen et al. 2005).

Among all the parameters (kaolin TEG K, α, and MA), the strongest correlations have been reported between TEG FF MA/ROTEM FIBTEM MCF and the plasma fibrinogen level (Kornblith et al. 2014; Harr et al. 2013), suggesting that these parameters are the most useful for monitoring the role of fibrinogen in the hemostasis of bleeding patients.

TEG/ROTEM-Guided Fibrinogen Replacement

ROTEM has been widely used to guide FC administration in different perioperative settings, including trauma surgery, cardiovascular surgeries, and liver transplantation obstetric hemorrhage. Retro- and prospective studies of cardiac surgery have shown that FIBTEM-guided fibrinogen replacement generally reduces transfusion (Williams et al. 2017).

TEG and ROTEM have been mostly implemented during active bleeding situations in the emergency room and during surgery. As summarized in Table 5, case reports (Schöchl et al. 2010c, d; Ziegler et al. 2013; Brenni et al. 2010;

Table 5 Summary of TEG/ROTEM-guided fibrinogen replacement in trauma

Study design	Guiding protocol for fibrinogen replacement	Main results	References
TEG			
Randomized study of 111 adult trauma patients with a median ISS of 30 (24–43) treated with MTP directed by TEG or SLT	Rapid TEG was performed upon MTP activation on native whole blood within 5 min after collection. If ACT ≥140 s, 2 U FFP, 10 U of cryoprecipitate, and 1 U PC were transfused; if ACT was 111–139 s, 2 U FFP was transfused; if a < 63°, 10 packs of cryoprecipitate were transfused; if MA < 55 mm, 1 U PC was transfused	Mortality at 28 days was lower in the TEG group than in the SLT group (19.6% vs. 36.4%, $p = 0.049$). Less plasma and platelets were required in the TEG group than in the SLT group in the first 2 h of resuscitation	Gonzalez et al. (2016)
Prospective study of 182 adult trauma patients with a median ISS of 17 (9–26) in a level 1 trauma center	Blood was sampled immediately upon admission and was kept at room temperature until analyzed by kaolin and rapid TEG and TEG FF at 1 h after sampling. When TEG FF MA < 14 mm, 20–20 mL FFP/kg body weight, cryoprecipitate pool (3–5 mL/kg) or FC (adults 1–2 g) was transfused	Non-survivors showed lower clot strength by kaolin TEG and TEG FF and lower rapid TEG a and LY30 than survivors. None of the TEG variables were independent predictors of massive transfusion or mortality	Johansson et al. (2013)
Retrospective study of 390 and 442 adult patients (age ≥ 15 years) who received more than 10 RBC transfusions within 24 h before and after the implementation of HCR	Kaolin TEG was used during resuscitation and in the operation room and ICU. When α < 52°, 2 U FFP or 1–2 g FC was considered; R = 11–14 min, 2 U FFP or 10 mL FFP/kg body weight was considered; R > 14 min, 4 U FFP, or 20 mL FFP/kg body weight was considered; MA = 46–50 mm, 1 U PC was considered; MA < 46 mm, 2 U PC was considered	PC transfusion within 24 h from admission was increased from 1.7 U to 5 U and 30- and 90-day mortality were reduced from 31.5% to 20.4% and from 34.6% to 22.4%, respectively, as a result of TEG-guided HCR	Johansson and Stensballe (2009)

(continued)

Table 5 (continued)

Study design	Guiding protocol for fibrinogen replacement	Main results	References
Retrospective study of 165 and 124 trauma patients receiving ≥6 U of RBC within the first 24 h from treatment with TEG-guided or MTP resuscitation, respectively	TEG was performed in the operating room or ICU. If a < 45°, 0.6 U/kg cryoprecipitate; MA = 41–48 mm, 5 U of platelets; MA ≤ 40 mm, 10 U of platelets were transfused. MTP involved transfusion with a 1:1:1	There were no differences in volumes of blood products or mortality between the two groups. The mortality of the penetrating trauma patients who received ≥10 U RBC decreased from 54.1% for MTP to 33.3% for TEG-directed resuscitation ($p = 0.04$)	Tapia et al. (2013)
Case report of trauma patients treated with MTP transfusion, with a 1:1:1 ratio of RBC, FFP, and platelets followed by TEG- guided transfusion	TEG was performed as soon as a blood sample could be obtained. If R > 8 min FFP; K > 4 min or a < 47°, cryoprecipitate; MA < 54 mm, platelets were transfused	TEG allowed for judicious and protocol-assisted utilization of blood components and allowed more effective management of blood products and resuscitation	Walsh et al. (2011)
Retrospective study of 1974 adult patients with a median ISS of 17	Blood was collected on admission and analyzed by rapid TEG. When K > 2.5 min or a < 56° or MA < 55 mm, cryoprecipitate or FC was transfused (dosage not specified)	Rapid TEG was superior over SLT (PT, PTT, INR, platelet count, and fibrinogen) and identified patients with an increased risk of early RBC, plasma, and platelet transfusions, and fibrinolysis	Holcomb et al. (2012)
Case report of three trauma patients treated with TEG- guided transfusion within MTP	Rapid TEG was performed in the ED. if ACT >110 s, 2 U of FFP; if a < 63°, cryoprecipitate (dosage not specified); MA < 55 mm, PC was transfused	TEG-directed therapy showed the potential to be both cost-effective and lifesaving	Sawyer et al. (2012)
Retrospective study of 80 trauma patients with an ISS of 29 ± 1	Native whole blood samples were analyzed by rapid TEG with 10 μL of rapid TEG solution (8% kaolin, human recombinant tissue factor, phospholipids, buffers, and stabilizers), used as an activator, being added to 0.36 mL of whole blood within 4 min of blood	Clot shear elasticity (G) was an independent predictor of massive transfusion. For the prediction of mortality, G had the greatest adjusted AUC (0.93) compared with the AUCs for base deficit (0.87), INR (0.88), and PTT (0.89)	Pezold et al. (2012)

(continued)

Table 5 (continued)

Study design	Guiding protocol for fibrinogen replacement	Main results	References
	collection, placed in cuvettes, and warmed to 37.3 °C. if α < 60, cryoprecipitate was transfused		
ROTEM			
Case report of a 52-year-old severely injured male trauma patient who suffered a high-velocity motorcycle accident	ROTEM (EXTEM, INTEM, and FIBTEM) was performed immediately after admission, during surgery, and in the ICU. FIBTEM MCF was 4 mm at admission. Accordingly, 12 g of FC (RiaSTAP/ Haemocomplettan P, CSL Behring GmbH, Marburg, Germany) was infused as three doses of 4 g during surgery to increase the FIBTEM MCF to 10 mm. According to FIBTEM MCF = 8 mm at 6 h after ICU admission, another 2 g of FC was administered	EXTEM results showed a slightly prolonged CT of 85 s and reduced MCF of 49 mm at admission. EXTEM CT remained in the normal range throughout the surgical procedure, suggesting normal thrombin generation. On ICU admission, EXTEM revealed CT = 77 s, MCF = 47 mm, and FIBTEM MCF = 13 mm. The patient was fully recovered upon release from the hospital, 60 days after the accident	Schöchl et al. (2010c)
Case report of a 7-year-old boy with severe abdominal and pelvic injuries	Immediately on ED admission and 1 and 2 h into surgery, blood samples were taken for EXTEM and FIBTEM. One unit of RBC concentrate (250 mL), 0.5 g of FC (Haemocomplettan P), and 250 mL of crystalloid were administered on ED admission. When FIBTEM MCF = 9 mm at 1 h, 0.5 g of FC was transfused; when FIBTEM MCF = 8 at 2 h, 1 g of FC was administered	FFP and PC transfusions were avoided, showing the application potential of preemptive fibrinogen supplementation followed by a goal-directed, theragnostic approach to hemostatic therapy in pediatric trauma	Ziegler et al. (2013)

(continued)

Table 5 (continued)

Study design	Guiding protocol for fibrinogen replacement	Main results	References
Case report of a 24- year-old man with a severe blunt abdominal trauma	ROTEM FIBTEM was performed 30 min after hospital admission indicating afibrinogenemia, and 4 g of FC (Haemocomplettan P) was intravenously administered. FIBTEM was performed 1 h after admission due to persistent afibrinogenemia, and an additional 8 g of FC was administered, followed by administration of 4 g of FC 1 h later, resulting in normal FIBTEM	ROTEM-guided FC treatment was successful and avoided FFP and platelet transfusions. ROTEM provided better guidance than INR and PTT for treatment decisions	Brenni et al. (2010)
Case report of a 68-year-old male patient with serious craniofacial trauma and massive hemorrhage	A blood sample was taken for ROTEM analysis (EXTEM, FIBTEM) on ED admission. EXTEM CT and CFT were prolonged by 167 and 739 s, respectively; MCF was below normal, at 29 mm. FIBTEM CA10 was only 2 mm, and a shorter clotting time was observed in APTEM than in EXTEM. Tranexamic acid (2 g) was administered to correct fibrinolysis. The patient was then treated with 1000 IU PCC (Uman complex DI), 5 g FC (Haemocomplettan P), and 2 U of PC	The patient's coagulation was normalized 2 h after admission in terms of EXTEM CT (62 s), MCF (50 mm), and FIBTEM MCF (10 mm), suggesting the success of ROTEM-guided CFC therapy for massive hemorrhage associated with craniofacial injury	Grassetto et al. (2012)
Retrospective study of 681 trauma patients with an ISS $\geq$ 16, AIS for thorax and/or abdomen and/or extremity $\geq$3, and AIS for head/neck $<$5	ROTEM analyses were performed on ED and ICU admission. Hemostatic therapy consisted of administration of 2–4 g of FC (Haemocomplettan P, CSL Behring GmbH,	RBC transfusion was avoided in 29% of patients in the FC-PCC group compared with only 3% in the FFP group ($p < 0.001$). PC transfusion was avoided in 91% of patients in the FC-PCC group	Schochl et al. (2011)

(continued)

Table 5 (continued)

Study design	Guiding protocol for fibrinogen replacement	Main results	References
	Marburg, Germany) when FIBTEM MCF < 10 mm and administration of 1000–1500 IU of PCC for patients showing prolonged EXTEM CT (>1.5 times normal)	compared with 56% in the FFP group ($p < 0.001$). Mortality was comparable between the two groups: 7.5% in the FC-PCC group and 10.0% in the FFP group ($p = 0.69$)	
Retrospective study of 131 trauma patients with a mean ISS of 38 ± 15 who received ≥5 U of RBC concentrate within 24 h	Blood was drawn on ED and ICU admission for ROTEM analysis, per the manufacturer's recommendations, and the analyses were started within 5 min of blood sampling. When FIBTEM MCF < 10 mm, 2–4 g of FC (Haemocomplettan P) was administered. Patients showing prolonged EXTEM CT (>1.5 times normal) received an additional 1000–1500 IU PCC	The observed mortality was 24.4% lower than the TRISS mortality of 33.7% ($p = 0.032$) and the RISC mortality of 28.7% ($p > 0.05$). After excluding 17 patients with traumatic brain injury, the difference in mortality was 14% observed vs. 27.8% predicted by TRISS ($p = 0.0018$) and 24.3% predicted by RISC ($p = 0.014$). These results supported ROTEM-guided hemostatic therapy, with FC as a first-line hemostatic therapy	Schöchl et al. (2010b)
Prospective study of 144 patients with major blunt trauma (ISS > 15). Patients who received FC and/or PCC alone (CF group) were compared with those who additionally received FFP transfusion	ROTEM was conducted with blood samples collected at ED admission and 4, 6, and 24 h thereafter. FC (Haemocomplettan P) was administered to correct low fibrinogen level and/or poor fibrin polymerization (fibrinogen level < 1.5–2.0 g/L, which equals FIBTEM MCF < 7 mm) at 25–50 mg/kg body weight	Patients treated with CF alone showed sufficient hemostasis and received significantly fewer units of RBC and platelets than those in the FFP group. Fewer patients developed MOF or sepsis in the CF group than those in the FFP group. Propensity score matching ($n = 28$ pairs) used to reduce the impact of treatment selection confirmed that additional FFP administration showed no benefit in restoring hemostasis but was associated with higher RBC and platelet transfusion rates	Innerhofer et al. (2013)

(continued)

Table 5 (continued)

Study design	Guiding protocol for fibrinogen replacement	Main results	References
Retrospective study of 157 trauma patients with a median ISS of 29 treated with FC alone (FC group), FC and PCC (FC + PCC group), or FC with PCC and FFP (FC + PCC + FFP group)	Blood samples for EXTEM and FIBTEM tests were drawn on ED admission. FC (Haemocomplettan P) was administered at 2–6 g (2–4 g if initial FIBTEM CA10 was 4–6 mm; 6 g if initial FIBTEM CA10 was 0–3 mm)	Plasma fibrinogen levels were maintained within the normal range in all patient groups. Transfusion requirements were the highest in the FC + PCC + FFP group and the lowest in the FC group	Schlimp et al. (2013a)
Prospective, observational, descriptive study of 77 trauma patients, with a mean ISS score of 25.6, separated into three cohorts: Patients who received no coagulation therapy (NCT group), patients treated with FC only (FC group), and patients treated with both FC and PCC (FC + PCC group)	ROTEM tests (EXTEM, FIBTEM, and INTEM) were performed on ED admission, during initial operative treatment and ICU stay and every morning thereafter, up to day 7. For patients with obviously severe coagulopathy upon admission, both FC (Haemocomplettan P) (6–8 g) and PCC (20–30 lU/kg body weight) were immediately administered. Additional fibrinogen was administered when FIBTEM CA10 was 0–3 mm, 6 g FC, and when FIBTEM CA10 was 4–6 mm, 3–4 g FC, to target 10–12 mm. If EXTEM CT remained prolonged (> 80 s) following FC treatment, PCC (Baxter, Vienna, Austria) was administered	ETP was higher in the FC + PCC group than that in the NCT group on days 1–4 and that in the FC group on days 1–3. Fibrinogen increased over time, with no significant between-group differences after ER admission. PT and PTT were prolonged in the FC + PCC group from admission until day 3–4	Schöchl et al. (2014)
Retrospective observational study of 96 trauma patients with a median ISS of 34.0 (25.0–44.5) treated with FC only (FC group), FC and PCC (FC + PCC group), or PCC only (PCC group)		Administration of FC resulted in reductions in EXTEM and FIBTEM CT and an increase in FIBTEM CA10 but had no effect on INTEM CT, CA10, and EXTEM CA10. Combined administration of FC and PCC increased FIBTEM MCF and normalized EXTEM CT but did not affect INTEM or FIBTEM CT. PCC therapy normalized EXTEM and FIBTEM CT and decreased CA10 in EXTEM, INTEM, and FIBTEM	Ponschab et al. (2015)
Retrospective observational study of 435 trauma patients treated with (treatment group) or without (control group) FC		In the treatment group (median FC dose 6 g), the fibrinogen level was lower than that in the control group on admission and up to	Schlimp et al. (2016)

(continued)

Table 5 (continued)

Study design	Guiding protocol for fibrinogen replacement	Main results	References
		day 2. In patients receiving high (≥10 g) doses of FC, the fibrinogen level was lower up to day 5 than that in the control group. At other time points, there was no difference between the groups	
Retrospective, observational study of 36 adult patients with ISS ≥ 15	ROTEM analysis was conducted at various time points from ED admission to 48 h after admission. FIBTEM CA5 < 10 mm in the setting of significant hemorrhage triggered fibrinogen replacement with FC	The median time from FIBTEM CA5 analysis to FC administration was 22 min (IQR, 17–30 min). The FIBTEM CA5 and Clauss fibrinogen levels were correlated (spearman correlation coefficient, 0.7–0.8), and both were significantly increased ($p < 0.05$) by 24 h after admission	Seebold et al. (2019)
Randomized-controlled trial of 100 trauma patients with ISS > 15 treated with FFP (15 mL/kg body weight, $n = 48$) or CFC (primarily FC) (50 mg/kg body weight, $n = 52$)	ROTEM analyses were conducted at the ED, at 24 h at the ICU. Patients received FC (CSL Behring, Marburg, Germany) at 50 mg/kg body weight when FIBTEM CA10 < 9 mm and four-factor PCC at 20 lU/kg body weight when EXTEM CT > 90 s or prothrombin time index <35%	A higher proportion of patients in the FFP group required rescue therapy than that in the CFC group (52% vs. 4%, $p < 0.000\ 1$) and had an increased need for massive transfusion (30% in the FFP group vs. 12% in the CFC group, $p = 0.042$) than that in the FFP group. There was no difference in MOF between the two groups	Innerhofer et al. (2017)

Abbreviations: *AIS* abbreviated injury scale, *AUC* area under the curve, *CA5* clot amplitude at 5 min after CT measurement, *CA10* clot amplitude at 10 min after CT measurement, *CFC* coagulation factor concentrates, *CPB* cardiopulmonary bypass, *CT* clotting time, *ICU* intensive care unit, *ED* emergency department, *FC* fibrinogen concentrate, *FFP* fresh froze plasma, *HCR* hemostatic control resuscitation, *INR* international normalized ratio, *MCF* maximum clot firmness, *MOF* multiorgan failure, *MTP* massive transfusion protocol, *PC* platelet concentrate, *PCC* prothrombin complex concentrate, *PT* prothrombin time, *PTT* partial thromboplastin time, *RBC* red blood cell, *RISC* revised injury severity classification, *ROC* receiver operating characteristics, *SLT*, standard laboratory test, *TRISS* trauma injury severity score, *ROTEM* rotational thromboelastometry, *CFT* clot formation time, *ISS* injury severity score, *ETP* endogenous thrombin potential, *ED* emergency department, *IQR* interquartile range, *TEG* thromboelastography, *ACT* activated clotting time, *FF* functional fibrinogen

Grassetto et al. 2012), retrospective (Ponschab et al. 2015; Schlimp et al. 2013a, 2016; Schöchl et al. 2010b, 2011; Seebold et al. 2019) and prospective clinical studies (Innerhofer et al. 2013; Schöchl et al. 2014), and randomized controlled trials (Innerhofer et al. 2017) have demonstrated that ROTEM FIBTEM has been successfully used to guide fibrinogen administration in trauma, leading to reduced allogeneic blood transfusion (Innerhofer et al. 2013; Ziegler et al. 2013; Schöchl et al. 2010d).

In contrast, there are few studies on TEG-guided fibrinogen replacement across various clinical settings, with most focusing on trauma. Kaolin-activated TEG (Johansson and Stensballe 2009; Tapia et al. 2013; Walsh et al. 2011) and rapid TEG (Gonzalez et al. 2016; Holcomb et al. 2012; Sawyer et al. 2012; Pezold et al. 2012) rather than TEG FF were used to guide fibrinogen supplementation in these studies, and TEG α was used to guide fibrinogen supplementation, whereas MA was used to guide platelet transfusion. Some of these studies used FFP (Johansson and Stensballe 2009) and cryoprecipitate transfusion guided by TEG (Walsh et al. 2011; Pezold et al. 2012) instead of FC. Disadvantages of FFP and cryoprecipitate include the requirement for cold storage and time for thawing (17 min on average) (Curry et al. 2015), risk of viral transmission, and large administration volume. FFP contains a low fibrinogen level, which can vary greatly between batches, and, when administered in large volumes, may dilute plasma fibrinogen (McNamara et al. 2015).

The clinically best-studied FCs in the USA and Canada are Haemocomplettan P and RiaSTAP (CSL Behring GmbH, Marburg, Germany); other commercially available FC products include Clottagen (LFB Biomédicaments, Les Ulis, France) (Roullet et al. 2015), Fibrinogen HT (Benesis, Osaka, Japan), and FibroRAAS (Shanghai RAAS, Shanghai, China) (Franchini and Lippi 2012). Fibryga (Octapharma, Lachen, Switzerland) is a new, highly purified, lyophilized FC (Schulz et al. 2018). In vitro and clinical studies have shown a higher factor XIII level (10.1 IU/mL vs. 7.2 IU/mL) (Haas et al. 2018), slower clearance (0.665 mL/h/kg vs. 0.804 mL/h/kg), and a larger volume of distribution (70.158 mL/kg vs. 76.631 mL/kg) for Fibryga than for RiaSTAP (Ross et al. 2018). Another clinical study reported an even lower clearance (0.53 mL/h/kg) and lower distribution volume (50.7 mL/kg) for Clottafact (Djambas Khayat et al. 2019).

While FC is generally administered by bolus intravenous injection, one study showed potential advantages of using continuous infusion, as it allows rapid adjustments in the delivery rate in response to changing plasma levels (Morrison et al. 2012). It avoids or reduces peaks and troughs in the plasma fibrinogen level and allows the maintenance of satisfactory hemostasis during surgery.

Different critical fibrinogen levels and cutoff values for TEG and ROTEM have been used to guide fibrinogen replacement therapy in trauma (Theusinger et al. 2014; Mengoli et al. 2017; Nardi et al. 2015; Schöchl et al. 2012, 2013b; Fries et al. 2009; Lier et al. 2013; Görlinger et al. 2012) (Table 6). Most of these thresholds are parts of the ROTEM- or TEG-guided transfusion algorithms for different blood products (RBC, FFP, platelets) (Johansson et al. 2013; Stensballe et al. 2014; Johansson et al. 2014). Fibrinogen supplementation has been recommended for a plasma fibrinogen level below 1 g/L (Miceli et al. 2016), which approximately corresponds to a TEG

Table 6 Summary of threshold values for TEG- and ROTEM-guided fibrinogen replacement in trauma

Triggers	Fibrinogen dosage	References
FIBTEM MCF < 7 mm, which equals fibrinogen level < 1.5–2.0 g/L	25–50 mg/kg BW	Innerhofer et al. (2013)
FIBTEM MCF < 10 mm	2–4 g	Schöchl et al. (2010b, 2011)
FIBTEM CA5 = 4–6 mm FIBTEM CA5 = 2–4 mm FIBTEM CA5 < 2 mm	25 mg/kg BW 50 mg/kg BW 75 mg/kg BW	David et al. (2016)
FIBTEM CA10 < 7 mm	3–8 g	Schöchl et al. (2014)
Blood loss >50% with diffuse bleeding and FIBTEM MCF ≤ 7 mm	Fibrinogen 2–4 g (maximally 3 × 2 g), after 6 g fibrinogen factor XIII was administered	Theusinger et al. (2014)
Blood loss >60% with ongoing diffuse bleeding, EXTEM/INTEM CT normal, MCF < 40 mm, and FIBTEM MCF < 7 mm	Fibrinogen up to 6 g, followed by factor XIII 15 U/kg BW	
FIBTEM CA10 ≤ 7 mm	2–4 g	Nardi et al. (2015)
FIBTEM CA10 = 0–3 mm FIBTEM CA10 = 4–6 mm	6 g 2–4 g Until FIBTEM CA10 = 10–12 mm	Schöchl et al. (2013a, b), Schlimp et al. (2013a)
FIBTEM CA10 < 7 mm	2–6 g until FIBTEM CA10 = 10–12 mm	Schöchl et al. (2012)
EXTEM CA10 < 45 mm and FIBTEM CA10 < 15 mm	2–6 g	Lier et al. (2013)
FIBTEM CA5 < 5 mm with bleeding or ongoing surgery and FIBTEM CA20 < 10 mm	50 mg/kg BW	Fries et al. (2009)
TEG FF MA < 14 mm	1–2 g	Johansson et al. (2013, 2014)
Rapid TEG K > 2.5 min, α < 56°	Unspecified	
TEG FF MA 7–14 mm TEG FF MA 0–7 mm	20 mg/kg BW 30 mg/kg BW	Stensballe et al. (2014)
FIBTEM MCF 6–9 mm FIBTEM MCF 0–6 mm	20 mg/kg BW 30 mg/kg BW	
EXTEM CA10 < 40 mm and FIBTEM CA10 < 10 mm	20–50–100 mg/kg BW	Görlinger et al. (2012)

Unless specified otherwise, TEG 5000 and ROTEM delta were used to guide fibrinogen replacement

Abbreviations: *BW* body weight, *CA* clot amplitude, *CA5/10/20* clot amplitude at 5/10/20 min after CT measurement, *MCF* maximum clot firmness, *CT* coagulation time, *TEG* thromboelastography, *K* kinetic time, *a* angle, *FF* functional fibrinogen, *MA* maximum amplitude

FF MA of 16 mm and ROTEM FIBTEM MCF of 8 mm (Peng et al. 2019). The abovementioned values of TEG FF MA and ROTEM FIBTEM MCF are both higher than the lower thresholds of the normal ranges for the TEG FF (11–24 mm) and

ROTEM FIBTEM (7–24 mm) tests recommended by the respective manufacturers. This agrees with a report that the frequently recommended threshold for fibrinogen substitution of 9 mm MCF in FIBTEM does not match the recommended threshold of $\leq$1.0 g/L plasma fibrinogen measured by the Clauss method, although there was a strong correlation between FIBTEM MCF and Claus fibrinogen ($r > 0.8$) (Requena et al. 2011). These discrepancies should be considered carefully when developing goal-guided fibrinogen replacement using TEG and ROTEM.

Fibrinogen levels of 0.8–2.0 g/L have been recommended as transfusion triggers in trauma and massive hemorrhage (Levy et al. 2014; Kaufner et al. 2016), with a level of 1 g/L being reported in most guidelines for fibrinogen replacement (McQuilten et al. 2017b). Accordingly, a range of CA10 and MCF values in FIBTEM, including CA10 < 7 mm (target FIBTEM CA10: 10–12 mm) (Ponschab et al. 2015; Schöchl et al. 2012) or MCF <7 mm in trauma (Innerhofer et al. 2013), CA < 8 mm in cardiac surgery (Weber et al. 2014), and MCF < 8 mm in liver transplantation (Goerlinger 2006), have been used to trigger fibrinogen replacement. Moreover, FIBTEM CA10 or MCF can be used to determine the FC dosage. For example, 2–4 g FC was required in trauma patients if FIBTEM CA10 was 4–6 mm; and 6 g FC was required if FIBTEM CA10 was 0–3 mm (Schlimp et al. 2013a). FC administration has also been based on the plasma fibrinogen level, with varying thresholds (Weiss et al. 2011; Danés et al. 2008). Specifically, the fibrinogen dosage can be calculated based on the desired increment in fibrinogen level, as follows (Lier et al. 2013):

$$\text{Fibrinogen dosage (g)} = 0.05 \times \text{desired increment} \left(\frac{g}{L}\right) \times \text{body weight (kg)}$$

There are fewer studies on TEG-guided fibrinogen transfusion in trauma (Gonzalez et al. 2016; Holcomb et al. 2012; Johansson and Stensballe 2009; Tapia et al. 2013; Walsh et al. 2011; Pezold et al. 2012). Compared with ROTEM FIBTEM, TEG FF, which uses a platelet inhibitor, has been less employed to measure fibrinogen levels and guide its administration. TEG FF MA < 14 mm has been used to trigger fibrinogen supplementation in patients with massive hemorrhage (Johansson et al. 2014), and MA $\leq$ 7 mm has been used in liver transplantation (De Pietri et al. 2016). Kaolin or rapid TEG K and α has been used to guide fibrinogen supplementation with cryoprecipitate in trauma (Schöchl et al. 2013a; Tapia et al. 2013; Brazzel 2013; Gonzalez et al. 2010; Stahel et al. 2009; Kashuk et al. 2009, 2012), but may not be as good as TEG FF MA, which is a more direct measure of the plasma fibrinogen level (Harr et al. 2013; Solomon et al. 2015).

Compared with ROTEM MCF, TEG α, in particular, kaolin-activated TEG α, is most commonly used to guide fibrinogen replacement (mostly using a cryoprecipitate), whereas TEG MA is generally used to guide platelet transfusion (Solomon et al. 2015). However, TEG MA could not distinguish fibrinogen from platelet deficiency when a single TEG test was conducted without platelet inhibitors; thus, its use in guiding fibrinogen transfusion may be limited (Kashuk et al. 2012).

These results underline the necessity to implement different individual triggers for fibrinogen supplementation, depending on the viscoelastic hemostatic tests used and the clinical settings. For example, in bleeding trauma patients, a FIBTEM CA10 $\leq$ 7 mm may trigger FC administration, with a target MCF of 10–12 mm. In contrast, when using TEG FF, MA $<$ 14 mm is recommended as a trigger (Schochl et al. 2016).

Cutoff values of kaolin TEG K $>$ 2.4 min, $\alpha < 60.6°$, and MA $<$ 51.2 mm have been recommended for the diagnosis and treatment of severe hypofibrinogenemia (fibrinogen $<$1 g/L) in trauma patients, whereas K could be used to guide early cryoprecipitate or FC transfusion (Chow et al. 2019).

One study suggested that the CA 10 min after R or CT reflects a more dynamic phase of the hemostatic process than MA/MCF and may lead to earlier goal-directed transfusion therapy (Meyer et al. 2014). FIBTEM and APTEM have been used in combination with EXTEM to guide platelet transfusion and the treatment of hyperfibrinolysis with tranexamic acid (Smith et al. 2020; Juffermans et al. 2019), respectively.

Mini-Dictionary of Terms

- *Trauma-induced coagulopathy (TIC). TIC normally refers to acute traumatic coagulopathy which consists of two core components: (1) trauma itself, tissue damage- and hypoperfusion-induced endogenous TIC and (2) resuscitation-associated exogenous TIC involving hypothermia, acidosis, and hemodilution.*
- *Viscoelastic hemostatic tests. These tests measure changes in viscoelastic properties of whole blood during clot formation, buildup, and degradation. The most commonly used devices are thrombelastography (TEG 5000) and rotational thromboelastometry (ROTEM delta).*
- *Conventional coagulation tests (CCTs). These tests also refer to standard laboratory tests typically including prothrombin time (PT) and activated partial thromboplastin time (aPTT), Clauss fibrinogen test, and platelet count.*
- *Fibrinogen concentrate. It is plasma-derived, highly purified concentrate of lyophilized human fibrinogen and needs to be reconstituted with sterile water for infusion.*
- *Hypofibrinogenemia. It is normally defined as plasma fibrinogen concentration below 1.5 g/L.*
- *Fibrinogen replacement. It is treatment of fibrinogen deficiency with exogenous fibrinogen via infusion of fibrinogen concentrate or cryoprecipitate.*

Key Facts of Trauma

- *Trauma is a major global public health issue, causing nearly six million deaths worldwide each year.*

- *It is the leading cause of death in people aged 18–39 years.*
- *Hemorrhage is the most common cause of preventable deaths after trauma.*

Summary Points

- *TEG and ROTEM tests play important roles in early diagnosis of TIC and its phenotypes, assessment, and guidance of fibrinogen replacement. Their potential clinical benefits are often inferred from trauma and cardiac surgery literature.*
- *ROTEM FIBTEM MCF has been mostly used to discriminate fibrinogen deficiencies and assess hemostatic effects of fibrinogen replacement compared to kaolin and rapid TEG, and TEG FF parameters including K, α, and MA.*
- *When using TEG FF and ROTEM FIBTEM to diagnose fibrinogen deficiency and guide fibrinogen administration, other variables, such as hematocrit, factor XIII levels, resuscitation fluids, and fibrinogen level ranges, should be considered.*
- *Since TEG FF and ROTEM FIBTEM test results have shown the strongest correlation with plasma fibrinogen level and provided the greatest discrimination of fibrinogen deficiencies, these tests are recommended for guiding fibrinogen replacement and monitoring its hemostatic effects.*
- *Studies comparing TEG FF and ROTEM FIBTEM suggest a stronger correlation of the latter with the plasma fibrinogen level, likely owing to its more effective elimination of the platelet contribution to clot strength.*
- *Studies supporting the use of TEG FF and ROTEM FIBTEM are limited to trauma and surgical bleeding patients. Even without robust clinical data, TEG and ROTEM are likely to remain popular for the hemostatic management of bleeding patients.*
- *Future studies comparing the different intervention thresholds for TEG and ROTEM and the therapeutic effects of predefined thresholds for fibrinogen augmentation are required to optimize fibrinogen administration (i.e., dosage and time of fibrinogen administration) to improve its efficacy and patient safety and to reduce costs in various clinical settings. Studies comparing preemptive and guided fibrinogen replacement are also warranted.*

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Radiotracers, Positron Emission Tomography Imaging and Traumatic Brain Injury

15

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Abstract

Over the last few decades, the expanded application of nuclear medicine has produced extensive knowledge of the pathophysiological processes conferred in traumatic brain injury. The use of metabolic radiotracers has shown to introduce hypermetabolism in the hyperacute phase of injury, and subsequent hypometabolism has shown to correlate to the degree of initial impact and gradual return to a baseline of cerebral activity. Similarly, the application of inflammatory radiotracers has suggested a notable increase in neuroinflammation in the acute period of traumatic injury. In the more chronic time course of traumatic brain injury, metabolic, inflammatory, and protein radiotracers have been able to uncover several pathophysiological processes including chronic levels of hypometabolism and neuroinflammation as well as increased prion deposition as linked to cognitive decline and neurodegenerative conditions. In totality, radioisotope imaging has shown to be the leading modality in forwarding the in vivo pathophysiological understanding of traumatic brain injury.

Keywords

Traumatic brain injury · TBI · Concussion · Positron emission tomography · PET · Single-photon emission computed tomography · SPECT · Chronic traumatic encephalopathy · CTE · Nuclear medicine · Neurology

Abbreviations

AD	Alzheimer's disease
alpha-syn	α-synuclein
APOE	epsilon4 allele-apolipoprotein E genotypes
APP	amyloid precursor protein
Aβ	amyloid-β
BACE	beta-site APP-cleaving enzyme
BBB	blood–brain barrier
CBF	cerebral blood flow
CSF	cerebral spine fluid
CT	Computed Tomography
CTE	chronic traumatic encephalopathy
DAI	diffuse axonal injury
DTI	diffuse tensor imaging

[18F]FDDNP	2-(1-{6-[(2-[18F]fluoroethyl)(methyl) amino]-2- naphthyl} ethylidene) malononitrile;
[18F]Florbetapi r	4-[(E)- 2-[6-[2-[2-(2-(18F)fluoranylethoxy)ethoxy]ethoxy] pyridin-3-yl]ethenyl]- N-methylaniline
[18F]FDG	2-deoxy2-(18F)fluoro-D-glucose;
[18F]Flortaucipir	7-(6-(18F)fluoranylpyridin-3-yl)-5Hpyrido[4,3-b]indole
MAO-A	monoamine oxidase A
MAO-B	monoamine oxidase B
Moderate TBI	moderate traumatic brain injury
MRI	Magnetic Resonance Imaging
mTBI	mild traumatic brain injury
NFT	neurofibrillary tangles
[11C]PBB3	[11C]pyridinyl-butadienyl-benzothiazole 3
[11C]PiB	2-(4′-[11C]methylaminophenyl)-6-hydroxybenzothiazole
PCS	post-concussive syndrome
PD	Parkinson's disease
PET	Positron emission tomography
PHF	paired protein filaments
PVC	partial volume correction
SD	spreading depression
SPECT	Single-photon emission computed tomography
sTBI	severe traumatic brain injury
SUV	standardized uptake values
SUVR	SUV ratio
[18F]THK-5351	(2S)-1-(18F)fluoranyl3-[2-[6-(methylamino)pyridin-3-yl] quinolin-6-yl]oxypropan-2-ol
TBI	traumatic brain injury
TDP-43	TAR DNA-binding protein 43

Introduction

Over the last few decades, the expanded application of nuclear medicine has produced extensive knowledge of the pathophysiological processes conferred in traumatic brain injury. The use of metabolic radiotracers has shown to induce hypermetabolism in the hyperacute phase of injury, and subsequent hypometabolism has shown to have correlated to the degree of initial impact and gradual return to a baseline of cerebral activity. Similarly, the application of inflammatory radiotracers has suggested a notable increase in neuroinflammation in the acute period of traumatic injury. In the more chronic time course of traumatic brain injury, metabolic, inflammatory, and protein radiotracers have been able to uncover several pathophysiological processes including chronic levels of hypometabolism and neuroinflammation as well as increased prion deposition as linked to cognitive decline and neurodegenerative conditions. In totality, radioisotope imaging has

shown to be the leading radiological modality in forwarding the in vivo pathophysiological understanding of traumatic brain injury.

Defining Traumatic Brain Injury

Traumatic brain injury (TBI) is a type of brain injury caused by a physical impact that leads to a temporary disturbance of neurological, behavioral, and cognitive functioning. The two main physical forces that can damage the brain are blunt and inertial forces (Ghajar 2000). Inertial forces cause mild TBI (mTBI), otherwise known as concussions. Conversely, blunt forces cause moderate to severe TBI (Ghajar 2000). Furthermore, shearing forces, which are caused by rotational movement, can cause TBI as well. To assess the severity of the TBI, several scales can be used and include the Canadian Head CT rule, the Glasgow Coma Scale, and New Orleans Criteria (Teasdale 2014). Generally, mTBI cases do not require the use of brain imaging techniques as they often resolve after a period of time. However, neuroimaging can assess the damage caused by moderate to severe TBI (Teasdale 2014).

Epidemiology of Traumatic Brain Injury

People who are in military combat or play sports have an increased likelihood of sustaining TBI because the nature of these activities increases the chances for physical impact (Langlois et al. 2006). Military combatants have increased chances of receiving mild, moderate, and severe TBI (Langlois et al. 2006). Athletes, particularly in soccer and American football, are at higher risk of receiving mTBI and repeated TBI. Furthermore, those who are involved in motor vehicle accidents also have an elevated risk of sustaining a TBI (Langlois et al. 2006). Outside of the type of activity being performed, gender and age also influence the likelihood of obtaining TBI (Langlois et al. 2006). While performing similar activities, females and older individuals have been found to more prone to mTBI than men (Ayubcha et al. 2021).

Acute Consequences of mTBI: Primary and Secondary Injury

The two types of injury that arise from mTBI are defined as “primary brain injuries” and “secondary brain injuries.” Primary brain injuries are those that refer to the brain damage of the impact that caused the mTBI and can refer to macroscopic or microscopic lesions (Greve and Zink 2009). Some examples of macroscopic primary brain injuries that can be imaged using computed tomography (CT) and magnetic resonance imaging (MRI) include axonal shearing, cranial fractures, and cerebral hemorrhages (Kim and Gean 2011). With respect to microscopic damage, diffuse axonal injuries (DAIs) are most encountered and can be assessed via diffusion tensor

imaging (DTI) MRI (Kim and Gean 2011). These DAIs can affect both the local area where they appear, as well as more distal regions via anterograde and retrograde neuronal degeneration (Kim and Gean 2011).

Secondary brain injuries refer to the indirect ramifications associated with the mTBI (Greve and Zink 2009). Physical brain trauma can produce abnormal ionic fluxes that lead to imbalances with sodium, calcium, magnesium, and potassium concentrations, which are important ions that generate neural signals across neurons (George et al. 2019). Furthermore, the initial brain trauma may also cause the indiscriminate release of excitatory neurotransmitters that can further exacerbate ionic imbalances (George et al. 2019). In particular, release of excitatory transmitters, such as glutamate, can activate NMDA receptors in neurons, which would cause widespread neuronal depolarization or spreading depression (SD) by allowing sodium and calcium ions into the neuron (George et al. 2019). Increased intracellular calcium concentrations promote mitochondrial calcium sequestration, which impairs mitochondrial function and causes the brain to enter an immediate state of hypermetabolism. As a means of restoring normal ion concentrations, hypermetabolic states overuse adenosine triphosphatase (ATPase) pumps (Lozano et al. 2015).

In this period of hypermetabolism, cerebral blood flow (CBF) is reduced, so that glycolysis is primarily responsible for satisfying metabolic needs during this hyperacute phase (Giza and Hovda 2014). After this immediate period of hypermetabolism, a less transient hypometabolic state occurs. Hypometabolic periods persist for up to 10 days as found in studies of rodent animal models (Giza and Hovda 2014) and up to 30 days in humans. Furthermore, this period is associated with abnormal neuronal functioning (e.g., axonal and mitochondrial disruptions), lingering cognitive impairment, and persistently decreased CBF. Many hypotheses have been proposed to explain this observed decrease in CBF, including abnormal vasculature morphology, blood-brain barrier (BBB) dysfunction, and parenchymal dysfunction (Zetterberg et al. 2013; Shetty et al. 2014; Sorby-Adams et al. 2017; Monson et al. 2019), but the exact contribution of each proposed etiology is presently unknown.

Acute Consequences of mTBI: Neuroinflammatory Responses

Another phenomenon that occurs during the hypometabolic state is acute activation of peripheral immune cells and microglial cell-mediated neuroinflammatory response pathways (Ramlackhansingh et al. 2011). The activation of these pathways causes the creation and proliferation of inflammatory cytokines, adhesion molecules, and growth factors, which can speed up neurological recovery and structural repair while also increasing neurotoxicity (Bennett et al. 2016). Following more severe TBI, inflammatory cytokines such as IL-6, IL-8, and IL-10 accumulate in the cerebral spinal fluid (CSF) and peripheral blood pool (Ziebell and Morganti-Kossmann 2010). However, only low amounts of these inflammatory biomarkers have been detected after mTBI (Zhang et al. 2016). Cell death from direct impact and cell-mediated apoptosis are two other primary and secondary aspects of TBI,

respectively. There is rarely enough force associated with single-event mTBIs to trigger cell death; hence there is no severe hemorrhage or other macrostructural cranial abnormalities. However, the activation of apoptotic pathways can cause modest cell death, albeit this level of cell death in mTBI patients does not hinder complete recovery (Giza and Hovda 2014).

Single-event mTBI typically does not result in chronic symptoms, but very rarely after an mTBI event, there may be long-term emotional, behavioral, and cognitive changes. Due to the subjectivity of symptoms and the influence of preexisting diseases (e.g., psychiatric disorders, depression, socioeconomic conditions), these patients are diagnosed with post-concussive syndrome (PCS), the cause of which is still unknown (Ryan and Warden 2003). Nonetheless, many patients with PCS have persistent aberrant neurophysiological states including low CBF and hypometabolism (Churchill et al. 2017), which usually improve within a year (Ryan and Warden 2003).

Repeat mTBI

Repeat mTBI is a phenomenon where a person suffers from successive TBIs within a 24-hour period after a single mTBI event. Immediately after a single mTBI event, a period of metabolic vulnerability occurs that lasts approximately 24 hours (Kamins and Giza 2016). A second TBI during this window of vulnerability may worsen all of the immediate physiological and anatomical repercussions of mTBI previously outlined (Kamins and Giza 2016). For example, when compared to the sum of individual mTBI recovery durations, a second mTBI occurrence can lengthen the period of hypometabolism before the return to baseline (Kamins and Giza 2016). Furthermore, the second mTBI increases the risk of cytoskeletal damage, DAI, apoptotic pathway activation, atrophy, and other structural effects (Prins et al. 2013). A repeat TBI has also been shown to postpone the restoration of adequate BBB function (Hay et al. 2015).

Repeat mTBI can lead to chronic conditions during or after the metabolic vulnerability phase. Axonal dysfunction, increased membrane fluidity, structural abnormalities, microglial-activated inflammation, and BBB failure are some of the chronic problems that result from repeated mTBI (Ramlackhansingh et al. 2011; Bailes et al. 2013). Furthermore, repeat mTBIs have also been linked to long-term cognitive impairment, neuroplasticity changes, and chronic neuroinflammation (Vascak et al. 2017). Existing evidence indicates that repeated trauma to the head can lead to chronic conditions, and shorter intervals between these concussions may intensify their consequences (Eisenberg et al. 2013).

Repeat mTBI: Neurodegenerative Disorders

The link between repeated mTBIs and neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and CTE is an emerging field of study. Repeat mTBIs have been linked to an increased risk of dementia. Furthermore, severe TBIs have been linked to the development of AD and PD (Mortimer

et al. 1991). Given these two observations, it is likely that mTBIs may be a risk factor for AD and PD (Mortimer et al. 1991). TBI and the development of Alzheimer's disease, Parkinson's disease, and other degenerative diseases are thought to be associated to aberrant protein proliferation, inflammatory processes, atrophy, and neuronal hypometabolism (Joie et al. 2012).

Chronic Traumatic Encephalopathy

Those with repeated mTBI events are more susceptible to developing a condition called chronic traumatic encephalopathy (CTE). CTE is a tauopathy, which is a type of disease where tau, a type of protein found throughout the brain involved in the stabilization of micro tumbles, among other processes, misfolds and forms aggregates that damage neurons (Omalu et al. 2010). Furthermore, tau aggregates are able to spread to different parts of the brain. Depression, memory and cognitive impairment, impulsivity, emotional instability, and substance addiction are all symptoms of CTE (Asken et al. 2016). CTE is diagnosed by the presence of one or more perivascular phosphorylated tau (p-tau) lesions in small blood vessels. Diagnosis of CTE is currently only done in a *postmortem* setting (Fig. 1). However,

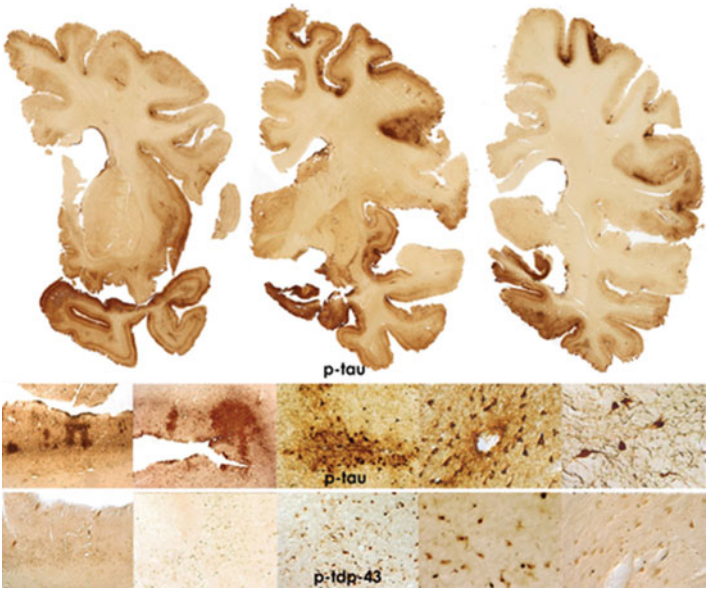

Fig. 1 Microscopic changes in stage IV chronic traumatic encephalopathy (CTE). Whole mount coronal sections in stage IV CTE show widespread p-tau pathology affecting most regions of the cerebral cortex and medial temporal lobe. Astrocytic tangles are prominent, and there is marked neuronal loss in the cortex, amygdala, and hippocampus. There are also widespread pTDP-43 abnormalities. All images: 50 μ tissue sections, CP-13 or p-TDP-43 immunostain. (The figure has been reproduced with permission of the copyright holder)

because the tau protein accumulated in CTE has a unique distribution in the brain, in vivo imaging techniques like PET may provide an avenue for diagnosing CTE in living patients (Table 1).

Table 1 Pathophysiological stages of chronic traumatic encephalopathy

Stage	Macroscopic observations	Microscopic observations
I	• Unremarkable	• Principal characteristic: One to two isolated perivascular focal epicenters of immunoreactive p-tau neurofibrillary tangles (NFT) and neurites (lesions) at the depths of the cerebral sulci in the parietal, frontal, and temporal cortices. • Beta-amyloid plaques are absent in subjects below 50 years of age. • P-tau-reactive microglia may exist
II	• Mild enlargement of lateral ventricles • Varying enlargement of the third ventricle • Pallor of the locus coeruleus and substantia nigra	• Principal characteristic: Multiple perivascular foci consisting of p-tau NFT, pretangles, and neurites are found in multiple cortices and the superficial cortical layers of surrounding regions such as the gyral crests and sulcal walls. • Active microglia exist in the subcortical white matter with surrounding axonal swelling. • Beta-amyloid plaques are absent in subjects below 50 years of age
III	• Decreased brain weight • Mild atrophy in the frontal and temporal lobes • Enlargement of the lateral and third ventricles • Potential cavum septum pellucidum, septal fenestration, or perforation in some patients	• Principal characteristic: Multiple perivascular neurites, p-tau NFT, pretangles, and neurofibrillary lesions of degeneration in the amygdala, hippocampus, entorhinal cortex, and perirhinal cortex. • Diminished myelinated nerve fibers, axonal dystrophy, and axonal loss can be observed. • Beta-amyloid plaques are rarely observed. • TAR DNA-binding protein 43 (TDP-43) immunopositive neurites may exist in some cases
IV	• Deceased brain weight • Cerebral atrophy within the temporal, frontal, and medial frontal lobes along with the anterior thalamus • Diffuse white matter and corpus callosum atrophy • Cavum septum, perforations, fenestration, or absence of posterior septum in most patients	• Principal characteristic: Dense p-tau foci ubiquitously distributed in the cerebrum, diencephalon, and brainstem. • Neuronal degeneration of the cortex, astrocytosis in the white matter, gliosis, loss of myelinated nerve fibers, axonal dystrophy, and TDP-43 immunopositive neurites are observed in most cases. • Beta-amyloid plaques may be observed

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Chronic Traumatic Encephalopathy: Theories

The primary risk factor for CTE is repeated mTBI (McKee et al. 2013). In TBI and trauma, the existence of neurofibrillary tangles (NFTs) and microglial activation is linked to the proliferation of different proteins (Gardner and Yaffe 2015). In addition, DAIs are linked to abnormal accumulations of amyloid precursor protein (APP), beta-site APP-cleaving enzyme (BACE), amyloid-(A), tau protein, alpha-synuclein (α-syn), TAR DNA-binding protein 43 (TDP-43), and Lewy body proteins [20].The primary protein associated with TBI is the tau protein. After TBI, tau accumulation and propagation are shown to occur at higher rates than those of Aβ plaques (Zetterberg et al. 2013). Notably, the predominance of tau NFTs and the absence of Aβ in CTE (McKee et al. 2013) are strikingly similar to the ratio of Aβ to tau fluid biomarkers in TBI-related DAI. Furthermore, α-syn fluid indicators are enhanced shorty after TBI, despite the fact that patients with CTE have been observed to have elevated concentrations of Lewy body proteins rather than α-syn deposits (Adams et al. 2018). In addition, the presence of TDP-43 in CTE has also suggested as a possible etiology of dementia connected to mTBI. In summary, mTBI is linked to CTE by abnormal protein growth and retention in response to a single or repeated mTBI (Smith et al. 2013), where the most distinctive protein pathology is the production of tau NFTs (Turner et al. 2013).

Another theory that links mTBI to CTE involves neuroinflammation, which proposes that a single or repeated TBI may chronically activate microglia-regulated inflammatory pathways, leading to inflammation, apoptosis, and tissue degradation (Johnson et al. 2013); these three effects are similar to atrophy and active microglial cells, as well as elevated cytokines, in CTE patients (Turner et al. 2013). One final theory proposes that there are hereditary variables that predispose people to both TBI and CTE sensitivity (Zhang et al. 2015). For example, studies have revealed that the existence of specific apolipoprotein E genotypes (e.g., the APOE epsilon4 allele) increases the risk of boxers developing more severe chronic neurological impairments and that these genotypes have recently been related to CTE and other neurodegenerative illnesses (Lenihan and Jordan 2015).

Positron Emission Tomography Imaging

Positron emission tomography (PET) is a molecular imaging technique that visualizes the in vivo spatial distribution of radiotracer molecules (Zimmer and Luxen 2012). Radiotracers are given to individuals, and a unique distribution of the radiotracer within the subject can be determined based on the subject's physiology and the radiotracer used (Le Bars 2006). The radiotracer then undergoes radioactive $\beta +$ decay, which is determined by the radioactive isotope's half-life. The PET scanner records this decay, and calculations are used to determine the spatial origin of the positron emitted [96]. PET scans are frequently used in conjunction with CT or MR images to improve picture contrast and spatial resolution, as well as to overlay radiotracer concentration and structural anatomy (Zimmer and Luxen 2012).

The most accurate type of PET is dynamic PET, which involves using dynamic PET scans and blood serum levels to gather continuous radiotracer activity data after injection (Huang (Henry) Sung-Cheng 2000). To evaluate tissue uptake, mathematical modelling is employed to account for transportation rates between the blood and tissue compartments, and the results are quantified using Ki values (Huang (Henry) Sung-Cheng 2000). Although dynamic PET produces superior data, it also requires lengthy picture acquisition times and complicated techniques. To overcome these drawbacks, semiquantitative approaches for tracking radiotracers are frequently used (Huang (Henry) Sung-Cheng 2000). The most often used approach is the standardized uptake value (SUV), which expresses radiotracer uptake normalized for radioactive decay and body weight, lean body mass, or body surface (Basu et al. 2011). SUV is determined by the ratio of nontarget tissue radiotracer uptake to the amount of radiotracer accessible in the blood plasma for target tissue uptake (Huang (Henry) Sung-Cheng 2000). SUV ratios (SUVR), which is the ratio of the SUV between the target anatomy and an anatomical structure not impacted by disease with stable SUV, can also be used to compare SUV among patients (Knešaurek et al. 2018). Though there is evidence of minor bias in SUV values when compared to Ki values, the two parameters are thought to be associated especially when collecting more scans for shorter time periods (Basu et al. 2011). SUV has been shown to be adequately repeatable in tissues with a high radiotracer uptake (Knešaurek et al. 2018).

Inflammatory Neuroimaging

Neuroinflammation has been implicated in a range of neurodegenerative and trauma-associated diseases, including dementia, psychosis, TBI, and multiple sclerosis, to name a few (Cagnin et al. 2002; Stefaniak and O'Brien 2016). Recent developments in PET imaging have provided highly detailed pictures of the neuroinflammatory environment. Imaging cerebral inflammation can work via several methods, including targeting immunocompetent cells, targeting a compromised blood-brain barrier, identifying immune cells that have passed through the blood-brain barrier which do not typically exist in the central nervous system, or imaging the end-stage consequences of neuroinflammation (e.g., apoptosis) (Deddens et al. 2012; Albrecht et al. 2016; Cumming et al. 2018) and will be the primary focus of this section.

In vivo imaging of neuroinflammation is dependent on the activity of cells, cytokines, and other proinflammatory molecules that circulate throughout the CNS. In particular, monocytes, ion channels, matrix metalloproteases, and microRNAs have been implicated in the propagation of cerebral inflammation. Existing research suggests that monocytes play several roles in neuroinflammation. They have been found to stimulate endothelial cells, thus allowing transmigration of lymphocytes across the blood-brain barrier (Man et al. 2012). Monocytes are also known to contribute to phagocytic activity within the brain (Man et al. 2012). Similarly, matrix metalloproteins (MMPs) are released by microglial cells and have been known to influence the permeability of the BBB, stimulate production of pro-inflammatory cytokines, and recruit astrocytes (in patients with

neuroinflammatory diseases), making MMPs a key player in neuroinflammation and a strong potential target for imaging studies (Haorah et al. 2008; Woo et al. 2008; Yang et al. 2015).

Current research suggests that TBI initiates neuroinflammatory processes through activation of glial cells, recruitment of immune cells that circulate in the periphery, and the release of pro-inflammatory mediators (e.g., cytokines or MMPs) in the brain (Xiong et al. 2018). TBI can cause macrophages outside of the CNS to infiltrate into the CNS (Loane and Kumar 2016), thereby stimulating inflammatory processes within the brain. Further, microglia, which often serve as neuroprotective and restorative cells after TBI, can become dysfunctional and produce proinflammatory mediators which prolong the effects of TBI (Loane and Kumar 2016). Further, those with repeated mTBI show prolonged inflammation, which can lead to the degenerative processes (Aungst et al. 2014) documented in dementia, CTE, and cognitive impairment (Fig. 2).

Neuroinflammation imaging has become a useful tool to study TBI. In one study using single-photon emission computed tomography (SPECT) to examine neuroinflammation after mTBI, the authors found significantly higher binding to the 18kD translocator protein (TSPO). TSPO is a molecule found primarily on the outer mitochondrial membrane implicated in many inflammatory processes in the brain, both during the 2 weeks after injury and 3–4 months post injury, with binding levels peaking in those with post-concussive syndrome (Ebert et al. 2019). Higher binding to TSPO has been confirmed in PET imaging studies as well, which find that binding of TSPO ligands remain chronically elevated years after TBI (Ramlackhansingh et al. 2011). Another study examining the effect of repeated TBI among former National Football League (NFL) players on brain inflammation and atrophy found significantly greater inflammation in the supramarginal gyrus and

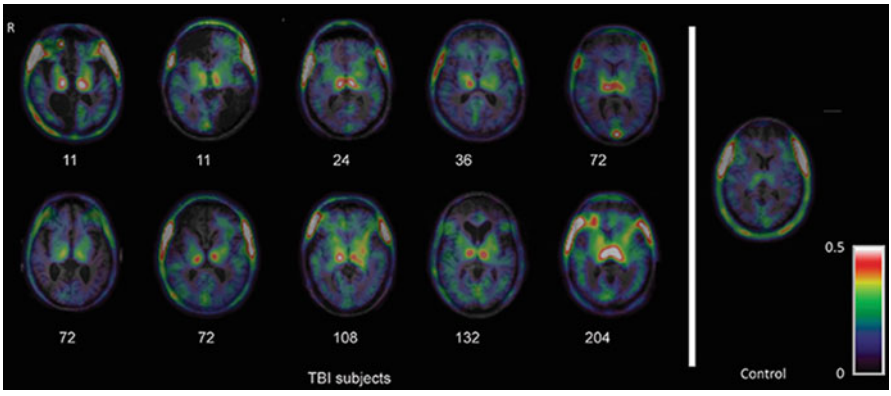

Fig. 2 Imaging of chronic microglial activation after TBI. Images of [11C]PK11195 PET images are shown superimposed on the T1 MRI scan at the level of the thalamus for 10 TBI patients, 11 months to 17 years after injury, and a representative control participant. Numbers indicate time since injury (months) R right (Ramlackhansingh et al., 2011). (The figure has been reproduced with permission of the copyright holder)

right amygdala compared to age-matched healthy controls (Coughlin et al. 2015). Although MRI is used to assess BBB integrity through administration of gadolinium (Rebeles et al. 2006), it is not used to directly assess neuroinflammation. It is, however, useful in identifying trauma associated with TBI (e.g., hemorrhage, contusion) (Thelin et al. 2017).

Metabolic Imaging

Radiotracer molecules' biological activity is critical for capturing the desired situation. For example, the glucose analog 2-deoxy-2(18F)fluoro-D-glucose ([18F]FDG) was created as a radiotracer to monitor the rate of cellular glycolysis, which indicates metabolic activity (Alavi and Reivich 2002). The scientific value and repeatability of [18F] FDG in measuring the numerous metabolic processes identified in TBI have been established by decades of broad and consistent use (Byrnes et al. 2014). The initial uptake of [18F] FDG into a cell is determined by the rates of local perfusion and cellular glycolysis (Byrnes et al. 2014). In addition, the radiotracer is scanned after a lag period to overcome transient inhomogeneities in local perfusion, allowing [18F]FDG absorption to be more directly linked to cellular glycolysis (Ayubcha et al., 2021). Furthermore, [18F]FDG-PET has been regularly employed in the investigation of TBI (Byrnes et al. 2014), with metabolism and CBF being used to measure morphological and functional alterations in the brain (Ayubcha et al., 2021).

The pathophysiological understanding of single-event TBI, namely, the hyperacute phase of TBI with hypermetabolism (Marklund et al. 2009) and the subacute phase of TBI with hypometabolism (Langfitt et al. 1986), has correlated well with [18F]FDG studies. Early [18F]FDG PET tests revealed widespread hypometabolism, especially in the occipital lobes, which gradually returned to normal metabolism (Alavi 1989). Hypometabolism with and without notable lesions has been identified in several acute phase [18F]FDG studies (Alavi 1989). Finally, some investigations were able to link local hypometabolism in specific grey and white matter areas to specific cognitive and behavioral parameters (Marklund et al. 2009). The implications of persistent hypometabolism have been studied further in animal models using [18F]FDG. Widespread hypometabolism in the hippocampus, corpus callosum, and amygdala along the ipsilateral and contralateral cortex has been linked to atrophic structural alterations, cell malfunction, and axonal injury in these investigations (Brabazon et al. 2017). Other [18F]FDG investigations in mTBI populations have linked cerebral hypometabolism to rapid eye movement (REM) cycle anomalies and aberrant gray to white matter metabolic activity ratios (Marklund et al. 2009).

Most early [18F]FDG PET studies focused on imaging severe TBI (sTBI) cases. Within a month after impact, [18F]FDG imaging revealed substantial structural damage and local hypermetabolism (Bergsneider et al. 1997). Furthermore, after sTBI, higher levels of [18F]FDG uptake were associated with better clinical status and long-term results (Yamaki et al. 1996). [18F] Hypermetabolism can arise 6 hours after a sTBI or moderate TBI but only occurs promptly after a mTBI, according to

FDG investigations (Yamaki et al. 1996). Nonetheless, mTBI, moderate TBI, and sTBI all have a long period of widespread hypometabolism, with the severity of the metabolic drop and the time of metabolic recovery correlated with the degree of TBI (Yamaki et al. 1996). [18F]FDG imaging has also been used to study repeat mTBI in animal models, showing that shorter time intervals between the initial and second mTBI impacts caused increased neuronal degeneration, impairment (i.e., cognitive and sensorimotor), microglial activation, and astrocyte activation (Yamaki et al. 1996). According to [18F]FDG imaging, repeat mTBI patients had considerably lower global [18F]FDG uptake, which was linked to worse symptoms and outcomes (Ayubcha et al. 2021) (Fig. 3).

Memory deficits, cognitive, auditory, somatic, and sensorimotor impairments have all been examined in [18F]FDG investigations following a single-event mTBI. The temporal lobes, hippocampus, and prefrontal cortex may exhibit decreased uptake, although the limbic system may show higher activity (Ayubcha et al. 2021). Other parts of the brain that may be injured by DAIs show decreased metabolic activity in the bilateral frontal lobes, temporal lobes, thalamus, and cerebellum (Kato et al. 2007). The injuries sustained after mTBI can cause symptoms such as worse memory performance, depression, and change in intelligence quotients depending on the region that is injured. Interestingly, [18F]FDG PET has scarcely been used in the context of CTE. Currently, CTE can only be diagnosed *postmortem*. [18F]FDG PET, on the other hand, is a promising approach that could be used to diagnose CTE in vivo by revealing significant neuronal, inflammatory, and even cognitive and behavioral problems (Ayubcha et al. 2021). However, it is important to note that [18F]FDG PET has some limitations that may interfere with its

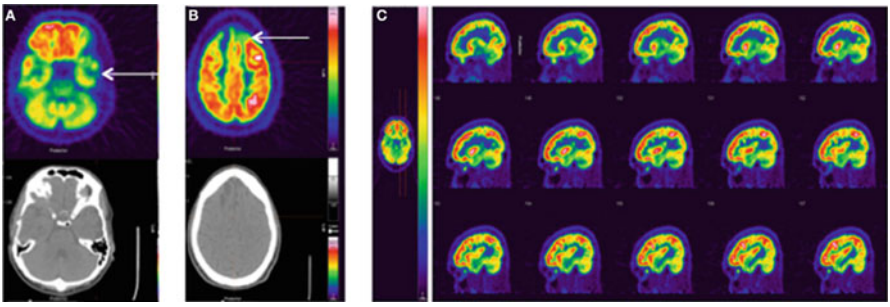

Fig. 3 (**a**) 25-year-old male, single non-blast moderate TBI, imaged 5 months post-injury. No recorded medications or sleep difficulties, pain/headache, or vision problems. FDG-PET shows left temporal hypometabolism associated with mild volume loss/encephalomalacia on the CT. (**b**) 28-year-old male, single non-blast severe TBI, imaged 12.5 months post-injury. No recorded medications or sleep difficulties, pain/headache, or vision problems. FDG-PET shows a more severe injury with prominent hypometabolism frontally (arrow) associated with encephalomalacia. (**c**) 35-year-old male, history of repeat exposure to blast-related mTBI, imaged 43 months post-injury. Pain medication (Ultram), mild body pain, and moderate sleep problems, no findings CT. FDG-PET shows prominent frontal hypermetabolism, which may be medication-related. The color bar displayed in (**c**) applies to all images with red representing greater FDG uptake (Byrnes et al. 2014). (The figure has been reproduced with permission of the copyright holder)

ability to be used in the diagnosis of CTE. [18F]FDG is a nonspecific marker for a variety of injuries. This suggests that [18F]FDG PET will detect damage caused by mTBI, such as inflammatory processes, cell dysfunction, atrophy, and other metabolic abnormalities that may be present in other tissues. [18F]FDG PET may not reflect a single process but rather the sum of numerous processes occurring in various tissues. Furthermore, PET imaging can only produce macroscopic images of the brain, so assessment of smaller brain regions will be difficult to obtain and reproduce.

Tau Imaging

Several neurodegenerative diseases including frontotemporal dementia (FTD), AD, and CTE are classified as tauopathies, a type of disease that involves the aggregation and propagation of pathogenic tau protein. Under normal conditions, tau proteins are produced in cells to stabilize microtubule structures (Rigney et al. 2021). Individual tau proteins are phosphorylated to allow them to form one of three structures: twisted, straight, and coupled protein filaments (PHF) (Rigney et al. 2021). However, tau proteins may become hyperphosphorylated, which causes a change in their conformation to make them more prone to aggregation into tau-based NFTs, which can be toxic to neurons. Furthermore, this aggregated form of the protein can spread to other neurons, thereby propagating the disease to different parts of the brain (Shoghi-Jadid et al. 2002). Interestingly, different tauopathies have characteristic propagation patterns of tau NFTs. In the case of CTE, pathogenic tau species accumulate in the medial temporal lobe and move via the cortical default mode network, with little or no involvement of subcortical regions (Omalu et al. 2010).

The goal of early research was to create an imaging agent that would only attach to fibrillar insoluble protein aggregates. 2-(1-[(2-[18F]fluoroethyl)(methyl) amino] 2-(1-[(2-[18F]fluoroethyl)(methyl) amino] 2-(1-[(2-[18F] [18F]FDDNP(−2-naphthyl ethyli- dene) malononitrile ([18F]FDDNP) was the first radiotracer to reflect prion accumulation in human patients (Shoghi-Jadid et al. 2002). The binding of [18F]FDDNP to NFT was initially thought to be extremely specific in vitro, but it was later demonstrated to have insufficient specificity and selectivity, since it was able to attach to numerous types of beta-sheet structures, including Aβ. As a result, a number of new radiotracers have been developed (Rigney et al. 2021). Individual radiotracers have distinct in vitro binding patterns, with certain tracers binding to specific tau proteins or aggregates (e.g., NFTs) but not others (e.g., pretangles). However, several tracers (e.g., MAO-A, MAO-B, A) still show considerable off-target binding (Leuzy et al. 2019). Furthermore, tracers in development for tau imaging are inconsistent among each other in terms of producing a characteristic pattern of tau pathology for specific tauopathies in vivo; although initial in vitro testing showed radiotracer binding to tau, in vivo work showed similar uptake in unrelated tissues without notable tau load (Leuzy et al. 2019).

Tau imaging has long been used to research dementia and other neurodegenerative diseases, but in recent years, more studies have employed tau imaging to

examine the brains of patients who have had multiple mTBIs and are thus at a higher risk of developing CTE. Unfortunately, the evidence is still restricted to case studies and small studies with insufficient statistical power to account for variability in uptake caused by tau tracers' poor resolution and binding specificity. Furthermore, several studies show that differences in uptake between TBI or suspected CTE groups occur in brain areas influenced substantially by the partial volume effect. As such, the use of tau imaging to diagnose CTE in vivo is still a developing field of study. More specialized tracers and validation methodologies should be developed in the future to confirm correct in vivo binding in CTE cases (Fig. 4).

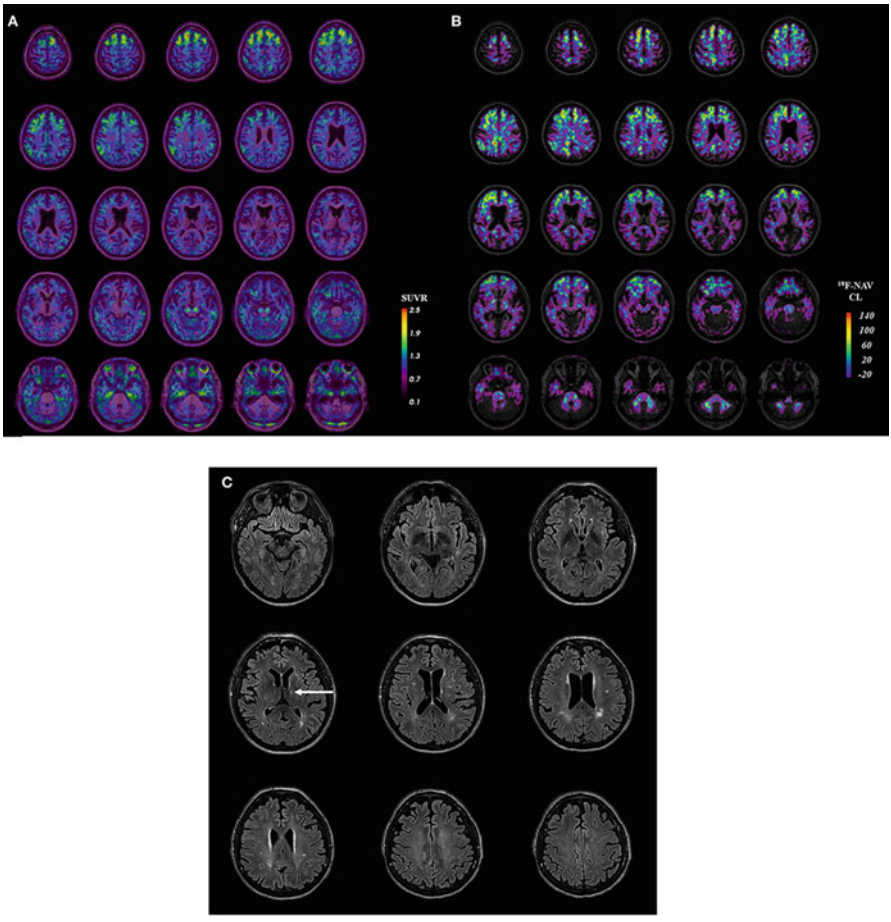

Fig. 4 Case participants. (**a**) Tau PET image. (**b**) Amyloid PET image. (**c**) Axial FLAIR image. Participant (**a**) 18F-MK6240 tau PET image in SUVR (standardized uptake value ratio; using the cerebellar cortex as the reference region) coregistered onto T1 MRI. (**b**) Participant's 18F-NAV4694 amyloid PET image in the Centiloid scale. (**c**) Participant's MRI (representative axial FLAIR sequences) with the arrow indicating his prior left thalamic infarct (Krishnadas et al. 2020). (The figure has been reproduced with permission of the copyright holder)

Amyloid Imaging

In addition to tau radiotracers, Aβ tracers are also being explored in imaging CTE. Aβ is an intrinsically disordered protein that can aggregate into cross beta-sheet fibrils that consist of a repeating linear array of peptide backbones (Lee et al. 2018). Although the presence and accumulation of Aβ in mTBI and CTE are extremely minimal, some radiotracers for Aβ are also being applied to CTE, including [11C]-Pittsburgh Compound ([11C]PiB), [18F]Florbetapir, and [18F]FDDNP (Ayubcha et al. 2021). As previously stated, [18F]FDDNP indiscriminately binds to both tau and Aβ NFTs. On the other hand, [11C]PiB and [18F]Florbetapir are intended to bind Aβ plaques exclusively(Mitsis et al. 2014; Lee et al. 2018).

The use of [11C]PiB and [18F]Florbetapir PET radiotracers in imaging CTE has been controversial, as they have been shown to exhibit off-site binding, which can lead to false positives for CTE in the PET scans being obtained. Furthermore, at imaging-level concentrations, these tracers have lower binding affinities, implying that they may create insufficient contrast between the bound Aβ protein and background tissues (Moghbel et al. 2012). Finally, similar to tau tracers, the efficacy of Aβ tracers may be influenced by the in vivo environment such that a tracer that is shown to work in vitro may have less pronounced or no effects in vivo (Moghbel et al. 2012). Furthermore, in vivo levels of Aβ in CTE are low, limiting the utility of Aβ-specific tracers in imaging early stages of CTE; however, advanced CTE does present with a few small Aβ centers in different brain areas. In view of PET's foundational needs, significant off-sight binding, minimal Aβ concentrations in CTE, and non-conducive in vivo environments limit the use of amyloid imaging for CTE. Unfortunately, limited studies on Aβ imaging for CTE also hamper the exploration of these tracers for use in CTE. Furthermore, the few studies that exist have failed to show that [18F]Florbetapir and [11C]PiB are useful for imaging CTE, as they have conflicting results regarding the uptake of these radiotracers when compared to control groups. Therefore, the use of Aβ tracers in CTE does not seem promising yet.

Conclusions

The development of PET has produced enormous knowledge regarding our understanding of disease processes. In the context of traumatic brain injury, such an effect is equally felt. The use of specific radiotracers to assess the prevalence of particular biomarkers associated with the processes of TBI. With evolving understandings of the chronic consequences of TBI in the form of neurodegenerative conditions such as CTE, AD and PD, there is even more opportunity for in vivo PET imaging to enhance our understanding of these conditions. Through the continued application of PET, the in vivo pathophysiological processes of TBI will become even more clear and further lay the basis for prospective therapeutic development and treatment monitoring. As such, PET will maintain its role as an indispensable imaging modality in the field of TBI.

Mini-Dictionary of Terms

- Traumatic brain injury – a type of brain injury caused by a physical impact that leads to a temporary disturbance of neurological, behavioral, and cognitive functioning.
- Positron emission tomography – a molecular imaging technique that visualizes the in vivo spatial distribution of radiotracer molecules.
- Chronic traumatic encephalopathy – a tauopathy, which is a type of disease where tau, a type of protein found throughout the brain involved in the stabilization of micro tumbles, among other processes, misfolds and forms aggregates that damage neurons.
- Tau proteins – intraneuronal proteins used to stabilize microtubule structures; pathological hyperphosphorylation can lead to prion formation.
- Beta-amyloid plaque – interneuronal accumulations of malformed beta-amyloid proteins that are insoluble.

Key Facts of Traumatic Brain Injury

- Traumatic brain injury is common in motor vehicle accidents, sporting activities, and falls.
- The severity of neurological trauma can be graded as mild, moderate, or severe.
- Widespread deficiencies in neurological functioning can occur in traumatic brain injury; however, the degree and number of sequalae correlate to the severity of impact.
- *Postmortem* studies have found that severe or repeated mild injury can be a significant risk factor for neurodegenerative diseases, namely, chronic traumatic encephalopathy.
- Molecular imaging modalities have been able to detect in vivo neurodegenerative changes that have occurred in those at higher risk for chronic traumatic encephalopathy.

Summary Points

- The use of metabolic radiotracers has shown hypermetabolism induced in the hyperacute phase of traumatic brain injury.
- Acute and subacute traumatic brain injuries are correlated to the degree of initial impact and gradual return to a baseline of cerebral neurometabolic activity.
- Inflammatory radiotracers suggest a notable increase in neuroinflammation in the acute period of traumatic injury.
- In severe traumatic brain injury and repeat traumatic brain injury, chronic levels of hypometabolism and neuroinflammation are observed.

- Tau radiotracers are found to accumulate in severe traumatic brain injury and repeat traumatic brain injury populations in conjunction with clinical symptoms of chronic traumatic encephalopathy.

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Circulating Polyunsaturated Fatty Acids (PUFAs) as Biological Indicators in Trauma

16

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Contents

Abstract

Dietary polyunsaturated fatty acids (PUFAs) play a vital role in cell growth, development, and function, especially in maternal and early child development. In particular, long-chain omega-3 (ω-3 or n-3) and omega-6 (ω-6 or n-6) PUFAs (≥20 carbons) like eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), and arachidonic acid (ARA, 20:4n-3) orchestrate critical cell membrane functions and trigger several inflammatory responses. With the

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increase in multi-omic-based studies and network-based analyses, the traditional silos for studying inflammation, coagulation, and physiologic responses to trauma as independent factors have been destroyed. In this chapter, we discuss the role PUFAs, specifically ARA, EPA, and DHA, play in modulating levels of inflammation and coagulation following trauma. We discuss what we have learned from past studies that aim to exploit the anti-inflammatory, antithrombotic, and pro-resolving properties of dietary n-3 PUFAs and highlight areas where further studies are needed to optimize the delivery of n-3 PUFAs for trauma care.

Keywords

PUFAs · Arachidonic acid · Omega-3 · EPA · DHA · Eicosanoids · Prostaglandins · Leukotrienes · Thromboxanes · Resolvins · Specialized lipid mediators · Traumatic brain injury · Trauma-induced coagulation · Inflammation

Abbreviations

ALA	Alpha-Linolenic Acid
ALI	Acute Lung Injury
ARA	Arachidonic Acid
ARDS	Acute Respiratory Distress Syndrome
ASCL6	Acyl-CoA Synthetase 6
COX	Cyclooxygenase
CYP450	Cytochrome P450
DGLA	Dihomo-Gamma-Linolenic Acid
DHA	Docosahexaenoic Acid
ELOVL	Elongase of Very Long Chain
EPA	Eicosapentaenoic Acid
FADS	Fatty Acid Desaturase
FC	Free Cholesterol
GLA	Gamma-Linolenic Acid
iPSC	Inducible Pluripotent Stem Cell
LA	Linoleic Acid
LC PUFA	Long-Chain Polyunsaturated Fatty Acid
LOX	Lipoxygenase
LPS	Lipopolysaccharide
LT	Leukotriene
LX	Lipoxin
mTBI	Mild Traumatic Brain Injury
NFLC	Neurofilament Light Chain
NSAID	Nonsteroidal Anti-Inflammatory Drug
PCS	Post Concussion Symptom
PEEP	Positive End-Expiratory Pressure
PG	Prostaglandin
PUFA	Polyunsaturated Fatty Acid
SNP	Single Nucleotide Polymorphism
SPMs	Specialized Pro-resolving Lipid Meditators

TBI	Traumatic Brain Injury
TH	Thromboxane

Introduction

Omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) are essential components of cell membranes and are precursors to several bioactive molecules in the body, regulating blood pressure, inflammation, and coagulopathic responses. There is increasing evidence that suggests omega-3 PUFAs can protect against heart disease, (Aung et al. 2018; Kromhout et al. 2012) traumatic brain injury, concussions (Barrett et al. 2014), prevent diabetes (Lee et al. 2014), and protect against certain kinds of cancer (Marventano et al. 2015; Gleissman et al. 2010; Simopoulos 2006). Understanding how endogenous and dietary PUFAs regulate key cellular events to promote repair and resolution from inflammation can be transformative to a wide array of diseases and health problems, including the treatment and management of acute traumatic injuries. In this chapter, we discuss the fundamental mechanisms regulating PUFA biosynthesis and metabolism and how endogenous and dietary PUFAs affect the inflammatory, coagulopathic, and metabolic responses following a traumatic injury. We also summarize key findings from clinical trials that highlight the importance of monitoring PUFAs as biological indicators for improved recovery after trauma.

PUFA Biosynthesis and Metabolism

Dietary polyunsaturated fatty acids (PUFAs) play a vital role in cell growth, development, and function, especially in maternal and early child development. In particular, long-chain omega-3 (ω-3 or n-3) and omega-6 (ω-6 or n-6) PUFAs ($\geq$20 carbons) like eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), and arachidonic acid (ARA, 20:4n-3) orchestrate critical cell membrane functions and trigger several inflammatory responses (Hester et al. 2014; Liu et al. 2012; Weaver et al. 2009). While these long-chain PUFAs (LC-PUFAs) cannot be synthesized de novo in mammals, they can be metabolized from essential dietary PUFAs linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3). Endogenous synthesis of these long-chain PUFAs largely occurs in the liver and is regulated by two types of enzymes: (1) fatty acid desaturase (i.e., FADS1 and FADS2) and (2) elongation of very long-chain fatty acids (i.e., ELOVL2 and ELOVL5), as illustrated in Fig. 1 (Zhang et al. 2016). Historically, the two desaturase steps (i.e., FADS1 and FADS2) have been considered the rate-limiting steps in this biosynthetic pathway, but over the past two decades, there is growing awareness that genetic variants influencing any *FADS* and *ELOVL* expression can significantly impact the production of long-chain PUFAs.

The long-chain n-6 PUFA: ARA is arguably the most important of all cellular PUFAs (Surette 2008). When cells are activated by external stimuli, ARA is released

Fig. 1 The PUFA biosynthetic pathway. Essential dietary n-6 PUFA linoleic acid (LA) and n-3 PUFA alpha-linolenic acid (ALA) are converted into respective n-6 (illustrated in orange rectangles) and n-3 (illustrated in blue rectangles) long-chain PUFAs (≥20C) through a series of elongation and desaturation steps. Conversion of the n-6 PUFA arachidonic acid (ARA) into pro-inflammatory eicosanoids (illustrated in red) occurs through the COX, 5-LOX, and 15-LOX enzymes. Conversely, these same pathways are utilized to convert ARA into anti-inflammatory lipoxins and act on n-3 PUFAs to produce the specialized pro-resolving lipid mediators (SPMs) illustrated in purple. Specifically, eicosapentaenoic acid (EPA) is the precursor for E-series resolvins, and docosahexaenoic acid (DHA) is converted by 5-LOX, 15-LOX, and COX-2 into D-series resolvins. The 12- and 15-LOX enzymes act on DHA to produce protectins and maresins, also SPMs. The SPMs formed from DHA are collectively known as docosanoids

from cell membranes and transformed into powerful ARA-derived metabolites through CYP450, cyclooxygenase (COX), and lipoxygenase (LOX) pathways, which provoke a cascade of pro-inflammatory and pro-thrombotic events, including activation of leukocytes and platelets (Hester et al. 2014; Garcia de Acilu et al. 2015; Chilton et al. 2014; Funk 2001; Sergeant et al. 2016; Jamieson et al. 2017). The importance of these pathways is evident by the number of anti-inflammatory drugs that target ARA metabolism (e.g., ibuprofen) and cyclooxygenase-2 (COX-2) inhibitors (e.g., rofecoxib, celecoxib) (Houston and Teach 2004; Loewen 2002). Recent evidence, however, demonstrates that dietary n-3 PUFAs can directly compete with ARA metabolism and ARA-derived metabolites by producing anti-inflammatory, antithrombotic, "pro-resolution" mediators (Weaver et al. 2009; Sergeant et al. 2016; Mathias et al. 2014; Arm et al. 2013). These n-3-derived anti-inflammatory metabolites are often referred to as specialized pro-resolving lipid mediators (SPMs) (Fig. 1) (Serhan and Levy 2018; Serhan et al. 2015a; Serhan et al. 2015b; Colas et al. 2014).

Dietary n-3 PUFAs including ALA, EPA, and DHA have been consistently associated with less inflammation and improved health outcomes. Dietary foods rich in n-3 PUFAs including fish, olive oil, and nuts continue to be recommended to counteract the "inflammatory" effects of n-6 PUFAs and ARA in particular. In fact, there are several studies that show the benefit of a lower n-6/n-3 ratio of PUFAs, such as in the Mediterranean diet which is approximately 3:1, unlike the modern Western diet where the ratio can be up to 15 or 20:1. This is largely due to the fact that n-3 and n-6 PUFAs are metabolized by the same enzymes, so by increasing the consumption of n-3 PUFAs, one can stack the pathway toward the n-3 arm, which subsequently generates more resolvins and docosanoids and less n-6-derived eicosanoids. Yet, the optimal tuning of n-3 PUFAs in one's diet to achieve the best pro-resolving properties remains unknown.

Despite consistent scientific literature supporting the concept that n-6 and n-3 PUFAs and their derived metabolites have different and often opposing effects, supplementation with dietary n-3 PUFAs and fish oil (which is rich in EPA and DHA) has produced mixed results. Some studies reveal benefits in patient outcomes after fish oil consumption, whereas others have failed to show any benefit. As a result, their use in clinical medicine remains controversial. Specific to trauma applications, dietary PUFAs have been administered as an adjuvant therapy to critically injured patients suffering from traumatic brain injury (TBI), concussions, and acute respiratory distress syndrome (ARDS), but there remains no consensus on their use (Garcia de Acilu et al. 2015; Parish et al. 2014; Sabater et al. 2011; Sabater et al. 2008; Li et al. 2015; Kagan et al. 2015; Zhu et al. 2014; Schott and Huang 2012; Rice et al. 2011). Given the heterogeneity of the patient populations and the injury types, as well as the genetic variability influencing PUFA metabolism, there is a need for improved clinical and translational studies that can help unlock the mechanisms by which PUFAs can be used for trauma care. We postulate that there will continue to be confusion in this important area until there is a much better understanding of the immunomodulatory effects of dietary PUFAs both during normal and injured states in humans.

The Role of PUFAs in Trauma-Induced Inflammation and Coagulation

Initiation of the acute inflammatory response after a traumatic injury is a complex process that involves various cell types including macrophages, leukocytes, platelets, endothelial cells, and tissues that experience the damage. Under hemorrhage, the vasculature responds to the change in blood pressure and flow by constricting through the release of vasopressin, epinephrine, and/or norepinephrine. Neutrophils migrate to the site of injury, and there is a storm of inflammatory cytokines that modulate T and B cells to respond to the injury. With the disruption of the vascular endothelium, there is also activation of platelets and formation of thrombus to stop bleeding. Trauma-induced coagulopathy is a common phenomenon, where changes in platelet reactivity, thrombin and fibrinogen production, and endothelial dysfunction affect the patient's response to injury (Cardenas et al. 2014; Chang et al. 2016).

At the cellular level, PUFAs orchestrate critical events in regulating both inflammation and coagulation responses (Fig. 2) (Hester et al. 2014; Liu et al. 2012; Weaver et al. 2009). This is because the cell membrane and more specifically the phospholipid bilayer of cells are rich in PUFAs. Upon damage or disruption, there is a mobilization of PUFAs and lipids, which give rise to the production of a number of biological active metabolites, eicosanoids, and lipoxins regulating inflammatory and anti-inflammatory pathways. For example, cell damage and disruptions to the cell membrane result in the release of free ARA. An increase in free ARA (which is typically low under normal conditions) results in the generation of prostaglandins, prostacyclins, thromboxanes, and leukotrienes. Most commonly, prostaglandin E2 (PGE2), prostaglandin I2, (PGI2), and leukotriene B4 (LTB4) are generated and trigger a series of inflammatory pathways (Fig. 2). The generation of prostaglandins occurs when ARA is metabolized by COX-2 in cells around the site of injury or infection. Prostaglandins are considered to have a pro-inflammatory nature and lead to some of the classic symptoms of inflammation: redness (rubor), swelling (tumor), pain (dolor), and heat (calor). The recruitment of neutrophils to a site of inflammation and subsequent passage across the endothelial barrier has been linked to prostaglandins, specifically PGD2 (Marion-Letellier et al. 2015). Despite this, prostaglandins also paradoxically display an anti-inflammatory nature through the stimulation of lipoxin (LX) productions or the suppression of the adaptive immune system, namely, helper T-cells. The balance between pro-and anti-inflammatory properties can contribute to improved wound healing, whereas imbalance in these two pathways can lead to chronic injury states and nonoptimal wound recovery.

Another type of eicosanoid derived from ARA is thromboxane, which is largely produced by activated platelets. Platelets convert prostaglandin H2 into thromboxane A2 (THA2) which locally promotes vasoconstriction and platelet activation. Therefore, as ARA levels increase, one would expect a concomitant increase in platelet activation and clotting. Given that hypercoagulability is commonly observed after trauma, modulating the level of ARA could be used to regulate platelet

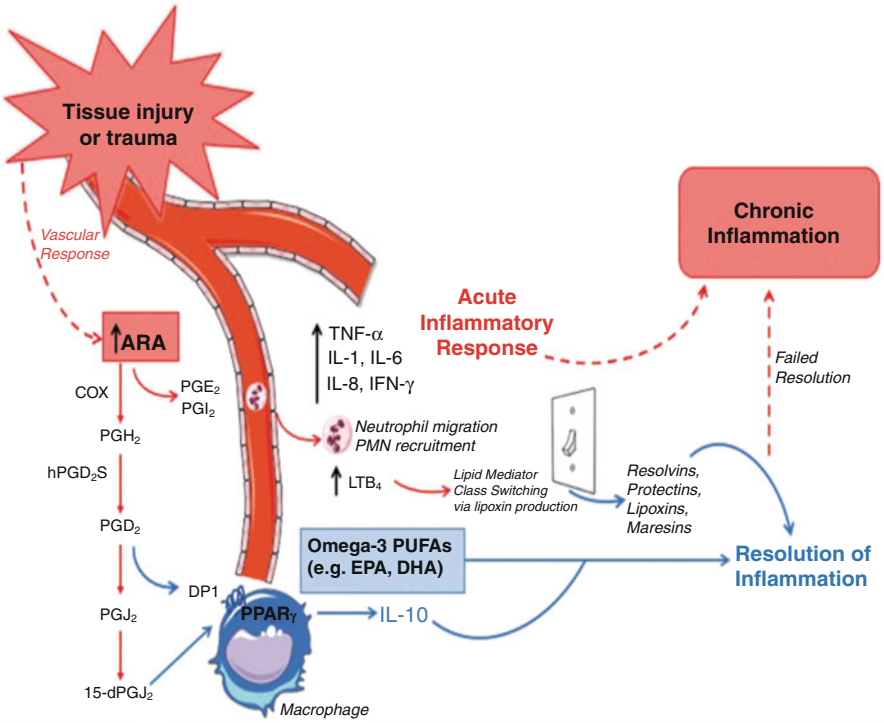

Fig. 2 Illustration of key n-6 and n-3 PUFAs, largely arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) pathways, involved in acute inflammation post-trauma and the role of lipid mediators in resolution or failure. Initiation of the acute inflammatory response after injury starts with the vascular response, often stimulated by PGE2 and PGI2, and LTB4, which are produced from ARA. The release of ARA from cell membranes is generally dependent on the extent of tissue damage. As ARA levels increase after trauma, there is a concomitant increase in interleukin (IL)-8 and IL-10 levels. IL-8 facilitates in the migration of neutrophils to the site of injury, whereas IL-10, often secreted by macrophages, inhibits inflammation and promotes a M2 phenotype. Therefore, a balance between both the acute inflammatory response and inflammation resolution phases is needed to ensure optimal wound recovery and tissue repair. (Reprinted with permission from Dr. Rahbar and Journal of Neurotrauma)

function. As mentioned earlier, dietary n-3 PUFAs are one possible method to attenuate the pro-inflammatory and pro-thrombotic response of ARA.

Freely circulating ARA can also be converted through LOX-mediated pathways into leukotrienes, a process that occurs primarily in leukocytes. Through the 5-lipooxygenase (5-LOX) pathway, leukotrienes are generated starting with the generation of leukotriene LTA4, leukotriene B4 (LTB4), and leukotriene E4 (LTE4). Leukotrienes are pro-inflammatory cytokines commonly produced by leukocytes and responsible for chronic inflammation in disease states such as asthma and heart disease (Peters-Golden and Henderson Jr 2007). In relation to trauma,

leukotrienes have been implicated in a number of post trauma/hemorrhagic shock states associated with poor patient outcomes such as acute kidney injury, ARDS, and neural inflammation (Corser-Jensen et al. 2014; Nunns et al. 2018; Stringham et al. 2014). Common anti-inflammatories such as NSAIDs or corticosteroids do not affect the 5-LOX pathway and have even been found to increase levels of leukotrienes when administered. Interestingly, dietary n-3 PUFAs have been shown to modulate leukocyte behavior and can be used to reprogram their response to inflammatory stimuli (Calder 2013; Calder 2006). This occurs either directly because n-3 long-chain PUFAs replace ARA as an eicosanoid substrate and inhibit ARA metabolism or indirectly via the change in expression of inflammatory genes through effects on transcription factor activation. Endothelial cells and neutrophils are also capable of producing leukotrienes and also responsive to dietary n-3 PUFAs; for example, DHA can inhibit neutrophil adhesion (Yates et al. 2011). Therefore, there is a delicate balance between n-6 and n-3 PUFA-derived metabolites that regulate the inflammatory and coagulopathic responses after injury. Table 1 provides a list of the ARA-derived eicosanoids and their primary functions in regulating inflammation and coagulation (Peters-Golden and Henderson Jr 2007; Yao and Narumiya 2019; Calder 2020; Braune et al. 2020; Innes and Calder 2018).

Table 1 ARA-derived eicosanoids and their primary functions

Eicosanoid family	Eicosanoid	Function
Prostaglandins	PGH_2	Precursor molecule to downstream PGs
	PGG_2	Precursor to PGH_2
	PGE_2	Endothelial permeability
		Inflammatory response (redness, swelling, pain)
	PGD_2	Produced mainly by mast cells in peripheral tissues
		Sleep regulation
		Allergic reactions
	PGI_2	Vasodilation
		Inhibits platelet aggregation
		Also referred to as prostacyclin
Leukotrienes	LTA_4	Starting molecule of LTx chain
	LTB_4	Immune cell recruitment and activation
		Increases vascular permeability
		Enhances leukocyte adhesion to endothelium
	LTE_4	Alternate product of LTA and most stable cysteinyl leukotriene
		Similar effects as LTB_4
		Upregulates COX-2 expression
		Increases production of PGE_2
Thromboxanes	TXA_2	Vasoconstriction
		Platelet aggregation
		Activation of endothelial inflammation
	TXB_2	Byproduct of TXA_2, inactive

Specialized Pro-Resolving Lipid Mediators (SPMs)

Converse to ARA, the n-3 long-chain PUFAs EPA and DHA are also release from cell membranes after cell damage and trauma. Release of EPA and DHA is subject to similar oxygenase pathways (i.e., COX and LOX) and generates a family of specialized pro-resolving lipid mediators (SPMs), including resolvins, maresins, protectins, and docasanoids.

Resolvins, derived from EPA (E-series) and DHA (D-series), are known to impact inflammation through downregulating the infiltration of macrophages and neutrophils (Abdolmaleki et al. 2020; Chiang and Serhan 2017). D-series resolvins are synthesized in neutrophils and macrophages through the formation of intermediates via COX-2. Specific to trauma, RvD1 has been demonstrated to be protective in the face of ischemia-reperfusion injury through the halting of neutrophil infiltration (Serhan and Levy 2018; Kasuga et al. 2008). Furthermore, the E-series resolving RvE2 is upregulated in hypoxic conditions (Serhan and Levy 2018). Maresins, or macrophage mediators in resolving inflammation, are derived from DHA via 12-LOX (Chiang and Serhan 2017). Marsin 1 (MaR1) has been identified as an activator of the LGR6 receptor, through which it stimulates phagocytosis and phosphorylation of downstream pathways (Chiang et al. 2019). Protectins are produced through 15-LOX oxidation of DHA and have been demonstrated to have neuroprotective effects in the face of TBI and ischemic stroke (Chiang and Serhan 2017). While the exact mechanisms by which n-3 PUFAs and their derived SPMs modulate inflammation are not clearly understood, we have evidence that they indirectly affect macrophage and leukocyte behavior through transcription factor changes and epigenetic modifications (e.g., hypomethylation of key CpG sites). There is a need for more studies to elucidate the exact mechanisms by which dietary PUFAs can be harnessed for immunomodulation.

It is also important to note that there are some byproducts of ARA that also have resolving properties, namely, lipoxins. A complete listing of SPMs has been provided in Table 2. Instead of inhibiting inflammatory action, SPMs actively contribute to the resolution of the inflammatory state and contribute significantly to the acute wound healing process. These and the SPMs produced from n-3 PUFAs are detailed in Table 2 (Serhan and Levy 2018; Innes and Calder 2018; Kwon 2020).

Effects of Dietary Supplementation with PUFAs in Trauma Populations

Traumatic injuries are the leading cause of mortality and morbidity in people between the ages of 1 and 45 years (Campbell et al. 2009; Kauvar et al. 2006). Common complications following a severe injury include acute respiratory distress syndrome (ARDS), sepsis, and multi-organ failure, often associated with a systemic inflammatory response. Therefore, it is not surprising that several attempts have been made to attenuate this inflammatory response via adjuvant and pharmacologic nutrition with n-3 PUFAs. Unfortunately, these clinical trials have led to mixed

Table 2 Specialized pro-resolving lipid mediators and their functions

PUFA precursor	SPM family	SPM	Function
EPA	E-series resolvins	RvE1	Inhibition of neutrophil migration
			Reduction of NF-κβ signaling
			Induces apoptosis of neutrophils
			Controls vascular inflammation
			Pain reduction
		RvE2	Upregulated during hypoxia
			Increases macrophage phagocytosis
			Inhibits neutrophil recruitment
		RvE3	Late-stage resolution
DHA	D-series resolvins	RvD1	Inhibits neutrophil infiltration through endothelium
			Stimulate macrophage phagocytosis
			Pain reduction
		RvD2	Inhibits neutrophil infiltration through endothelium
			Stimulates NO release
			Induces M2 macrophage phenotype
		RvD3	Blocking neutrophil migration
			Increases macrophage phagocytosis
		RvD4	Clot resolution
			Stimulate macrophage phagocytosis
		RvD5	Induces M2 macrophage phenotype
			Regulation of NF-κβ and TNF-α
	Maresins	MaR1	Increases macrophage phagocytosis of apoptotic neutrophils
			Neuroprotection
			Induces M2 macrophage phenotype
			Alleviation of inflammatory pain
	Protectins	PD1	Activated during ischemia-reperfusion injury
			Neuroprotective
			Renal protective
ARA	Lipoxins	LXA_4	Limits neutrophil infiltration and vascular adhesion
			Reduction of vascular inflammation during ischemia
			Modulates memory B-cell responses
		LXB_4	Limits neutrophil infiltration and vascular adhesion
			Stimulate monocyte recruitment and adhesion

findings and confusion (Garcia de Acilu et al. 2015; Li et al. 2015). In this section we summarize some of the main clinical trials that have investigated the use of dietary PUFAs in trauma populations and shed light on potential reasons for failure. We also provide some insights regarding the genetic contributions to PUFA metabolism and areas that future studies should focus on for trauma care.

Traumatic Brain Injury (TBI) and Concussions

Long-chain PUFAs have long been recognized to be essential for brain development and implicated to play a major role in memory and cognitive function (Barrett et al. 2014; Desai et al. 2014; Hasadsri et al. 2013; Cheatham et al. 2011). Long-chain PUFAs are an integral component of neuronal membrane phospholipids and have been shown to demonstrate anti-inflammatory effects. In particular, deficiencies in n-3 LC-PUFAs have been associated with impaired memory, inflammation, and delayed neuronal repair after mTBI (Barrett et al. 2014; Desai et al. 2014; Hasadsri et al. 2013; Wu et al. 2007; Schuchardt et al. 2016; Cooper et al. 2015). A metabolomic panel tested by Hogan et al. found that in the case of TBI within rodent models, free PUFA levels of ARA, DPA, and DHA were significantly higher compared to sham groups and that levels of oxidized PUFAs dropped (Hogan et al. 2018). This indicates that PUFAs have a notable role in the inflammatory and recovery process following TBI and could act as useful biomarkers in the diagnosis of TBI and mTBI.

LC-PUFAs have been recognized to be vitally important for brain development in early childhood. Data from the Rahbar research lab and others have shown that LC-PUFAs may continue to play a critical role in neuronal development and repair beyond these early years (Miller et al. 2016; Gow and Hibbeln 2014; Gow et al. 2009). Deficiencies in LC-PUFAs have been associated with several neuropsychiatric disorders, attention deficit disorders, lapses in memory, and impaired cognition (Strike et al. 2016; Eriksdotter et al. 2015; Agrawal and Gomez-Pinilla 2012; Boucher et al. 2011; Brookes et al. 2006). Alternatively, higher n-3 LC-PUFA levels have been shown to be associated with improved cognition, memory, reduced inflammation, and neuroprotective properties (Barrett et al. 2014; Hester et al. 2014; Cooper et al. 2015; Strike et al. 2016; Kulzow et al. 2016; Frensham et al. 2012).

As a result, the use of n-3 LC-PUFA supplements, such as DHA or fish oil, has been suggested for improving outcomes and mitigating post-concussion symptoms (PCS). Recently, Oliver et al. observed marked increases in circulating neurofilament light chain (NFLC) peptide levels in starting football college athletes and striking reductions in NFLC after taking n-3 LC-PUFA supplements over the duration of a single season (Oliver et al. 2016a; Oliver et al. 2016b). This data implies that LC-PUFAs and/or its metabolites may be linked to neuronal injury biomarkers and inflammation during mTBI recovery and PCS. A recent meta-analysis by Patch et al. found that in a number of rodent models ($n = 18$) that a diet supplemented with n-3 PUFAs led to statistically significant improvements in cognitive abilities and lowered signs of inflammation in rats with induced mTBI (Patch et al. 2021).

Conversely, there is literature reporting no effect of n-3 LC-PUFA supplements on patient outcomes and PCS (Rice et al. 2011; Phillips et al. 2015). There is even evidence that n-3 PUFA supplements may be detrimental to patient health when combined with prescribed blood thinners and could lead to excessive hemorrhage, but such cases are extremely rare and would require much more extensive investigation to indicate that PUFA supplements were the cause (Gross et al. 2017). A recent study by Fernandez et al. revealed that acyl-coA synthetase 6 (Acsl6) is

needed for the retention of DHA within the rodent brain (Fernandez et al. 2021). Hence, we believe these mixed findings from the clinical trials are due to our rudimentary understanding of the complex metabolic and lipidomic responses to concussive injuries and mTBIs and often the exclusion of genetic/epigenetic factors. It is likely that a "one-size-fits-all" approach is not sufficient. This is explained in greater detail at the end of this chapter.

Acute Respiratory Distress Syndrome (ARDS)

Acute lung injury (ALI) and its more severe form acute respiratory distress syndrome (ARDS) are inflammatory disorders characterized by decreased lung compliance, hypoxemia, capillary leakage, and pulmonary edema (Butt et al. 2016; Parekh et al. 2011; Rubenfeld and Herridge 2007). This disorder develops as a result of trauma, sepsis, pneumonia, and a number of other local or systemic factors and is associated with a high rate of morbidity and mortality in patients who develop the disorder. In the United States, roughly 150,000 patients develop ARDS per year, and despite improvements in treatment focused on continuous positive end-expiratory pressure (PEEP) and the administration of corticosteroids, the mortality rate for patients with ARDS is still high at roughly 40% (Butt et al. 2016; Parekh et al. 2011; Rubenfeld and Herridge 2007; Zhou et al. 2017). In patients suffering from ARDS and those at risk of developing ARDS, Kumar et al. found that circulating n-3 and n-6 PUFA levels were significantly lower compared to controls (Kumar et al. 2000). Omega-3 PUFAs have repeatedly demonstrated immunomodulatory and anti-inflammatory effects. Due to these properties, they have been investigated as possible treatments for patients with ALI or ARDS to mixed results.

The OMEGA trial was a large Phase 3 clinical trial with 44 enrolling hospitals of the NHLBI ARDS Clinical Trials Network. They hypothesized that enteral supplementation of n-3 PUFAs EPA and DHA, n-6 PUFA gamma-linolenic acid (GLA), and antioxidants would improve patient outcomes and reduce time on the ventilator (i.e., improvement in VENT-free days) (primary outcome) in ARDS patients. However, the study was terminated when an interim analysis showed no difference in VENT-free days between the treatment and placebo groups ($N = 272$ enrolled). Plasma, urine, and DNA samples from this trial are available for secondary analysis and currently stored at the NIH BioLINCC repository.

In some cases, it was found that supplementation of n-3 PUFAs was actually detrimental to favorable patient outcomes (Rice et al. 2011; Stapleton et al. 2011). These studies did note however that the methods of delivery for their dietary supplements differed compared to those that found more favorable results. These studies utilized a bolus delivery method as opposed to continuous enteral feeding. The possible difference in application of PUFA supplements for patients with ARDS could be a contributing factor to the mixed results characterizing the past decade of research into PUFA-based dietary treatment of ARDS. Different types and rates of deliveries could disrupt the balance of pro- and anti-inflammatory markers within the patient, both of which are important to recovery.

Despite these findings, research into possible mechanisms and applications using PUFA dietary supplements for patients with ALI and ARDS is continuing. Numerous studies have found that n-3 PUFA diets have decreased mortality compared to controls in murine models simulating sepsis-induced ARDS (Rice et al. 2011; Chang et al. 2017; Zhu et al. 2020). Zhu et al. found that in the case of intestinal reperfusion injury, pretreatment with n-3 PUFAs led to higher survivability in murine models. Perhaps the addition of n-3 PUFA supplements as a treatment for patients with ALI/ARDS is time-sensitive and possibly more effective based on the form of fatty acid and lipid emulsion provided. There is a need for more focused studies on better identifying the optimal timing, dosing, and method of administration of dietary PUFAs for ALI/ARDS patients. Additionally, other studies have found that in order to gain anti-inflammatory effects, proper ratios of n-6 and n-3 PUFAs need to be maintained rather than just supplementing with n-3 PUFAs alone (Chang et al. 2017). Considering that a large fraction of ARDS cases are caused as a secondary effect of sepsis, this could make sense. Unnecessary suppression of the immune response could limit the body's ability to combat the infection. Conversely, allowing rampant inflammation within the lungs could lead to ARDS. These conflicting findings between animal models and clinical studies are most likely due to different genetic factors within the observed populations and we discuss this in greater detail in the subsequent sections.

Orthopedic Trauma and Arthritis

Dietary PUFAs have also been investigated is bone health and repair following fracture. A 2015 study by Harris et al. concluded that n-3 PUFA consumption in late life corresponded to a decrease in fracture risk in older men as well as women in the middle stages of their lives (Harris et al. 2015). More recently, PUFA ratios and concentrations have been investigated as biomarkers in patients undergoing surgery to repair femoral neck fractures. It was found that both n-6 and n-3 PUFA levels were lower compared to controls within hours following both the initial fracture and surgery (Arsic et al. 2020a). The decrease in n-6 levels is thought to result from the increased generation of prostaglandins which are released in response to injury and are created as previously discussed. Specifically, PGE2 is important in regulating bone resorption and formation depending on the circulating levels of the molecule. N-3 PUFAs are important in the generation of SPMs which play a role in mitigating prostaglandin activity as well as in the promotion of insulin-like growth factors and in calcium absorption, a key process in bone repair.

Genetic and Epigenetic Determinants of PUFA Biosynthesis and Metabolism

One potential explanation for the mixed results regarding the efficacy of dietary PUFAs in human clinical trials is the lack consideration of genetic variants. Over the past two decades, there is growing evidence that the production of long-chain

PUFAs is considerably affected by genetic variants located within the fatty acid desaturase (*FADS*) gene cluster (11q12–13.1) and elongation of very long-chain fatty acids 2 (*ELOVL2*) (6p24.2) and *ELOVL5* (6p12.1) (Hester et al. 2014; Chilton et al. 2014; Mathias et al. 2014; Cormier et al. 2013; Sergeant et al. 2012; Glaser et al. 2010). These FADS and ELOVL variants have been shown to be associated not only with circulating and tissue levels of PUFAs but also complex diseases (Mathias et al. 2014; Cui et al. 2016; Mathias et al. 2011a; Howard et al. 2014).

The desaturase enzymes encoded by the *FADS* gene cluster have long been recognized as the rate-limiting steps in long-chain PUFA biosynthesis. Comprising of three genes (*FADS1*, *FADS2*, and *FADS3*), this is a region of high linkage disequilibrium (LD) (Mathias et al. 2014; Rahbar et al. 2017). There have been ~25 studies (Malerba et al. 2008; Martinelli et al. 2008; Rzehak et al. 2009; Schaeffer et al. 2006; Mathias et al. 2011b; Mathias et al. 2010; Sergeant et al. 2012; Xie and Innis 2008; Xie and Innis 2009; Porenta et al. 2013; Hong et al. 2013; Harsløf et al. 2013; Li et al. 2013; Morales et al. 2011; Gillingham et al. 2013; Freemantle et al. 2012; Lattka et al. 2013; Lattka et al. 2011; Steer et al. 2012; Koletzko et al. 2011; Kwak et al. 2011; Rzehak et al. 2010; Bokor et al. 2010) confirming that *FADS* variants account for large variation in circulating and cellular long-chain PUFA levels. New studies from the Rahbar lab and others indicate that the methylation status of specific CpG sites within the *FADS* cluster (specifically within the *FADS2* promoter and a region with between *FADS1* and *FADS2* with an enhancer signature) impacts the transcription of *FADS* cluster genes, PUFA metabolism, and, in one study, both immediate and delayed memory performance in toddlers (Cheatham et al. 2015; Hoile et al. 2014; Lupu et al. 2015). Taken together, these studies suggest that *FADS* genetic and epigenetic factors may not only contribute to differential levels of PUFAs and metabolites but also inadvertently be associated with altered inflammatory and physiologic responses. Moreover, these differential PUFA levels and genetic variants and epigenetic modifications may be important confounding variables impacting the efficacy of n-3 PUFA supplements, particularly in ethnically diverse populations where the allele frequencies are drastically different.

The most prominently studied of these variations is the single-nucleotide polymorphism (SNP) rs174537, which is located downstream of FADS1 on chromosome 11. There are three genotypes associated with rs174537, GG, GT, and TT and the frequency with which each genotype appears in the population is dependent on racial/ethnic background and geographical location, as illustrated in Fig. 3 (Mathias et al. 2014). For example, within those of European ancestry, the G allele frequency is 0.651, while the T allele frequency is 0.349. For those of African ancestry, the G and T allele frequencies are 0.975 and 0.025, respectively, while those of American ancestry display frequencies of 0.412 and 0.588, respectively (Mathias et al. 2011b). Genotype at rs174537 has been linked to circulating and tissue PUFA levels, as well as eicosanoid formation. Specifically, individuals who carry the major allele (i.e., GG and GT) are rapid metabolizers of n-6 PUFAs and convert DGLA to ARA at a faster rate than those homozygous with the minor allele (i.e., TT), and therefore we hypothesize that they may be more susceptible to a pro-inflammatory response due to elevated levels of ARA. As genotype is highly dependent upon race and

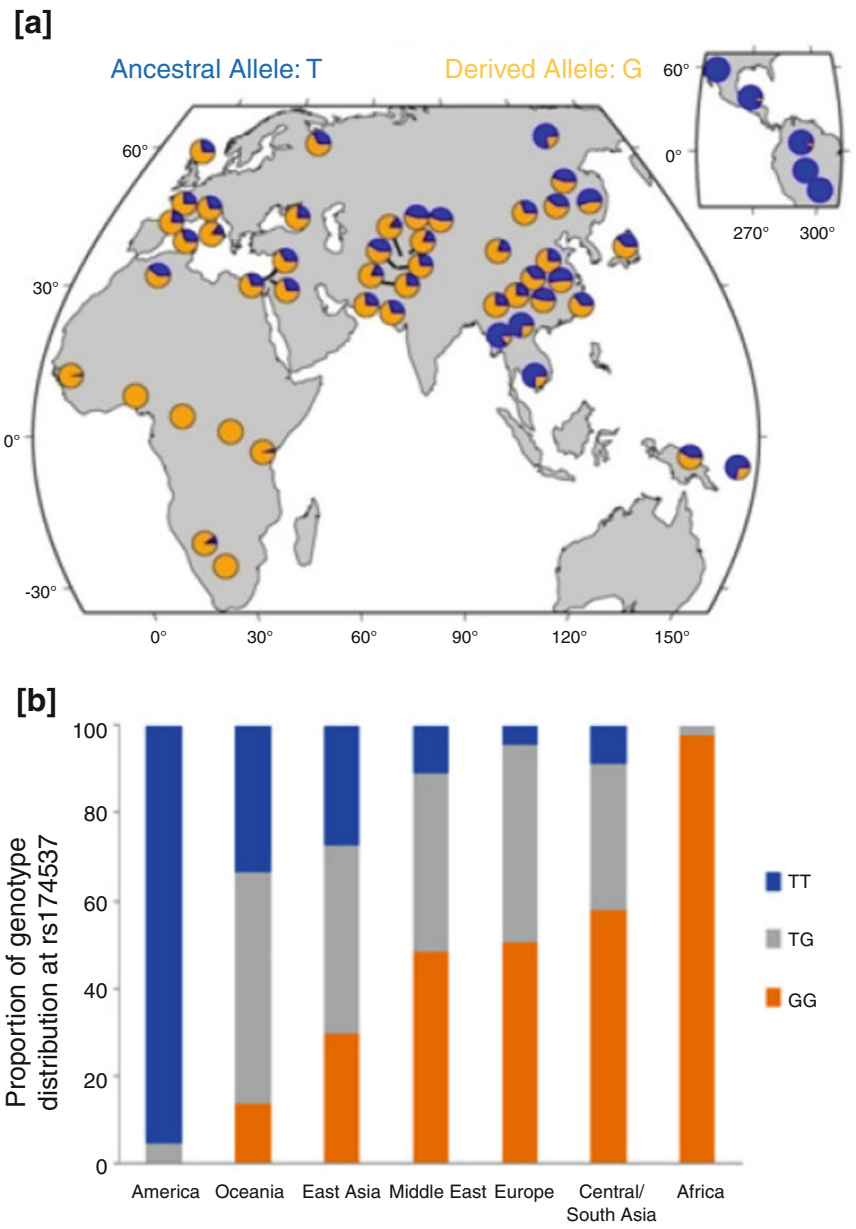

Fig. 3 Allele frequency of SNP rs174537 globally. The allele frequency of rs174537 varies tremendously by geographic and racial/ethnic populations. Individuals with African ancestry are predominantly homozygous with the major allele (i.e., GG), whereas individuals from Peru and South American ancestry are largely homozygous with the minor allele (i.e., TT). Caucasian and European ancestral populations tend to exhibit a more balanced allele frequency distribution. The reason for this variation is presumed to be an evolutionary trait from the population's primary diet,

geographical location, this is one mechanism through which race-based health disparities could be exacerbated (Chilton et al. 2014).

In particular, the OMEGA randomized clinical trial evaluated the effect of enteral dietary PUFA blend (GLA + EPA + DHA) in a cohort of critically ill patients with ARDS and was terminated early due to futility (Rice et al. 2011). One potential explanation for these unsatisfactory results may be due to the genetic variability within a population impacting their PUFA metabolic conversion capacities since both n-6 and n-3 PUFA was included in the blend. Given that *FADS* variants contribute to differential PUFA levels, we postulate that they may also inadvertently be associated with altered responses to dietary supplements and subsequent inflammation and coagulation after injury via the generation of PUFA-derived bioactive metabolites. In a secondary analysis of the OMEGA trial, Dosso et al. discovered that rs174537 had a significant impact on circulating DHA levels and urinary isoprostane levels (Dosso et al. 2020). While they were unable to detect a statistically significant effect of genotype at rs174537 on patient outcomes due to the relatively small patient population, they did observe some differences between African American and Caucasians warranting the need for larger ethnically diverse studies that can investigate the gene-diet interactions on inflammatory outcomes.

Another confounding factor influencing the mixed results in clinical studies is the use of dietary n-3 PUFAs in isolation vs. in dietary blends that include n-6 PUFAs like gamma-linolenic acid (GLA). A recent prospective clinical trial performed by the Chilton group has provided additional validation to these gene-diet interactions in healthy subjects. Supplementation with the n-6 PUFA GLA results in highly variable responses, and the varied efficiency of *FADS1* associated with genotype at rs174537 is proposed as the reason for this inconsistency. Upon supplementation with GLA, the PUFA is converted rapidly into DGLA through the ELOVL5 enzyme; FADS1 converts DGLA to the pro-inflammatory ARA at variable rates. The Chilton group explored the variable effects of GLA supplementation by feeding soybean oil (50% LA) or borage oil (37% LA and 23% GLA) to a cohort of healthy non-Hispanic white individuals genotyped at rs174537. After 4 weeks of dietary supplementation, analysis of circulating PUFA levels indicated that GLA feeding altered circulating levels of ARA and DGLA in a genotype-dependent manner; TT individuals had increase fold changes of DGLA in response to 4 weeks of GLA supplementation, which is consistent with decreased *FADS1* activity. Additional study is necessary to identify the links between this variability in PUFA levels and eicosanoid production, which directly impacts the inflammatory response (Sergeant et al. 2020).

Fig. 3 (continued) such that those who primarily eat fish and vegetables are prone to be more TT rather than those who eat red meat and favor a GG genotype. This genetic variation may potentially explain some of the health disparities in drug studies (e.g., COX inhibitors) and chronic diseases that are dependent on ARA and PUFA metabolism. (Reprinted with permission from Drs. Sergeant, Chilton and BMC Genomic Data)

Future Directions

As we look to the future, there is a need for larger clinical studies that include multi-omic biomarkers inclusive of genomic, metabolomic, lipidomic, and proteomic data. Clinical trials that consider evaluating the use of dietary PUFAs must perform their analyses stratified by race and adjust for genetic variation. For too long, we have ignored the role of ethnicity and gender on the effectiveness of nutritional supplements. While randomized clinical trials will continue to be a gold standard to evaluate efficacy of these dietary supplements, there is a need for alternative platforms that can assess the human response to PUFAs after injury.

A major contributor to our poor understanding of the complex human PUFA metabolic pathway and its implications on inflammation and immunity is the heavy reliance on single cell in vitro systems, or in vivo animal models, that have failed to translate to humans. For example, studies that evaluate PUFA exposure on isolated macrophages or in mouse models do not replicate human metabolism (Gutierrez et al. 2019; Kiecolt-Glaser et al. 2016). We suggest that using 3D tissue engineering and organoid-based platforms where primary human cells or inducible pluripotent stem cells (iPSCs) are cultured can be used to study the underlying mechanisms by which dietary PUFAs exert protective effects.

It is also important to consider that PUFAs and dietary supplements may also be affected by other factors such as age, obesity, diabetes, and other concomitant diseases. Thus, there is a need for future basic science and translational studies to consider the effects of these comorbidities in the altered response not only to trauma but dietary PUFA supplementation after injury. For example, the OXBIO trial was designed to study the effects of marine- and plant-sourced n-3 PUFAs on inflammation in female obese populations (Rodway et al. 2021; Hatchimonji et al. 2020).

Finally, while there have been over 25 studies (Malerba et al. 2008; Martinelli et al. 2008; Rzehak et al. 2009; Schaeffer et al. 2006; Mathias et al. 2011b; Mathias et al. 2010; Sergeant et al. 2012; Xie and Innis 2008; Xie and Innis 2009; Porenta et al. 2013; Hong et al. 2013; Harsløf et al. 2013; Li et al. 2013; Morales et al. 2011; Gillingham et al. 2013; Freemantle et al. 2012; Lattka et al. 2013; Lattka et al. 2011; Steer et al. 2012; Koletzko et al. 2011; Kwak et al. 2011; Rzehak et al. 2010; Bokor et al. 2010) confirming that fatty acid desaturase (*FADS*) variants account for a large variation in circulating and cellular PUFA levels in humans, highlighting the variability in PUFA metabolism not only by genetic variants but also racial/ethnic backgrounds, there are variants within *ELOVL* that may be just as important. Based on the current state of literature, differential PUFA levels (especially ARA and DHA levels) are driving the chronic inflammatory processes. Thus, genetic variants influencing these PUFA levels may be important confounding variables impacting the efficacy of n-3 PUFA supplements in human studies, especially in ethnically diverse populations. Ultimately, we need more multidisciplinary teams that can bridge the gaps between nutrition, metabolism, inflammation, coagulation, and trauma to identify new treatment and management strategies for trauma populations.

Applications to Prognosis, Other Diseases, and Conditions

In this chapter we discuss how circulating and tissue levels of PUFAs can be used to monitor the inflammatory and coagulopathic response to traumatic injuries. One of the primary proposed uses for PUFAs in terms of disease prognosis is measuring their relative balance following trauma. As we will discuss, measuring the levels of n-3, n-6, and the n-3/n-6 PUFA ratio in a patient's blood has been found to be a useful metric in predicting outcomes for a variety of different trauma-induced states (Colas et al. 2014; Chang et al. 2017; Arsic et al. 2020b). An imbalance toward the n-6 PUFA side could be indicative of a chronic inflammatory state, whereas a more n-3 heavy or balanced ratio of circulating PUFAs could be a sign of better outcomes and less chance of recurrent disease and comorbidity. Analysis of PUFAs as biomarkers is still a relatively new field, so there is no definitive guide to how to interpret circulating PUFA levels. This is further complicated by the fact that n-6 PUFAs do not fit neatly into an exclusively pro-inflammatory state. For instance, ARA predominantly generates prostaglandins which are pro-inflammatory but is also capable of being converted into the lipoxin molecule class which serves in an anti-inflammatory role. More in-depth metabolomic and lipidomic analyses are needed to determine which PUFA-derived biomarkers are most predictive of patient status and outcomes.

In terms of applications outside of trauma, PUFAs have been identified as important biomarkers in evaluating heart disease, neurodegenerative disorders, and cancer. The importance of chronic inflammation in the development of several chronic disease states has begun to be researched more extensively within the past 10 years and has been hypothesized to contribute to higher incidence of these chronic disease states. Higher circulating of n-3 PUFAs and their subsequent role in the generation of anti-inflammatory biomolecules generally correlates with lower incidence of chronic illness (Marventano et al. 2015; Calder 2006; Gu et al. 2015).

Glossary: Mini-Dictionary

Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) – inflammatory illness group categorized by inflammation in the alveoli and decreased oxygen exchange. These conditions occur in response to sepsis or traumatic injury.

Arachidonic acid (ARA, 20:4n-6) – a long-chain omega-6 polyunsaturated fatty acid containing 20 carbons. It is synthesized from linoleic acid and acts as a precursor molecule for several proinflammatory molecules, namely, prostaglandins, thromboxanes and leukotrienes.

Cyclooxygenase pathway (COX) – pathway that converts ARA into prostaglandins. A common target for anti-inflammatory drugs.

Docosahexaenoic acid (DHA, 22:6n-3) – a long-chain omega-3 polyunsaturated fatty acid consisting of 22 carbons. Can be synthesized from α-linolenic and is a precursor molecule for several anti-inflammatory biomolecules.

Eicosanoids – A family of biomolecules derived from arachidonic acid and other similar 20 carbon PUFAs. Eicosanoids serve primarily as signaling molecules multiple pathways such as inflammation, immune responses, pain, and many more.

Eicosapentaenoic acid (EPA, 20:5n-3) – a long-chain omega-3 polyunsaturated fatty acid consisting of 20 carbons. Can be synthesized from α-linolenic acid and is a precursor molecule for several anti-inflammatory biomolecules.

Fatty acid desaturase (FADS) – enzymes responsible for creating double bonds in fatty acids.

Leukotrienes (LTEs) – inflammatory molecules derived from ARA processed by the LOX pathway.

Lipoxins – an abbreviation of lipoxygenase interaction products. Lipoxins are biomolecules within the specialized pro-resolving mediator family. They act as signaling molecules and exhibit an anti-inflammatory effect.

5-Lipoxygenase pathway (5-LOX) – pathway by which ARA is synthesized into leukotrienes.

Polyunsaturated Fatty Acid (PUFA) – any fatty acid with more than one double bond within their backbone structure.

Prostaglandins – a family of ARA-derived biomolecules. Generally act as pro-inflammatory molecules.

Single nucleotide polymorphism (SNP) – the substitution of a single nucleotide at a specific point in the genome.

Thromboxanes – an eicosanoid family molecule. Thromboxanes play an important role in platelet activation and vasoconstriction.

Key Facts of PUFAs

- PUFAs are significant components of cellular membranes and important substrates in the synthesis of numerous signaling molecules.
- Dietary PUFAs, namely, ARA, EPA, and DHA play a critical role in early development of cell and brain tissues (e.g., maternal and prenatal health).
- The liver is the primary organ responsible for PUFA biosynthesis and metabolism.
- Omega-6 (n-6) PUFAs are generally pro-inflammatory and are precursors of biologically active metabolites that exert pro-inflammatory and pro-thrombotic effects.
- Omega-3 (n-3) PUFAs are generally anti-inflammatory and are precursors of biologically active metabolites that aid in the resolution of inflammation.
- ARA and associated pathways are common targets for anti-inflammatory drugs.
- Disruptions in the PUFA metabolism and biosynthesis can lead to serious chronic diseases and impairments in cellular function.

Summary Points

- PUFAs are a relatively new and promising field of investigation for biomarkers and are hypothesized to play an important role in multiple conditions and disease states.
- PUFAs play a significant role in the onset and resolution of inflammation, immune response, and clotting following trauma.
- Treatments using dietary n-3 PUFAs have shown promise in the treatment of injury following trauma in animal models and some clinical trials but have remained inconclusive in clinical trials.
- Balancing ratios of n-3 to n-6 PUFAs may be more important than just supplementing one and removing the other for the resolution of inflammation.
- Investigation into the role of genetic variants in the processing of PUFAs in different populations could explain the variation in effectiveness of treatment using n-3 PUFA supplements. This investigation could help unlock potential health disparities in response to PUFA supplementation.
- Genotype at rs174537 is associated with variable clinical outcomes in response to treatment using dietary PUFA supplementation. This is due to differing levels of enzymatic efficiency based on haplotype and how that affects downstream production of either pro- or antiinflammatory biomolecules.

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Part IV

Biomarkers in Specific Conditions

Blood Count Profiles as Biomarkers in Burns: Red Cells, Platelets, and Beyond

17

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Abstract

The search for biomarkers that help physicians in medical practice has been a topic of great interest in the last decades. Burned patients represent a particular challenge due to the non-infectious systemic inflammatory response that characterizes thermal injury and the high incidence of sepsis in this population. Procalcitonin, a classic biomarker of infection, has demonstrated utility for identification of sepsis in burned patients. In contrast, C-reactive protein does not seem to improve infection diagnostic accuracy in this population. Diverse cytokines have also been studied as potential biomarkers in burned patients. Among these, interleukin-6 and interleukin-8 have shown a predictive value for different outcomes. Considering the importance of availability of biomarkers in everyday clinical practice, parameters derived from complete blood count have raised as interesting tools. Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and RDW-to-platelet ratio (RPR) have demonstrated usefulness in burned patients. A dynamic profile of these biomarkers can be observed, with significant differences between survivors and non-survivors. The integration of some of these biomarkers with clinical scores could help physicians to better understand and treat burned patients.

Keywords

Burn patient · Blood count · Biomarkers · Red blood cells · Platelets · White blood cells · Neutrophil- to- lymphocyte ratio · Platelet-to-lymphocyte ratio

Abbreviations

AKI	Acute kidney injury
ARDS	Acute respiratory distress syndrome
AUC	Area under the curve
BALF	Bronchoalveolar lavage fluid
CBC	Complete blood count
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
FEV1	Forced expiratory volume in 1 s
FVC	Forced vital capacity
Hb	Hemoglobin
Htc	Hematocrit
IL-6	Interleukin-6
IL-10	Interleukin-10
IL-1a	Interleukin-1a
IL-8	Interleukin-8
LAP	Latency-associated peptide
MCP-1	Monocyte chemoattractant protein 1
MLR	Monocyte-to-lymphocyte ratio
MODS	Multiple organ dysfunction syndrome

MPV	Mean platelet volume
NF-κB	Nuclear transcription factor kappa B
NGAL	Neutrophil gelatinase-associated lipocalin
NLR	Neutrophil-to-lymphocyte ratio
PC	Platelet count
PCT	Procalcitonin
PLR	Platelet-to-lymphocyte ratio
RBC	Red blood cell count
RDW	Red blood cell distribution width
RPR	Ratio between RDW and platelet count
SIRS	Systemic inflammatory response syndrome
TBSA	Total body surface area
TNF-α	Tumor necrosis factor-α
WBC	White blood cells

Diverse biomarkers have been studied during the last decades trying to improve our diagnostic capabilities. Among these, many of them require complex and costly techniques. Therefore, physicians have tried to find biomarkers that can be accessible and with low cost. Complete blood cell count and its derived parameters have raised as an interesting alternative in different clinical scenarios, especially in diseases characterized by a pro-inflammatory profile.

Usefulness of CBC-Derived Biomarkers in Different Clinical Situations

Sepsis and Septic Shock

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection with a high mortality (Singer et al. 2016). It is a major public health problem and a leading cause of hospitalization and mortality worldwide. Only in the USA, every year more than 1.5 million persons are diagnosed with sepsis and 250,000 die. Further, patients with sepsis had a 2.2-fold relative increase in late mortality, and this mortality is not fully explained by age, sociodemographics, or health status before sepsis (Prescott et al. 2016).

An early diagnosis is mandatory in patients with sepsis and septic shock to achieve a rapid administration of antibiotics and deliver a guided resuscitation. A prompt and specific treatment has shown improved outcomes among these patients (Seymour et al. 2017).

However, the diagnosis of sepsis is often not straightforward. That is why investigators are constantly looking for tools that allow us to approach the diagnosis and severity of sepsis. In this sense, different biomarkers have been tested, including procalcitonin, CRP, NGAL, as well as NLR.

The interest in having a biomarker derived from the blood count is its wide availability and low cost compared to other biomarkers.

Leukocytes play a crucial role on infection with an association between number and function of neutrophils and lymphocytes and outcome in patients with sepsis and septic shock. Leukocyte increase and lymphocyte depletion were associated with mortality in septic patients. Several causes explain the relationship between leukocyte count and survival in septic patients. Low circulating neutrophils were related to mortality due to ineffective innate immune response. Low circulating neutrophil count is produced by different reasons including neutrophil adhesion to the vascular endothelium. This sequestration in capillary beds can lead to microvascular occlusion and subsequent tissue ischemia with a critical role in the development of organ failure. Additionally, neutrophils release a variety of substances that are known to affect endothelial barrier integrity including reactive oxygen species and proteolytic enzymes (Fox et al. 2013; Bermejo-Martin et al. 2014).

According to the evidence, blood count profile was associated with sepsis and septic shock prognosis (Guell et al. 2019; Li et al. 2021).

Huang Z et al. in a recent meta-analysis including 9 studies comprising 10,685 patients evaluated the prognostic value of NLR in patients with sepsis and showed that higher NLR was associated with poor prognosis (Li et al. 2021).

In an interesting study, Djordjevic D et al. showed significant differences in mean platelet volume-to-platelet count ratio, monocyte-to-lymphocyte ratio, and platelet-to-lymphocyte ratio values regarding the nature of bacteremia. In this study, the lowest levels were found in patients with Gram-positive blood cultures (Djordjevic et al. 2018).

In small studies, neutrophil- to- lymphocyte ratio has shown a significant correlation with other more expensive inflammation and sepsis markers like PCT or CPR (Rehman et al. 2020).

ARDS

Numerous studies have shown that a high NLR can be an independent predictor of prognosis in various clinical situations. In the last years, studies among ADRS patients were conducted. In this sense, Huang Y et al. in a retrospective study including 275 ARDS patients showed an association between lower NLR and survival in this group of patients (Huang et al. 2019). In other study by Li W et al., when the ARDS patients were stratified by the NLR quartile, the highest NLR values quartile was associated with the highest mortality, while the lowest NLR values quartile showed the highest survival possibility (Wang et al. 2018).

Recently, among 81 ARDS related to COVID-19 patients, Ma A et al. showed an NLR area under the ROC curve more accurately than for procalcitonin or CPR (Ma et al. 2020). The high NLR group showed a higher incidence of ARDS and a higher invasive mechanical ventilation utilization (Li et al. 2019).

Cancer

An elevated NLR has been recently recognized as a poor prognostic indicator in various cancers (Ethier et al. 2017; Zhou et al. 2018).

In this sense, a meta-analysis conducted by Templeton et al. including 100 studies comprising 40,559 patients with solid tumors shows a consistent effect of an elevated NLR on survival among various disease subgroups and across disease stages (Templeton et al. 2014).

The relationship between blood count parameters and cancer prognosis in patients under chemotherapy or chemoradiotherapy was evaluated in small studies. In this sense, Hirahara T et al. showed that the NLR-PLR score was an independent prognostic factor for prediction of overall survival (Hirahara et al. 2019).

However, the evidence is constructed based on small studies. Many aspects must still be studied in largest studies.

COVID-19

Several studies reported that severe cases of COVID-19 were likely to have higher neutrophil count but lower lymphocyte count compared with non-severe patients. In this sense, NLR behaves as an independent risk factor of the in-hospital mortality for COVID-19 patients (Hirahara et al. 2019). The inflammatory response stimulates the production of neutrophils and favors lymphocyte apoptosis. In this scenario, patients with more severe clinical pictures show higher NLR (Liu et al. 2020; Ma et al. 2020).

COPD

There were significant positive correlations between NLR, stable COPD, and acute exacerbations of COPD. Several studies showed that the NLR is a valuable predictor of acute exacerbations of COPD and mortality in these patients. Furthermore, NLR is even more accurate than more complex and expensive markers like CRP and calprotectin. Additionally, several NLR cutoff values have been identified depending on the prediction purposes.

Other blood count elements were evaluated with interesting results. COPD is a systemic inflammation state and should deform erythrocytes and platelet membranes reducing the survival of these cells. High RDW and MPV have been associated with increased inflammatory activity in several diseases and particularly among COPD patients (Paliogiannis et al. 2018).

In an elegant study, Gao X et al. evaluated the relationship between NLR changes and pulmonary changes among patients examined up to 13 times each. NLR increase was associated with a decrease in FEV1, FVC, and FEV1/FVC. Changes in NLR up to approximately 10 years were associated with corresponding longitudinal changes in lung function. Furthermore, an increase in NLR was associated with higher development of COPD for participants without COPD at baseline (Gao et al. 2020).

Cardiovascular Diseases

Inflammatory and oxidative stresses have a role in the pathogenesis of cardiovascular disease, and many investigations are targeted to inflammatory markers. The NLR has been shown to predict cardiac arrhythmias and short- and long-term mortality in patients with acute coronary syndromes. NLR also presents a good correlation with acute coronary syndrome risk prediction models. On the other hand, a higher NLR has also been associated with frequent congestive heart failure decompensation and long-term mortality (Afari and Bhat 2016).

Inflammation and Biomarkers in Burn Injury

Inflammation is the cornerstone to understand the pathophysiology of burn injury. Burn injuries trigger both local and systemic responses that lead to multiple organ dysfunction syndrome (MODS) and death. Patients with severe burns are likely to suffer from sepsis that can rapidly develop into a systemic inflammatory response syndrome. Moreover, the systemic inflammatory response triggered by burns mimics sepsis presentation and complicates early sepsis diagnosis.

Therefore, a prompt sepsis diagnosis and the immediate initiation of antimicrobial therapy are needed to reduce morbidity and mortality. However, the unnecessary administration of antimicrobials is often associated with adverse effects, increased costs, and the emergence and spread of antimicrobial resistance.

The ability to identify or monitor organ function, infections, clinical trajectory, or patient outcome in severely burned patients would enable early intervention, reduce morbidity and mortality, and significantly lower the cost of clinical care.

Consequently, over the years, there has been an urgent need to find a sufficiently sensible and specific laboratory biomarker, which could allow distinguishing between a non-infectious SIRS and sepsis particularly in burn injury patients.

In this context, a wide variety of biomarkers have been explored and used in burn injury with the objective of predicting the risk of death and indicating an antibiotic therapy and diagnosis of sepsis.

Procalcitonin (PCT)

PCT is a member of the calcitonin gene-related peptide-amylin procalcitonin-adrenomedullin family. It is the precursor of the hormone calcitonin, composed of 116 amino acids (MW, 14 kDa), and is encoded by gene CALC-1. It is synthetized in neuroendocrine thyroid C cells in low concentrations under normal conditions. During systemic infections, CALC-1 is upregulated and consequently expressed in all organism cells, leading to the release of elevated amounts of PCT in the circulation.

PCT has a half-life of ~22–29 h, and, during bacterial infections, its levels start to rise 4 h after onset and reach the peak between 12 and 24 h. PCT levels decrease by 50% every 1–1.5 days (half-life) when the infectious process is controlled.

PCT levels are highly correlated with bloodstream infections, and PCT non-clearance was related with an increased risk of sepsis and a higher mortality rate. PCT is accurate for sepsis diagnosis, and its kinetics exhibit good correlation with sepsis severity. In critically ill patients, PCT is a reliable marker to assist intensivists in the decision to stop antibiotics. PCT-guided antibiotic duration is a validated approach to prevent antibiotic overconsumption in the intensive care unit setting. However, the utility of PCT in burn patients was questioned because of the high rate of false-positive results from the systemic inflammatory response induced by burn injury. Burn patients are generally excluded from sepsis studies and clinical trials based on the assumption that PCT levels are always elevated as a result of the non-septic inflammatory systemic response related to burn trauma. However, several studies consistently demonstrated different PCT kinetics in burn patients based on the presence or absence of systemic infection. In a retrospective observational study, *Cabral L* et al. described the kinetics of PCT before and after surgery. The authors demonstrated that PCT levels in the first 5 days after burn injury were significantly higher in patients who developed at least one sepsis episode ($n = 85$) compared with patients who did not develop sepsis ($n = 60$). PCT values >1.00 ng/mL were clearly associated with sepsis. PCT consistently showed good potential to discriminate between septic and non-septic patients, particularly when frequent PCT assays were performed and when its kinetics were dynamically assessed (Cabral et al. 2018). In the same study, they found that PCT levels increased modestly and rapidly returned to basal levels after the second postoperative day in patients with no preoperative or postoperative sepsis episodes. Patients with increased preoperative PCT values that corresponded to preoperative sepsis exhibited PCT kinetics with a higher peak on the second postoperative day, which was presumably related to the additive increment of PCT of surgical trauma. PCT values returned to the initial values when antimicrobial therapy was administered. PCT levels in patients who only developed sepsis after surgery exhibited a parallel evolution to the already septic patients but generally with lower absolute values. Accordingly, PCT is useful for sepsis diagnosis in cases of surgical intervention when preoperative PCT values are known because PCT kinetics follow the same pattern of evolution in cases of sepsis as in other critically ill patients.

In burn injury, the immediate inflammatory burst elevates PCT levels independently of infection and correlates with TBSA, but it rarely surpasses 2.0 ng/mL. The maximum PCT value is reached within 24–48 h in the absence of sepsis and returns to normal values (1.0–1.5 ng/mL or less) by the end of the third day. PCT levels continue increasing in the presence of sepsis and rapidly reach values greater than 5–100 ng/mL (Sachse et al. 1999; Lavrentieva et al. 2012). Lavrentieva A et al. evaluated the diagnostic and prognostic performance of inflammatory markers for septic and non-septic (localized) bacterial infections in patients with severe burns. Data of 145 patients were prospectively included in this study. Serum procalcitonin and other inflammatory markers were measured within 24 h after burn and daily

thereafter. Maximum procalcitonin was an independent predictor of outcome in logistic regression analysis. PCT thresholds of 1.5 ng/ml, 0.52 ng/ml, and 0.56 ng/ml had adequate sensitivity and specificity to diagnose sepsis, respiratory tract, and wound infections, respectively (Lavrentieva et al. 2012). Egea-Guerrero et al. analyzed serum levels of CRP and PCT at admission and every 48 h thereafter until intensive care unit discharge or death. They analyzed 157 determinations from 17 severe burn injury patients. ROC curve analysis revealed that ΔPCT could predict positive sepsis samples (area under the curve 0.75 [95% CI 0.58 to 0.90], P = 0.003) (Egea-Guerrero et al. 2015).

In an elegant study, Kim HS et al. demonstrated that PCT concentrations could be a useful prognostic indicator for sepsis and mortality in burn patients. Positive blood culture and mortality rates correlated significantly with procalcitonin concentrations within the first 48 h after burn injury. The area under the ROC curve for procalcitonin related to mortality was 0.844. Survival analysis revealed that the mortality rate was significantly higher in patients with procalcitonin concentrations ≥2 ng/mL than in patients with procalcitonin concentrations <2 ng/mL. Multivariate analysis demonstrated that procalcitonin was an independent prognostic factor for burn patients (Kim et al. 2012).

Three meta-analyses also validated the use of PCT for sepsis diagnosis in these patients; Cabral L et al. published a meta-analysis showing that overall pooled area under the curve (AUC) is 0.83 (95% CI = 0.76 to 0.90); the estimated cutoff is 1.47 ng/mL (Mann et al. 2011; Ren et al. 2015; Cabral et al. 2016).

Recently, Chen Z et al. published a meta-analysis whereby 10 studies and 704 patients were included. They evaluate the diagnostic value of serum PCT in adult burn sepsis and conclude that PCT may be used as a useful biomarker for the early diagnosis of burn sepsis (Chen et al. 2021).

Finally, results demonstrate that serum PCT may be used as a useful biomarker for the early diagnosis of burn sepsis in adults. PCT levels coupled with rigorous clinical monitoring and blood cultures as the diagnostic cornerstone may help to confirm or exclude sepsis in patients during the acute phase after burn trauma. The use of PCT dosing may inclusively reduce healthcare costs and avoid the superfluous use of antimicrobials and consequent increments on microbial resistance.

C-Reactive Protein (CRP)

Measurement of the serum level of CRP is a simple, rapid, and inexpensive procedure and has consequently become routine clinical practice in the follow-up of patients hospitalized with severe infections or burns. Induction of CRP releasing is mediated by the stimulation of pro-inflammatory cytokines, such as interleukin-6 (IL-6), IL-1, as well as tumor necrosis factor-α (TNF-α). Therefore, the synthesis and secretion of CRP from hepatocyte usually reflect pro-inflammatory cytokine production. CRP secretion began 4–6 h after inflammation stimulus, doubled every 8 h, and peaked at 36–50 h. CRP has a short half-life of 19 h, so as soon as the stimulus is stopped, it falls rapidly (Pepys and Hirschfield 2003; Sproston and Ashworth 2018).

Elevation of serum CRP is well documented in burn injury. Dynamic changes in CRP in the acute phase of burn injury are dependent on time from injury and TBSA (Jeschke et al. 2013). High concentrations of CRP occur with inflammation; however, this response is not specific (Barati et al. 2008). The value of CRP to predict the risk of sepsis has been studied in pediatric burn population. Although CRP is a marker of the inflammatory response post burn, CRP fails to predict infection or sepsis in pediatric severely burn patients.

Cytokines

Cytokines play a major part of the immunological response and the pathophysiological process following burn injury, contributing to increased risk of multi-organ failure and death. The levels of cytokines have a variable course after burn injury. However, a handful of these are strongly associated with morbidity and mortality.

Among cytokines which were increased in patients with severe burn injury as compared to moderate injury, IL-10, LAP (latency-associated peptide), and IL-1a displayed peak concentrations within the first 12 h after injury. Other cytokines (IL-6, IL-8, and MCP-1) displayed peak concentrations in these patients more than 24 h after injury.

Usually burn injury is complicated by inhalation injury (II), which contributes to mortality. Patients with II have an odds of dying nearly three times higher than those without inhalation injury. Besides that, the severity of inhalation injury involves an immune response measurable at a systemic level. In a study conducted by Davis CS et al., several plasma immune mediators measured early after injury were associated with mortality. The concentrations of several plasma immune mediators were increased with worse inhalation injury severity, even after adjusting for age and % TBSA. Of these, IL-1RA seemed to have the strongest correlation with injury severity and outcome measures (Davis et al. 2013). It has also been observed that bronchoalveolar lavage fluid (BALF) in burn patients with inhalation injury may present different inflammatory patterns according to the severity of the inhalation injury (Albright et al. 2012). Moreover, in a prospective observational study in 43 burn patients conducted by Jones SW, high early levels of IL-10 and low levels of IL-12p70 in the central airways show association with acute lung injury in patients intubated after acute burn/inhalation injury in the multivariate analysis (Jones et al. 2013). Finally, elevated hyaluronic acid, double-stranded DNA, and IL-10 levels in bronchial washings obtained early (the first 72 h after II) were significantly associated with positive bacterial respiratory cultures obtained during the first 14 days post injury in population of 72 burned patients (Maile et al. 2015).

IL-6

The production of pro-inflammatory cytokines, especially IL-6, is a hallmark of SIRS in the immediate phase after burn injury. Despite the fact that other

pro-inflammatory cytokines such as IL-8 and TNF-α have been identified as important mediators of the inflammatory changes that transpire after burn injury, the generally accepted notion is that IL-6 is the primary cytokine responding to burn injury (de Bandt et al. 1994; Fuchs et al. 2009).

Interleukin-6 is a pro-inflammatory cytokine secreted by various cells including inflammatory cells, keratinocytes, fibroblasts, and endothelial cells. It regulates the acute-phase response, and its main role involves the host response to infection. Even though it is predominantly a pro-inflammatory cytokine, in some cells, IL-6 can have regenerative and anti-inflammatory effects through the activation of membrane-bound IL-6 receptor signaling (Scheller et al. 2011).

IL-6 serum levels are significantly elevated early after burn injury and that IL-6 levels correlate with survival in adults (Jeschke et al. 2014). There is also a positive correlation between TBSA with IL-6 serum levels in the first 24 h after burn injury, supporting the fact that wounded skin is a likely source of early elevated IL-6 serum levels. Especially in the early phases of the burn trauma, when the pathogenic influences of bacteria are absent, the skin has to be considered as a possible source of IL-6 levels (Hager et al. 2018).

Although the initial spike in IL-6 elevation reflects the early anti-inflammatory response, chronic and excessive increases in serum IL-6 concentrations following burn injury are associated with a higher incidence of infection, sepsis, and death (Finnerty et al. 2007; Jeschke et al. 2014).

IL-8

IL-8 is primarily produced by macrophages, recruits leukocytes to primary sites of injury, and has been correlated with mortality caused by multi-organ failure. Aside from the recruitment of neutrophils, IL-8 signaling is implicated in the mechanisms underlying angiogenesis, cell growth, and tissue remodeling. IL-8 production is induced by cellular dysfunction and exogenous stimuli such as bacteria and viruses and via TNF-α or nuclear transcription factor kappa B (NF-κB) (Petzelbauer et al. 1995).

Kraft R et al. aimed to determine whether IL-8 expression could be used to predict post-burn sepsis, infections, and post-burn mortality in 468 pediatric burn patients. ROC analysis identified a cutoff level of 234 pg/ml for IL-8 for survival. Regression analysis revealed a significant predictive value of IL-8 to percent of total body surface area (TBSA) burned and incidence of MOF. High levels of IL-8 correlated with increased MOF, sepsis, and mortality. These data suggest that serum levels of IL-8 may be a valid biomarker for monitoring sepsis, infections, and mortality in burn patients (Kraft et al. 2015).

Recently, Bergquist M et al. demonstrated that although IL-6 and IL-8 concentrations peaked in plasma at 36–48 h after the injury occurred, these cytokines performed similar to Baux score and modified Baux score at predicting outcome at the time of admission. Moreover, IL-6 and IL-8 were higher in survivors compared to non-survivors on the second day after burn injury, suggesting that there is a

relationship between the inflammatory response, organ failure, and outcome (Bergquist et al. 2019).

Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Acute Kidney Injury (AKI)

AKI is a well-known complication of severe burns and is an important cause of increased mortality. Early detection of AKI and subsequent adequate treatment could improve patient outcomes. However, AKI is determined mainly by creatinine and urine output which are insensitive and nonspecific diagnostic factors that reflect renal function. Plasma and urine neutrophil gelatinase-associated lipocalin (NGAL) is known to rise 24 to 48 h before the serum creatinine levels increase. NGAL has shown good performance as an AKI biomarker in several critical and emergency care populations. NGAL is normally produced by neutrophils during inflammation and is renally cleared. During AKI, plasma NGAL increases (>100 ng/mL), and interestingly, renal tubular cells also produce NGAL. To this end, plasma and urine NGAL levels are elevated in patients at risk for renal injury. NGAL has been proven to be associated with development of early acute kidney injury and mortality (Yang et al. 2014).

Blood Count Profiles in Burns

Several components of complete blood count (CBC) can be affected in patients with burn injuries. Given the great complexity of burned patients, dynamic changes in blood components could account for the initial injury, systemic inflammation, blood loss, infections, or surgery. Although all these factors must be kept in mind when interpreting CBC, characteristic patterns have been described in burned patients. Importantly, certain blood count profiles have been associated with outcome of burned patients. In this section, we will review some of the most characteristic patterns of blood count and derived parameters in burned patients (Table 1).

Red Blood Cells

A progressive reduction in red blood cell count (RBC), hemoglobin (Hb) content, and hematocrit (Htc) has been consistently described in burned patients requiring hospitalization (Qiu et al. 2017; Sen et al. 2019; Angulo et al. 2020; Qiu et al. 2021). Differences between survivors and non-survivors in these parameters have been reported, although findings have been contradictory. Nevertheless, Osuka et al. found that reduction in RBC (during the first 5 days of admission) was an independent risk factor for mortality (Osuka et al. 2019).

Besides the absolute number of red blood cells and hemoglobin content, red blood cell distribution width (RDW) can provide information about cell

Table 1 Studies that analyze blood count profiles as biomarkers in burns

Author	Year Type	Population	n	TSBA (%)	Results
Karakaya E	2021 Retrospective	Adults	172	43	In a model to estimate risk of acute kidney injury in severe burn patients, increased NLR and platelets >160,000 were associated with AKI
Kim HY	2018 Retrospective	Adults	471	N/A	Multivariate logistic regression analysis revealed that increased presurgery NLR was independently associated with AKI. Optimal cutoff 11.7
Hu L	2020 Retrospective	Adults	271	55	NLR at day 1 > 14, NLR at day 2 > 13, and NLR at day 3 > 7.5 were associated with mortality In a multivariable logistic regression, increased NLR at admission was independently associated with mortality
Guo F	2012 Retrospective	Adults	148	N/A	Platelet count decline during the first 3 days was significantly associated with 30-day mortality.
Angulo M	2020 Retrospective	Adults	88	14	NLR >13 at day 1, RPR >0,07 at day 3, and PLR >60 at day 3 were associated with an increase in ICU mortality
Sen S	2019 Retrospective	Adults	191	32	Increase in mean platelet volume on day 7 was independently associated with mortality
Temiz A	2020 Retrospective	Pediatric and adults	133	>15	Increase in mean platelet volume and NLR values and decrease in PLR values were found to be associated with mortality
Osuka A	2019 Retrospective	Adults	280	37	Day 3 lymphocyte count and day 10 monocyte count were risk factors for mortality. Low platelet counts from day 3 to day 30 following injury were a predictor of mortality
Qui L.	2021 Retrospective	Adults	577	67	NLR < 10,5 on the third day was associated with higher mortality
Cato LD	2017 Retrospective	Adults	145	30	Platelet peak was the strongest predictor of mortality

(continued)

Table 1 (continued)

Author	Year Type	Population	n	TSBA (%)	Results
Qui L	2017	Adults	610	N/A	RDW, PLT, and RPR values on the third and seventh day were significantly associated with the outcomes
Xiao C	2019 Retrospective	Adults	610	52	RDW were independently associated with the development of ARDS
Huang X	2017 Retrospective	Adults	206	Burn index >50	A less mean daily increase in the platelet count was associated with increased 30-day mortality
Takkar RK	2018 Retrospective	Pediatric	90	17	Decreased lymphocytes between 72 h and 7 days post injury were associated with infection and ICU length of stay

homeostasis. Interestingly, RDW is increased in non-survivors but remains stable in surviving patients (Guo et al. 2016; Sen et al. 2019; Angulo et al. 2020). However, after adjusting for diverse confounders, Qiu et al. could not demonstrate an independent predictive value for mortality of RDW in adult burned patients (Qiu et al. 2017). Notwithstanding, an increased RDW has been reported as an independent risk factor for developing acute respiratory distress syndrome (ARDS) in severely burned patients (Xiao et al. 2019).

Platelets

Thrombocytopenia is among the most characteristic CBC abnormalities observed in patients suffering major burns. Platelet count describes a particular pattern in this population. Patients admitted to the hospital usually present a nearly normal platelet count that is consistently reduced after the first day, reaching a nadir on days 3 to 4 (Guo et al. 2012; Marck et al. 2013; Huang et al. 2019; Osuka et al. 2019; Sen et al. 2019; Angulo et al. 2020). Multiple factors could explain thrombocytopenia in burned patients, including hemodilution resulting from fluid resuscitation, platelet consumption and reduced production as a cause of bone marrow depression, as well as the use of certain drugs such as silver sulfadiazine or heparin. Thrombocytopenia is followed by a progressive increase in platelet count that could result in a transient thrombocytosis by day 15 (Marck et al. 2013). Numerous studies have demonstrated differences in platelet count between survivors and non-survivors after thermal injury. While a similar pattern can be observed in both groups of patients, non-survivors present a more profound and persistent degree of thrombocytopenia (Guo et al. 2012; Marck et al. 2013; Qiu et al. 2017; Cato et al. 2018; Huang et al. 2019; Osuka et al. 2019; Sen et al. 2019; Angulo et al. 2020). In fact, the magnitude

of thrombocytopenia has been reported as an independent risk factor for mortality in severely burned patients by different authors (Marck et al. 2013; Cato et al. 2018; Osuka et al. 2019 ; Qiu et al. 2021). Interestingly, a lower platelet count has also been associated with sepsis in adult burned patients (Cato et al. 2018).

In addition to absolute platelet count, mean platelet volume (MPV) is believed to reflect the inflammatory and thrombotic status. In adult burned patients, a decrease in MPV has been described by days 5 and 7 after hospital admission (Angulo et al. 2020). However, by the end of the first week, MPV is significantly higher in non-survivors compared to survivors (Sen et al. 2019; Angulo et al. 2020). Importantly, Sen et al. reported MPV on day 7 to be an independent risk factor for mortality in patients with severe burn injuries (Sen et al. 2019).

White Blood Cells

Pronounced and dynamic alterations can be observed in white blood cells' (WBC) total count and formula in burned patients. Thermal injury and the resulting inflammatory response probably account for these phenomena during the initial phase of burned patients' evolution. However, in the following days (and thereafter), as infection risk rises, WBC count abnormality interpretation represents a major challenge for physicians. As a general rule, burned patients requiring hospitalization present increased WBC count on admission, probably as part of the systemic inflammatory response to a major trauma (Thakkar et al. 2018; Osuka et al. 2019; Sen et al. 2019; Qiu et al. 2021). This is followed by an abrupt decrease in WBC levels from days 2 to 4, to gradually normalize thereafter (Osuka et al. 2019; Sen et al. 2019). Some differences have been reported in total WBC count between survivors and non-survivors, with the latter presenting higher levels during the first days post injury (Osuka et al. 2019; Sen et al. 2019). However, other authors found no statistically significant differences in total WBC count between survivors and deceased burned patients (Qiu et al. 2017; Temiz et al. 2020).

Specific populations of WBC also present characteristic patterns and implications in burned patients. Neutrophil kinetics follow a similar pattern than WBC (Osuka et al. 2019; Angulo et al. 2020). Non-survivors had higher neutrophil levels than survivors on admission (Angulo et al. 2020; Qiu et al. 2021; Steinvall et al. 2021). Lymphocytes and monocytes decrease rapidly after admission in all patients, with non-survivors presenting lower levels than survivors according to some reports (Osuka et al. 2019; Qiu et al. 2021). However, Qiu et al. did not find any statistically significant differences on total WBC, neutrophil, or lymphocyte count between survivors and non-survivors on days 3 and 7 after thermal injury (Qiu et al. 2017). In one study including 133 burned patients, eosinophil count was reduced in deceased patients compared to survivors (Temiz et al. 2020).

Interestingly, in a study including over 600 patients with severe burns, higher WBC, neutrophil, and lymphocyte count were associated with ARDS development, although none constituted an independent risk factor on multivariate analysis (Xiao et al. 2019).

Complete Blood Count-Derived Parameters

Given the heterogeneous and dynamic patterns of different blood cells in burned patients, the analysis of indices derived from complete blood cell count has raised particular interest during the last years. As previously described, NLR, PLR, and RPR have been demonstrated to represent the inflammatory status in diverse scenarios, being associated with patients' outcome in different pathological states. Thermal injury has not been an exception, although studies are still scarce.

After a relatively normal NLR on admission, a significant decrease in NLR has been described in adult burned patients after hospital admission (Angulo et al. 2020). The NLR value on day 1 presents a significant correlation with total body surface area (TBSA) burned, TBSA third-degree lesions, and patient severity (abbreviated burn severity index) (Angulo et al. 2020). Moreover, a negative correlation exists between NLR and ventilator-free days at day 28 (Hu et al. 2021). Finally, a higher preoperative NLR constitutes an independent risk factor for development of acute kidney injury after burn surgery (Kim et al. 2019; Karakaya et al. 2022).

Not surprisingly, different authors reported deceased burned patients having higher NLR than survivors (Angulo et al. 2020; Temiz et al. 2020; Qiu et al. 2021; Steinvall et al. 2021). Our group identified an admission NLR $\geq$ 13.0 as a risk factor for hospital mortality (sensitivity of 69.2% and specificity 76.0%) (Angulo et al. 2020). Importantly, similar cutoff values were observed in a larger study, with NLR $>$ 14.0 on day 1 and NLR $>$ 13.0 on day 2 being associated with higher mortality (Hu et al. 2021). In that study, Hu et al. reported that higher admission NLR was an independent risk factor for mortality after adjusting for diverse possible contributing factors. Accordingly, in a study involving 577 patients with TBSA burned $>$30%, a higher NLR on day 3 was found to constitute an independent risk factor for 90-day mortality (Qiu et al. 2021). However, in a recent study, although high NLR was associated with increased mortality in burned patients, its addition to the Baux score failed to improve mortality prediction (Steinvall et al. 2021).

Very little information exists regarding other CBC-derived indices in burned patients. In a study involving two cohorts of adult burned patients, PLR significantly decreased on day 3, returning to baseline levels by day 5. Non-survivors presented lower PLR than survivors (Angulo et al. 2020). The authors found a PLR on day 3 $<$ 60 to predict patient mortality with a 54.5% sensitivity and a 95.8% specificity. Temiz et al. also reported deceased burned patients to have a lower PLR than survivors (Temiz et al. 2020). Finally, the ratio between RDW and platelet count (RPR) in burned patients has been described in a couple of studies. In adult burned patients, a rise in RPR is observed between days 3 and 5, returning to baseline levels afterward (Angulo et al. 2020). No differences are observed between survivors and non-survivors on admission; however, a progressive increase is seen in deceased patients from day 3, while a decrease trend is described in survivors. Therefore, non-survivors present significantly higher RPR on days 3 to 7. In fact, higher RPR has been reported as an independent risk factor for 90-day mortality in adult patients suffering thermal injury (Qiu et al. 2017).

Summary Points

- Biomarkers have been a topic of great interest in the last decades.
- Burned patients represent a particular challenge due to the non-infectious systemic inflammatory response.
- Procalcitonin has demonstrated utility for identification of sepsis in burned patients.
- Other biomarkers like C-reactive protein do not seem to improve infection diagnostic accuracy in this population.
- Interleukin-6 and interleukin-8 have shown a predictive value for different outcomes.
- Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and RDW-to-platelet ratio (RPR) have demonstrated usefulness in burned patients.
- A dynamic profile of these biomarkers can be observed, with significant differences between survivors and non-survivors.
- The integration of some of these biomarkers with clinical scores could help physicians to better understand and treat burned patients.

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The Opportunity of Surfactant Protein D as a Potential Biomarker for Detecting Acute Lung Injury

18

Anna Surgean Veterini

Contents

Abstract

Acute lung injury is a medical problem that causes a high mortality rate if it is not detected early and followed by appropriate treatment. The detection of acute lung injury is generally by clinical, radiological, and arterial blood gas analysis. This is a little late because when clinical symptoms appear, the cure rate will decrease. We need an early diagnosis of acute lung injury so that, as clinicians, we can anticipate further organ damage. One idea to detect acute lung injury is by examining the serum surfactant protein D. Although it still has to be combined

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with clinical examination and other examinations, at least there is a simple technique to increase the sensitivity and specificity of the overall diagnostic method if done holistically before more extensive organ damage occurs.

Keywords

Biomarker · Diagnostic · Surfactant protein D · Acute lung injury · Acute respiratory distress syndrome · Alveolar type II pneumocyte · Clara cells · Collectin family · Collagen-containing C-type lectin · External lung injury

Abbreviations

ALI	Acute lung injury
Ang-2	Angiopoietin-2
ARDS	Acute respiratory distress syndrome
AT2	Alveolar type II pneumocyte
BAL	Bronchoalveolar lavage
BMI	Body mass index
CD4	Cluster of differentiation 4
CD91	Cluster of differentiation 91
CF	Cystic fibrosis
COPD	Chronic obstructive pulmonary disease
CRD	Carbohydrate recognition domain
ELISA	Enzyme-linked immunosorbent assay
IPF	Interstitial pulmonary fibrosis
KGF	Keratinocyte growth factor
KL-6	Krebs von den Lungen 6
LPS	Lipopolysaccharides
MAPK	Mitogen-activated protein kinase
MBL	Mannose-binding lectin
NF-κB	Nuclear factor kappa light chain enhancer of activated B cells
PAMPs	Pathogen-associated molecular pattern molecules
PARS	Pediatric acute respiratory distress syndrome
PTB	Pulmonary tuberculosis
RAGE	Receptor for advanced glycation end products
SARS	Severe acute respiratory syndrome
SNPs	Single-nucleotide polymorphisms
SP-A	Surfactant protein A
SP-B	Surfactant protein B
SP-C	Surfactant protein C
SP-D	Surfactant protein D
sRAGE	Soluble receptor for advanced glycation end products
Th1	T helper 1 cells
VEGF	Vascular endothelial growth factor
VWF	von Willebrand factor

Definitions of Words and Terms

Acute Lung Injury	A diffuse heterogeneous lung injury characterized by widespread capillary leakage, low lung compliance, non-cardiogenic pulmonary edema, and hypoxemia and a milder form of ARDS
Acute Respiratory Distress Syndrome	A severe lung injury that allows fluid to leak into the lungs which is characterized by impaired carbon dioxide excretion, increased permeability pulmonary edema, and severe arterial hypoxemia
Chronic Hypoxemic Respiratory Failure	An ongoing condition of not enough oxygen in the bloodstream, but the levels of carbon dioxide are close to normal
Pulmonary Infiltrates	A substance denser than air that lingers within the lung parenchyma
SP-D, Surfactant Protein D	A collagen-containing C-type lectin which is produced by alveolar type II and Clara cells and known to play a role in surfactant homeostasis and pulmonary immunity

Key Facts

Key Facts for Acute Lung Injury

- Considered as a condition of acute inflammation which causes the endothelial and epithelial barriers in the lungs to be disrupted.
- Biomarkers present on the epithelium and endothelium, as well as those that are involved in the inflammatory and coagulation cascades, are used to predict morbidity and mortality in ALI patients.

Key Facts for Surfactant Protein D

- SP-D is mainly produced by alveolar type II and Clara cells.
- The presence of SP-D in the plasma of ALI/ARDS patients is thought to indicate permeability and damage to the alveolar epithelial barrier.
- The detection of lung damage can be aided by measuring serum SP-D levels.

Introduction

ARDS cannot be diagnosed by a single laboratory examination. The risk of establishing a false-positive ARDS diagnosis is high since no specific biomarkers for ARDS have been reported. This is due to the ease with which ARDS can be identified in patients with transient or chronic hypoxemic respiratory failure due to an underlying disease and bilateral pulmonary infiltrate. One of the most significant challenges in diagnosing and effectively treating ARDS is the lack of specific biomarkers. Biomarkers are being developed to better understand the risk and severity of ARDS in patients. The perfect biomarker will be able to distinguish patients who are at risk of developing ARDS as their lung injury progresses. The use of biomarkers to predict or track the progression of ARDS is hoped to make it easier for clinicians to collect data from the therapy that has been administered. This has aided clinical practice as well as the advancement of innovative testing technologies and medications that can improve outcomes (Isabel García-Laorden et al. 2017).

Surfactant protein D (SP-D) is synthesized by type II pneumocytes and belongs to the collectin family, and its primary function is to recognize pathogen-associated molecular patterns allowing microbial elimination. SP-D participates in the neutralization and clearance of influenza viruses, given its molecular affinity to viral hemagglutinin. SP-D levels correlate with pro-inflammatory immune responses, mainly when alveolar macrophages interact with the trimeric form through their CD91 receptor, leading to the activation of the p38 MAPK signaling pathway that elicits Th1 responses. Various lung disorders influence SP-D production, and its role as a biomarker of lung inflammation has been described (Table 1). Based on the central role of SP-D in the pulmonary host defense and the regulation of inflammatory responses and its dysregulation in lung diseases, we hypothesize that circulating levels of SP-D are modified as a result of lung tissue damage in critically ill A/H1N1-infected patients and that SP-D is a valuable biomarker to predict poor outcomes in ARDS patients with A/H1N1 infection. According to recent studies, the analysis of circulating levels of SP-D is helpful as a diagnostic tool in severe sepsis patients with ARDS and to evaluate the progression of lung injury in critically ill patients with mechanical ventilation in whom circulating levels of this protein positively correlate with the lung injury score as a parameter to measure the pathophysiological features of ARDS (Delgado et al. 2015).

Biomarker Combination

Several studies on markers of epithelial and endothelial injury, coagulation, and inflammation have shown that combining multiple biomarkers can predict mortality better than clinical or single biomarkers alone. The use of combined biomarkers is superior to clinical risk factors alone in predicting mortality in ARDS and is helpful for ARDS diagnosis. In severe sepsis, a combination of biomarkers such as RAGE, SP-D, and Club cell protein 16 is more sensitive in diagnosing ARDS (Spadaro et al. 2019; Ware et al. 2013).

Table 1 Serum SP-D levels as a biomarker in acute lung injury/acute respiratory distress syndrome

References	Main points
Eisner et al. (2003)	ALI/ARDS is a disorder in which the lung reacts severely to various forms of injuries to the lungs ranging from trauma to drug abuse. SP-D level in alive subjects was 73 ng/ml which is ~40% increase to the control serum level. However, increased SP-D levels (101 ng/ml) in postmortem subjects demonstrate the relationship between the substantial increase in lung SP-D and a greater risk of death. A higher level of plasma SP-D early in the course of ALI/ARDS is linked to a worse clinical outcome
Punsawad et al. (2019)	The mean levels of SP-D in the plasma were significantly elevated in the malaria-infected mice with ALI/ARDS (24.79 ± 0.23 ng/mL) compared with those in the malaria-infected mice in the non-ALI/ARDS group (6.24 ± 0.69 ng/mL) and the mice in the control group (5.86 ± 0.64 ng/mL) ($p = 0.009$). An increased level of plasma SP-D was observed in the malaria-infected mice with ALI/ARDS compared with that in the malaria-infected mice in the non-ALI/ARDS group and the mice in the control groups. This finding suggests that serum SP-D could be useful for evaluating and monitoring signs of ALI
Determann et al. (2010)	SP-D levels were not significantly different between ALI/ARDS patients and patients without lung injury. In ALI/ARDS patients, SP-D and KL-6 levels increased over time, which was attenuated by lung-protective mechanical ventilation using lower tidal volumes. Plasma levels of SP-D rise with potentially injurious ventilator settings and thus may serve as biological markers of VALI in patients with ALI/ARDS
Ware et al. (2010)	A combination of biomarkers and clinical predictors is superior to clinical predictors or biomarkers alone for predicting mortality in ALI/ARDS and may be useful for stratifying patients in clinical trials. Across all models tested, the two best performing biomarkers were IL-8 and SP-D, confirming the importance of these pathways in the pathogenesis of clinical ALI/ARDS
Park et al. (2017)	Patients with ARDS had higher SP-D levels (20.8 ng/ml) in plasma than those without ARDS (7.9 ng/ml). The results showed that plasma SP-D has acceptable discriminatory ability for the diagnosis of ARDS. This findings seem to provide sufficient support for further consideration of SP-D as a promising biomarker for diagnosis of ARDS

A biomarker can take many forms, from physiologic parameters (e.g., blood pressure) to radiographic findings (e.g., carotid intima-media thickness), cell-based markers (e.g., CD4 levels), and molecules (e.g., low-density lipoproteins). In recent years, genetic profiles, single-nucleotide polymorphisms (SNPs), and protein expression patterns have increasingly been viewed as potentially valuable biomarkers (Walter et al. 2014).

What Is Surfactant Protein D?

SP-D is a collagen-containing C-type lectin that belongs to the collectin family and is known to play a role in surfactant homeostasis and pulmonary immunity. Alveolar type II and Clara cells are the main producers of SP-D, which is secreted into the lungs' airspace. A cysteine-rich N-terminus, a triple-helical collagen region

consisting of Gly-X-Y triplet repeats, an a-helical coiled neck region, and a C-terminal C-type lectin or carbohydrate recognition domain (CRD) form its primary structure. SP-D can interact with a variety of pathogens (Table 2), activating clearance mechanisms against viruses, bacteria, and fungi, as well as apoptotic cells, as an innate immune molecule (Hsieh et al. 2020).

SP-D belongs to the C-type lectin family, which has four structural domains: a cysteine-rich domain at the N-terminus, a collagenous domain at the center, a neck region, and a carbohydrate recognition domain (CRD) at the C-terminus (Seaton et al. 2010). SP-D is involved in innate host defense and inflammatory response control in a variety of infectious diseases. SP-D knockout (KO) mice have demonstrated an increased resistance to Gram-negative and Gram-positive bacteria, viruses, and fungi-induced lung infection. Endotoxemia caused increased inflammation and lung damage in SP-D KO mice (King and Kingma 2011). SP-D facilitates the uptake and clearance of pathogens by phagocytes and epithelial cells, the clearance of apoptotic cells, and the modulation of inflammatory processes through the NF-κB pathway by binding to pathogen-associated molecular patterns (PAMPs) (Waters et al. 2009; Du et al. 2018).

SP-D is a 43-kDa collectin superfamily member that is found in lung surfactant and has been shown to play an important role in the innate immune system as a pattern recognition molecule (Korfhagen et al. 1998; Crouch and Wright 2001). Surfactant protein D (SP-D) is known to bind to various bacterial, fungal, and viral surfaces and immune cells, playing an important role in host defense and regulation of immune responses and lung phospholipid levels (Korfhagen et al. 1998). SP-D also affects the role of lymphocytes and neutrophils by promoting chemotaxis of antigen-presenting cells. SP-D is a protein formed almost exclusively by type II cells of the alveolar epithelial system. The presence of SP-D in the plasma of patients with ALI/ARDS is thought to reflect on damage to the alveolar epithelial barrier and increased permeability (Eisner et al. 2003; Fig. 1). The composition of pulmonary surfactant is 90% lipid and 10% protein. Surfactant-associated proteins include two collagenous carbohydrate-binding glycoproteins (SP-A and SP-D), as well as two tiny hydrophobic proteins (SP-B and SP-C).

The extreme acute respiratory syndrome (i.e., SARS virus infection) has also been shown to bind to SP-D. SARS is an enveloped virus that belongs to the *Coronaviridae* family of viruses that infect both humans and animals. In 2002 and 2004, there were two self-limiting SARS outbreaks that resulted in a highly infectious and potentially life-threatening form of pneumonia. The spike protein, also known as S-protein, is a trimerized virus fusion protein found in the SARS virus. SP-D was discovered to bind to recombinant trimeric proteins, with the binding being calcium dependent and inhibited by maltose, exhibiting the characteristics of a classic C-type lectin-carbohydrate relationship. Purified MBL did not interact with the S-protein, indicating that the interaction was unique to SP-D and highlighting that the collectins have different ligands (Watson et al. 2018).

A complex interaction of inflammation, injury to alveolar cells types I and II, injury to bronchiolar and endothelial cells, and activation of coagulation causes acute lung injury in the initial (or exudative) process of ALI/ARDS (Fig. 2) (Mokra

Table 2 Surfactant protein D interacts with various pathogens (Nayak et al. 2012)

Pathogen	Target	Implication	References
Virus			
SARS coronavirus	S-protein	nd	Leth-Larsen et al. (2007)
Human immunodeficiency virus	Glycoprotein 120 (gp120)	Neutralization	Meschi et al. (2005)
Respiratory syncytial virus	G protein	Neutralization	Hickling et al. (1999)
Rotavirus (bovine)	VP7 glycoprotein	Agglutination, neutralization	Reading et al. (2004)
Fungi			
Candida albicans	Mannose, maltose	Agglutination, growth inhibition, and inhibition of phagocytosis	Van Rozendaal et al. (2000)
Aspergillus fumigatus	Mannose, maltose, 45 and 55 kDa	Binds to conidia forms, agglutination, attachment to phagocytes, and enhanced uptake	Madan et al. (1997)
Blastomyces dermatitidis	1,3-β-Glucan	Binds to yeast form	Lekkala et al. (2006)
Saccharomyces cerevisiae	1,6-β-Glucan	Agglutination	Allen et al. (2001)
Gram-negative bacteria			
Escherichia coli	LPS	Agglutination, enhanced uptake, and growth inhibition[b]	Kuan et al. (1992), Wu et al. (2003), Hartshorn et al. (1998)
Enterobacter aerogenes	LPS	Inhibits growth[a]	Wu et al. (2003)
Legionella pneumophila	LPS	Inhibits growth	Sawada et al. (2010)
Gram-positive bacteria			
Bacillus subtilis	Lipoteichoic acid	nd	Van de Wetering et al. (2001)
Staphylococcus aureus	Peptidoglycan	Enhanced uptake	Hartshorn et al. (1998), Van de Wetering et al. (2001)
Mycobacterium tuberculosis	Lipoarabinomannan	Reduces uptake by macrophages	Ferguson et al. (1999)
Streptococcus pneumoniae	nd	Agglutination[b] and enhanced uptake[b]	Hartshorn et al. (1998), Jounblat et al. (2004)

(continued)

Table 2 (continued)

Pathogen	Target	Implication	References
Protozoa			
Schistosoma mansoni	nd	nd	Van De Wetering et al. (2004)

nd not determined
[a]Strain dependent
[b]rough/smooth LPS – Strain dependent

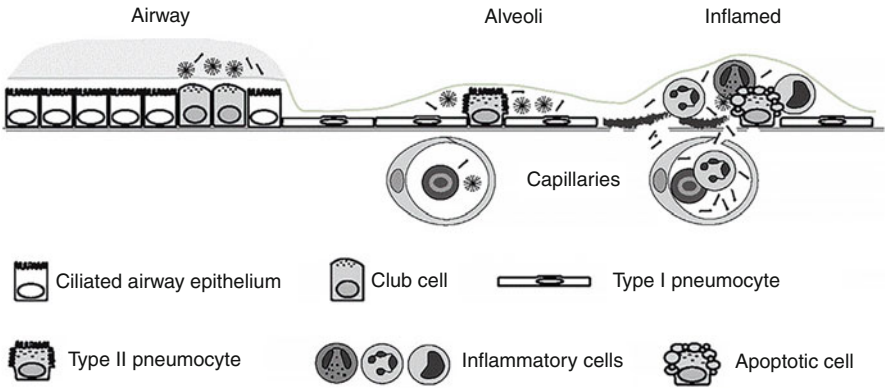

Fig. 1 Circulatory spillover of pulmonary surfactant protein D (SP-D) in inflammatory disease. SP-D is synthesized by Club cells, type II alveolar cells, and endothelial cells, and the levels of SP-D multimers and trimers in the serum are highly genetically determined. In the inflamed lung, the production of trimeric SP-D is increased, due to various chemical modifications and proteolytic breakdown of the protein, and loss of air-blood barrier integrity allows spillover of pulmonary SP-D into the circulation. For simplicity, only alveolar damage is illustrated. Moreover, only fuzziball SP-D multimers are depicted (Sorensen 2018)

and Kosutova 2015). The measurement of serum SP-D levels is a useful tool for detecting lung damage, and it can be used in both toxicological and pharmacological studies (Murata et al. 2016).

Respiratory Epithelium Markers

Respiratory epithelium markers include surfactant proteins (SP), Krebs von den Lungen 6 (KL-6) protein, vascular endothelial growth factor (VEGF), and soluble receptor for advanced glycation end products (sRAGE) (Spadaro et al. 2019).

(a) Surfactant proteins (SP) are generally increased in ARDS, and SP-B can cross the damaged alveolar-capillary membranes (Greene et al. 1999). SP-D blood levels have been shown to correlate with ARDS mortality (Ware et al. 2010; Spadaro et al. 2019).

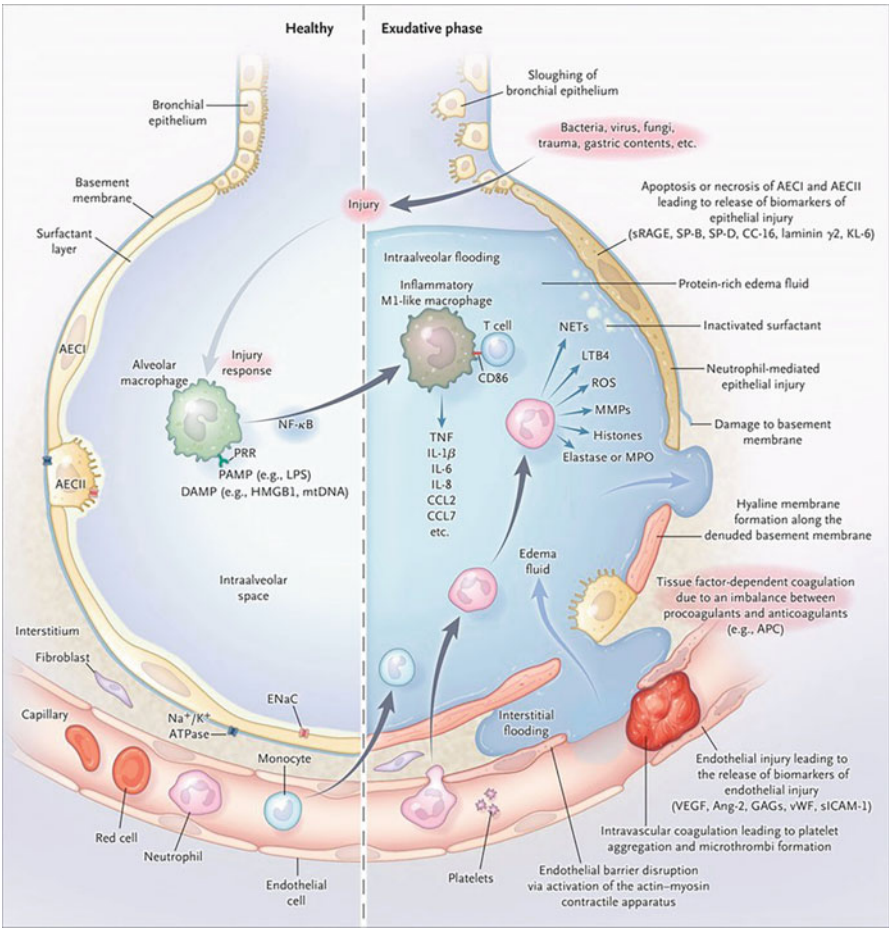

Fig. 2 The healthy lung is shown on the left, and the exudative phase of ARDS is shown on the right. Injury is initiated by either direct or indirect insults to the delicate alveolar structure of the distal lung and associated microvasculature. In the exudative phase, resident alveolar macrophages are activated, leading to the release of potent pro-inflammatory mediators and chemokines that promote the accumulation of neutrophils and monocytes. Activated neutrophils further contribute to injury by releasing toxic mediators. The resultant injury leads to loss of barrier function, as well as interstitial and intra-alveolar flooding. Tumor necrosis factor (TNF)-mediated expression of tissue factor promotes platelet aggregation and microthrombus formation, as well as intra-alveolar coagulation and hyaline membrane formation (Thompson et al. 2017)

(b) KL-6 levels have been associated with ARDS mortality (Sato et al. 2004). KL-6, lactate dehydrogenase, sRAGE, and von Willebrand factor were found to be correlated with a diagnosis of ARDS in a high-risk population in a meta-analysis of plasma biomarkers for ARDS that analyzed 54 studies in 2014 (Terpstra et al. 2014).

(c) The levels of vascular endothelial growth factor (VEGF) and keratinocyte growth factor (KGF) are linked to the seriousness of the disease and the patient's outcome (Koh et al. 2008).
(d) RAGE utility as a biomarker is still questionable. Higher RAGE levels have been linked to impaired alveolar fluid clearance in ARDS patients and thus represent the seriousness of the pulmonary epithelial injury, according to several reports. Soluble RAGE (sRAGE) helped diagnose ARDS in a high-risk population in a meta-analysis review, but it was not linked to mortality (Isabel García-Laorden et al. 2017).
(e) Angiopoietin-2 (Ang-2) and endothelial dysfunction markers are examples of endothelial markers. Ang-2 levels that are elevated in ARDS patients and at-risk patients are predictors of mortality. Furthermore, the von Willebrand factor (VWF) tends to be linked to mortality in ARDS patients (Spadaro et al. 2019).

The Advantage and Disadvantage of Using Circulating SP-D

SP-D levels in the blood have been studied to see whether they could be used as a biomarker for dermatitis, acute lung injury (ALI)/acute respiratory distress syndrome (ARDS), periodontitis, interstitial pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD) (Sin et al. 2007), emphysema, cystic fibrosis (CF), coronary disease, sclerosis, cancer, and sarcoidosis. There have also been research looking at SP-D levels in people with Turner syndrome and paraquat toxicity and swimming in always treated lakes, lung transplant patients, patients undergoing neurosurgical procedures, drowning victims, and people with polymyositis/dermatomyositis, dementia, lupus, and sleep apnea (Bratcher and Gaggar 2014).

The following are some of the advantages of the circulating SP-D test for detecting acute lung injury:

1. Collection of specimens is quick and easy.
2. The enzyme-linked immunosorbent assay (ELISA) method makes inspection relatively simple.
3. The effects of circulating SP-D levels, which serve as a warning that "something" is wrong with the body's organs, especially the lungs (Kuroki et al. 1998).

The following are some of the SP-D examination disadvantages for detecting acute lung injury:

1. Levels of SP-D have also been linked to genetic factors (Heidinger et al. 2005), body mass index (BMI) (Sorensen et al. 2006) (Zhao et al. 2007), age (Engström et al. 2012), circadian rhythm (Bratcher and Gaggar 2014), and particle exposure and cigarette smoking habits (Mutti et al. 2006; Vinod et al. 2019).
2. If AT2 cells are damaged (e.g., as a result of the SARS COV-2 virus), an increase in circulating SP-D levels cannot be used as a marker for tissue damage. A

decrease in circulating SP-D levels is most likely a sign of lung organ injury (Shtepa 2018).
3. While SP-D has been reported to be able to predict external lung injury, further research is needed to determine how responsive and specific it is for extra-pulmonary detection.

The Rationalization of Using Circulating SP-D as Biomarker Diagnostic for Acute Lung Injury Based on Literature Study

1. In children with acute respiratory failure, elevated circulating SP-D levels are linked to extreme pediatric acute respiratory distress syndrome (PARS) and poor outcomes (Dahmer et al. 2020).
2. Diagnosing and differentiating pulmonary pathology can be difficult. Lower concentrations of surfactant proteins A and D in bronchoalveolar fluid and higher concentrations in plasma have been linked to more severe lung damage and poor clinical outcomes in patients intubated for acute lung injury. Pulmonary pathology is sometimes challenging to diagnose and differentiate. Lower concentrations of surfactant proteins A and D in bronchoalveolar fluid and higher concentrations in plasma have been linked to more serious lung damage and poor clinical outcomes in patients intubated for acute lung injury (Czechowski et al. 2008).
3. Increased permeability of lung vessels in inflammatory conditions, such as ARDS, may result in an alveolar-to-vascular leakage of SP-D; the integrity of alveolar concentrations of surfactant proteins A and D in bronchoalveolar fluid and higher concentrations in plasma have been linked to more serious lung damage and poor clinical outcomes in patients intubated for acute lung injury. Epithelial secretory cells may be compromised in pulmonary inflammation, resulting in an efflux of SP-D from epithelial cells into the alveoli and alveolar vessels, and since SP-D is less tightly associated with surfactant lipids than the other surfactants, it may be effluxed. High levels of SP-D in the blood may be due to a slower clearance rate of SP-D from the bloodstream in inflammatory states; epithelial surfaces of many nonpulmonary organs secrete SP-D and are possible sources of SP-D in the blood (Heinrich et al. 2006).
4. The outward intravascular leakage of secreted lung proteins and inward edematous flooding in the interstitium and airspaces were caused by a loss of air-blood barrier integrity. Thus, in acute and chronic lung injury, a concentration gradient of SP-D causes SP-D to be synthesized in the respiratory tract and leak into the bloodstream (Sorensen 2018).
5. In patients infected with SARS-CoV, a related coronavirus that causes severe acute respiratory syndrome (SARS), studies have shown that SP-D levels increase with disease severity and IgG levels (Dahmer et al. 2020; Park et al. 2017; Kerget et al. 2020).

6. SP-A and SP-D have also been found in serum and could be used as biomarkers for lung disease, especially when alveolar epithelial integrity is compromised (Park et al. 2017).
7. Surfactant protein D (SP-D) and the receptor for advanced glycation end products (RAGE) are validated alveolar epithelial biomarkers for lung epithelial injury (Johnson and Matthay 2010).
8. Three of the top 5 biomarkers in the current study were lung epithelial injury biomarkers (Ware et al. 2013). SP-D is a natural component of surfactant that is almost entirely formed by alveolar epithelial type II cells. Compared to patients with hydrostatic pulmonary edema, patients with ARDS have higher levels of SP-D in their blood (Cheng et al. 2003).
9. SP-D levels in both BAL and serum rose in response to pro-inflammatory stimuli and during acute inflammation. During the 3 hours of LPS exposure, SP-D nearly instantly translocated from the lungs to the bloodstream. The immediate intravascular leakage is most likely caused by increased endothelial permeability, in which the disruption of SP-D multimeric structure may exacerbate into relatively low-molecular-weight single subunits. Extrapulmonary synthesis of SP-D is unlikely to be the source because it needs extrapulmonary synthesis to react to inflammatory stimuli faster than pulmonary cells, which are known to be the primary source of SP-D synthesis and are located at the site of inflammation (Gaunsbaek et al. 2013).
10. In patients with acute respiratory distress syndrome (ARDS), idiopathic interstitial lung diseases, and alveolar proteinosis, serum levels of SP-A and SP-D are elevated (Honda et al. 1995). In diffuse lung disease, serum levels of both proteins may be used as biomarkers. SP-D tends to be more sensitive and precise than other tests (Fujita et al. 2005). When rats are given acid or bleomycin, their serum SP-D levels increase (Pan et al. 2002). A lack of SP-A harms the host protection against viruses and certain bacteria, but the surfactant system is unaffected. Chronic inflammation, macrophage activation, and alveolar damage are all symptoms of a lack of SP-D. SP-D deficiency is also linked to a decreased ability to clear viruses and bacteria, as well as apoptotic cells (LeVine et al. 2001).
11. Surfactant proteins A, B, and D, as well as interleukin-8, all showed substantial increases in plasma during acute lung injury. Changes in the phosphatidylcholine profile, surfactant proteins, and inflammatory markers of bronchoalveolar lavage fluid and plasma in children with acute lung injury are consistent with alveolar/blood leakage and inflammatory cell membrane degradation. Damage to the alveolar-capillary membrane and cellular infiltration are the causes of these changes (Todd et al. 2010).
12. SP-D is a potential biomarker useful to distinguish severe pandemic influenza A (H1N1) from COVID-19 and other chronic infectious or inflammatory lung conditions such as PTB and COPD. The serum SP-D levels of COVID-19, PTB, and COPD patients were reported as significantly lower than severe pandemic influenza patients. Despite the severity of COVID-19, it was also indicated that the alveolar-capillary membrane of lungs infected with SARS-CoV-2 maintains

its selective permeability for SP-D and other proteins (Choreño-Parra et al. 2021).

13. Serum SP-A and SP-D level might be useful as biomarkers of COVID-19 pneumonia severity as it was reported that lung-specific serum SP-A and SP-D levels, which can be detected from relatively early pneumonia, increased with the aggravation of symptoms and disease severity indicated by radiological findings (Saito et al. 2020).
14. SP-D might play a key role in the lung's defense against Gram-negative bacteria. Rat and human BAL caused Ca++ − dependent agglutination of *E. coli* which was dose dependent and inhibited by competing saccharides or anti-SP-D. SP-D was efficiently and selectively adsorbed from rat BAL by incubating it with *E. coli*, and it was revealed that SP-D is the primary *E. coli*-binding protein secreted by freshly isolated cells in culture after incubation of *E. coli* with radiolabeled rat type II cell medium (Kuan et al. 1992).

Summary

- Surfactant protein D has a chance as a marker in cases of acute lung injury even though it cannot stand alone.
- Circulating surfactant protein D levels can strengthen clinical symptoms that appear to play a role in acute respiratory distress syndrome therapy management.
- Surfactant protein D as a biomarker of acute lung injury makes it easier for clinicians to establish the presence of lung epithelial damage.
- The results of circulating surfactant protein D level's measurement may be used as a further rationale for surfactant therapy.
- It is necessary to further investigate the cells that produce SP-D other than AT2, since if AT2 is damaged, the circulating SP-D levels can describe ALI.

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Troponin as a Biomarker: Use in Non-cardiac Surgery

19

Bruno Caramelli and Fábio de Souza

Contents

Abstract

The growing demand for surgical procedures associated with an aging population raises concerns about the risk of perioperative complications. In general, people undergo non-cardiac surgery to treat diseases to protect or improve their quality of life. Anyway, the expectation is for a better state of health, so perioperative complications that compromise survival do not make sense in this equation. Many scores have been developed and revised in recent decades, with the aim

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of predicting risk of complications, among which perioperative myocardial infarction has always been one of the most feared, precisely, because it is associated with increased mortality. Troponins are traditionally known biomarkers of myocardial damage. It was noticed, especially after the development of more sensitive dosage methods, that troponin may vary in the perioperative period, regardless of the classic symptoms of infarction. Most importantly, perioperative troponin raising predicts complications and therefore has prognostic value with a huge impact on the survival after non-cardiac surgeries. In this chapter, we discussed the clinical meaning of perioperative troponin elevations and its pathophysiology and prognosis.

Keywords

Troponin · Non-cardiac surgery · Cardiovascular risk · Perioperative care · Perioperative period · Postoperative complications · Myocardial injury · Myocardial ischemia · Biomarkers · Prognosis

Abbreviations

ASA	Acetylsalicylic acid
BP	Blood pressure
CI	Confidence interval
cTn	Cardiac troponin
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
HR	Hazard ratio
hs-cTn	High-sensitivity cardiac troponin
hs-cTnI	High-sensitivity cardiac troponin I
hs-cTnT	High-sensitivity cardiac troponin T
MACE	Major cardiovascular event
MI	Myocardial infarction
MINS	Myocardial injury after non-cardiac surgery
OR	*Odds* ratio
PMI	Perioperative myocardial injury
RCRI	Revised Cardiac Risk Index
URL	Upper range limits

Introduction

Surgical treatment is an essential part of medicine, related to treatment of several diseases and associated with increased life expectancy. Worldwide estimates indicate that about 230 million procedures were performed by the early 2000s. This number seems very high, but it will continue to increase in the coming decades around the world (Meara et al. 2015; Weiser et al. 2015). Perioperative care needs to keep up with this demand, and, in this context, there is a growing concern with event rates,

especially cardiovascular complications, which can compromise postoperative survival. In this context, myocardial ischemia is the leading cause of death within a month after non-cardiac surgery (Devereaux and Sessler 2015).

The Revised Cardiac Risk Index (RCRI) is one of the most used and certainly the easiest to calculate preoperative risk. It is a risk stratification score, which, like others, has only moderate accuracy (Lee et al. 1999; Bilimoria et al. 2013). Troponin is the most remarkable and useful among cardiac biomarkers that have been studied so far, with consistent evidence adding to and improving traditional scores used for risk prediction in non-cardiac surgery (Weber et al. 2013; Roshanov et al. 2021). Many studies have shown that, in surgical context, patients with elevated troponins have a worse prognosis with higher mortality, independent from other ischemic symptoms (Devereaux et al. 2012, 2017; Botto et al. 2014; Puelacher et al. 2018). Consequently, this evidence has brought a new understanding of the importance of troponin monitoring. In this case, to differentiate from classical myocardial infarction, some authors used the terms perioperative myocardial injury (PMI) and myocardial injury after non-cardiac surgery (MINS). Both concepts, although not synonymous, point to patients with some grade of myocardial damage associated with the surgical procedure, with prognostic relevance.

Current studies, as well as the first ones, continually bring new evidence that supports the prognostic value of troponin, reinforcing recommendations for its systematic use in the diagnosis and management of myocardial damage in perioperative setting (Park et al. 2020; Gualandro et al. 2021). Thus, we objectively discuss some definitions currently applied in perioperative assessment and the diagnostic, pathophysiological, and prognostic implications of increased troponin after non-cardiac surgeries.

Myocardial Infarction or Injury?

- Exploring different concepts from perioperative setting, which can generate confusion and sometimes can be used in an interchangeable meaning.

Elevated troponin levels in postoperative period, generally described as those above the reference level recommended by the manufacturer, can represent different clinical scenarios. We found interesting to determine firstly how some concepts have been used in the literature.

The classical myocardial infarction that can occur in perioperative period is the so-called perioperative myocardial infarction, and it is associated with poor prognosis (Devereaux et al. 2011a, b, 2012). It is related with ischemic causes (associated or not with coronary disease) and satisfies other diagnostic criteria beyond troponin, including other ischemic clinical features, electrocardiography, or echocardiography findings, as defined by the Fourth Universal Definition of Myocardial Infarction (Thygesen et al. 2018). Conversely, myocardial injury is defined, in a general context, as troponin raised without signs or symptoms of acute ischemic

manifestations independently of the clinical situation involved and observing predetermined cutoff levels for troponin (Thygesen et al. 2018).

On the other hand, but specifically in the perioperative care period, the term MINS (**m**yocardial **i**njury after **n**on-cardiac **s**urgery) was first proposed by Botto in 2014 (Botto et al. 2014). It is caused by ischemia (that may or may not result in necrosis), has prognostic relevance, and occurs within 30 days after non-cardiac surgery. This term includes patients with myocardial damage due to ischemia but who do not necessarily fulfill the diagnostic criteria for acute myocardial infarction (Thygesen et al. 2018). Briefly, MINS represents the finding of elevated troponin levels regardless of any other ischemic symptom or electrocardiographic finding.

Other term that has been commonly used is PMI (**p**erioperative **m**yocardial **i**njury) which can be defined more generically than MINS and includes patients with non-ischemic causes of troponin raising. Non-ischemic causes can be classified in cardiac (as a result of tachyarrhythmia or heart failure) or extracardiac etiology as severe sepsis, stroke, or pulmonary embolism (Puelacher et al. 2018).

Basically, in the absence of other signs or symptoms of classical ischemic event (e.g., chest pain, new or presumably new significant ST-T segment alteration), we must use preferentially the term myocardial injury, although it includes myocardial infarction. In addition, with the introduction of new troponin assays, with a higher sensitivity, the diagnosis of myocardial injury has substantially increased (McCarthy et al. 2019). As highlighted in the subtitle of this section, the concepts can be found in the literature with interchangeable meaning (Nagele 2020a, b). Here we seek to clarify their interpretation in the perioperative context, as illustrated in Fig. 1.

Furthermore, none of these definitions should apply to chronic troponin elevations, which are frequently found in end-stage renal failure and many chronic myocardiopathies. Nonetheless, chronic troponin elevations represent chronic myocardial injury and are related to worst prognosis in the postoperative period (Weber et al. 2013; Park et al. 2020).

Cardiac Troponin Assays

- Differences and similarities.

Troponin participates in a protein complex involved in muscle contraction (skeletal and cardiac). The cardiac subunits T (cTnT) and I (cTnI) are specific. In fact, more frequently elevated cTnT/I levels are related to acute coronary syndromes. However cTnT/I are heart-specific structural proteins but they are not disease-specific markers. Therefore, currently they are the biomarkers of choice for the diagnosis of acute MI and risk-outcome stratification (Apple et al. 2017; Thygesen et al. 2018; Wu et al. 2018; McCarthy et al. 2019).

Previous studies reporting perioperative myocardial injury were performed with a cTnT assay (also known as fourth-generation) in which the results are reported in ng/ml. A peak postoperative concentration $\geq$ 0.03 ng/mL predicted a nearly fourfold

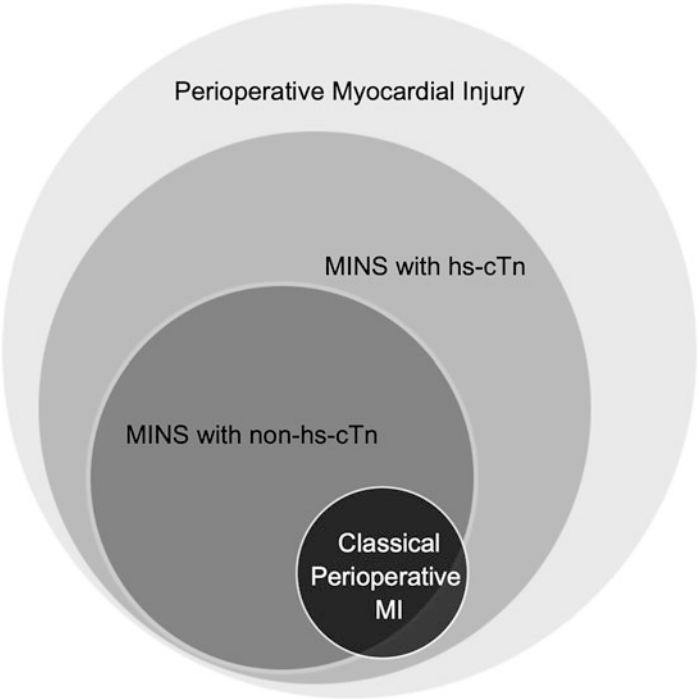

Fig. 1 Different concepts used in the literature to refer to elevated troponin in non-cardiac surgery (Abbreviations: hs-cTn, high-sensitivity cardiac troponin; MI, myocardial infarction; MINS, myocardial injury after non-cardiac surgery; non-hs-cTn, non-high-sensitivity cardiac troponin)

increase in 30-day mortality, and this threshold was considered to define MINS (Devereaux et al. 2012; Botto et al. 2014).

Successively, it has become available in high-sensitivity troponin (hs-cTn) assays. A "high sensitivity" allows to detect lower levels of troponin variations with clinical meaning. One of the obligatory requesting to affirm that an assay has "high sensitivity" is to be able to identify levels of troponin upper limit of detection in at least 50% of healthy controls (Apple et al. 2017). Especially in perioperative context, hs-cTn assays exactly quantify the amount of myocardial injury and consequently must be interpreted as quantitative variables and never as categorical form (positive or negative) (Gualandro et al. 2019). Since that has been used for perioperative setting, it caused an obvious and expected increasing in diagnosis of myocardial injury, and its incidence will likely increase more considering aging population, cardiovascular comorbidities, and continuous greater sensitivity of troponin assays (Devereaux et al. 2017; McCarthy et al. 2019).

To avoid equivocal "false-positive" interpretations of acute myocardial injury in the perioperative period, it is recommendable to obtain preoperative dosage or at least two postoperative dosages to show a minimal variation between them. Unfortunately, the lack of standardization among different troponin assays is remarkable.

In general, the recommendation is to use the 99th percentile of upper range limits (URL) considering levels detected in healthy patients. It is also recommended to use the ng/L units to refer to hs-cTn results to differentiate from former non-hs-cTn assays (Thygesen et al. 2018). In addition, the use of different gender-specific cutoff points has been discussed where women have lower thresholds than men (Apple et al. 2017; Giannitsis et al. 2020).

Comparisons regarding performance and sensitivity for diagnosing myocardial injury were performed between cTnT and cTnI. When evaluated in the same group of patients, PMI was less common using hs-cTnI versus using hs-cTnT. Nevertheless, injury diagnosed by hs-cTnI has also important prognostic significance. Regardless of the differences, until now, there is no evidence supporting preference for one of the two hs-cTn, and both can be used for routine screening (Gualandro et al. 2021).

Pathophysiology

- Thrombosis vs mismatch.

As with spontaneous myocardial infarction, different pathophysiological processes may underlie the development of MINS or PMI (Gualandro et al. 2012; Puelacher et al. 2015). By definition, MINS is related to ischemic causes, while PMI encompasses non-ischemic etiologies as well. Despite the differences in these concepts, most patients (about eight in ten) who present elevated perioperative troponin levels have ischemic etiologies (Puelacher et al. 2018; Devereaux and Szczeklik 2020).

Several factors in preoperative, intraoperative, and postoperative period are involved with ischemia which can occur due to two situations: thrombosis and unbalance between artery supply and oxygen demand known as mismatch (Devereaux and Sessler 2015). Some of these factors (e.g., chronic conditions identified in preoperative period as coronary disease, heart failure, or renal disease) are clearly involved providing substrate for cardiac complications after surgery, and they are easily captured by main risk scores (Lee et al. 1999; Gupta et al. 2011; Botto et al. 2014; Devereaux and Sessler 2015). Nevertheless, many other conditions can play equally important role in this process including intraoperative and postoperative data (Table 1). Sympathetic activation, inflammation, hypercoagulability, bleeding, hypotension, tachycardia, hypoxemia, and pain are all associated with an increased risk of cardiac complications and myocardial injury (Devereaux and Sessler 2015; Devereaux and Szczeklik 2020).

According to these possible causes, mismatch is much more frequent than thrombosis to explain ischemic substrate in perioperative setting. Additionally, coronary angiography performed in patients with non-ST elevation myocardial infarction after non-cardiac surgeries has demonstrated thrombus as culprit lesion in only 13% of the perioperative myocardial infarctions compared to 67% of the non-operative myocardial infarctions (Sheth et al. 2018). On the other hand, atherosclerotic coronary disease was common in patients with perioperative myocardial

Table 1 Possible causes and predictors of perioperative myocardial injury in non-cardiac surgeries

Preoperative	Intraoperative	Postoperative
Previous condition related to characteristics, comorbidities, or urgency of the procedure:	**Conditions related to anesthesia and surgery:**	**Conditions inherent to postoperative period or complications:**
Age Urgent/emergent surgery Trauma Coronary artery disease[a] Renal insufficiency[a] Heart failure[a] Cerebrovascular disease[a] Peripheral arterial disease Diabetes[a] Hypertension	Sympathetic stimulation Hypotension Tachycardia Hypothermia Hypercoagulability Bleeding Inflammation	Pain Tachycardia Bleeding Inflammation/infection Venous thromboembolism Hypotension Hypoxemia

[a]Characteristics that can point to higher scores on the RCRI (Revised Cardiac Risk Index)

infarction. A prospective cohort study using preoperative coronary tomography demonstrated absence of coronary disease in only 4% of patients who had a perioperative myocardial infarction (Sheth et al. 2015). Although these studies have not addressed specifically MINS, it seems to be unlikely that pathophysiological mechanism in myocardial injury would be different.

Intraoperative hypotension has been associated with adverse clinical outcomes after non-cardiac surgery including myocardial infarction, stroke, and cardiovascular death (Sessler et al. 2018; Wesselink et al. 2018). Retrospective data suggest that organ damage (including myocardial injury) might occur when mean arterial pressure decreases <80 mmHg for ≥ 10 minutes and that this risk increases with blood pressure (BP) becoming progressively lower (Gregory et al. 2021). Interestingly, despite the strong association between intraoperative hypotension and postoperative major cardiovascular events (MACE), targeting higher intraoperative BP was not associated with a reduction in the incidence of 30-day MACE or acute myocardial injury including troponin evaluation in the first three postoperative days, compared with standard intraoperative BP management (Wanner et al. 2021).

Prognosis and Predictors

- Mortality at 30 days and later.

The **VISION** (**V**ascular events **I**n non-cardiac **S**urgery pat**I**ents c**O**hort evaluatio**N**) Study was a large observational and prospective cohort study, where authors evaluated the prognostic value of myocardial injury measuring cTnT in postoperative period (three first days) after non-cardiac surgery. Eligible patients for the VISION Study were those who were at least 45 years of age, received a general or regional anesthetic, and underwent elective, urgent, or emergency non-cardiac surgery. It is the largest one in number of patients and enrolled altogether about 40,000 patients

from several countries (Devereaux et al. 2012, 2017; Botto et al. 2014). The first 15,000 patients were evaluated with non-hs-cTnT, and it was demonstrated a positive correlation between postoperative troponin levels and 30-day mortality. Patients with a peak cTnT value of 0.01 ng/mL or less, 0.02, 0.03–0.29, and 0.30 or greater had 30-day mortality rates of 1.0%, 4.0%, 9.3%, and 16.9%, respectively (Devereaux et al. 2012). The authors defined, for the first time, a diagnostic criterion for MINS as a peak of cTnT (non-hs-cTnT assay) of 0.03 ng/ml or greater, presumably due to myocardial ischemia. According to this criterion, MINS was identified in 8% of patients in whom approximately 60% did not experience any other ischemic feature. The 30-day mortality rate was 9.8% among patients who suffered MINS and 1.1% among patients who did not suffer MINS, i.e., a tenfold increase (*odds* ratio (OR) 10.07; 95% confidence interval (CI), 7.84–12.94). In those with and without an ischemic feature, 30-day mortality rates were 13.5% and 7.7%, respectively. The authors highlighted the fact that for every 13 patients with MINS without another ischemic characteristic (identified only by troponin monitoring), 1 died within 30 days. It represented a seven- to eightfold increase compared to patients without MINS (Botto et al. 2014).

Posteriorly, the VISION Study evaluated patients with hs-cTnT measured until the third postoperative day. Raised postoperative hs-cTnT measurement was defined as 20 to less than 65 ng/L with an absolute change of at least 5 ng/L or an hs-cTnT level of at least 65 ng/L. MINS was presented in approximately 18% of individuals, in whom about 20% fulfilled the universal definition of myocardial infarction (i.e., an elevated hs-cTnT with $\geq$1 ischemic feature). As expected, due to the use of assays with higher sensitivity, it was quite different from previously reported, with a higher percentage of patients demonstrating MINS. The peak postoperative hs-cTnT concentration was significantly associated with 30-day mortality, and the higher the hs-cTnT concentration, the higher the mortality (even for patients with concentrations within the normal range, when compared with patients with undetectable values). Again, it was quite relevant that most patients who had MINS (more than 90%) did not experience an ischemic symptom, and myocardial injury would not be suspected without troponin monitoring. All cardiovascular complications were increased among patients who had MINS, including a composite of nonfatal cardiac arrest, congestive heart failure, coronary revascularization, and mortality within 30 days (risk among MINS was 7.3% compared to 0.9% without MINS; unadjusted OR, 8.47; 95% CI, 6.94–10.34) (Devereaux et al. 2017).

Notably, previous evidences support that peak of troponin occurs more often in the first postoperative days (Devereaux et al. 2012), but chronic elevation of troponin can be present in several clinical situations. For this reason, some studies proposed an approach of including preoperative measurement, to determine the true impact of surgery itself on prognosis. The VISION Study (Devereaux et al. 2017) included preoperative troponin, but the obtainment of preoperative hs-cTnT measurements was implemented after the study had started, and only 40% of patients had a preoperative measurement.

The BASEL-PMI Study enrolled 2546 consecutive patients either older than 65 years or older than 45 years with atherosclerosis disease, measuring hs-cTnT

concentrations before and for 2 days after non-cardiac surgery. Different from VISION and as explained before, this protocol used PMI definition instead of MINS. To clearly distinguish acute from chronic troponin raises, PMI was defined as an absolute increase in hs-cTnT of 14 ng/l (the 99th percentile URL for the assay studied) above preoperative concentrations or between two postoperative concentrations, which occurred in 16% of surgeries. Similarly, as reported in the VISION Study, most patients with PMI (82%) did not experience any symptoms of ischemia; therefore, it can only be detected by routine screening. At 30 days, 9.8% of patients with PMI versus 1.6% without PMI had died. Considering cardiovascular death, it occurred in 4.9% versus 0.5% of patients with and without PMI, respectively. Late mortality, evaluated at 1 year, was also increased in patients with PMI (22.5% versus 9.3%). In multivariate regression analysis, PMI was associated with adjusted hazard ratio (HR) of 2.7 (95% CI, 1.5–4.8) and 1.6 (95% CI, 1.2–2.2) for mortality at 30 days and 1 year, respectively (Puelacher et al. 2018). According to the concept of PMI, the BASEL-PMI Study results made possible to establish the prognosis associated with non-ischemic and extracardiac causes of troponin raised in perioperative setting. The mortality at 30 days was 6.1% versus 32.5% in patients with PMI from cardiac and extracardiac subtype, respectively. Therefore, PMI related to a primarily extracardiac disease associated with cardiomyocyte damage, such as severe sepsis, had a worse prognosis (Puelacher et al. 2018).

A large registry study from Korea (retrospective observational analysis of acquired data from Samsung Medical Center Troponin in Non-cardiac Operation – SMC-TINCO registry) enrolled more than 20,000 non-cardiac surgeries reporting acute and chronic myocardial injury with cTnI, regardless of the etiology. Compared with the normal group, acute and chronic myocardial injury groups both showed significantly higher 30-day mortalities (Park et al. 2020).

The characteristics and results of the main studies mentioned are summarized in Table 2.

In addition to defining the prognosis of MINS or PMI, it is also important to look for their own predictors, as this would make it possible to find better criteria to indicate who should benefit from troponin monitoring. In general, patients with MINS/PMI were older, had more cardiovascular risk factors, and had known cardiovascular disease. Beyond that, end-stage renal failure, diabetes mellitus, urgent or emergency surgery, and extracardiac causes of perioperative myocardial injury, as sepsis, were also associated with mortality (Devereaux et al. 2012, 2017; Botto et al. 2014; Puelacher et al. 2018; Costa et al. 2021). Considering some preoperative features, it seems reasonable that RCRI could predict perioperative myocardial injury (see Table 1). Nevertheless, data from the VISION Study evaluating elective surgeries demonstrated that one third of patients who suffered MINS were previously classified in RCRI class I, i.e., despite no RCRI risk factors. Moreover, considering the primary outcome as a composite of MINS, myocardial infarction, nonfatal cardiac arrest, or cardiac death at 30 days after surgery, 35% of events occurred in patients in RCRI class I (Roshanov et al. 2021).

Based on these findings, it is currently recommendable an individual screening for myocardial injury using hs-cTnT/I in high-risk patients such as those older than

Table 2 Perioperative studies assessing prognostic value of myocardial injury after non-cardiac surgery

Author/year (study)	Patients enrolled	cTn assay (manufacturer)	cTn threshold	Parameter evaluated	Main results (prevalence and mortality)
Devereaux et al. (2012) (VISION Study)	15,133	Non-hs-cTnT (Elecsys, Roche® fourth-generation)	0.02 ng/ml	Postoperative peak troponin (first 3 days after surgery)	cTnT >0.02 ng/ml occurred in 11.6% of patients; cTnT value of 0.01 ng/mL or less, 0.02, 0.03–0.29, and 0.30 or greater had 30-day mortality rates of 1.0%, 4.0%, 9.3%, and 16.9%, respectively
Botto et al. (2014) (VISION Study)	15,065	Non-hs-cTnT (Elecsys, Roche® fourth-generation)	0.03 ng/ml	MINS	1200 patients (8.0%) suffered MINS MINS was an independent predictor of 30-day mortality (adjusted HR, 3.87; 95% CI, 2.96–5.08)
Devereaux et al. (2017) (VISION Study)	21,842	hs-cTnT (Elecsys, Roche®)	20–65 ng/L (with absolute change of at least 5 ng/L) or > 65 ng/L	MINS	Among the 3904 patients (17.9% of total) that suffered MINS, 3633 (93.1%) did not experience an ischemic symptom. An elevated postoperative hs-cTnT without an ischemic feature was associated with 30-day mortality (adjusted HR, 3.20; 95% CI, 2.37–4.32)
Puelacher et al. (2018) (BASEL-PMI 2018)	2018 (2546 surgeries)	hs-cTnT (Elecsys, Roche®)	14 ng/L[a]	PMI	PMI occurred after 397 of 2546 surgeries (16%). Mortality at 30 days was 8.9% in patients with PMI versus 1.5% in patients without PMI (adjusted HR, 2.7; 95% CI, 1.5–4.8). Difference was also evaluated at 1 year, with mortality rates of 22.5% versus 9.3%, respectively

Gualandro et al. (2021) (BASEL-PMI 2021)	2455 (3111 surgeries)	hs-cTnI (Architect, Abbott®) hs-cTnT (Elecsys, Roche®)	26 ng/L[a] 14 ng/L[a]	PMI/MINS	The incidence of overall PMI[b] was lower using hs-cTnI versus hs-cTnT, 9% and 15%, respectively. However, the prognostic impact of PMI diagnosed by hs-cTnI was comparable to the hs-cTnT, at 30 days and 1 year
Park et al. (2020) (SMC-TINCO registry 2020)[c]	22,969	cTnI (ADVIA, Siemens®)	40 ng/L[c]	PMI	Acute injury (22.5%) and chronic injury (0.5%) had higher 30-day mortalities compared with the normal group (0.8% vs. 8.0%; HR, 11.0; 95% CI, 9.0–13.3; and 0.8% vs. 7.6%; HR, 10.5; 95% CI, 5.3–20.7, respectively)

Abbreviations: *cTn* cardiac troponin, *hs-cTnI* high-sensitivity cardiac troponin I, *hs-cTnT* high-sensitivity cardiac troponin T, *MI* myocardial infarction, *MINS* myocardial injury after non-cardiac surgery, *PMI* perioperative myocardial injury

[a]Absolute increase above preoperative values or between 2 days' postoperative measurements if preoperative value was missing

[b]Total rate of PMI included infarction and injury

[c]Retrospective study enrolled patients with serial cTnI measurements within 30 days before and after surgery. Acute myocardial injury was defined with an initial cTnI value greater than the 99th percentile of the URL (an increase of at least 50% of the 99th percentile value or a change greater than 20% relative to the initial value) and compared with chronic injury

65 years or older than 45 years with documented coronary, cerebral, or peripheral artery disease, before and in the first three postoperative days, if undergoing major non-cardiac surgery (Duceppe et al. 2017; Gualandro et al. 2017).

Future Perspectives

- What we should do next?

From the strong evidence discussed above, there is no doubt that elevated troponin values, measured in the perioperative period, independently predict mortality after non-cardiac surgery. In addition to looking for predictors to select high-risk patients in the preoperative period, once MINS/PMI is detected, it is also possible to take measures to protect patients in the postoperative period.

Previous observational data indicate a possible benefit of acetylsalicylic acid (ASA) and statin therapy reducing perioperative myocardial infarction and demonstrating lower risk of death at 30 days after non-cardiac surgery (Devereaux et al. 2011a, b; Foucrier et al. 2014). Retrospective and interventional studies have demonstrated benefit with statin therapy (Durazzo et al. 2004; O'Neil-Callahan et al. 2005; Paraskevas et al. 2006), but this evidence is difficult to reproduce and confirm today, mainly due to the widespread use of statins in the population and the ethical aspects of having a placebo non-statin arm in the study (Durazzo et al. 2004; Sanders et al. 2013; Marcucci et al. 2020).

Beyond ASA and statins, more recently, the potential of dabigatran to prevent major vascular complications among patients with MINS was addressed by the MANAGE trial (Management of Myocardial Injury After Noncardiac Surgery), a large international study, which enrolled 1754 patients in more than 80 countries. Eligible patients were those who were at least 45 years of age, had undergone non-cardiac surgery, and were within 35 days of MINS. They were randomly assigned (1:1) to receive dabigatran 110 mg orally twice daily or matched placebo. The primary efficacy outcome was the occurrence of a composite of vascular mortality and nonfatal myocardial infarction, non-hemorrhagic stroke, peripheral arterial thrombosis, amputation, and symptomatic venous thromboembolism. The primary safety outcome was a composite of life-threatening, major, and critical organ bleeding. At a median follow-up of 16 months, primary efficacy outcome occurred in 15% of the placebo group and 11% of patients randomized to the dabigatran group (HR, 0.72; 95% CI, 0.55–0.93) without difference in primary safety outcome, allowing the authors to conclude that among patients who had MINS, dabigatran 110 mg twice daily reduced the risk of major vascular complications, without a significant increase in major bleeding (Devereaux et al. 2018).

Regardless of these evidences and results, there is no consensus in guidelines from the different societies that recommend systematically treatment to patients identified with MINS/PMI to date (Fleisher et al. 2014; Kristensen et al. 2014; Gualandro et al. 2017). Exception for Duceppe et al. that guide the prescription

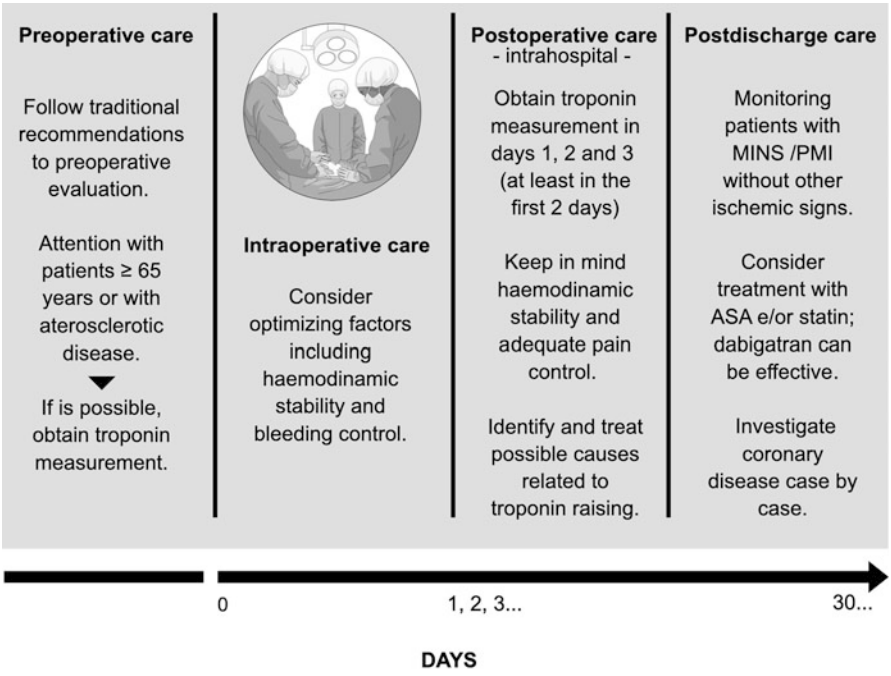

Fig. 2 Summary of main actions in the perioperative care in non-cardiac surgery related to MINS/PMI (Abbreviations: ASA, acetylsalicylic acid; MINS, myocardial injury after non-cardiac surgery; PMI, perioperative myocardial injury)

of ASA and statin, both as strong recommendations and moderate quality of evidences (Duceppe et al. 2017).

We briefly summarize the main actions and precautions currently recommended for MINS/PMI in Fig. 2.

Applications to Prognosis in General Population and Other Diseases

In this chapter, we have discussed that myocardial injury is the most common cause of elevated troponin in perioperative period. Nevertheless, we have also highlighted that after the development of high-sensitivity assays, troponin could be detected at lower levels in different clinical settings, including individuals with no clinical signs of myocardial damage or previous cardiovascular disease (McCarthy et al. 2019). *Consequently, its prognostic value could be tested in population studies. The results of a study involving more than 8000 Americans aged 45–64 years, followed by ≈15 years, concluded that elevated hs-cTnI was strongly associated with an increase in cardiovascular disease in the general population, regardless of traditional risk factors. Detectable levels of hs-cTnI were observed in 85% of subjects, and, in*

*adjusted models, comparison of the highest hs-cTnI quintile (≥3.8 ng/L) with the lowest hs-cTnI (≤ 1.3 ng/L) demonstrated an approximately twofold increase in all-cause mortality (HR, 1.83; 95% CI, 1.56–2.14) (*Jia et al. 2019*). These results are completely in agreement with meta-analysis that included more than 150,000 participants from 28 prospective studies in different countries, with no previous cardiovascular event. In the general population, high cardiac troponin concentration (cTnT/I) within the normal range is associated with increased cardiovascular disease risk, and this association is independent of conventional risk factors* (Willeit et al. 2017). *Similarly, it has already been demonstrated that high levels of hs-cTnI or hs-cTnT improve cardiovascular and mortality risk stratification in adults with diabetes even after adjustment for traditional risk factors* (Tang et al. 2020).

Mini-dictionary of Terms

- *Cardiac troponin: structural protein of myocyte (heart muscle).*
- *Mismatch: unbalance between demand and offer of oxygen.*
- *Myocardial injury: myocyte damage identified by serum elevation of cardiac troponin levels.*
- *MINS: term used to define myocardial injury that occurs in perioperative period, related to ischemic etiology.*
- *Perioperative period: period of time surrounding the surgical intervention, usually defined from preoperative to the 30th postoperative day.*

Key Facts of Troponin as a Biomarker in Non-cardiac Surgery

- *Elevated troponin values, measured in the perioperative period, independently predict mortality after non-cardiac surgery.*
- *It is noteworthy that the majority of patients who present elevated levels of troponin with clinical significance are asymptomatic and therefore only can be screened by routine troponin monitoring.*
- *To distinguish chronic from acute myocardial injury, we must obtain preoperative dosage or at least two dosages in postoperative period to show a minimal variation between troponin values.*

Summary Points

- *Elevated troponin levels in postoperative period, generally described as above cutoff range limit, can represent different clinical scenarios.*
- *Many studies have shown that, in surgical context, patients with elevated troponins have a worse prognosis with higher mortality, independent from other ischemic symptom.*

- *In the absence of other signs or symptoms of classical ischemic event (e.g., chest pain, new or presumably new significant ST-T segment alteration), we must use preferentially the term injury.*
- *MINS (myocardial injury after non-cardiac surgery) includes patients with myocardial damage due to ischemia but who do not fulfill the diagnostic criteria for acute myocardial infarction.*
- *PMI (perioperative myocardial injury) also includes patients with non-ischemic causes of troponin raising*.
- *Several factors in preoperative, intraoperative, and postoperative period are involved in the pathophysiology of injury. Related to all possible causes, mismatch is much more frequent than thrombosis to explain elevated troponin in perioperative setting.*
- *Traditional risk scores are not sufficient to predict MINS/PMI. Many adverse cardiac events including myocardial injury occur in patients with low scores.*
- *Based on evidence so far, we should perform systematic screening for myocardial injury with hs-cTn, before and in the first three postoperative days, in patients over 65 years or over 45 years with documented atherosclerosis disease.*

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Glucose Variability Measures in Critical Care 20

Ofer Sadan and C. L. Hall

Contents

Abstract

Hyperglycemia commonly occurs in critically ill patients. Research over the past 20 years has evolved to indicate that glucose variability – not solely hyperglycemia or hypoglycemia – is associated with patient outcomes in critical care settings. However, many unknowns remain, such as the optimal definition of variability, the underlying pathophysiologic mechanisms of injury, and, most importantly, whether optimizing glucose variability leads to improved patient outcomes. In the current chapter, we review recent data accumulated in the field.

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Specifically, we describe the different glucose variability measurements, physiological effects of glucose variability, and clinical data regarding specific patient populations and diseases, and finally, we summarize the ongoing questions and directions for future research.

Keywords

Glucose variability · Glucose management · Critical care · Intensive care unit · Postoperative · Sepsis · Cardiac arrest · Stroke · Traumatic brain injury · Subarachnoid hemorrhage · Outcome prediction · Patient outcomes

Abbreviations

ANZICS	Australian and New Zealand Intensive Care Society
BG	Blood glucose
CACP	Consecutive absolute change percentage
CGM	Continuous glucose monitoring
CNS	Central nervous system
CV	Coefficient of variation
DFA	Detrended fluctuation analysis
GLI	Glycemic lability index
GLP-1	Glucagon-like peptide 1
HbA1c	Hemoglobin A1c or glycated hemoglobin
HBGI	High blood glucose index
ICU	Intensive care unit
LBGI	Low blood glucose index
MAG	Mean absolute glucose
MAGE	Mean amplitude of glucose excursions
mg/dL	Milligrams per deciliter
NIHSS	National Institutes of Health Stroke Scale
SAH	Subarachnoid hemorrhage
SD	Standard deviation
TBI	Traumatic brain injury
TIR	Time in range
TTM	Targeted temperature management

Introduction

Hyperglycemia commonly occurs in patients needing admission to intensive care units. Within 48 h of admission, approximately 77% of critically ill patients have become hyperglycemic, defined as fasting blood glucose (BG) >126 mg/dL or random BG >200 mg/dL. This finding is not limited in patients with diagnosed or previously undiagnosed diabetes: of 1000 patients prospectively reviewed, 22% had a prior diabetes diagnosis, 5.5% had unrecognized diabetes, and the 49.8% majority had critical illness or "stress" hyperglycemia (Plummer et al. 2014). The presence of

hyperglycemia during an ICU stay is strongly and consistently related with mortality and morbidity, a finding that holds across medical, cardiac, neurologic, surgical, and cardiothoracic ICU populations (Egi et al. 2008; Krinsley 2006).

However, until the landmark "Leuven" trial by van den Berghe et al. (2001), the negative impact of hyperglycemia on ICU outcomes was generally underappreciated. In a surgical (~60% cardiovascular) ICU population, intensive insulin therapy targeting BG between 80 and 110 mg/dL was associated with a lower ICU mortality compared with the control arm targeting BG between 180 and 200 mg/dL. A later study from Leuven group focusing on medical ICU patients failed to show overall mortality benefit but did improve ICU length of stay and decrease acute kidney injury. A mortality benefit was seen in the subgroup ICU patients requiring over 3 days of ICU care (van den Berghe et al. 2006). These discrepant results were met with criticism, and multiple following investigations failed to replicate the mortality benefits seen in the initial trial (Brunkhorst et al. 2008; Preiser et al. 2009; Schultz et al. 2010). The single largest study to date, the Normoglycemia in Intensive Care Evaluation-Survival Using Glucose Algorithm Regulation (NICE-SUGAR) trial, was organized to clarify the risk-benefit profile of intensive insulin therapy defined as 81 mg/dL to 108 mg/dL. In contrast to the van den Berghe study, intensive insulin therapy increased mortality (Finfer et al. 2009). NICE-SUGAR found that severe hypoglycemia was associated with a 2.6% absolute increase in mortality at 90 days – in both *intensive and standard care* groups. A subsequent meta-analysis analyzing results from 25 studies that included over 13,500 patients determined that intensive insulin therapy to maintain glucose control did not improve mortality for all "general" ICU patients (Griesdale et al. 2009).

Consistent with this concern, the 2016 Surviving Sepsis Campaign suggested an upper limit of 180 mg/dL but did not propose a lower glucose target level given the lack of clear evidence to support a single unified target range across all septic ICU patients. Further, avoidance of hypoglycemia was strongly suggested given the concern for increased mortality (Rhodes et al. 2017). Additionally, the American Association of Clinical Endocrinologists and the American Diabetes Association support a BG range of 140 to 180 mg/dL for most ICU patients (American Diabetes Association 2021).

Despite the significant association between dysglycemia and ICU outcomes, glucose control in critically ill patients remains controversial (Preiser and Straaten 2016; Marik 2016). This likely reflects a narrow focus on average BG values generalized across too broad of a target population in early studies. Questions remain about the appropriate glucose targets, the risk-benefit of hypoglycemia, the role of glucose fluctuations, the applicable target populations, and the impact of how BG targets are attained. These questions are fueled by the lack of clear-cut outcome benefits in numerous randomized trials of intensive glucose control. Lately, the triad of hyperglycemia, hypoglycemia, and glycemic variability has gained an increasing focus as a lens through which to view glucose control in critically ill patients (Brunner et al. 2012a, b).

Glycemic variability describes fluctuations of BG over time. Glycemic variability has been suggested as an additional measure for glucose control in ambulatory

patients with diabetes (Brunner et al. 2012a, b). As several trials have linked increased glycemic variability to mortality in critically ill patients, this measure has been suggested as an important measure for glucose control in this population as well (Ali et al. 2008; Dossett et al. 2008; Egi et al. 2006; Hermanides et al. 2010; Krinsley 2008; Mackenzie et al. 2011). This chapter will examine the role that glycemic variability may play in understanding outcomes in critically ill patients.

Blood Glucose Variability Measures

Glucose variability refers to the fluctuation in BG levels over time (Fig. 1). BG variability has been recognized as an important aspect of diabetes control in ambulatory patients for over 50 years. Common statistical measures such as standard deviation (SD) and the coefficient of variation (CV) have been used to quantify variability, as well as measures developed specifically for glucose fluctuations such as the M-value introduced in 1965 and, later, the mean amplitude of glucose excursions (MAGE) and mean amplitude of glucose change (MAG) (Kovatchev and Cobelli 2016). A description of several commonly used variability measures is listed in Table 1.

The earliest studies exploring glucose variability in the critical care populations utilized standard deviation and coefficient of variation (Egi et al. 2006; Krinsley 2008), with later studies exploring additional measures such as the mean absolute glucose change per hour, maximum absolute change, and inter-measure percentage change (Dossett et al. 2008; Hermanides et al. 2010; Sadan et al. 2020). In Egi et al. (2006) and Krinsley (2008), SD was the measure most strongly associated with increased ICU mortality. The SD is calculated as the square root of the average of the squared differences between individual glucose values and the mean. SD does not account for the order of BG measurements nor the timing. Though it is the most common measure, SD can mask several meaningful aspects of glucose variability

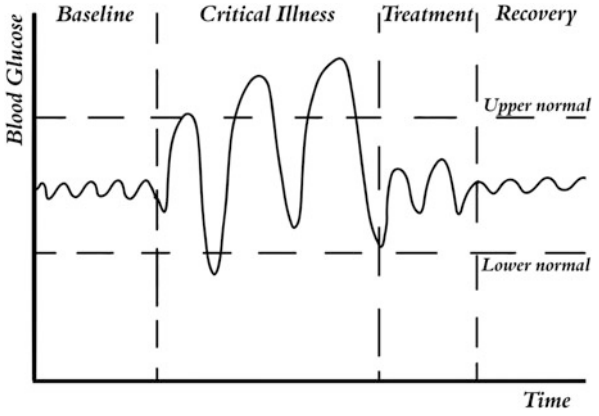

Fig. 1 Change in glucose variability during critical illness. The changes in blood glucose increase during critical illness, while treatment (treating the cause and/or correcting glucose with medications such as insulin) gradually brings the variability closer to normal. Upon recovery, blood glucose can return to its baseline form

Table 1 Measures to characterize glucose variability

Measure	Description
Amplitude-based measures of glucose variability	**Typically measured over hours to days**
SD	Variation around a mean blood glucose
CV	Magnitude of variability relative to mean blood glucose (CV=SD/mean)
MAGE	Mean amplitude of glycemic excursion which are above one SD or below the mean blood glucose level
GLI	
LGBI/HGBI	Measure of frequency and magnitude of hypoglycemia (LGBI) or hyperglycemia (HGBI)
CACP	Measures calculated based on the percentage of absolute change between two consecutive glucose measurements, with assessment of average percentage change or median percentage change
Time-based measures of glucose variability	**Typically measured over minutes to hours, commonly based on CGM data**
TIR	Includes time in range, above range, or below range. Provides a picture of overall glucose control
MAG	Total absolute changes in glucose levels between successive pairs of points, divided by the total time interval
Multiscale entropy and complexity	Various measures that demonstrate high vs. low complexity across a times series, with lower complexity being associated with more profound disease states
Absolute change by time difference	Calculating change between measurements while controlling for the duration of time between measures

Abbreviations: *CACP*, consecutive absolute change percentage; *CGM*, continuous glucose monitoring; *CV*, coefficient of variation; *GLI*, glucose lability index; *HBGI*, high blood glucose index; *LBGI*, low blood glucose index; *MAG*, mean absolute glucose (change over time); *MAGE*, mean amplitude of glycemic excursions; *SD*, standard deviation
Definitions of glucose variability calculations
Adapted from Sadan et al. (2020) and Umpierrez and Kovatchev (2018)

(Hermanides et al. 2010; Meynaar et al. 2012). For example, two different populations of patients could have the same mean and SD in BG but have large differences in variability depending on how widely these repeated values fluctuate. Further, SD does not indicate how rapidly successive values shift (Meynaar et al. 2012) nor does it in the setting of glucose measurements represent a true normal distribution of values. This last point relates to the fact that ICU protocols nearly always include countermeasures to prevent hypoglycemia, thus creating a lower boundary that limits a truly normal distribution.

Dossett et al. (2008) addressed some of these shortcomings by looking at the variability between successive BG values in a population of critically ill surgical patients. Survivors had mean increases in successive BG values of 54 mg/dL compared with 70 mg/dL in non-survivors. This pattern held when looking at successive decreases in BG as well with −70 mg/dL vs −77 mg/dL in survivors

versus non-survivors, respectively. Tellingly, survivors and non-survivor groups had identical mean and SD values. Thus, not all measures of glucose variability are linked to mortality risk. In this study, value-to-value differences in BG, not SD, are associated with increased mortality in the ICU: the more extreme the excursion, the higher the risk for death.

Hermanides et al. (2010) also addressed the limitations of using SD as a measure of variability by investigating the mean absolute glucose (MAG) change per hour. This measure attempts to address a second principle of variability beyond changes in amplitude: changes in time. The MAG is determined by finding the sum of all absolute glucose changes during the admission and dividing this by the total time spent in the ICU in hours. The MAG takes BG order and time into account. They found that glucose variability as determined by MAG was highly associated with death in the ICU in populations with high *and* low mean BG levels. In a multicenter cohort study of over 20,000 patients across Dutch surgical and medical ICUs, SD, MAGE, MAG, and the glycemic lability index (GLI) were evaluated for their association with mortality (Eslami et al. 2011). The GLI is calculated by assessing the squared difference between consecutive BGs per unit of actual time between those samples. The results of this prospective study found that in the surgical ICU population, SD, MAGE, and MAG, but not GLI, were associated with mortality; however, in medical ICU patients, only the SD was associated with mortality. The reason for an association of multiple measures of variability with mortality in the surgical ICU but not the medical ICU population is unclear. The measures MAG, MAGE, and GLI take order of measurements into account, but only MAG and GLI consider the time that elapsed between measurements. Standard deviation, however, does not take sequence or time into account. Despite its limitations, SD is the measure of glucose variability that has been studied most extensively, and, except for one study, SD has been found the most frequent independent predictor of mortality (Ouattara et al. 2006). Whether the distinct properties of these measures capture aspects unique to specialty ICU populations is unclear. However, the differences in outcome between surgical ICU and medical ICU patients are a recurrent theme in BG research.

While glucose variability measures can be calculated using conventional BG measurements captured every 1–6 h, continuous glucose monitoring (CGM) allows time as an additional measure of glucose variability. The data derived from CGM devices offer much higher density data and necessitates more complex variability analyses and statistical approaches, so far limiting their routine clinical use (Danne et al. 2017).

Glucose complexity has been proposed as a marker of glucose regulation. Complexity analysis of CGM data could reveal patterns of endogenous regulation not detectable by analysis of intermittent BG values (Lundelin et al. 2010). Brunner et al. (2012a, b) analyzed CGM data from medical ICU patients for both common variability measures such as SD, CV, and GLI and time series complexity using detrended fluctuation analysis (DFA). DFA is a unitless metric that specifies internal correlations within a time series. High complexity is thought to represent a healthy regulatory system, whereas low complexity is proposed to indicate that a system is no longer able to adequately regulate glucose fluctuations (Lundelin et al. 2010;

Brunner et al. 2012a, b). Interestingly, their analysis demonstrated that intensive insulin therapy guided by CGM did not decrease glucose variability, and there were no differences in glucose variability between survivors and non-survivors. However, there was a loss of glucose complexity in non-survivors, associating low complexity of CGM data with increased mortality.

One time-based variability measure gaining acceptance is the time in targeted BG range (TIR). The TIR measure provides a metric for the time a patient spends within, above, and below the target BG range (Kovatchev and Cobelli 2016). Clinically, TIR can demonstrate how hypoglycemic or hyperglycemic excursions respond to treatment over time. In the ambulatory setting, the time spent out of range can guide the clinical plan, establishing levels of urgency or degree of response needed to increase TIR (Kovatchev and Cobelli 2016). In a mixed ICU setting, TIR has been associated with mortality (Lanspa et al. 2019). Mortality was lower in those with TIR $> 80\%$ (BG values 70–139 mg/dL in this study) compared with those with TIR $\leq 80\%$. As CGM becomes more ubiquitous in ICUs, exploring time series analyses such as measures of complexity (Brunner et al. 2012a, b), entropy (Meyfroidt et al. 2010), and TIR (Lanspa et al. 2019) can provide valuable insights into the impact of glucose variability, especially when combined with more common variability measures (Meynaar et al. 2012).

With growing interest in glucose variability, there has been an explosion in the number of variability measures. However, there remains no gold standard, particularly in the critical care population. This has led to confusion and likely hinders a more organized research effort surrounding the clinical impact of glucose variability. A recent international consensus statement for CGM recommends using CV (which is the SD divided by the mean) as the primary amplitude measure, with SD as a secondary measure because of its familiarity to physicians (Umpierrez and Kovatchev 2018; Meyfroidt et al. 2010). Additionally, TIR, specifically the time spent outside of the targeted range, should be evaluated and reported in clinical studies as a time-based measure of glucose variability (Meyfroidt et al. 2010).

The assertion that various measures of glucose variability are associated with ICU mortality is clear. However, establishing a clear causal role for glucose variability is difficult. Is BG variability simply an epiphenomenon of an underlying state of critical illness, overall inflammation, or evolving insulin resistance? How might the approach to glucose control with the use of insulin over the duration of an ICU stay contribute to increased glucose variability and outcomes (Krinsley 2008; Honiden and Inzucchi 2015)? Several well-described mechanisms may support the link between glucose variability and poor outcome in critical illness.

Insulin resistance in the setting of critical illness is commonly experienced at the bedside as elevated BG values. Critical illness induces state of insulin resistance associated with impaired glucose uptake, higher circulating concentrations of insulin, and increased hepatic gluconeogenesis. This is further exacerbated by catecholamines and hormonal regulation of hepatic glucose production (Duska and Andel 2008). The resulting hyperglycemia often seen in critical illness has downstream immunomodulatory effects and promotes micro- and macrovascular inflammation (Skrha et al. 2016), endothelial injury (Langouche et al. 2005), and oxidative stress

(Choi et al. 2008). Glucose fluctuations compared with a stable but hyperglycemic cellular milieu induce reactive oxygen species generation (Quagliaro et al. 2005). These authors conclude that variability in glucose could be more deleterious to cells than constant high BG, although constant levels of near-normal BG would be least damaging of all.

In the ICU, we lack complete understanding of the factors contributing to extreme glucose excursions. Those recognized include medication effects such as glucocorticoid administration, rapidly changing nutrition administration, and infectious and post-surgical processes, which rekindle inflammatory responses. Indeed, typical ICU protocols for hypoglycemic management may play a role in promoting injury as well. When patients develop hypoglycemia on intensive insulin therapy, most protocols call for administration of intravenous dextrose (e.g., an ampule of d50). Blood glucose rebounds immediately after administration of the dextrose, resulting in glucose variability. In a study of healthy volunteers and patients with type 1 diabetes, hypoglycemia was found to induce endothelial dysfunction, oxidative stress, and inflammation. Interestingly, this study also demonstrated that correction of hypoglycemia may also influence vascular injury markers. When recovery from hypoglycemia quickly reaches normoglycemia, the deleterious effects of the previous hypoglycemic episode are mainly counterbalanced. However, when hypoglycemia is aggressively corrected and results in rebound hyperglycemia, markers of endothelial dysfunction, oxidative stress, and inflammation are produced beyond those levels seen with "gentle" correction to normoglycemia (Ceriello et al. 2012). Additionally, acute increases in BG after hypoglycemia have also been associated with neuronal cell (Suh et al. 2007).

As noted by Honiden and Inzucchi (2015), this finding of how ICU management practices of hypoglycemia may promote injury allows us to reevaluate both our ICU populations and clinical trials. When hypoglycemia rates are high, as seen in several notable trials of intensive insulin therapy (van den Berghe et al. 2001, 2006), this "yo-yo" effect may be negating the potential beneficial effects of glucose control. As glucose variability is increasingly linked to biological mechanisms of cellular and vascular injury, promotion of inflammation, and deleterious modulation of the immune system, we get closer to a mechanistic linking of glucose variability with adverse ICU outcomes.

Blood Glucose Variability in the Surgical Intensive Care Unit

Post-Surgical and Trauma ICU

In surgical ICU patients, perioperative hyperglycemia increases risk of postoperative mortality, as well as cardiovascular and infectious morbidity (Doenst et al. 2005; Duncan et al. 2010). Hyperglycemia is common during and after major surgery due to the hypermetabolic stress response, which is characterized by hyperglycemia and insulin resistance (Ljungqvist 2010). This response involves an increased level of endogenous hepatic gluconeogenesis, while insulin-stimulated peripheral glucose

uptake becomes impaired. This hyperglycemic response has been referred to as critical illness or "stress" hyperglycemia (Plummer et al. 2014; Marik and Bellomo 2013). The variability of hyperglycemic excursions in response to major surgery are associated with preoperative glucose regulation as measured by glycated hemoglobin (HbA1c) (Cely et al. 2004) and the extent of the surgery (Thorell et al. 1999). Thus, surgical patients are predisposed to hyperglycemia, the risk of which increases with more extensive surgery, and exacerbated with baseline diabetes or glucose intolerance. This may explain some of the discrepancy of outcomes between patients in surgical and medical ICUs, though this is unclear. The impact of glucose excursions on postoperative patients admitted to surgical ICUs has been investigated. Dossett et al. (2008) conducted a retrospective analysis of critically ill surgical and trauma ICU patients to determine if blood glucose variability was associated with mortality. They found that, despite no difference in mean glucose values between survivors and non-survivors, multiple measures of glucose variability were associated with mortality. This important investigation was one of the early studies to confirm that increased glucose variability was independently associated with increased risk of mortality. Interestingly, this leads the way for the Leuven group to perform a retrospective review on their two intensive insulin therapy trials to investigate the impact of glucose variability, a concept which was not recognized in the initial trials. They found measures of variability, including mean amplitude variation and pattern irregularity (as measured by jack-knifed approximate entropy), were associated with mortality. Importantly, intensive insulin therapy was not associated with reduced glucose variability and, instead, tended to increase variability in both surgical ICU and medical ICU cohorts (Brunner et al. 2012a, b).

The utility of glucose variability in surgical ICU patients goes beyond mortality associations. Kaufmann et al. (2011) investigated surgical ICU patients on intravenous insulin to maintain tight glucose control for predictors of hypoglycemic events. They calculated blood glucose variability as measured by SD and median absolute change in successive BG values (current BG value to previous BG value) in those with hypoglycemic excursions versus those without. Using an index event such an episode of hypoglycemia, they then evaluated glucose variability in the 24 h prior to the event. Analysis of this time association suggested that glucose variability increases in the 24 h preceding a hypoglycemic event. Thus, monitoring glucose variability over the duration of an ICU stay could indicate of increasing risk for hypoglycemic events.

Cardiac and Vascular Surgery

In cardiac surgery patients, multiple patient and procedural factors contribute to hyperglycemia including heparin administration (Lee et al. 1988) and administration of glucose-containing cardioplegic solutions (Werb et al. 1989). In cardiac surgical patients, postoperative glucose variability was significantly higher in non-survivors compared with survivors, and increased postoperative glucose variability was found to be an important risk factor for adverse postoperative outcomes (Duncan et al.

2010). Interestingly, significant intraoperative glucose variability was similar in both survivors and non-survivors and was not associated with mortality. However, severe intraoperative glucose excursions into the hypoglycemic range were a strong predictor of morbidity and mortality (Doenst et al. 2005). Intraoperative glucose variability is thought to be increased due to development of insulin resistance which is exacerbated by frequent intraoperative administration of glucose-containing cardioplegic solution. The reasons for an association between increased glycemic variability and mortality could be related to activation of an oxidative stress effect due to excessive glucose fluctuations as noted above (Duncan 2012).

Poor preoperative glucose control is associated with increased sternal wound infections, respiratory complications, renal failure, and stroke, in addition to worse long-term survival (Halkos et al. 2008; Gatti et al. 2016). Hemoglobin A1c (HbA1c) is recognized as a significant preoperative risk factor driving adverse events, and glucose control postoperatively mitigates complications (Desai et al. 2012). Clement et al. (2019) investigated the impact of glucose variability on perioperative complications in patients undergoing coronary artery bypass surgery. In contrast to the established view, the association of HbA1c becomes non-significant when adjusted for postoperative glucose variability (measured by SD and MAGE) and mean glucose levels. Major adverse events including postoperative cardiac arrest, pneumonia, acute kidney injury, stroke, sepsis, unplanned reoperation, and 30-day mortality were associated with higher levels of glucose variability. Specifically, these events were associated with glucose variability at 24 h but not 12 h postoperatively. Every 10% increase in 24-h glucose variability was associated with a 22% increase in adverse events. Additionally, mean glucose at 12 and 24 h were also associated with adverse events, demonstrating that with a 10 mg/dL increase in mean glucose levels at 12 and 24 h, adverse event increases by 13.8% and 18.4%, respectively. The authors question the impact of early transitions from insulin infusions to subcutaneous insulin as a possible contributor to the variability finding. Though HbA1c was not associated with major adverse events, it was – along with insulin-dependent diabetes and emergency surgery – associated with increased glucose variability. These findings indicate preoperative risk factors for increased variability and suggest a role for tailoring of postoperative insulin strategies based on this risk.

Glycemic variability is linked to the postoperative adverse event of delirium (Lin et al., 2021). ICU delirium is characterized by episodes of confusion, inattention, and altered level of consciousness (Abelha et al. 2013). It is increasingly recognized as a postoperative and ICU complication as it is linked to prolonged mechanical ventilation, increased ICU and hospital length of stay, and mortality (Kahn et al. 2020). Postoperative delirium is recognized in up to 55% of cardiac surgery patients (Smulter et al. 2013) and up to 34% of vascular surgery patients with acute aortic dissection (Liu et al. 2017). In a prospective observational study of vascular surgery patients with acute aortic dissection, higher glucose variability (as measured by SD) was associated with postoperative ICU delirium (Lin et al. 2021). Those with delirium experienced longer mechanical ventilation times, two additional ICU days, and three additional days of hospitalization.

Glucose variability is increasingly recognized as a measure of interest in the postoperative management of surgical ICU patients. Preoperative factors associated with glucose dysregulation and insulin resistance predispose patients to increased glucose levels and variability in the setting of surgical stress. In addition to surgical patients with trauma, cardiac, and vascular surgery, the association of glucose variability with adverse postoperative events has also been demonstrated in orthopedic and abdominal surgery (Shohat et al. 2018) but not gynecologic surgery (Polderman et al. 2016). Recognizing the association with preoperative risk factors for glucose variability and the link of variability with postoperative adverse events allows clinicians to tailor their glucose management to mitigate these events. To date, however, there are no trials focusing on limiting glucose variability in the postoperative setting to determine if this strategy is truly beneficial.

Blood Glucose Variability in the Medical Intensive Care Unit

Critically ill patients due to a systemic illness can manifest symptoms and signs outside of the primary system from which the acute illness originated. The loss of homeostasis occurs at many levels, and glucose, which is tightly regulated under normal conditions, becomes variable. This is often a sign of severity of illness, and therefore, it is of no surprise that extreme glucose levels are tied with worse outcomes. For example, a large patient cohort from the Australian and New Zealand Intensive Care Society (ANZICS) Adult Patient Database on 66,184 adult admissions demonstrated how increased glucose variability (and, independently, hypoglycemia) occurred more frequently in patients with a higher illness severity score. Increased glucose variability was associated with ICU requirement and with hospital mortality (Bagshaw et al. 2009). A practical application of the loss of homeostasis lies within setting the glucose goals for critically ill patients. As mentioned above, based on the findings in the recent NICE-SUGAR clinical trial (Finfer et al. 2009), the current practice allows BG of up to 180 mg/dL, before correcting it in critically ill patients. We will review the association between glucose variability and patient outcomes in two common causes for medical ICU admission, namely, sepsis and cardiac arrest.

Sepsis

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection (Rhodes et al. 2017). It is a common indication for ICU admission and one which carries a significant mortality rate of about 25% (Rhodes et al. 2017). Due to lack of data suggesting otherwise and further data pointing at risk with intensive insulin management in these settings, the current recommendation is to avoid extreme hyperglycemia or hypoglycemia in the settings of sepsis (Rhodes et al. 2017). Glucose variability is common in septic patients, in those with and without a diagnosis of diabetes (Preechasuk et al. 2017). Moreover,

increased glucose variability is associated with higher rates of mortality (Ali et al. 2008; Waeschle et al. 2008). Interestingly, in ICU patients admitted with burns, who are at high risk for infection and sepsis, increases in glucose variability preceded the onset of sepsis, even if glucose was within normal range (Pisarchik et al. 2012). However, given that reducing glucose variability per se has not been targeted in a clinical trial, it is unclear if reducing it would improve overall outcomes.

Post-Cardiac Arrest

Another common and often devastating cause for an ICU admission is for patient who survived cardiac arrest. Naturally, cardiac arrest and the aggressive resuscitation needed to reverse it take physiology to its limits. Most patients require ongoing resuscitative support with catecholamine and inotropic infusions to maintain the cardiac function and systemic perfusion (Girotra et al. 2015). Similar to other critical conditions, dysglycemia is associated with poor outcomes and mortality in the post-cardiac arrest syndrome (Pitcher et al. 2018; Steingrub and Mundt 1996), although contradicting results have also been reported (Lee et al. 2013).

Glucose variability is a predictor of mortality in cardiac arrest survivors treated with targeted temperature management (TTM). In a retrospective review of cardiac arrest patients treated with TTM, variability measures were collected during the entire period of TTM from induction, maintenance, and rewarming phases (Lee et al. 2013). This study found that increased glucose variability (as measured by MAG) was higher during the 24-h maintenance phase of TTM for non-survivors at 6 months and poor neurologic outcome at 30 days. There was no difference between groups during the rewarming phase. This finding is interesting, and the authors speculate that variability decreased over time (from maintenance to rewarming phases) due to achieving BG targets *over time*. This raises the speculation that glucose variability as a predictive factor may be more accurate if it is measured as early as possible after ROSC. Additionally, an important aspect of this study is the use of the MAG to calculate the variability. Calculating the difference between glucose measurements and how they change over time offers a practical treatment target, as opposed to standard deviation, which can only be calculated in hindsight.

In addition to the dysglycemia after return of circulation, the patient's own pre-cardiac arrest variability as measured by the HbA1c is a key contributor to poor outcomes. A single-center retrospective analysis of comatose cardiac arrest survivors undergoing TTM found that a positive correlation between higher HbA1c on admission was associated with poor outcome. Patients with high HbA1c were more common in the unfavorable group (defined as Glasgow-Pittsburgh Cerebral Performance Category scales 3–5 at 6 months) than in favorable group. Further, higher HbA1c was found to increase the odds of a poor outcome by 1.5 times and was associated with higher rates of hyperglycemia throughout the cooling, maintenance, and rewarming phases of TTM. It is of no surprise that patients with elevated

HbA1c had more frequent incidences of hyperglycemia, yet hyperglycemia on its own did not appear to be correlated with poor outcome (Lee et al. 2020).

Blood Glucose in Neurocritical Care Unit

Acute injuries to the central nervous system (CNS) are somewhat unique from a glucose management standpoint. The brain is the main regulator of glucose, by maintaining its homeostasis using various hormonal axes such as neuronally produced insulin, leptin, and glucagon-like peptide (GLP) 1 (Lam et al. 2009). In addition, neurons are highly dependent on glucose as the main energy source. In fact, the brain consumes ~20% of the body's glucose, although the brain contributes only 2% of the body's weight (Mergenthaler et al. 2013). Therefore, it is of no surprise that prior studies showed how hyperglycemia and especially hypoglycemia are associated with worse outcomes in patients suffering from acute brain injuries. Worse outcomes are observed in a "U" or "J shape" where the ends of the target BG range (hypo- and hyperglycemia) are most strongly associated with poor outcomes (Bilotta et al. 2019). However, the question remains whether this risk is related to the absolute number or its variability. The latter was addressed in several acute neurologic injuries. Herein, we will explore three common pathologies within the neurocritical care patient population and the data related to BG variability in these populations.

Traumatic Brain Injury

Traumatic brain injury (TBI) is a common acute cerebral injury. According to the Centers for Disease Control and Prevention (CDC), in the USA, mortality related to TBI is approximated at 61,000 yearly. Apart from a short-term high rate of mortality, TBI also has a long-lasting effect on function and cognition (Masel and DeWitt 2010). Glucose dysregulation is a relatively common systemic side effect in critically ill TBI patients. Dysglycemia in this patient population was shown to be an important predictor for poor outcome (Prisco et al. 2012).

Blood glucose treatment targets in patients with TBI are not well elucidated. A few clinical trials have addressed appropriate glucose ranges and a management approach in the acute phase of brain injury. The evidence that does exist is limited and demonstrates mixed results. While a single study showed an association between tight glucose control and improved long-term functional outcome (Yang et al. 2009), other studies did not demonstrate any effect (Coester et al. 2010; Bilotta et al. 2008). Therefore, the most recent brain trauma foundation guidelines do not have a specific recommendation regarding glucose management (Carney et al. 2017).

Going beyond the mean BG values as typically targeted in intensive insulin therapy regimens, higher glucose variability measured by SD does independently predict worse functional outcomes (Matsushima et al. 2012). In another prospective study, Xue et al. (2018) compared two methods of glucose control: standard and an

intervention protocol which specifically aimed at reducing glucose variability. The latter was shown feasible and indeed resulted in lower variability. Interestingly, targeting variability and not just the range resulted in lower rates of hypoglycemic events.

Stroke

Stroke, being the fifth cause of death in the USA, is another common neurological emergency that requires critical care level of treatment for many of the patients. In this patient population, hyperglycemia was shown to predict worse patient outcomes, and therefore, the general recommendation for treatment during the acute phase post stroke is to maintain BG between 140 and 180 mg/dL (Powers et al. 2019). Several studies attempted to identify the optimal treatment goal for these patients, and the latest and largest clinical trial was the Stroke Hyperglycemia Insulin Network Effort (SHINE) study (Johnston et al. 2019). This study failed to demonstrate an advantage to aiming at a tighter glucose range, like prior, smaller clinical trials (Gray et al. 2007; Walters et al. 2006). However, a post hoc analysis demonstrated the importance of the patient's baseline glucose variability as measured by the HbA1c (de Havenon et al. 2021). Indeed, a correlation between baseline glucose variability and the risk for a stroke and mortality was previously suggested (Lee et al. 2020).

Following a stroke, the glucose variability is as predictive as the baseline National Institutes of Health Stroke Scale (NIHSS) score when trying to anticipate mortality risk (Cai et al. 2020). In another study, the glucose variability was predictive of worse cognitive outcome, yet interestingly only in patients with normal baseline HbA1c (Lim et al. 2018). The latter was not reproduced in a large observational study, which showed worse outcomes in correlation with higher glucose variability regardless of the baseline HbA1c (Kim et al. 2017). Taken together, these data led to the thought that glycemic variability could be a therapeutic target of its own following an ischemic stroke, rather than aiming at an absolute range (González-Moreno et al. 2014).

Subarachnoid Hemorrhage (SAH)

Subarachnoid hemorrhage (SAH) is a life-threatening type of hemorrhagic stroke, with in-hospital mortality rates as high as 20% (Rincon et al. 2013; Udy et al. 2017). Long term, ~40% of survivors are unable to go back to work, and even fewer can return to their previous occupations (Hafeez and Grandhi 2019). Glucose dysregulation was shown to occur in about a third of the patients in the acute phase and to be correlated with poor outcomes, including higher risk for mortality (Rincon et al. 2013; Pappacena et al. 2019). Current evidence is insufficient to dictate a specific absolute glucose goal for these patients (Schmutzhard and Rabinstein 2011).

SAH patients often require multimodal monitoring including invasive cerebral microdialysis. The goal of monitoring is to detect delayed cerebral ischemia, worsening intracranial pressure, and hypoperfusion which can happen days after the initial injury. Multimodal monitoring allows a unique peek into the relationship between the tissue glucose and the systemic one. In an observational data across over 3000 h of monitoring, the data showed a clear correlation between increased glucose variability and cerebral tissue metabolic crisis defined as a pyruvate/lactate ratio > 40 (Kurtz et al. 2014). These findings give insight into a possible mechanism which entwines the systemic glucose variability and the delayed cerebral injury leading to the poor outcomes mentioned. Still, the cause for the glucose dysregulation remains obscure, while one small case series demonstrated lower serum ghrelin and higher serum leptin concentrations in SAH, hinting toward a possible understudied mechanism (Kubo et al. 2014).

In a different study, covering 2451 SAH patients, several forms of glucose variability descriptors were found to be highly predictive of in-patient mortality (Sadan et al. 2020). Increase in percentage change between measurements, even though these were not taken at strict intervals, had an odds ratio of 5.2 (confidence interval 95% [1.4–19.8]) of increased mortality, showing how the change between one measurement to the other could make a difference. Interestingly, and similar to some of the findings from the acute ischemic stroke literature, this effect was driven solely from patients with normal admission HbA1c (either non-diabetics or with well-controlled disease).

Applications to Prognosis, Other Diseases, or Conditions

In this chapter, data describing the relationship between BG and glucose variability with patient prognosis and outcome was summarized. Specifically, the correlation between glucose variability and critically ill patient mortality was shown across multiple disease conditions, for example, in the postoperative period (Dossett et al. 2008; Duncan et al. 2010), sepsis (Ali et al. 2008; Waeschle et al. 2008), post-cardiac arrest (Lee et al. 2013), and acute neurological injuries (Sadan et al. 2020; Cai et al. 2020).

Higher glucose variability in the ICU is not only associated with higher mortality but also with ICU-related complications such as delirium (Lin et al. 2021) and postoperative adverse events (Shohat et al. 2018). Even the deterioration into critical condition and the requirement for an ICU admission were found to be correlated with increased glucose variability (Bagshaw et al. 2009).

It remains unknown, however, if glucose variability as a biomarker could be utilized to identify patients at higher risk for immediate deterioration. If an association between pre-critical illness increased glucose variability and a clinical deterioration leading to an ICU admission will be robustly shown across multiple disease processes, this could be a game-changer in the application of glucose variability. One can imagine how an electronic medical record could analyze the variability in BG

measurements in real time and alert the clinical teams regarding a patient increased risk for deterioration.

Conclusion

Herein, the information available describing glucose variability in critically ill patients was summarized. The data demonstrated that across the board, in critically ill patients of various etiologies, increased glucose variability is associated with patient outcomes. However, despite this literature supporting the association between glucose variability and outcomes in critically ill patients, this is not a settled topic. There remain several challenges and unanswered questions before glucose variability can be clinically used as a treatment goal.

Variability is an inherent trait of normal physiology. There are systems that are more tightly controlled, while others are more variable. For example, while healthy non-diabetic subjects' glucose is tightly controlled, the heart rate and blood pressure change incredibly through the day based on one's activity and needs. As proposed by Godin and Buchman (1996), healthy organs behave as biological oscillators, and they communicate to maintain and adjust to one another during normal physiology. That oscillation, and probably more so the uncoupling between organs, is disrupted during critical illness and organ dysfunction. This idea was shown to be relevant across many organ systems in sepsis (Buchan et al. 2012). Glucose variability is a clear example of this phenomenon. During normal physiology, there are multiple systems communicating to maintain tight glycemic control even in settings of fasting or postprandially. However, when one or more of these systems become dysfunctional as in critical illness, the result is dysglycemia.

Measuring glucose variability is an attractive clinical target, since it is easily obtainable and frequently measured as part of standard of care. However, before attempting to incorporate glucose variability into clinical care, there are several outstanding questions. The most important question is straightforward but has a complex answer: how to define and measure glucose variability? As described above, there are numerous measures of glucose variability, each with advantages and disadvantages (Table 1). The optimal variability measure should allow for real-time decision-making on the one hand and correlated with patient outcomes on the other. Targeting changes between successive measurements accomplishes these two aspects. Indeed, the absolute difference between each two measurements or the percentage change between them did demonstrate a correlation to patient outcome in some patient populations (Sadan et al. 2020; Lee et al. 2013). Targeting the rate of change, and not just an absolute threshold, resembles the current approach to hypertensive emergencies, in which the recommendation is to reduce the blood pressure by no more than 25% per hour (Whelton et al. 2018). These are measures that can be obtained with higher-frequency BG checks (e.g., every 4–6 h) as is common in the critical care setting.

A second open question is the frequency of sampling needed to address glucose variability. As described in the "Blood Glucose Variability Measures" section, the time between measurements has a role in determining variability in a clinically applicable manner. Yet whether variability measures can rely on a strict schedule (e.g., every 6 h) versus event related (e.g., before and after meals or before and after correction of a value) remains unclear. CGM offers a way forward offering high-frequency measurements and offers additional glucose variability measures (Krinsley et al. 2017). Although technically feasible to incorporate CGM into ICU management, to date, it remains little utilized. More widespread adoption could clarify if CGM offers a level of clinically useful resolution to optimize BG and glucose variability. Future studies will need to investigate the optimal frequency of measurement.

A third open question remains regarding the underlying cause and impact of increased variability in critical illness. Understanding the pathophysiology may help determine if glucose variability is simply an epiphenomenon indicating an underlying process. It could also improve our understanding of whether variability reduction is a viable treatment target. In sepsis, for example, patients deteriorate and develop hypotension and shock, which is life-threatening. Although maintaining adequate blood pressure fluid and vasopressor support is necessary for survival, without the appropriate antimicrobial therapy, the patient is unlikely to survive regardless of the aggressive blood pressure support. Hence, treating the cause (infection) is at least as important as treating the result (hypotension). Similarly, if glucose variability increases due to critical illness, addressing underlying mechanisms may reduce variability regardless of the direct treatment administered, such as insulin. However, there are many potential causes for the increased variability which differ between patient populations. Understanding the unique pathophysiologic changes in different disease processes, and not simply treating glucose management as a one-size-fits-all approach, will align with the recent understanding of the importance of precision medicine approach in critical care (Buchman et al. 2016). Unfortunately, current standard of care is not patient specific as discussed in sections "Blood Glucose Variability in the Surgical Intensive Care Unit, " "Blood Glucose Variability in the Medical Intensive Care Unit," and "Blood Glucose in Neurocritical Care Unit." Indeed, the evidence in glucose management is increasingly supportive of differentiating management practices based on a host of underlying patient and illness factors (Krinsley et al. 2017) (Fig. 2).

Lastly, in order to determine if glucose variability is indeed an independent treatment target, clinical trials will be needed to answer this question. Such future trials will face numerous challenges as they must address many of the questions above, such as which variability measure to utilize, the appropriate frequency of measurement, and how to define the most relevant patient population. Future studies are needed to determine if glucose management in our ICUs will move beyond overly simplistic targets and discover whether glucose variability can pave itself a path into clinical use or will remain only a statistical calculation.

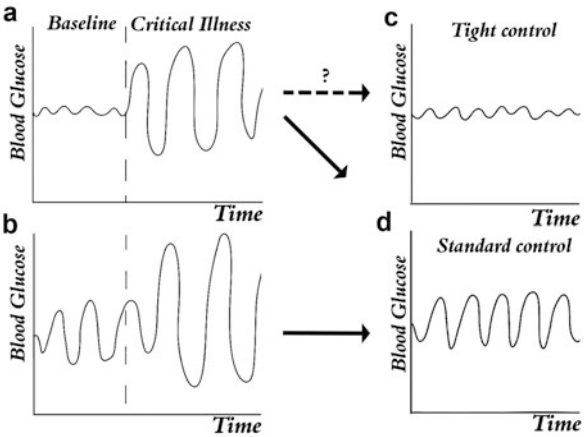

Fig. 2 Different patients may require different glucose goals. (**a**) A patient without diabetes or with well-controlled diabetes may develop increased variability during critical illness. (**b**) Patients with uncontrolled diabetes will have higher baseline variability. Treatment during critical illness could be aimed at tight (**c**) glucose control, yet there is no good evidence to support this approach. Most critically ill patient treatment goal is the standard one (**d**) which allows variability. Current standard care does not account for variability nor baseline patient characteristics. Future studies may identify specific patient populations which may benefit from a tighter, less variable glucose control, such as non-diabetic or well-controlled diabetic patients

Mini-Dictionary of Terms

- Blood glucose – A measurement of the concentration of glucose within a blood sample. Normal values (fasting) are 70–99 mg/dL which corresponds to 3.9–5.5 mmol/L.
- Critical illness – A condition or disease which is defined as life-threatening. There are many causes for critical illness, such as sepsis, respiratory failure, postoperative recovery, trauma, and more. The commonality is the need for intensive care and monitoring which usually occur in an intensive care unit (ICU).
- Variability – In statistics, this represents the dispersion of the data. In addition to describing the central tendency (e.g., with the mean or median), one can describe its dispersion with common descriptors such as variability, standard deviation, etc. In this chapter, other more sophisticated measures of variability are discussed.
- Continuous glucose monitoring – New technology that allows measuring blood glucose in a continuous manner, either by attaching to an existing intravenous catheter or transdermally. Continuous glucose monitoring differs from the current standard in critical care, in which glucose is measured every few hours. This offers increased data on blood glucose fluctuations.
- Insulin – A peptide hormone secreted from the beta cells of the pancreas. It regulates the body's carbohydrate metabolism and serves as the main anabolic

hormone. It serves as a medication as well to control hyperglycemia in many clinical scenarios, including critical illness, with or without diabetes mellitus.

Key Facts of Glucose Variability Measures in Critical Care

- During critical illness, blood glucose often becomes dysregulated. Both hyperglycemia (too high) and hypoglycemia (too low) are common and are associated with poor outcomes.
- Despite the association between dysregulated glucose levels and poor outcomes, clinical trials exploring glucose control have shown mixed outcomes.
- There is growing evidence that fluctuations in glucose or its variability play a role in patient outcomes and may explain the mixed outcomes found in clinical trials.
- Glucose variability may be a predictive biomarker indicating complications and increased mortality in critically ill patients.

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Fluid Biomarkers in Sports-Related Mild Traumatic Brain Injuries: Current Status and Novel Trends

21

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Abstract

Mild traumatic brain injury (mTBI) or concussion is the most common type of head injury reported worldwide, with millions of injuries occurring yearly due to traffic accidents, high impact sports, or during military services. Although reliable biomarkers in serum, plasma, or cerebrospinal fluid (CSF) have been developed for several neurological and psychiatric diseases to date, post-concussion biomarkers are less developed, especially long-term following an injury. We have utilized neuron-derived exosomes (NDEs) to explore changes in collegiate athletes with or without a history of one or several mTBIs. Our findings strongly suggest that NDEs can be used to reliably explore long-term alterations in brain health occurring after one or multiple mTBIs in athletes. This chapter is focused on the latest findings on blood biomarkers for mTBIs, including neuronal, glial, and inflammatory classical markers of mTBI, as well as highlighting novel biomarker candidates, including miRNAs and exosomal cargo.

Keywords

Concussion · Mild traumatic brain injury · High impact sports · Chronic traumatic encephalopathy · Blood-based biomarkers · Extracellular vesicles · Exosomes

Abbreviations

AD	Alzheimer's disease
ADE	Astrocyte-derived exosome
ALS	Amyotrophic lateral sclerosis
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
Aβ42	Amyloid-beta 42
BBB	Blood-brain barrier
CNS	Central nervous system
CTE	Chronic traumatic encephalopathy
EV	Extracellular vesicle
GABA	Gamma-aminobutyric acid
GCS	Glasgow coma scale
GFAP	Glial fibrillary acidic protein
IFNγ	Interferon gamma

IL	Interleukin
LOC	Loss of consciousness
LP	Lumbar puncture
MCI	Mild cognitive impairment
miRNA	microRNA
mTBI	Mild traumatic brain injury
NDE	Neuron-derived exosome
NFL	National Football League
NF-light	Neurofilament-light
NFT	Neurofibrillary tangle
NSE	Neuron-specific enolase
PCS	Post-concussion syndrome
PD	Parkinson's disease
pTau	Phosphorylated Tau protein
PTSD	Post-traumatic stress disorder
ROS	Reactive oxygen species
S100B	S100 calcium-binding protein B
SNTF	Calpain-cleaved all-spectrin N-terminal fragment
TBI	Traumatic brain injury
TNFα	Tumor necrosis factor alpha
UCH-L1	Ubiquitin C-terminal hydrolase L1
VOMS	Vestibulo-ocular motor screening
VOR	Vestibulo-ocular reflex

Introduction

Traumatic brain injury (TBI) is a complex and heterogeneous injury caused by a bump, a blow, or a jolt to the head, resulting in structural and physiological damage that can affect brain function. Classification of TBI severity can range from mild to severe and is based on self-reported or clinical presentations and structural findings observed with brain imaging (Maas et al. 2008). While moderate to severe TBI creates structural changes in the brain visible using conventional neuroimaging modalities (computerized tomography and magnetic resonance imaging), a mild TBI (mTBI) usually does not elicit structural damage (Bigler et al. 2016) but can result in microstructural injury to neural tissue that can be detected using advanced imaging techniques (Koerte et al. 2016). However, despite a more widespread use of these imaging techniques, the lack of standardization is hampering proper diagnosis. Consequently, the diagnosis of mTBI still relies mostly on clinical and self-reported symptoms.

An mTBI is typically diagnosed after a mild non-penetrating insult to the head that can lead to a brief period of loss of consciousness (LOC, less than 30 min) and up to 1 day of amnesia, as defined by the American Congress of Rehabilitation Medicine in 1993 (Kay et al. 1993). Other clinical symptoms can vary broadly among individuals and include cognitive (memory problems and poor

concentration), physical (headache, sensitivity to light/noise, fatigue, dizziness, nausea), and emotional symptoms (irritability, anxiety, sadness). Symptoms may appear right away or hours to days after the injury, rendering the diagnosis challenging. For most people, symptoms improve shortly after the injury. However, a subset of individuals will develop post-concussion syndrome (PCS), which is defined as the occurrence of clinical symptoms lasting for weeks or months after head trauma (Polinder et al. 2018; Tagge et al. 2018). PCS-associated symptoms are highly variable and likely dependent on psychological, social, and physiological factors in addition to severity of the initial insult, and the number of insults sustained (Polinder et al. 2018). The mechanisms underlying PCS are still poorly understood, and the potential contribution of social, emotional, and psychological factors remains a controversial issue.

Several review studies have reported that TBI of any severity is associated with a higher risk of developing neurodegenerative conditions, including Alzheimer's disease (AD) (e.g., McAllister and McCrea 2017; Snowden et al. 2020). In recent years, a devastating neurodegenerative condition called chronic traumatic encephalopathy (CTE) that has been mostly reported in former high impact sport athletes and military veterans has attracted media attention as well as research interests (Alosco et al. 2021). A single or repetitive mTBI may also trigger other neurodegenerative diseases including Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) (Gupta and Sen 2016), although there are less quantifiable data available to firmly connect mTBIs with the incidence of either PD or ALS to date. The increased awareness of the potential long-term consequences of single or repetitive mTBI has led several research groups to investigate the mechanisms underlying the shift from acute and subacute physiological changes occurring after mTBI to neurodegenerative conditions such as CTE, PD, or AD. In this context, biomarker-based studies could contribute to a better understanding of the pathophysiological mechanisms likely driving the development of neurodegenerative disorders long-term after mTBI.

In this book chapter, we provide a brief overview of the acute physiological events following an mTBI before describing the biological mechanisms hypothesized to play a role in the shift from acute/subacute changes into the long-term consequences. Next, we describe the current knowledge of fluid biomarkers for mTBIs, with an emphasis on new blood biomarkers and emerging exosomal biomarkers.

Mild TBI: Prevalence and Risk Factors

Concussions, also known as mTBIs, are the most common form of TBI and account for more than 70% of all treated brain injuries in the USA and Canada (Cassidy et al. 2014). The annual international incidence rate of mTBI is estimated to range between 100 and 300 per 100,000, with a recent meta-analysis finding the incidence rate to be 224 per 100,000 (Nguyen et al. 2016). Military personnel and high impact sports athletes are at higher risk for sustaining an mTBI (McKee et al. 2014). Several

risk factors have been identified in sports-related mTBI (reviewed by Abrahams et al. 2014). Briefly, athletes with a history of previous sports-related mTBIs have a higher risk of sustaining another mTBI, and also having more long-term problems. Women appear to be at greater risk for sustaining an mTBI but this seems to be dependent on the type of sport played (Abrahams et al. 2014). Further, evidence suggests that having more than one concussion in the past increases the risk of having more pronounced and longer-lasting physical, cognitive, and emotional symptoms with every successive injury (Marklund et al. 2019). It is estimated that the total cases are largely underestimated since many mTBIs are not detected, reported, or treated, resulting in probably only 50% of mTBIs being accounted for (Meier et al. 2015). In sports-related mTBIs, underreporting varies largely between sports and many competitive athletes underreport PCS and/or fail to report concussive injuries (Ferdinand Pennock et al. 2020). As a result, they do not receive the clinical attention needed to promote recovery and avoid short- and long-term consequences. This is especially prominent in high-impact sports that can lead to professional careers, such as ice hockey or football (Baugh et al. 2020).

Acute Pathophysiology of mTBI and Associated Symptoms

An mTBI occurs when an impulsive force is transmitted to the head causing the brain to stretch and deform within the skull (McKee et al. 2014). The hallmark of a concussion is the presence of neurological symptoms without visible anatomic damage to the brain tissue (Giza and Hovda 2014), but these forces do cause microstructural stress injuries to neuronal cell bodies, axons, dendrites, blood vessels, and glial cells (McKee et al. 2014). This damage leads to the immediate initiation of a complex cellular signaling cascade involving neurochemical, metabolic, and inflammatory processes. Animal studies have contributed to a better understanding of subtle acute neuropathological mechanisms and complex cellular signaling cascades that accompany mTBI (Bigler 2015). Generally, the microstructural primary injuries contribute to secondary injuries and include excitotoxicity, neuroinflammation, axonal injury, and possibly apoptotic cell death. While the primary injuries are usually not visible using CT scans or other imaging techniques in the case of an mTBI, the consequences of the secondary injuries can be detected based on the self-reported symptoms and changes in cognition and balance over the years (Barkhoudarian et al. 2011).

Excitotoxicity

Excitotoxicity is a phenomenon by which excitatory neurotransmitters, primarily glutamate, cause prolonged activation of glutamate receptors ultimately leading to loss of neuronal function and cell death (Dong et al. 2009). The sudden changes in ionic fluxes caused by the tearing of brain cells after brain injury lead to the indiscriminate release of glutamate and other neurotransmitters into the synapse in

an attempt to maintain homeostasis (McKee et al. 2014; Choe 2016). This results in reduced neuronal firing, which activates ATP-requiring membrane pumps in an effort to restore ionic equilibrium but deplete cellular energy reserves in the process. The increased demand for energy coupled with the reduced cerebral blood flow seen after injury creates a mismatch between energy supply and demand (Giza and Hovda 2014). In addition, it has been shown that excessive glutamate release directly affects neuronal health, leading to an imbalance between toxic reactive oxygen species (ROS) and the antioxidant systems in the brain and an imbalance between glutamate and GABA transmitters after a TBI (Guerriero et al. 2015). Unfortunately, the environment immediately post-injury can overwhelm the cells causing the activation of several cell death/apoptotic pathways (Zhou et al. 2012).

It is hypothesized that there is a window of vulnerability after a single mTBI where a subsequent mTBI will worsen the outcome (Prins et al. 2013). Investigations into a single mTBI have shown minimal neuronal cell loss and relatively little resulting cognitive impairment. However, repeated mTBIs experimentally have shown significant increases in neuronal cell damage and death (Longhi et al. 2005). This could be caused by glutamate toxicity, as described above. Furthermore, it is hypothesized that chronic excitotoxicity may play a role in numerous neurodegenerative diseases such as AD (Lewerenz and Maher 2015). In athletes, repetitive mTBIs have been shown to have cumulative effects on cognitive performance (McAllister and McCrea 2017). Overall, repetitive mTBI appear to lead to chronic persistent neurological symptoms due to neurodegeneration (Aungst et al. 2014).

Axonal Injury

Axonal injury is a common neuropathological finding in mTBIs and is a major contributor to the morbidity after injury (Choe 2016). Cytoskeletal damage is a direct result of the impact-acceleration forces that cause the microtubules supporting the cell structure to undulate and break interfering with bidirectional microtubule function, which affect neurotransmission in severe cases. Axonal injury does not uniformly affect all axonal populations; smaller, unmyelinated axons may be more susceptible to damage from concussive forces and to more functional impairment after injury than larger myelinated axons (Reeves et al. 2005). In animal models, repetitive mTBIs exacerbated axonal degeneration and this degenerative process was ongoing in subcortical white matter tracts for up to 24 months post-injury, suggesting long-term damage (Mouzon et al. 2014, 2018). Progressive white matter changes (i.e., loss in white matter neurite density) among individuals with mTBI have also been reported in a prospective cohort (Palacios et al. 2020), suggesting that repetitive mTBIs can induce chronic and progressive axonal degeneration, which likely contributes to cognitive, balance, and perhaps emotional deficits post-mTBI (Spitz et al. 2017; Dailey et al. 2018).

Neuroinflammation

Recent studies have indicated that, acutely after an mTBI, the blood–brain barrier (BBB) undergoes subtle changes, potentially allowing peripheral immune cells like leukocytes to infiltrate the brain (Tagge et al. 2018). Moreover, the neuronal damage caused by the injury promotes the activation and recruitment of microglia to the injury site to clear debris (Choe 2016). The presence and activation of microglia and astrocytes increase the synthesis of a variety of pro-inflammatory cytokines and chemokines (Choe 2016), promoting the neuroinflammatory response. Additionally, microglial cells appear to be sensitive to mechanical signals induced by the head injury (Bollmann et al. 2015) and could become activated, further exacerbating neuroinflammation (Verboon et al. 2021). In clinical reports, peripheral inflammatory biomarkers are elevated in patients with TBI weeks to months after injury (Vedantam et al. 2021). Interestingly, in preclinical models, neuroinflammatory changes in the absence of behavioral deficits after sub-concussive injuries have been reported (Shultz et al. 2012), suggesting that sub-concussive impacts alone and in combination with a history of concussion may contribute to a cumulative neuroinflammatory response. Given the role of neuroinflammation in neurodegenerative diseases like AD, TBI-associated neuroinflammation is undoubtedly contributing to the onset of neurodegenerative diseases later in life. While the progression from TBI to such diseases as AD is still not fully elucidated, *post mortem* neuropathological studies have reported elevated inflammatory markers up to 18 years post-injury, suggesting a potential involvement of neuroinflammation in the neurocognitive and neurodegenerative changes observed later in life (Johnson et al. 2013).

Connection to Clinical Symptoms

While there is a lack of studies directly connecting the pathophysiological changes after an mTBI to clinical symptoms, there is some circumstantial evidence linking these two elements (Giza and Hovda 2014). For example, the flux of ions and energy crisis described previously may contribute to the presence of migraines (Mihalik et al. 2005). The typical symptoms associated with PCS mirror those commonly associated with migraine, such as headache, nausea, and light and noise sensitivity. In some clinical settings, a history of headache or migraine is considered a risk factor for more severe symptoms and prolonged recovery period (Mihalik et al. 2005). Axonal injury and altered neurotransmission may contribute to the slowed cognition and impaired balance observed after an mTBI (Hellstrøm et al. 2017). Clinical studies using advanced imaging techniques demonstrated changes in white matter integrity after mTBI, which have long been associated with impaired cognition (Boroda et al. 2021). In addition, dizziness, loss of balance, and vestibulo-ocular reflex (VOR) are short- or long-term clinical consequences of mTBIs (Kolev and Sergeeva 2016). Novel testing methods for concussion now include eye tracking and vestibulo-cochlear function, such as vestibulo-ocular motor screening (VOMS),

allowing for a court-side diagnosis of more subtle signs of acute mTBIs in sports (Doperak et al. 2019).

Transition from Acute to Chronic Pathophysiology

Increasing evidence from epidemiological studies suggest that repetitive mTBI can increase the risk for AD and other dementias decades after the injuries were sustained. Guskiewicz et al. (2005) documented earlier than typical onset of AD in a cohort of retired American football players. In that study, the football players who reported three or more mTBIs had five times increased frequency of mild cognitive impairment (MCI) diagnoses and three times increased frequency of reported memory problems compared to players who did not report a history of mTBIs. In a large cohort study of US veterans, it was found that mTBI without LOC was associated with more than a two-fold increase in the risk of dementia diagnosis later in life (Barnes et al. 2018). A recent meta-analysis study compiling 21 studies reported that individuals were 1.96 times more likely to be diagnosed with dementia if they had prior mTBIs (Snowden et al. 2020). CTE is a complex and heterogeneous neurological disorder characterized by executive dysfunction, depression, explosivity, aggression, poor impulse control, memory impairment, and dementia, among other types of cognitive and affective dysfunctions (Stern et al. 2013). CTE and AD share several pathological features, such as the presence of hyperphosphorylated Tau forming neurofibrillary tangles (NFTs) and amyloid-beta plaques. A number of *post mortem* studies have identified CTE in professional athletes who participated in boxing (McCrory et al. 2007), American football (Mez et al. 2020), soccer (Ling et al. 2017), and rugby (Lee et al. 2019), although in some cases, other co-occurring neuropathologies were reported. Further, 16% of neuropathologically confirmed cases of CTE had no reported history of mTBI (Stein et al. 2015), highlighting the potential long-term consequences of sub-concussive injuries which do not cause any acute symptoms, but may lead to increased risk for neurodegeneration in the long-term.

Animal models and human *post mortem* studies have yielded a better understanding of neuropathological mechanisms underlying the progression from mTBI to chronic neurodegenerative conditions such as AD, PD, and CTE. Understanding the direct and indirect pathways by which acute effects of mTBI can contribute to age-related neurodegeneration and dementia could allow for implementing preventative therapies.

Axonal injury is prevalent after a single mTBI and even more so after repetitive mTBIs (Xu et al. 2021). As mentioned above, chronic white matter atrophy takes place after mTBI and is believed to be implicated in neurodegenerative processes (Graham and Sharp 2019; Brett et al. 2021). Disturbances to microtubule structure resulting from the shearing forces of the injury promote aberrant phosphorylation and accumulation of microtubule-associated protein Tau, which becomes hyperphosphorylated, resulting in the formation of NFTs (Tagge et al. 2018). Moreover, axonal injury is causing protein accumulation in the axon, including amyloid

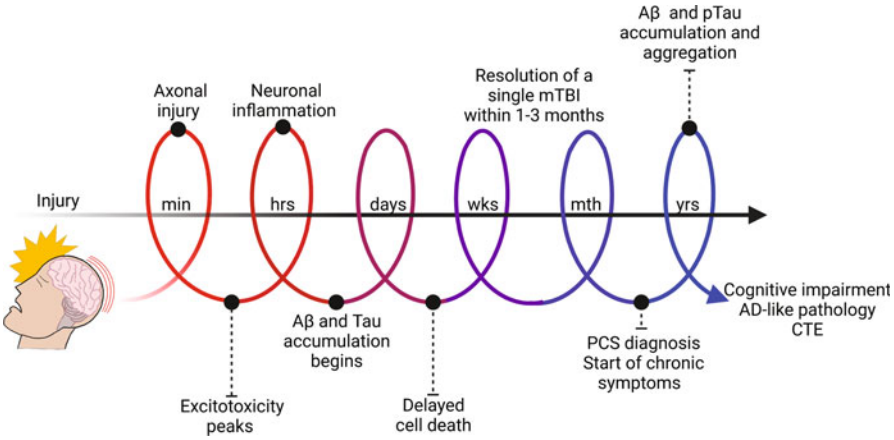

Fig. 1 Possible timeline of events after mTBI. While no official timeline of events exists, it appears the impact to the head initiates microstructural injuries to the neurons. Within minutes, excitatory signals overwhelm the cells as they try to maintain homeostasis. Within hours, the immune cells have built a strong inflammatory response to combat the injury. Experimental data have shown that Aβ and p-Tau accumulations begin hours after injury. The onslaught of excitotoxic and inflammatory responses are believed to delay progressive cell death mechanisms. Most single mTBI patients fully recover in 1–3 months. However, some may experience symptoms lasting longer than 3 months and are then diagnosed with PCS. The combination of increased neuroinflammation with Aβ plaque deposition and p-Tau aggregation can lead to dementia-like pathology observed years later. Created with BioRender.com

precursor protein (APP) which can be cleaved to form toxic amyloid-beta deposits (Iwata et al. 2002). Overall, the accumulation of the toxic aggregation-prone forms of Tau and Aβ can induce long-term neuropathological changes similar to those found in neurodegenerative diseases, such as AD or CTE (Edwards 3rd et al. 2017; Johnson et al. 2017).

Chronic neuroinflammation has been observed in animal models subjected to a single or repeated close-head injuries (Sauerbeck et al. 2018). While direct evidence of neuroinflammation in more challenging to prove in human studies, several findings point toward a prolonged neuroinflammatory response after mTBI (Johnson et al. 2013; Vedantam et al. 2021). Under pathological conditions, the complex crosstalk between the central nervous system and the peripheral and central immune systems is disrupted, promoting an unresolved inflammatory response of microglia, the CNS-resident immune cells that might initiate and propagate the progression of neurodegeneration (Scheiblich et al. 2020; Verboon et al. 2021) (Fig. 1).

Challenges with Diagnosis of mTBI

The main challenges in mTBI management are correctly diagnosing the injury and determining whether a person has fully recovered. The reliance on self-reporting and the heterogeneity of symptoms only exacerbate the difficulty (Meyer et al. 2020).

Currently, general guidelines for diagnosis and management of sports-related concussions include an assessment of symptoms and disposition after the injury (McCrory et al. 2017). Unfortunately, 87% of athletic trainers report that they will return an asymptomatic athlete to play based on self-reported symptoms alone (Covassin et al. 2009); a practice that can be dangerous for collegiate and professional athletes that have financial incentives to continue playing. In symptomatic athletes, prognosis and recovery times are often predicted based on perceived severity of the injury and clinical symptoms; however, the extent to which these assessments correlate to short-term and long-term outcomes is unclear (Ledreux et al. 2020). Calls for a highly specific and objective test to diagnose and monitor concussion have led to an increased focus on identifying noninvasive biomarkers. While recent advances in neuroimaging allow for a more comprehensive diagnosis of mTBI, longitudinal imaging studies are needed to better characterize the acute and potential long-term effects of mTBI. Even though many who have sustained an mTBI exhibit symptoms for more than a month after injury, many will return to play prematurely, causing more harm. Novel and more sensitive imaging techniques, such as functional magnetic resonance imaging (fMRI), susceptibility-weighted imaging (SWI), and diffusion kurtosis MRI (dMRI) along with more sensitive and rapid court-side testing such as VOMS, can be used to examine longer-term PCS in high-impact sports (Lunkova et al. 2021). However, an area that is currently under rapid development in this field is blood biomarkers for long-term PCS and ensuing neurodegenerative conditions.

In the next section of this book chapter, we focus on noninvasive fluid biomarkers, with an emphasis on blood and extracellular vesicle (EV) biomarkers in the context of sports-related mTBI.

Blood Biomarkers for mTBI

Fluid biomarker research has gained traction in recent years for its promise to determine the severity of injury, when it is safe for athletes to return to play, and the long-term prognosis of brain health after mTBIs (O'Connell et al. 2018). Three classes of biomarkers are commonly used to acutely identify mTBIs: (1) neuronal/axonal damage biomarkers such as aII-Spectrin N-terminal fragment (SNTF), neurofilament light polypeptide (NF-light), ubiquitin C-terminal hydrolase L1 (UCH-L1), and Tau/pTau; (2) glial activation markers such as glial fibrillary acidic protein (GFAP) and S100B; and (3) inflammatory biomarkers that occur due to neuronal injury including interleukin (IL)-1, IL-12, and IL-18, tumor necrosis factor alpha (TNFα), and interferon gamma (IFNγ) (Ledreux et al. 2020). Increased biomarker levels (i.e., those associated with neuronal/axonal damage and glial activation) have been consistently observed in mTBI patients regardless of age and injury mechanism indicating their utility in diagnosis and management guidelines for mTBIs (Kawata et al. 2016). The current best candidates for acute diagnosis of mTBIs have been extensively covered (Kawata et al. 2016; Ledreux et al. 2020; McCrea et al. 2020), thus we will only summarize the key findings here (see Fig. 2).

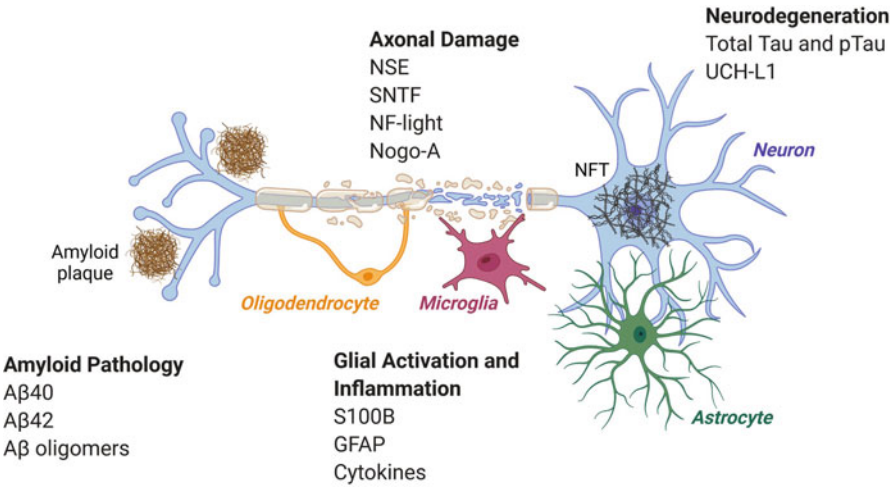

Fig. 2 Blood biomarkers for mTBI. Neuropathological mechanisms implicated in mTBI and associated fluid biomarkers. Created with BioRender.com

Neuronal Damage Biomarkers

Neuron-specific enolase (NSE) is a marker for neurons and neuroendocrine cells (Marangos and Schmechel 1987) and was the first biomarker explored for its role in mTBI (Stalnacke et al. 2004). NSE is involved in regulating axonal transport and signal propagation (Mondello and Hayes 2015) as well as initiating the inflammatory response to neural injury (Kawata et al. 2016). Several research groups assessing mTBI in amateur boxers reported not only an increase in serum NSE levels after direct punches to the head but also that those levels remained significantly elevated even after a 2-month rest period (Zetterberg et al. 2009). This suggests that repetitive head trauma may lead to sustained neuronal damage. Although after a more severe TBI, a poor prognosis significantly correlated to higher NSE levels, there are wide variations between studies making cutoff values and sampling determinations difficult (Cheng et al. 2014).

Although not a commonly used biomarker, **calpain-cleaved α-spectrin N-terminal fragment (SNTF)** is released from degenerating neurons and has been identified as a potential biomarker for axonal damage (Johnson et al. 2016). SNTF levels have been shown to rapidly increase within the first hour of an mTBI and may remain elevated for up to 6 days (Siman et al. 2015). SNTF has also been shown to reliably predict delayed recovery after mTBI (Gan et al. 2019) and may provide some insights once it is more thoroughly investigated.

Neurofilament-light (NF-light) is another potential marker for axonal damage. NF-light levels are increased in retired and active adult athletes with PCS (Shahim et al. 2017). However, in adolescents with uncomplicated mTBI, no detectable changes in NF-light levels were seen within 14 days after an mTBI, suggesting that the severity of the injury might have been too low to induce axonal damage

(Wallace et al. 2018). Interestingly, this biomarker has become a hot topic for analysis in neurological diseases, such as AD, PD, and ALS (Gaetani et al. 2019; Lee et al. 2020), reliably showing an increase in both plasma and CSF as the condition worsens and correlating significantly between CSF and plasma, suggesting that this marker for neurodegeneration can hold promise also for long-term brain health after mTBIs.

Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is an enzyme highly expressed in neurons. UCH-L1 interacts with both phosphorylated tau and amyloid beta, making it a promising target for the development of therapies for neurodegenerative diseases (McCrea et al. 2020). Studies in concussed athletes have documented elevated UCH-L1 levels in the serum shortly after injury but returning to baseline levels 48 h later, suggesting its potential use as a biomarker for acute but not chronic phases of injury (McCrea et al. 2020).

Nogo-A is an important inhibitor of axonal growth, present in neurons and oligodendrocytes (Pernet and Schwab 2012). Several animal studies have pointed to Nogo-A as an important player in mediating neural plasticity and functional recovery after a TBI (e.g., Chao et al. 2012). Serum Nogo-A levels of patients with TBI continuously increased for several hours after injury, peaking at 72 h post-injury. Additionally, serum Nogo-A levels were able to distinguish between patients with severe or mild TBIs with peak serum levels correlating to GCS scores. While more studies are needed, Nogo-A might be a promising biomarker for the acute phase after injury.

A correlation between increased plasma levels of **Tau** and greater exposure to repetitive head impacts was found in a cohort of 96 former NFL players (Alosco et al. 2017), suggesting long-term effects of repetitive head injury on axonal damage. **Phosphorylated Tau (p-Tau)** accumulates in the brain within NFTs in both AD and in CTE (Tracy and Gan 2018) and is therefore considered an important biomarker for examining long-term consequences of mTBIs in terms of risk for developing dementias (Brett et al. 2021). In neuron-derived EVs (see section below) enriched from blood of athletes with previous mTBIs, levels of p-Tau (T181 and S396) exhibited long-term changes (Goetzl et al. 2019), strongly suggesting that this novel method for assessing biomarkers in EVs of neuronal origin may provide an important insight into brain-specific events after injury, and could be considered as a "liquid biopsy" of brain tissue using a non-invasive blood sample.

Glial Activation Markers

S100 calcium-binding protein B (S100B) and **glial fibrillary acidic protein (GFAP)** are both astrocytic proteins that have gained attention as glial activation biomarkers following an mTBI (Kawata et al. 2016; Bogoslovsky et al. 2017). Levels of S100B have been shown to help distinguish between an mTBI and a severe TBI as well as help improve prediction accuracy of long-term outcomes (Dey et al. 2017). However, S100B is also produced by peripheral cells such as adipocytes, reducing its specificity as an injury marker with regard to sports-related mTBI

(Diaz-Romero et al. 2014). GFAP is almost exclusively produced by astrocytes and is one of the strongest candidates for use as an mTBI biomarker (Okonkwo et al. 2020). However, research groups have seen conflicting results with several studies reporting serum elevations of GFAP after an mTBI while others did not see increased GFAP levels even in athletes with diagnosed concussions (Kou et al. 2013). This may be due to differences in sampling time after injury between the studies. One study reported elevated GFAP levels immediately after arriving at the hospital post-injury (Bogoslovsky et al. 2017), suggesting that GFAP may be a useful marker within the first hours after injury.

Inflammatory Markers

Release of inflammatory cytokines after mTBIs reflects the activation of glial cells in the brain but also reflects the activation of the peripheral immune system, which can indirectly affect neuroinflammation (Patterson and Holahan 2012). While the initial and short-lived activation of glial cells is beneficial for injury repair, prolonged activation can promote neuroinflammation and is detrimental to neuronal cells. Thus, neuroinflammation is often considered the driving force behind a secondary (or delayed) wave of injury occurring hours or days after the initial injury. The severity of the second wave is highly influential on prognosis and long-term outcomes (Jassam et al. 2017). Pro- and anti-inflammatory cytokines correlate with days to recovery timelines and can discriminate between concussed and non-concussed athletes (Nitta et al. 2019). However, taken alone, their clinical significance may be hampered by their lack of specificity. If used in conjunction with other biomarkers, examination of inflammatory markers could further our understanding of the physiological consequences of mTBIs.

Despite their utility for acute diagnosis, these blood-based biomarkers have significant limitations in their prognostic value for long-term consequences of concussion. While these biomarkers are exponentially increasing their expression in the brain and bloodstream during the acute phase of neuronal damage and gliosis, the majority are susceptible to protease degradation and filtration by the kidney and liver resulting in short plasma half-lives (<6 hrs) (Kawata et al. 2016). Additionally, plasma and serum biomarker levels vary between athletes with mTBI and may not accurately reflect changes occurring in the brain. For example, data suggest that the glymphatic clearance system, part of the brain's waste management system that removes toxins and soluble proteins from the interstitial space (Jessen et al. 2015), has a 60% reduction in clearance efficiency up to 1 month after injury (Iliff et al. 2014). Due to the compromised clearance, it is possible that lower levels of blood biomarkers could be detected following an mTBI potentially leading to the underestimation of injury severity, although this has yet to be confirmed in clinical studies (Plog and Nedergaard 2015). Inflammatory cytokines measured in serum or plasma are largely not specific to brain inflammation but are also activated with peripheral injuries as well. When measured in CSF, cytokines are an excellent indicator of neuroinflammation. However, the difficulty of CSF serial sampling in clinical settings and the risks associated

with repeated lumbar punctures (LPs) can pose significant challenges. Blood levels of NF-light, GFAP, UCH-L1, Tau/p-Tau, and/or inflammatory biomarkers are promising for identifying the acute phase of mTBI but these blood biomarkers have limitations for quantifying the chronic effects of repetitive mTBIs (Agoston et al. 2017). To overcome some of these limitations, examination of the cargo of small extracellular vesicles (also called exosomes) has emerged as a novel, noninvasive way to detect changes in the brain after injury (Marazioti et al. 2019).

Extracellular Vesicle Biomarkers

All cells release a variety of extracellular vesicles (EVs) that can be isolated from body fluids, including blood, CSF, urine, saliva, breast milk, semen, amniotic fluid, and bile (for a review, see Yanez-Mo et al. 2015). A specific type of small EVs (also called exosomes) are formed by the inward budding of the endosomal membrane, creating multivesicular bodies (MVBs) that contain intraluminal vesicles (ILVs) and fuse with the plasma membrane to release exosomes in the extracellular space (see Fig. 3). Exosomes are nanosized vesicles (40–150 nm) that contain proteins, lipids, and nucleic acids and participate in cell-to-cell communication (Gurung et al. 2021). Because of their small size, secreted exosomes diffuse into biological fluids and can cross the blood–brain barrier (BBB) in both directions (Marazioti et al. 2019). Exosomes contain surface markers and cargo proteins as well as different types of RNA (Gurung et al. 2021), all reflective of the physiological state of their cell of origin. Thus, they represent a novel and powerful source of biomarkers for various conditions including cancer and neurodegenerative conditions (Hornung et al. 2020; Mosquera-Heredia et al. 2021).

A major challenge in the field is the specific isolation of exosomes from different populations of cells (e.g., neuron-derived, glial-derived, etc.) in a complex body fluid such as blood. Indeed, serum/plasma proteins, liposomes and components of the extracellular compartments can contaminate the sample and significantly affect biomarker measurements. Enrichment of exosomal preparations into subsets from cells of different origins can be done via immunoprecipitation. For example, neuron-derived exosomes (NDEs) can be enriched from blood by immunocapture using the neuron-specific protein L1CAM (Mustapic et al. 2017; Sun et al. 2017) while astrocyte-derived exosomes (ADEs) can be enriched using astrocyte-specific proteins such as GLAST1 (Goetzl et al. 2020). This relatively specific, noninvasive separation of circulating EV subsets is considered a "liquid biopsy" of distinct cellular populations in the brain and can help with the diagnosis and prognosis of neurological conditions such as AD (Kapogiannis et al. 2019).

Exosomal Biomarkers in mTBI

As mentioned above, blood biomarkers are promising aids in identifying the acute phase of mTBI but their reliability waivers when attempting to quantify prolonged and chronic symptoms or recovery time (Agoston et al. 2017). On the other hand,

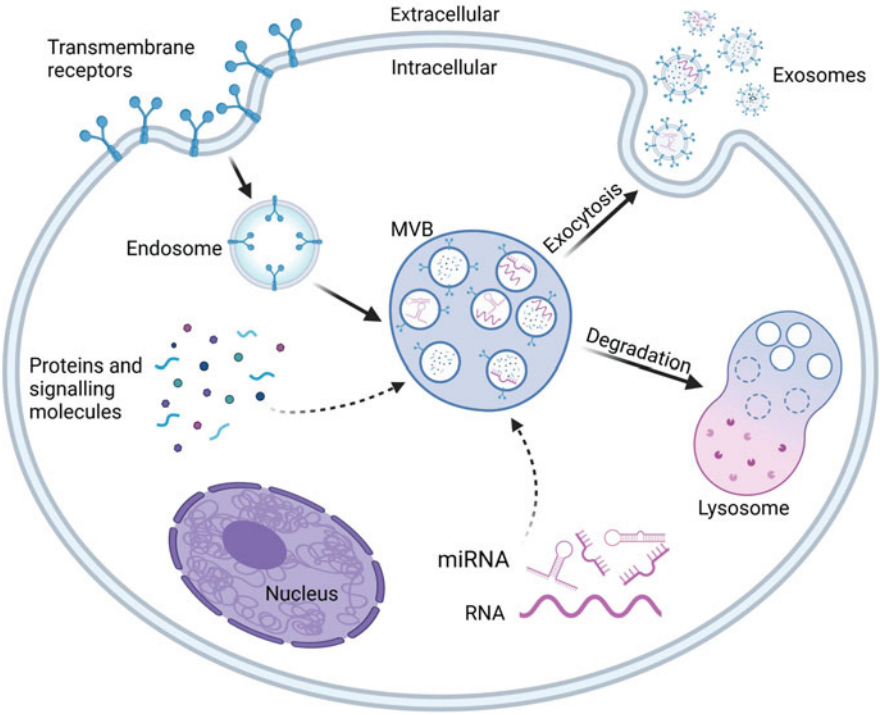

Fig. 3 Exosome Biosynthesis. The plasma membrane invaginates and buds, creating an endosome. The inward budding of the endosomal membrane creates a multivesicular body (MVB) containing small intraluminal vesicles. These vesicles are marked either for degradation through the lysosomal pathway or for exocytosis where proteins, signaling molecules, and nucleic acids are then packaged for release into the extracellular space as exosomes. Created with BioRender.com. Adapted from Schorey et al. (2015)

exosomes are emerging as a promising complement to blood-based biomarkers. Cells secrete exosomes under both normal and pathological conditions where they engulf a variety of surface and cargo proteins (and microRNA), effectively protecting them from degradation in the blood or filtration in other organs (Kalani et al. 2014). This exosomal cargo can provide specific information on what is happening in the brain acutely after injury as well as months later. Moreover, their specific surface proteins allow brain-derived exosomes to be enriched from the blood, making them excellent candidates for continued monitoring of mTBI progression and bypassing the limitation of other fluid and tissue biopsies (Cheng et al. 2019). This combination of information makes them attractive targets for accurate and objective diagnostic, prognostic, and predictive outcomes in single and repetitive mTBIs.

Overall, there is evidence to suggest that exosomes may be useful in understanding and predicting the recovery and long-term consequences of concussion. In veterans with a history of mTBI, total exosomal and plasma NF-light levels were

found to be highest in those experiencing chronic PCS and PTSD (Guedes et al. 2020). Interestingly, in former NFL players, Tau levels in total exosomes isolated from plasma were 80% accurate in predicting patients suffering with CTE compared to healthy controls. The elevated Tau levels were also associated with lower memory test scores but not changes in mood or behavior (Stern et al. 2016). In military personnel who suffered mTBI from blast injuries, neuron-derived exosomes were found to have elevated Tau and IL-10 levels, which also correlated to the incidence of PCS and PTSD (Gill et al. 2018). This suggests that injury mechanisms may uniquely contribute to the heterogeneity of symptoms in mTBI and that exosomal markers may be better able to predict conversion to chronic symptoms and better reflect post-concussive changes in the brain than blood biomarkers. Recent studies by our group demonstrated increases of annexin VII, UCH-L1, claudin-5, aquaporin 4, and synaptogyrin-3 in neuron-derived exosomes, acutely after an mTBI. Interestingly, in athletes that sustained a concussion 12 months prior to blood sampling, proteins commonly associated with AD and neurodegeneration (Aβ42, p-tau T181, p-tau S396, IL-6) were elevated in NDEs, suggesting sustained neuronal damage and inflammation with a shift in the pathological processes behind the neuronal damage sometime after injury (Goetzl et al. 2019).

While proteins provide front-line insights into current injury mechanisms, other cargo, particularly microRNA (miRNA), can provide insights into genetic modifications that may modulate key injury or recovery mechanisms. MicroRNAs are small non-coding, single-stranded RNA molecules comprising of around 22 nucleotides (Zhang et al. 2015) that can impact the post-transcriptional regulation of genes (Kalani et al. 2014). Although this field is still in its infancy, altered miRNA levels have been observed in blood and saliva following a TBI with several identified as potential biomarkers (Atif and Hicks 2019). Brain-derived exosomal miRNAs have been able to accurately identify TBI patients from healthy patients with the aid of new diagnostic and machine-learning technologies (Ko et al. 2018). Distinct signatures in brain-derived exosomal miRNA cargo were able to accurately classify injury severity, whether there was a history of injury, and the timing of those injuries (Ko et al. 2018). Despite promising evidence, research is still in its infancy and longitudinal studies as well as method standardization for exosomal analysis are needed to examine the changes in exosomal cargo and how they relate to symptom presentation, recovery timelines, and long-term effects after mTBI.

Conclusion and Future Directions

A single or repeated mTBIs can cause short-term symptoms (collectively called PCS) and may also increase the risk for long-term neurodegenerative conditions including CTE, AD, or other neurodegenerative or neuropsychiatric conditions. The shift in our understanding of mTBI from an “inconsequential” and mild injury to a

profoundly more impactful event for brain health is largely due to recent findings related to the potential long-term consequences of mTBI. While diagnosis of mTBIs relies still mostly on self-reported symptoms, blood-based biomarkers represent a safe, noninvasive, and relatively inexpensive method to monitor mTBI and associated symptoms. Although more research is needed to determine the most sensitive and specific biomarkers for long-term brain health, it seems appropriate to include a panel of both neuroimaging and blood biomarkers in mTBI protocols (Graham and Sharp 2019; McCrea et al. 2020). Further, while the study of EVs is still in its infancy, recent work highlights the potential of EVs isolated from peripheral blood as promising mTBI biomarkers.

Several research groups have recently shown that exosomes may also serve as a targeted delivery mechanism for treatment of TBI as well as other diseases. Exosomes and nanoparticle drug delivery systems may also provide a more targeted approach enabling more successful interventions and prevention of long-term brain damage following one or multiple TBIs (Kaijzel et al. 2017). Although cancer research has been a driving force behind exosome based-therapies, the results produced can have broader applications for the development of new therapies in other fields including TBI.

Applications to Prognosis, Other Diseases or Conditions

Evidence suggests that a single mTBI can induce a metabolic cascade, leading to short- and long-lasting effects that can include PCS and neurodegenerative diseases. In this book chapter, we reviewed the current knowledge about blood-based biomarkers for mTBI. A study in military veterans reported an elevation in exosomal and serum levels of NF-light associated with repetitive mTBIs and with PCS. Interestingly, this study also showed that exosomal NF-light levels continued to increase with number of years since the first mTBI (Guedes et al. 2020) whereas serum NF-light levels in boxers and hockey players returned to normal within 3 months of rest (Shahim et al. 2017). This suggests that exosomal NF-light levels may present a more accurate picture of the processes still occurring in the brain long-term but also presents NF-light as a possible way to distinguish between patients with rapidly resolving symptoms from those with prolonged PCS (Shahim et al. 2017). Moreover, while evidence is still sparse, NF-light has been shown in neurodegenerative diseases to correlate to cognitive function (He et al. 2021). Few studies have focused on biomarkers for mTBIs either in the pediatric population or in people that are 65+. It will be interesting in the continuation to examine whether younger children who experience a TBI are more likely to bounce back faster, and with more rapid return to "normal" biomarker levels, and whether older adults exhibit increased PCS symptoms and biomarkers as they age to a greater extent. In a longitudinal cohort of cognitively normal individuals, analysis of NDEs predicted AD diagnosis (Kapogiannis et al. 2019). These results are excellent motivation for the mTBI field to continue developing our understanding of acute and chronic biomarkers to better

prevent, treat, and manage sports-related injury as well as the long-term health risks our young and professional athletes are taking.

Mini-Dictionary of Terms

- CTE is a neurodegenerative disease that occurs as a result of repetitive head impacts. CTE has become a public health concern largely affecting athletes and military veterans. Symptoms can include violent and explosive behavior, emotional disturbances, and cognitive changes. The pattern of neurodegeneration is unique, distinguishing it from other neurodegenerative conditions but can only be diagnosed by *post mortem* examination.
- PCS is a term used to describe the persistence of the cognitive, emotional, and physical symptoms of an mTBI for longer than 3 months. In most cases, prolonged PCS will resolve within a year but in rare instances, PCS can persist for years.
- Tau is a microtubule-associated protein that plays an important role in axonal stabilization and neuronal development. Under pathological conditions, Tau becomes hyperphosphorylated and forms aggregates that evolve in neurofibrillary tangles (NFTs), causing the death of the affected neurons.
- Amyloid-beta (Aβ) is a 38–43 amino acid peptide produced by the cleavage of the amyloid precursor protein (APP) by β- and γ-secretase enzymes. It is a hallmark of Alzheimer's disease.
- Mild TBI is a type of mild closed-head injury caused by an impact to the head or by a sudden hit to the body that causes the head and brain to jerk back and forth. Also referred to as a concussion.
- Exosomes are nanosized vesicles released from the cell into the extracellular environment. They carry DNA, RNA, and other signaling molecules in order to communicate with surrounding cells.
- Neurodegeneration is the progressive loss of neuronal cells. It can occur in any brain region and is the hallmark of diseases such as Alzheimer's and Parkinson's diseases.

Key Facts of mTBI

- Mild TBI is the most common form of TBI, representing more than 70% of all diagnosed TBIs.
- mTBI is caused by a hit to the body that causes the head and brain to move quickly back and forth.
- Symptoms of mTBI are heterogeneous and include cognitive, physical, and emotional disturbances that can last for several years.
- High contact sport athletes and military personnel are at higher risk of sustaining an mTBI.

- Long-term consequences of mTBI may include neurodegenerative disorders later in life.

Summary Points

- An mTBI is caused by a mild non-penetrating insult to the head, leading to physical, cognitive, and emotional disturbances that can resolve quickly or last for months/years.
- Mild TBI cases account for more than 70% of all treated brain injuries in North America but underreporting is a significant issue that can increase the risk for repetitive mTBIs and prevent full recovery.
- An mTBI acutely causes significant microstructural injuries, causing shearing and tearing of the brain cells and possibly transient damage to the blood–brain barrier. This leads to a cascade of metabolic, neurochemical, and inflammatory events in an attempt to restore homeostasis.
- Epidemiological studies suggest that repetitive mTBI can increase the risk for Alzheimer's disease and other dementias decades after the injuries were sustained.
- Objective diagnostic markers are needed to better monitor injury and recovery; fluid biomarkers may present a solution to better determine the severity of injury, when it is safe for athletes to return to play, and the long-term prognosis of brain health after mTBI.
- Blood and other fluid biomarkers are useful in the acute phase after injury but exosomal biomarkers may provide more accurate information and present a better avenue to monitor injury progression and recovery long-term.

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Biomarkers in Hypoxic Brain Injury: Methods, Discoveries, and Applications

22

Shreyas Gutte, Afzal Azim, and Rupali Patnaik

Contents

Abstract

The brain uses a significant amount of energy compared to its weight and size. It is highly metabolically active and exquisitely sensitive to hypoxia and hypoperfusion. Cellular injury can begin within minutes, and permanent brain injury will follow if prompt intervention does not occur. For that reason, it is critical to understand the clinical presentation, pathophysiology, and management options. Hypoxic brain injury can result from interruption of blood flow to the brain, such

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as cardiac arrest or strangulation, or from systemic derangements that affect the oxygen content of the blood. Following hypoxic brain injury, biomarkers are released in blood and cerebrospinal fluid (like NSE, S100β, miRNA, etc.), which can be used for prognostication and management purpose. This chapter goes on to discuss the methodology, discoveries, and application of biomarkers in hypoxic brain injury.

Keywords

Hypoxic brain injury · Biomarker · NSE · S100β · miRNA · Glutamate · MMP · Apoptosis · Necrosis · Excitotoxicity · Mitochondrial dysfunction · COVID-19

Abbreviations

CNS	Central nervous system
CT	Computed tomography
dl	Deciliter
DTNB	5,5′-Dithiobis-(2-nitrobenzoic acid)
ELISA	Enzyme-linked immunosorbent assay
Fmol	Femtomole
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
Glu	Glutamate
GluR	Glutamate receptor
gm	Gram
GSH	Glutathione
GST	Glutathione-S-transferase
HRP	Horseradish peroxidase
K2-EDTA	Dipotassium salt of ethylenediaminetetraacetic acid
l	Liter
LPO	Lipid peroxidation
M	Molar
min	Minutes
ml	Milliliter
mM	Millimolar
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MW	Molecular weight
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NMDA	N-Methyl-D-aspartic acid
NR2B	NMDA receptor subunit
OD	Optical density
OS	Oxidative stress
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
ROS	Reactive oxygen species

Introduction

The brain is one of the most important biologic organ in the human body. However, as with all living things, we are not invincible, and we remain susceptible to a host of medical disorders, some of which are related to the malfunction of our brains. Over the last decade, an increasing interest in the use of biomarkers that are disease-specific to improve the diagnostic, prognostic, and therapeutic approach of different pathologies has been observed. Biomarkers have to be measurable in body fluids and able to address unmet clinical needs for the detection of a tissue injury. In particular, biochemical markers of brain damage, both in adult and in pediatric population, have been object of growing interest. In reality, however, many neurodegenerative diseases show similar symptoms and features, and the task of diagnosis is often challenging. Therefore, much research has been undertaken to both the clinical and the molecular mechanisms that cause these illnesses in order to identify characteristics. A biomarker is a measurable attribute associated with the clinical status of a patient. The National Institutes of Health (NIH) defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or biologic responses to a therapeutic intervention" (Strimbu and Tavel 2010). The emphasis on biomarker utility is dependent on the particular brain disorder, and this will be discussed in detail in later sections of this chapter. However, it is unlikely that a single biomarker will have value in diagnostic and/or measuring response to treatment. Hence, it is expected that a panel of several biomarkers will be required to serve these different tasks.

Discovery

Over the period of the last two decades, adult critical care medicine has evolved in management of hypoxic brain injury, and the focus of management has been changed from prevention of mortality to prevention of morbidity along with mortality. In the acute period after the presentation to the hospital, laboratory and radiological evaluations of a patient with hypoxic brain injury are dictated by the underlying cause of the injury. Initial studies should include basic blood work, including blood glucose, electrolyte panel, a complete blood count, a blood urea nitrogen, serum creatinine, and liver function studies. An arterial blood gas is often indicated to evaluate the acid-base status and rule out hypercarbia. Clinical management is focused on supportive care, treatment of the underlying cause of the hypoxia, and prevention of ongoing brain injury. A variety of neuroprotective strategies have been evaluated in an effort to prevent cell death by interrupting or attenuating the cascade of events by which hypoxia precipitates neuronal apoptosis and necrosis. Hence different biomarkers came into light to recognize severity, prognostication, and management purpose.

Pathophysiology

Anoxic and hypoxic brain injury can occur whenever oxygen delivery to the brain is compromised. Oxygen delivery is a function of the blood flow to the brain and the oxygen content of the blood (Arora and Tantia 2019). In the United States, cardiac arrest is the most common cause of hypoxic brain injury. Other causes include traumatic vascular injuries; near-drowning; smoke inhalation or carbon monoxide poisoning; shock, including hemorrhagic and septic shock; drug overdoses; and acute lung injury. The brain depends on a constant energy supply provided by glucose and oxygen but is unable to store energy. With the cessation of blood flow, intracellular production of adenosine triphosphate is diminished. This results in dysfunction of energy-dependent ion channels, which contributes to intracellular sodium accumulation and cytotoxic edema. Ongoing ischemia results in the release of glutamate, an excitatory neurotransmitter, which promotes calcium influx through N-methyl-D-aspartate (NMDA) receptors. Calcium influx exacerbates neuronal injury by activating lytic enzymes, precipitating free radical formation, and interfering with mitochondrial function. This process, known as excitotoxicity, can ultimately lead to cell death (Geocadin et al. 2008; Sekhon et al. 2017). After hypoxic brain injury, four major processes will release biomarkers which are as follows:

1. Apoptosis
2. Necrosis
3. Excitotoxicity
4. Mitochondrial dysfunction

Methodology

In this section, we will discuss the major pathways by which biomarkers are released and the detailed procedure of biomarker assay (Supplementary Table 1).

1. **Apoptotic Pathway**

Programmed cell death plays an important role in early brain development and neurodegenerative diseases (Gorman 2008). Studies have shown that a large number of cells die by the apoptotic pathway after hypoxia-ischemia and that there is a relationship between neuronal loss and decrease in high-energy phosphate in brain tissue (Thornton et al. 2017). Cerebral hypoxia results in increased activity of poly (ADP-ribose) polymerase (PARP) (a nuclear enzyme that participates in DNA repair) in neuronal nuclei of newborn piglets, indicating activation of the DNA repair pathway (Mishra et al. 2003). Free radical (FR)-mediated DNA damage during hypoxia may lead to increased PARP activity. It has also been shown that NO or peroxynitrite can trigger oxidative damage to DNA and activate PARP (Islam et al. 2015). However, FRs and peroxynitrite may also chemically modify the enzyme by nitration or nitrosylation of PARP protein, which may result in increased

PARP activity. Ca2+ is an important inducing agent in the mitochondria-dependent apoptotic pathway (Figs. 1 and 2). Increased free cytosolic Ca2+ may lead to uncoupling of mitochondrial oxidative phosphorylation, inducing the mitochondrial MPT state and hence the release of cytochrome C (Guo et al. 2013). Once released from mitochondria, cytochrome C specifically activates caspase 3, which triggers a biochemical cascade involving activation of many other caspases and other substrates, including PARP and DNA-dependent kinase (Elmore 2007). Severe cerebral ischemia also results in a major increase in intracellular Ca2+, which is closely related to NO; both play a fundamental role in signal transduction – controlling cell processes such as proliferation. NO has several important physiologic functions in the central nervous system (CNS). These include control of central and peripheral functions, modulation of synaptic plasticity, perception of pain and neuronal damage, and protection. High NO levels may be neurotoxic and induce apoptosis or necrosis. Intracellular Ca2+ concentration in the CNS regulates the expression of genes responsible for the activation of extracellular-regulated MAP kinases, ERK1 and ERK2, mediated by the Ras/Raf/ERK cascade, which is the major signal transduction pathway regulating the cell cycle. Changes in intracellular Ca2+ or NO may alternatively lead to blockade or activation of the cell cycle, and the decision whether the cell lives or dies is presumably a well-regulated phenomenon in which the duration and intensity of the Ca2+ and NO signals may play a fundamental role. Identification of enzymes and substrates of these phosphorylation pathways would shed light on hypoxia-induced processes leading to cell recovery or death. This observation may clarify some aspects of the activation of apoptosis

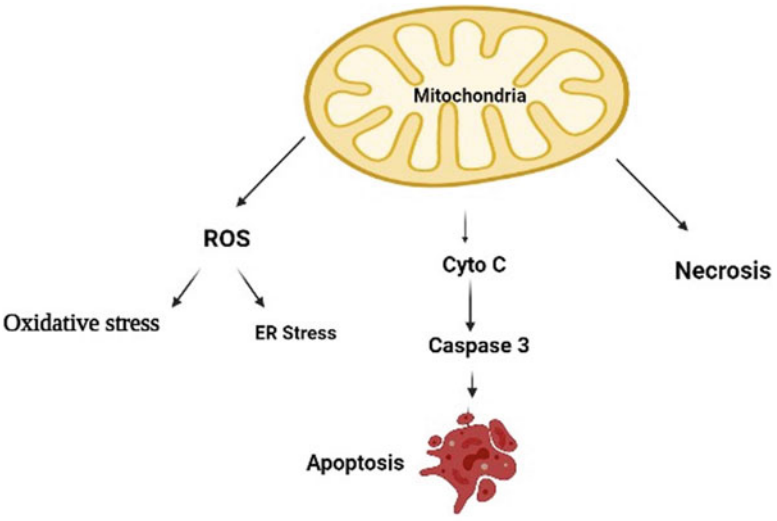

Fig. 1 A schematic demonstrating the various cellular pathophysiologic consequences which occur during the hypoxic brain injury (HBI) (ROS reactive oxygen species, ER stress endoplasmic reticulum stress)

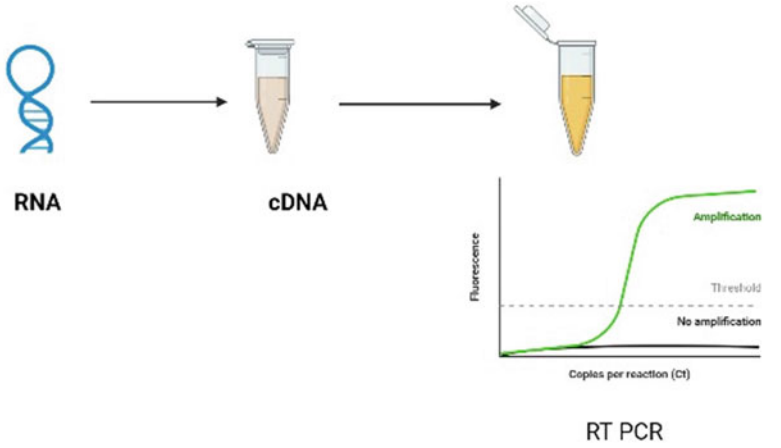

Fig. 2 Formation of cDNA from RNA and expression of different gene by RT-PCR (RNA ribonucleic acid, cDNA complementary deoxyribonucleic acid, RT-PCR reverse transcription-polymerase chain reaction)

because VDAC proteins play an essential role in the increase of mitochondrial membrane permeability. It is generally accepted that the increase in outer mitochondrial membrane permeability is a central event in apoptotic cell death and is regulated by the Bcl-2 family. Anti-apoptotic Bcl-2 family members close VDAC proteins, whereas certain pro-apoptotic members interact with VDAC proteins to generate a protein-conducting channel through which cytochrome C may pass. Although VDAC proteins have an important role in regulating mitochondrial membrane permeability, this permeability may also be modified by other mechanisms, such as mitochondrial swelling followed by membrane rupture. Whether protein modification depends on the degree of FR release remains to be clarified. However, an increasing amount of data demonstrates abundant FR release during hypoxia. Recently, elevated FR products have been reported in the spinal fluid of infants with cerebral white matter injury. An abundance of carbonyl groups, which are well-known expressions of oxidative stress in the protein molecule, was found. These are helpful for the detection of initial stimuli and possible protein damage responsible for the initiation and completion of the various stages of apoptosis.

A. **Active caspase-3 assay**

Recent study showed that hypoxic brain injury can cause significant increase of caspase-3 expression and increase of neuronal apoptosis in the brain of neonatal mice (Deng et al. 2019a; b). The amount of active caspase-3 in serum and CSF was measured using a caspase assay kit according to the manufacturer's protocol (Elabscience Biotechnology, Beijing, China). The absorbance at 450 nm was measured using a microplate reader.

B. **Cytokine mRNA expression**

Recent study in rats showed that TNF-α and IL-6 promote neuronal apoptosis after hypoxic brain injury, while IL-10 antagonizes it, so measurement of these cytokines can be used as biomarkers for hypoxic brain injury within 24 to 72 hours after initial insult (Li et al. 2014). Real-time PCR (RT-PCR) was used for mRNA expression of cytokines. Expression of all genes was normalized to the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a housekeeping gene. The Cp value of gene is used for calculation of fold expression of gene. The 10 μl of SYBR Green (TaKaRa), 1 micromolar forward and 1 micromolar reverse primer, 1 μl cDNA, and 7 μl of water were used for amplification of 20 μl one-well reaction. RT-PCR was performed using a Roche system (LightCycler 480). The primers for TNF-α, IL-6, and IL-10 are presented in Supplementary Tables 2 and 3.

C. **XIAP assay**

Recent study in rats showed that overexpression of XIAP protein after hypoxia is an apoptosis resistance marker and showed improved neuronal survival (Deng et al. 2019a, b).

The amount of XIAP protein in the serum and CSF was measured by using a human-specific enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's protocol (FineTest kit, Wuhan Fine Biotech). The absorbance at 450 nm was measured using a microplate reader.

D. **MicroRNAs (miRNAs)**

MicroRNAs (miRNAs) are novel biomarkers in hypoxic brain injury, and recent literature showed that miRNA 210 and 155 are biomarkers released after hypoxic brain injury (Bruning et al. 2011). MicroRNAs (miRNAs) are a type of highly conserved non-coding RNAs (ncRNAs), which consist of 21–25 nucleotides in length and usually lead to suppression of target gene expression. MicroRNAs 210, 155, and 592 are useful in hypoxic brain injury. These markers were detected by RT-PCR.

2. **Necrosis**

It is the name given to accidental death of cells and living tissue. Necrosis is less orderly than apoptosis, which is part of programmed cell death. In contrast with apoptosis, cleanup of cell debris by phagocytes of the immune system is generally more difficult, as the disorderly death generally does not send cell signals which tell nearby phagocytes to engulf the dying cell. This lack of signaling makes it harder for the immune system to locate and recycle dead cells which have died through necrosis than if the cell had undergone apoptosis. The release of intracellular content after cellular membrane damage is the cause of inflammation in necrosis.

Morphological Markers

I. Always pathological
II. Involves sheets of cells
III. Energy independent
IV. Cell swelling and mitochondrial damage leading to rapid depletion of energy levels
V. A breakdown of homeostatic control
VI. Cell membrane integrity lost
VII. Nuclei lost, no DNA cleavage
VIII. Cell release of the intracellular contents, leading to an inflammatory response
IX. Dead cells ingested by neutrophils and macrophages

A. **C-Reactive protein**

C-reactive protein (CRP) is an annular (ring-shaped) pentameric protein found in blood plasma whose circulating concentrations rise in response to inflammation, an important prognostic marker of necrosis. A rise in CRP levels suggests poor neurological outcome in the absence of infective etiology.

B. **Beta-Glucuronidase, ALT, FK-18, miR-122, and HMGB1**

They are also markers of necrosis. These are not widely studied in hypoxic brain injury but can be potential biomarkers, and further research is needed.

3. **Excitotoxicity**

The brain depends on a constant energy supply provided by glucose and oxygen but is unable to store energy. With the cessation of blood flow, intracellular production of adenosine triphosphate is diminished. This results in dysfunction of energy-dependent ion channels, which contributes to intracellular sodium accumulation and cytotoxic edema. Ongoing ischemia results in the release of glutamate, an excitatory neurotransmitter, which promotes calcium influx through N-methyl-D-aspartate (NMDA) receptors. Calcium influx exacerbates neuronal injury by activating lytic enzymes, precipitating free radical formation, and interfering with mitochondrial function. This process is known as excitotoxicity.

A. **Glutamate**

Glutamate is an important excitotoxin having important role in brain function in health and disease. Excess of glutamate has been associated with poor outcome.

Among the N-methyl-D-aspartate (NMDA) receptors, NR1, NR2A, and NR2B are the main component of heteromeric NMDA receptor which are mainly distributed in frontal and hippocampal cortex.

Plasma Glutamate and Its Receptor Assay
Plasma glutamate level will be measured using the Amplex Red Glutamic Acid/ Glutamate Oxidase Assay Kit (cat no. A12221, Invitrogen, USA). For glutamate measurement, L-glutamic acid is oxidized by glutamate oxidase to produce α-ketoglutarate, NH3 and H2O2. L-Alanine and L-glutamate-pyruvate transaminase will be included in the reaction to regenerate L-glutamic acid by transamination of α-ketoglutarate, resulting in multiple cycles of the initial reaction and a significant amplification of the H2O2 produced. The hydrogen peroxide reacts with 10-acetyl-3,7-dihydroxyphenoxazine (Amplex® Red Reagent) in a 1:1 stoichiometry in the reaction catalyzed by horseradish peroxidase (HRP) to generate the highly fluorescent product, resorufin, and was determined by a fluorescence microplate reader (BioTek Synergy HT, software Gen5) using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ± 17.5 nm.

B. **RT-PCR study for glutamate receptors**

RNA will be isolated from the blood samples by kit method (Nucleo-Pore GRNA blood kit), and the concentration and integrity of the RNA will be determined by measuring the absorbance at 260 and 280 nm by spectrophotometer. Total RNA will be reversely transcribed to cDNA using a high-capacity cDNA reverse transcription kit (Thermo Scientific RevertAid First Strand cDNA Synthesis Kit). Equal amount of mRNA will be taken from all samples to convert cDNA and amplified by RT-PCR. Receptor expression by reverse transcription-polymerase chain reaction (RT-PCR) and Amplified cDNA will be analyzed in real-time machine after optimizing the primer conditions, and the PCR cycle will be carried out by the following steps:

1. Denaturation 94 °C for 10 min
2. Amplification for 45 cycles (95 °C for 10 s, 56 °C for 10 s, and 72 °C for 20 s)

Expression of all genes will be normalized to the expression of GAPDH (housekeeping gene). The Cp value of gene will be used for calculation of fold expression of gene. The 10 μl of SYBR Green (Applied biosystem), 0.5 micromolar forward and reverse primer, 5 μl cDNA, and 4 μl of water are used for amplification of 20 μl one-well reaction. RT-PCR was performed on Roche system (LightCycler 480).

4. **Mitochondrial Dysfunction**

Mitochondrial dysfunction will be discussed under two subheadings as oxidative stress markers and endoplasmic reticulum dysfunction markers.

A. **Oxidative stress**

Reactive species are chemical species with one or more unpaired electrons in their outer orbit. They readily accept electrons from iron and other metals to form more reactive radicals, which attack other biomolecules, especially lipids, proteins, and nucleic acids, generating more radicals that damage the developing brain. The relationships between FR generation and brain damage in the perinatal period are complex. During hypoxia, there are a number of potential mechanisms of FR generation:

1. Accumulation of intracellular Ca2+ (Liao et al. 2011) and subsequent activation of phospholipase A2 leading to increased generation of oxygen FRs from cyclo-oxygenase and lipoxygenase pathways, nitric oxide (NO) synthase leading to peroxynitrite formation and generation of FRs, proteases leading to conversion of xanthine oxidase resulting in increased FR generation, and phospholipase C leading to IP3 formation resulting in release of Ca2+ from intracellular stores
2. Reduction of electron transport chain components including ubiquinone (a component that undergoes auto-oxidation to produce free radicals) (Zorov et al. 2014)
3. Release of iron from ferritin under the condition of depleted cellular high-energy compounds
4. Increased degradation of ATP during hypoxia and increased substrate for the xanthine oxidase reaction

Measurement of Antioxidants and Oxidative Stress Markers
Much literature is not available on lipid peroxidation, glutathione level, and catalase after hypoxic brain injury; current studies are oriented to its application.

(a) **Lipid peroxidation:** Malondialdehyde level is the end product of lipid peroxidation. For measuring MDA, plasma was mixed with EDTA, ascorbate (10 mM), and FeSO4 (16.7 mM) and incubated at 37 °C for 60 min. The reaction was stopped by adding ice-cold 10% trichloroacetic acid (TCA). The mixture was centrifuged at 2000 × g for 10 min. The supernatant was aspirated and mixed with equal volume of 0.67% thiobarbituric acid (TBA), which was kept in a boiling water bath for 15 to 20 min. Malondialdehyde level was determined with the absorption coefficient of MDA-TBA complex at 532 nm using spectrophotometer and can be used for hypoxic brain injury biomarker.

(b) **Glutathione:** Plasma GSH was measured by a spectrophotometer at 412 nm (30). Plasma was added to 10% trichloroacetic acid (TCA) and allowed to stand at 4 °C for 2 h, after which the mixture was centrifuged at 2000 × g for 15 min. The supernatant was added to Tris-HCl buffer (0.4 M, pH 8.9) containing EDTA (0.02 M). 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) (0.01 M) was added to the mixture, which was reduced into the yellow colored product, 5-thio-2-

nitrobenzoic acid (TNB), and was measured using spectrophotometer at 412 nm. A standard curve of reduced GSH was plotted to measure the amount of GSH in the plasma.

(c) **Catalase:** Plasma catalase activity was determined by the spectrophotometer. The enzyme reaction was initiated by the addition of 10 μl of plasma sample in 2.99 ml of 30 mM H_2O_2 in a cuvette. The decreased absorbance was measured for 3 minutes at 240 nm, and the activity was expressed in U/ml.

B. **Endoplasmic reticulum stress (ER stress)**

The ER stress/unfolded protein response (UPR) genes were consist of molecular chaperone BiP/Grp78, indicator of the onset of the UPR, as well as key regulators of the UPR pathway including transcription factors ATF4 and XPB1. Expression of the housekeeping gene GAPDH was served as an internal positive control in each assay performed. The primer sequences for genes are given in Table 4.

Other Biomarkers in Hypoxic Brain Injury

A. **Matrix Metalloproteinase Expression**

All samples were analyzed using the Milliplex Multi-Analyte Profiling Human MMP-9 analyte premixed kit (Millipore, St Charles, MI), according to the manufacturer's instructions. All samples were assayed in duplicate wells (25 l per well), and the mean of these results was used. Plates were read using a Luminex 200 analyzer (Luminex Corporation, Austin, TX) running STarStation software (Applied Cytometry Systems, Sheffield, UK). Protein concentrations were calculated by reference to an eight-point spline fit curve for each MMP.

B. **Neuron-Specific Enolase (NSE)**

It is an enzyme expressed mainly in neurons and neuroectodermal cells, which anaerobically converts glucose to metabolites suitable for oxidation (Bottoni and Scatena 2015; Marangos and Schmechel 1987). The serum NSE level is normally low in healthy people but increases significantly in cases of neuronal tissue damage, such as traumatic brain injury and stroke, and so is used as a biomarker for brain damage. The serum NSE level also increases in cases of hypoxic brain damage; the serum concentration is proportional to the extent of brain damage. As near-hanging injury causes anoxic brain injury, serum NSE level is expected to predict the neurological outcome of near-hanging patients. NSE levels > 33 ng/mL determined within 48 h in patients not treated with hypothermia were identified as a reliable marker for poor outcome in the large Prognosis in Postanoxic Coma (PROPAC) study (Zandbergen et al. 2006).

Method

NSE is measured by a non-radioactive automated immunoassay (Kryptor, Brahms France, Saint Ouen, France) using TRACE (time-resolved amplified cryptate emission) technology (Zuber et al. 1997).

1. **S-100β**

S-100β concentrations were determined with an immunofluorometric sandwich assay as described earlier. In brief, all measurements were set up in duplicate. Microtiter plates coated with 10 mL of anti-S-100β-chain (Sigma Chemical) in 20 mL of phosphate buffer (0.05 mol/L, pH 8.6) were incubated with 200 mL per well of S-100 calibrators, controls, or samples for 120 min. Biotin-labeled rabbit anti-S-100 antibody (DAKO) in a Tris (0.05 mol/L), NaCl (0.15 mol/L), CaCl2 (10 mmol/L), and NaN3 (0.15 mmol/L) buffer was added, and the plates were incubated for another hour. After the plates had been washed, 200 mL of streptavidin-europium in assay buffer (0.05 mol/L Tris; 0.15 mol/L NaCl; 1 g/L bovine serum albumin; 0.5 g/L bovine g-globulin, both from Sigma; and 0.15 mmol/L NaN3) was added to each well, and the plates were incubated for 30 min. The resulting fluorescence was measured with a DELFIA 1234 fluorometer (Wallac).

2. **Nogo-A**

Hypoxic ischemic brain damage can downregulate the expression of Nogo-A receptor in the central nervous system. NEP1–40 contributes to the regeneration of axon and repair of brain damage, thus exerting a neuroprotective effect. This marker is detected by RT-PCR.

Application to Other Diseases

1. **MicroRNA**

It is now well documented that upregulation or downregulation of miRNAs occurs in various human cancers. Overexpressed miRNAs may function as both oncogenes (through downregulation of tumor suppressor genes) and regulator of cellular processes such as cell differentiation or apoptosis (Zhang et al. 2007).

A. **MicroRNA in inflammatory disease**

Inflammation is an essential component of host defense system and a major response to infection and injury, which is believed to contribute to multiple acute and chronic diseases (Ross et al. 2008).

B. **MicroRNA in neurodevelopment disease**

MicroRNAs are highly expressed in human and other mammalian brains relative to other organs (Babak et al. 2004). The expression of miRNAs in the brain changes during brain development. Therefore, some miRNAs are expressed more abundantly during early development in the mammalian brain, and some are expressed less during later development (Miska et al. 2004); Smirnovaet al. (Smirnova et al. 2005) have shown that some miRNAs are differentially expressed in neuronal nuclei and/or different cell populations in the brain.

C. **MicroRNA in Down syndrome**

Down syndrome (DS) results from triplication of all or part of human chromosome 21 and affects 1 in 700 newborns and is manifested with variable phenotypes such as congenital heart defects, craniofacial abnormalities, and cognitive impairment. Recently, bioinformatic analyses of chromosome 21 have revealed that two miRNAs (miR-125b-2 and miR-155) are encoded on this chromosome (Epstein et al. 2001).

D. **MicroRNA in Huntington's disease**

Huntington's disease (HD) is a neurodegenerative disease resulted from CAG expansion in the gene encoding the protein huntingtin (Htt). The manifestations of HD include cognitive defects and motor control impairment which lead to neuronal dysfunction characterized by progressive loss of cortical and striatal neurons. How this process is regulated is not precisely known; however, many potential miRNA targets have been predicted in the brains of HD sufferers. MicroRNAs displayed significant reduction in expression (Johnson et al. 2008).

2. **C-reactive protein**

C-reactive protein predicts myocardial necrosis after successful coronary stenting in patients with stable angina (Goldberg et al. 2006). It is an important prognostic marker of pancreatic necrosis with the highest sensitivity and negative prognostic value given the cutoff is 110 mg/l. The patients with C-reactive protein below 110 mg/l are low risk to develop pancreatic necrosis (Barauskas et al. 2004).

3. **Beta-glucuronidase**

It is a lysosomal hydrolase, which is a possible serological marker for histological hepatic cell necrosis and to predict the histological progression of hepatitis (Ohta 1991).

4. **Application of ER stress marker**

A. **Alzheimer's disease** – Prolonged ER stress response and elevated level of CHOP can trigger pathologic cell death. Recent reports have indicated that

the UPR is activated in the AD brain. Increased expression of the ER chaperone Grp78, which is indicative of UPR activation, is found in AD cases compared with controls (Hoozemans et al. 2005).

B. **Amyotrophic lateral sclerosis** – Amyotrophic lateral sclerosis (ALS), also known as Charcot's or Lou Gehrig's disease, is characterized by muscle weakness, atrophy, and paralysis. The pathologic feature of ALS is the selective degeneration of brain and spinal cord motoneurons. Markers of ER stress are upregulated in the spinal cords of patients with ALS. Moreover, mutations in the superoxide dismutase 1 (SOD1) gene have been linked to the familial form of the disease, and mouse models with this mutation show improper folding of SOD1 and SOD1 aggregate-induced UPR activation (Saxena et al. 2009).

C. **Nonalcoholic fatty liver disease** – The liver is one of the major secretory organs and has essential roles in carbohydrate and lipid metabolism. Hepatic lipogenesis is activated upon uptake of excess carbohydrates and is controlled at the transcriptional level by sterol regulatory element-binding protein-1c (SREBP-1c). Nonalcoholic fatty liver disease (NAFLD) is a progressive disorder characterized by aberrant lipid storage in hepatocytes. Studies in humans have pointed out the role of de novo lipogenesis in the excessive accumulation of lipids in the livers of patients with NAFLD. SREBP-1c is an ER-localized transcription factor, which, like ATF6, requires processing in the Golgi organelle to become active when ER stress is relieved through Grp78 overexpression, SREBP-1c induction is suppressed, and hepatic steatosis is decreased. Additionally, the link between ER stress and lipogenesis has been shown in genetic ablation studies in which genes involved in the UPR were silenced. In one scenario, mice deficient in XBP1 specifically in the liver exhibited downregulation in key lipogenic enzymes (Lee et al. 2008).

5. **NSE in other disease**

A. **Cerebral infarction and intracerebral hemorrhage** NSE concentrations in plasma preceded as a diagnostic parameters in cerebral infarction and intracerebral hemorrhage with secondary neuronal destruction (Schaarschmidt et al. 1994; DeGiorgio et al. 1995).

6. **S-100β**

A. **S-100β protein in neurological disorders**

The S-100β is a **calcium**-binding peptide and is used as a parameter of glial activation and/or death in many disorders of the central nervous system (CNS) (Yardan et al. 2011).

B. **S-100β expression in Alzheimer's disease (AD)**

These results provide evidence that (over-)expression of S100-β acts to accelerate AD-like pathology and suggest that inhibiting astrocytic activation by blocking S100-β biosynthesis may be a promising therapeutic strategy to delay AD progression (Mori et al. 2010).

C. **Role of S-100β in schizophrenia**

Research studies on S100-β concentrations and schizophrenia clinical diagnosis are very consistent; patients with schizophrenia showed higher S100-β concentrations than healthy controls (Monji et al. 2009).

7. **Matrix metalloproteinase**

A. **Matrix metalloproteinase levels in inflammatory bowel disease**

The abundance and activation of matrix metalloproteinases significantly increases in ulcerative colitis and Crohn's mucosa. Inhibitors of these proteolytic enzymes may therefore be of therapeutic value in the treatment of inflammatory bowel disease (Baugh et al. 1999).

B. **Matrix metalloproteinase-9 in Alzheimer's disease**

Circulating levels of MMP-9 are increased in AD and may contribute to disease pathology (Stefan Lorenzl et al. 2003).

8. **Nogo-A**

A. **Nogo-A in neuroregeneration**

The Nogo-66 receptor (NgR), a membrane protein which binds to Nogo, may play an important role in signal transduction for several myelin-associated inhibitors. The discovery of the Nogo family and the NgR provides an opportunity to develop interventions to promote axonal regeneration in the CNS after brain injury (Wang et al. 2012).

Nogo-A expression found in neurons is involved in plasticity of the CNS, including the hippocampal formation and the cerebral cortex (Schmandke et al. 2014).

9. **COVID-19**

Recent pandemic of COVID-19 has caused neurological disturbances after 3 months of discharge from hospital. Approximately 55 percent of hospitalized COVID-19 patients have been reported to have neurological disturbances. The pathophysiology of CNS infection by SARS-CoV-2 and the associated neurologic sequelae of this infection remain poorly understood. Elevations of biomarkers have been reported in CSF and blood of COVID-19 patients (DeKosky et al. 2021). Brain hypoxia may be another prominent contributor to CNS injury, especially in patients presenting with significant respiratory symptoms and having experienced prolonged periods of hypoxia. "Silent hypoxemia," oxygen levels incompatible with life without dyspnea in COVID-19 patients, has been of concern as well, in spite of difficulties in conducting reliable assessments using pulse oximetry (Tobin et al. 2020). Histopathological examination of the brain from 18 patients revealed only hypoxic changes and not encephalitis or other specific changes referable to COVID-19 virus (Solomon et al. 2020). A recent observational study and limited case reports detected increased CSF and/or plasma levels of glial fibrillary acidic protein (GFAP), neurofilament light polypeptide (NfL), tau, and several inflammatory markers in COVID-19 patients (Benameur et al. 2020). Increased staining of GFAP was also detected in the postmortem analysis of the brain of a COVID-19 patient. There are a number of currently well-characterized biomarkers of hypoxic brain injury that can be incorporated in studies of COVID-19, GFAP, UCH-L1, S100-β, and NfL,

proteins extensively described in studies of acute hypoxic brain injury. Still research is going on for biomarkers for hypoxia after COVID-19 infection.

Key Facts

- Pathophysiology of hypoxic brain injury has been the focus of considerable research to identify therapeutic target of biomarker.
- Biomarkers are key tools and can provide special information on the complex cascade of events.
- Profile of different classes of biomarkers reflects pathologic mechanisms to identify and characterize the hypoxic brain injury.
- Biomarker assay (for cytokines, miRNA, NMDA receptors, etc.), validation, and FDA approval are required before widespread use of these biomarkers.
- Research is still going on in COVID-19-induced hypoxic brain injury biomarkers.

Summary

- **Hypoxic brain injury** is one of the main causes of morbidity and mortality in adults. Because of high concentrations of sensitive immature cells, free radicals, non-saturated fatty acids, and low concentrations of antioxidant enzymes, the brain requires high levels of oxygen supply and is, thus, extremely sensitive to hypoxia.
- Strong evidence shows that oxidative stress plays an important role in pathogenesis and progression; reactive oxygen species (ROS) production rapidly increases and overwhelms antioxidant defenses.
- ROS will degenerate cell membranes, proteins, lipids, and DNA and lead to a cascading inflammatory response and protease secretion.
- Multiple pathways like **apoptosis, endoplasmic reticulum stress pathway, mitochondrial dysfunction, and necrosis** finally lead to injury.
- In this present chapter, we highlight the molecular mechanism for oxidative stress, apoptosis, different plasma marker, **S-100β**, and **NSE**, summarize current research on therapeutic strategies utilized in combating oxidative stress, and try to explore new clinical and biological approaches.

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D-Dimer Levels, Stroke, and Critical Care

23

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Contents

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Abstract

Biomarkers are important tools in medical decision-making. D-Dimer levels show diagnostic and promising prognostic implications in several diseases including stroke, venous thromboembolism, and COVID-19.

Can D-dimer be the ideal biomarker or one among panels to diagnose critical stroke and predict its outcome? What are other important clinical implications of D-dimer?

Keywords

Acute ischemic stroke · Biomarkers · Prognosis · D-Dimer levels · D-Dimer tests · Mortality · Pulmonary embolism · DVT · Trauma · Intensive critical unit · COVID-19

Abbreviations

AF	Atrial fibrillation
AIS	Acute ischemic stroke
AMI	Acute myocardial infarction
CTPA	Computed tomography pulmonary angiography
DD	D-Dimer
DVT	Deep vein thrombosis
FDPs	Fibrin degradation products
ICU	Intensive care unit
INR	International normalized ratio
PE	Pulmonary embolism
PT	Prothrombin time
VTE	Venous thromboembolism

Introduction

Biomarker is defined according to the official National Institutes of Health (NIH) as "a characteristic that is objectively measured and evaluated as a marker of normal biological functions, pathogenic processes, or pharmacologic responses to a therapeutic interference" (NIH Biomarkers Definitions Working Group 2001).

A biomarker can be a molecule or fragments measured in blood, CSF, or tissue, a recording such as an electroencephalograph (EEG) or electrocardiogram (ECG), or a radiological imaging test (Jickling and Sharp 2011).

Biomarkers are widely used in clinical trials and well accepted in clinical medicine. The initiation of lipid lowering drugs as well as further adjustment (LDL-C, low-density lipoprotein cholesterol plasma levels) and establish the

diagnosis of acute coronary syndromes (with high-sensitivity cardiac troponins (CTns) and in earlier times cardiac enzymes), excluding suspected pulmonary embolism (via D-dimer levels). Biomarkers might aid physicians in several steps of stroke management (Foerch et al. 2009).

Biomarkers in AIS help support a clinical diagnosis of stroke, classify patients according to risk of complications and outcome, and guide treatment (Saenger and Christenson 2010).

Although most diagnostic approaches for the management of acute stroke rely on neuroimaging techniques, an alternative strategy would be the evaluation of biochemical markers of tissue injury and inflammation. Biomarkers may guide the triage and early management of other medical emergencies. A key role is provided by hs-CTns, CK-MB, D-dimer, and B-type natriuretic peptide in the diagnosis and treatment of acute myocardial ischemia, pulmonary embolism, and congestive heart failure, respectively (Gibler et al. 2003).

The etiology of ischemic stroke, even with a thorough evaluation, remains undetermined [cryptogenic] in 25–39% of patients. The recruitement of biomarkers for etiologic assessment might reduce this percentage of cryptogenic strokes and prescribe the most appropriate secondary managements (Amarenco et al. 2009).

Ideal Criteria of Biomarkers for brain disorders

Ultimate stroke biomarkers should yield diagnostic specificity and sensitivity to cerebral infarcts, discriminate between cerebral hemorrhage and ischemic stroke, early steady release after Central Nervous System (CNS) infarction, allow risk stratification, and can guide management protocols while readily accessible and cost-effective (Saenger and Christenson 2010).

Biomarkers could be examined in specimens derived from patients (e.g., plasma, CSF, tissue biopsy, brain micro-dialysate) (Iadecola et al. 2006).

A combined biomarker panel might be more reasonable than a single biomarker for management due to the complex pathophysiology of stroke (Kaplan et al. 2008). Biomarker panel incorporates biomarkers from each stroke etiology or combines biochemical markers with bio-imaging markers as an alternative approach (Marcovina et al. 2007).

The ischemic cascade of glial activation and ischemic neuronal injury in stroke is far more complex than myocardial ischemia and less appropriate to use as a single biochemical marker (Jickling and Sharp 2011).

During stroke, the blood-brain barrier (BBB) integrity is jeoparded by endothelial cell death, and the cytosolic contents released from the injured brain tissue have the potentiality to cross the BBB. Therefore, the measurement of brain-derived proteins in blood could be used to monitor stroke onset and severity (Kashyap et al. 2006).

Use of a Panel of Serum Protein Markers

Unfortunately, no single biomarkers are included in routine assessment of AIS diagnosis, etiological classification, or prognosis. Multiple biomarker panels have been developed in an attempt to improve the diagnostic accuracy of individual biomarkers and yield better rapid results. Multiple biomarker panels need to be readily available, easy to use, and cost-effective (Saenger and Christenson 2010).

Serum Biomarkers for Stroke Diagnosis

A number of serum biomarkers of cerebral ischemia showed promise to aid in AIS diagnosis (Table 1). Many are related to the underlying pathophysiology of ischemic stroke, including acute thrombosis, CNS tissue ischemia, and inflammatory reaction (Jickling and Sharp 2011).

Biomarkers for the Diagnosis of Ischemic Stroke

Currently, the diagnosis of AIS relies on clinical examination combined with brain imaging. A blood test could guide the triage of AIS in a pre-hospital setting and speed the transfer to specialized centers as well as speed the CT imaging to initiate timely thrombolytic therapy. Additionally, a blood biomarker can support the physician clinical diagnosis of stroke in stroke mimic conditions which remains unclear in spite of clinical examination and neurological imaging (Jickling and Sharp 2011).

A multiple panel of four biomarkers (S100B, matrix metalloproteinase 9 (MMP-9), D-dimer, and brain natriuretic peptide (BNP)) was able to differentiate ischemic stroke from stroke mimic conditions among 1146 patients including hemorrhagic stroke with 85% sensitivity; yet, specificity was limited (34%) (Laskowitz et al. 2009).

Biomarkers of Ischemic Stroke Etiology

Ischemic stroke is often classified by etiology into cardioembolic, large vessel, small vessel, and cryptogenic causes. This classification system is unable to determine an etiology of ischemic stroke in as many as 30% of patients (Jickling and Sharp 2011).

In 707 AIS patients, BNP levels >76 pg/ml were able to predict cardioembolic stroke origin with sensitivity and specificity (72% and 69%, respectively). D-Dimer >0.96 g/mL was able to predict cardioembolic origin with a sensitivity and specificity of 56% and 64%, respectively. The combination of both BNP and D-dimer yields a PPV of 70% in cardioembolic stroke (Montaner et al. 2008). D-Dimer has been linked with cardioembolic origin in stroke in several researches (Ageno et al. 2002; Tombul et al. 2005; Isenegger et al. 2010). Similarly, BNP serum levels were

Table 1 Different biomarkers for stroke evaluation

Mechanism and biomarker	Physiological function	References
Inflammation		
CRP	Acute-phase reactant biomarker of hepatic origin, part of innate immune system response	Andersson et al. (2009) and Kaplan et al. (2008) Elbelkimy et al. (2019) Kim et al. (2011)
VCAM-1	Binds monocytes and lymphocytes	Lynch et al. (2004)
Micro-RNAs	Important for regulation of gene expression	Kadir et al. (2020)
MCP-1	Potent mononuclear cell produced by endothelial and smooth muscle cells	Reynolds et al. (2003)
IL-6	Mediates several biological functions, including pro-inflammatory defense of the immune system, regenerative functions, metabolism, bone homeostasis, cardiovascular system protection, and neural function	Shaafi et al. (2014) Aref et al. (2020)
TNF-α	Regulates inflammation, cell survival, apoptosis, proliferation, and differentiation	Bokhari et al. (2014)
Dyslipidemia/ endothelial damage		
ApoC1	Smallest apolipoproteins. Constitutes 2% of HDL and 10% of VLDL, involved in plasma lipoprotein remodeling, inhibits CETP	Allard et al. (2004)
ApoA1	Apo A1 has antiinflammatory role in addition to the atheroprotective and antioxidants effects	Eldeeb et al. (2020)
ApoC3	Component of VLDL, HDL, and LDL; attenuates lipoprotein/hepatic lipase activity in triglyceride hydrolysis; attenuates endothelial function	Allard et al. (2004)
Adiponectin	Plays a role in anti-inflammatory, anti-atherogenic, and insulin-sensitizing properties	Tu WJ et al. (2020)
BNP	Biomarker released from cardiomyocytes. BNP is a natriuretic, diuretic, and vasodilator polypeptide	Mäkikallio et al. (2005) and Montaner et al. (2008) Mohamed et al. (2019)
FABP	Cytoplasmic protein that modulates lipid metabolism; involved in fatty acid oxidation	Wunderlich et al. (2005) and Pelsers et al. (2004)
Growth factors		
BDNF	Important for growth, maturation, survival, and maintenance of nerve cells	Reynolds et al. (2003) Lasek-Bal et al. (2015)
Brain damage		
MBP	Neuro-biomarker main constituent of myelin. Produced by oligodendroglia neural cells	Jauch et al. (2006) and Hill et al. (2000)
NSE	Glycolytic enzyme in nerve cells and neuroendocrine cells	Undén et al. (2009), Anand and Stead

(continued)

Table 1 (continued)

Mechanism and biomarker	Physiological function	References
		(2005) Shash et al. (2021)
S100B	Regulates calcium balance, stimulates neuritis growth, and promotes neuronal survival, involved in energy metabolism and inflammation	Elting et al. (2000)
Coagulation/ fibrinolysis		
D-dimer	Fibrin degradation product, reflects a global activation of coagulation and fibrinolysis	Laskowitz et al. (2009) and Barber et al. (2006) Nezu et al. (2018) X Yang et al. (2021) Abbas et al. (2021)
Von Willebrand factor	A blood clotting glycoprotein factor important for hemostasis specially platelet adhesion	Barber et al. (2004) and Folsom et al. (1999) M Menih et al. (2017)

Modified from Saenger and Christenson (2010)
VCAMs Vascular cell adhesion molecules; *MCP* monocyte chemotactic protein; *Apo* apolipoprotein; *FABP* fatty acid-binding protein; *BDNF* brain-derived neurotrophic factor; *MBP* myelin basic protein; *NSE* neuron-specific enolase; *LDL* low-density lipoprotein; *VLDL* very-low-density lipoprotein

linked to cardioembolic stroke in atrial fibrillation patients (Nakagawa et al. 2005; Shimizu et al. 2002).

Biomarkers of Final Infarct Volume and Outcome

Serum levels of S100B, MMP, IL-6, TNF-alpha, ICAM-1, and glutamate were predictor of final ischemic infarction volume and outcome (M. Rodríguez-Yáñez et al. 2012).

D-dimer levels higher than 310 ng/mL can predict infarction size >1.5 cm in diffusion-weighted brain MRI with sensitivity and specificity (100 and 83%, respectively) as well as admission of D-dimer (D0) at cutoff concentration of 350 ng/mL and D1 at cutoff value of 370 ng/mL that are predictors of complicated course with sensitivity and specificity (100 and 84.6%, respectively) (Abbas et al. 2021). However, it should be noted that infarction size may not always predict neurologic outcome, as even small infarcts in critical anatomical regions such as the brainstem can be fatal (Jickling and Sharp 2011).

The prognostic impact of D-dimer on mortality among acute ischemic stroke patients is contradictory (Kim et al. 2021, Squizzato et al. 2006; Di Napoli and Papa 2002).

Furthermore, an elevated D-dimer within the first weeks of ischemic stroke is an independent predictor of poorer survival regardless of age, stroke type, or severity (Feinberg et al. 1996). D-Dimer levels are independent factor of short-term functional outcome and mortality, and the levels correlated with infarct volume and NIHSS scores (Yao et al. 2019; Abbas et al. 2021).

Biomarkers of Hemorrhagic Transformation

Risk stratification of AIS patients could help lessen the incidence of hemorrhagic transformation. Clinical variables (age, hypertension, anticoagulant treatment, hyperglycemia) and radiological factors (diffusion-perfusion mismatch, infarction volume, proximal arterial occlusion, leukoaraiosis which is an abnormal appearance of the subcortical white matter of the brain on neuroimaging) correlated with increased hemorrhagic transformation risk. Several biomarkers, including MMP-9, c-FN, PAI-1, TAFI, and S100B, were also linked with an increased risk of hemorrhage after tPA thrombolytic agent (Jickling and Sharp 2011).

Early parenchymal hematomas were linked to an increase in FDPs at 2 hours after lysis in 157 patients in the Lyon rt-PA trial. FDP levels >200 mg/L 2 hours after lysis, indicating massive fibrin and fibrinogen lysis, increased the risk of parenchymal hematoma (Trouillas et al. 2004).

FDP and D-dimer levels were correlated with clinical outcome in 80 acute stroke patients before rt-PA treatment, 2 hours after thrombolysis, and 24 hours after thrombolysis. Between h0 and h2, FDP and D-dimer levels increased, with a tendency to return to initial levels at 24 h post-lysis. None of these values, however, predicted a poor outcome after 3 months (Sun et al. 2015).

Plasma D-dimer levels were significantly associated with unfavorable outcome at 3 months in 159 patients after starting rt-PA but within 24 hours of stroke onset. After intravenous rt-PA treatment, an elevated D-dimer level was found to be an independent predictor of symptomatic intracerebral hemorrhage (Hsu et al. 2016).

Biomarkers of Arterial Recanalization

In acute cerebral ischemia, recanalization of arterial blood flow is an important predictor of a positive prognosis.

A biomarker could help identify patients who are most likely to benefit from recanalization therapy. PAI-1 (plasminogen antigen inhibitor 1) is a fibrinolysis marker linked to recanalization resistance (Ribo et al. 2004).

In 80 stroke patients, FDP and D-dimer levels were assessed before rt-PA treatment, 2 hours after thrombolysis, and 24 hours after thrombolysis, and the results were linked with overall prognosis (Sun et al. 2015).

D-Dimer and Ischemic Stroke

D-Dimer Formation

Coagulation

In response to vascular damage, clotting processes are initiated in a cascade, resulting in the formation of an insoluble fibrin-platelet plug at the site of the vessel wall defect, which stops blood loss and restores vascular integrity.

The release of numerous chemicals required for platelet aggregation, activation, and beginning of the coagulation cascade results in the production of cross-linked fibrin, which ensures the development of a solid blood clot at the injury site (Hoffman and Monroe III 2001).

The blood coagulation system is made up of inactive clotting enzyme and cofactor precursors that get activated and then activate the next enzyme in the sequence, thus the name "coagulation cascade."

Despite the fact that the initial stimulus may be little, a considerable amount of fibrin (factor I), the final component in the cascade, is generated (Casey 2003).

The coagulation cascade is triggered by tissue factor (TF) exposure, which activates FIX and FX with factor (F) VIIa, which then stimulates thrombin production and subsequent conversion of FI fibrinogen to fibrin with FVIII and FV as cofactors, respectively.

FXI, FVIII, and FV are activated by thrombin, increasing the coagulation signal.

Tissue factor pathway inhibitor (TFPI) blocks the TF/FVIIa pathway once the TF/FVIIa/FXa complex is produced, rendering coagulation dependent on the amplification loop through FIX/FVIII (Fig. 1).

Fibrinolysis

Fibrinogen is a soluble plasma glycoprotein that, when cleaved by thrombin, transforms into extremely self-adhesive fibrin monomers (Blomback et al. 1978).

Plasma fibrinogen is converted to fibrin monomers by thrombin.

Factor XIII, which circulates attached to fibrinogen, is activated by thrombin, which stays coupled with fibrin.

Factor XIIIa is produced while fibrin polymerizes and continues to be produced after fibrin has solidified into an insoluble gel.

The sequential action of thrombin, FXIIIa, and plasmin results in the formation of the D-dimer antigen (Adam et al. 2009).

Thrombin cleavage reveals a previously cryptic polymerization site on fibrinogen, which facilitates the binding of another fibrinogen or a monomeric fibrin molecule in the initial phase of D-dimer formation.

Fibrin monomers spontaneously polymerize by attaching to one another half-staggered (overlapping) to create two molecule-thick proto-fibrils (Doolittle and Pandi 2007).

During the fibrinolysis process, plasmin cleaves fibrin at specific locations on the molecule, resulting in the formation of different fibrin fragments.

The plasmin cleavage sites for fibrin are identical to those for fibrinogen.

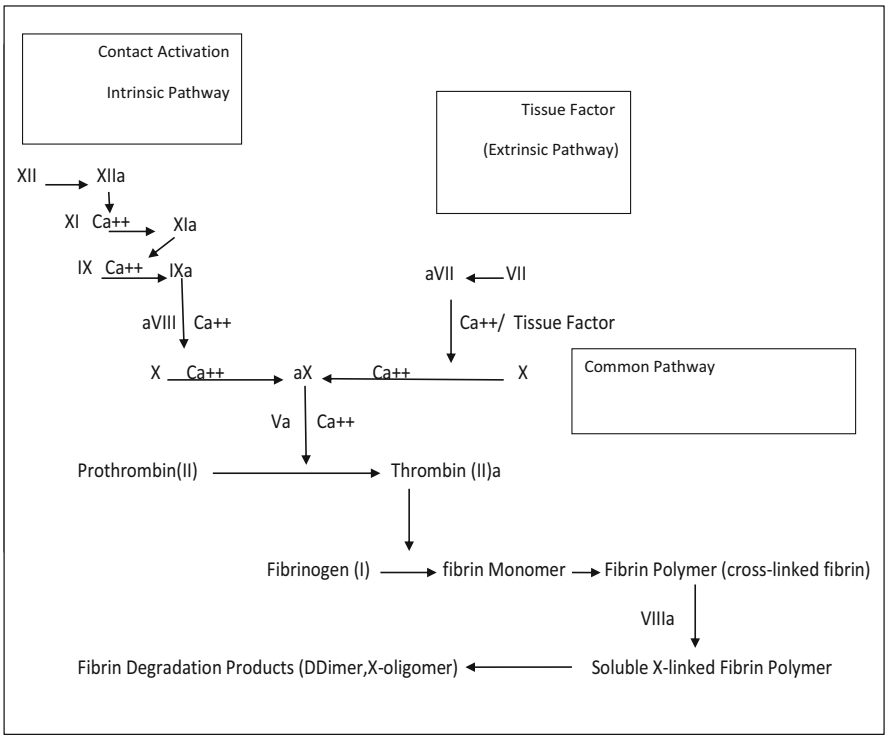

Fig. 1 The coagulation cascade (intrinsic pathway and extrinsic pathway). Coagulation requires calcium and takes place on phospholipid surfaces, usually the activated platelet membrane. (Modified from Manzoor et al. 2021)

Before and after the formation of a fibrin insoluble gel, plasmin releases D-dimer antigen from fibrin polymers. Thus, D-dimer antigen identified by commercially available tests can be produced from soluble fibrin polymers before absorption in the clot or be the result of cleavage of cross-linked fibrin clot by plasmin.

D-Dimers are a very precise test of fibrin (rather than fibrinogen) breakdown that may be detected in plasma (Adam et al. 2009).

The strength tension of the fibrin mesh is enhanced by factor XIIIa, which cross-links adjacent fibrin monomers. Plasminogen activation into plasmin is enhanced with fibrin formation. Plasmin digests the individual fibers. Plasmin cleavage between the D and E domains yields (D-d) E, the non-covalent complex of D-dimer (D-d) and fragment E. Further proteolysis separates fragment E from D-d (J.I. Weitz et al. 2017).

D-Dimer assays are sensitive markers of blood clot formation.

In practically every patient of acute VTE, D-dimer levels rise.

However, every medical condition that increases fibrin formation or breakdown, such as inflammation, cancer, trauma, or surgery, also raises D-dimer levels.

According to a recent study, there are 30 commercially available D-dimer assays that utilize 20 distinct monoclonal antibodies (Longstaff et al. 2016).

The D-dimer epitope targeted by the antibody, the technique of capture and detection, the instrumentation required, and the calibration standard all differ among the assays.

Specific monoclonal antibodies are required to identify D-dimer antigen.

Monoclonal antibodies detect an epitope present in the factor XIIIa-cross-linked fragment D domain of fibrin, but not in fibrinogen degradation products or non-cross-linked fibrin degradation products, in all recent commercial D-dimer tests.

It is worth noting that each monoclonal antibody for detection has its own specificity.

A portion of the polypeptides in the D-domain that shows conformational reaction after factor XIIIa and plasmin have modified the protein has been epitope-mapped by several monoclonal antibodies, and the antigenic determinant recognized is a portion of the polypeptides in the D-domain that shows conformational reaction after factor XIIIa and plasmin have modified the protein (Adam et al. 2009) (Table 2).

For D-dimer detection, different qualitative (positive or negative), semiquantitative, and quantitative approaches such as ELISA or latex immunoassay (LIA) which use a monoclonal antibody specific to distinct D-dimer epitopes are available (Olson JD et al. 2013; Lippi G et al. 2015).

The fundamental challenge connected to D-dimer testing is that many D-dimer assays are not standardized due to the variety in methodological variables, including the use of different monoclonal antibodies and calibrators for testing (CLSI 2011; Oude Elferink et al. 2015).

As to date no standard reference preparation (i.e., international standard) is available, measurement units are still not standardized meaning that results, reference intervals, and clinical cutoff values cannot be extrapolated between procedures.

Thus, D-dimer data must be carefully interpreted based on the test utilized (CLSI 2011; Douma et al. 2012).

On the test report along with the D-dimer results, it is required to point out the measurement method (e.g., measurement method enzyme-linked fluorescence assay, ELFA) (Bronić et al. 2019).

D-d levels offer significant advantages over other measurements of thrombin production, because it is resistant to ex vivo activation, is rather stable, and has a long half-life (Lowe 2005).

D-Dimer Test Collection and Panels

Serum, plasma (with sodium citrate anticoagulant, 3.2%)

Blood is obtained with routine venipuncture. A citrate-containing tube must be properly filled and mixed via inversion. It is transported to the laboratory within 3 hours. If this is not possible, the plasma is separated with centrifugation, frozen, and transported to the laboratory on dry ice (Pagana et al. 2019).

Table 2 Brief description of D-dimer (D-d) tests

Type of test	Short description	Time of results
ELISA	Test with the highest accuracy. It is not practical for diagnosing VTE in individual patients because it is laboratory intensive. Availability in most centers is limited	≈ 8 h
Quantitative rapid ELISA	This test uses an antibody to D-d that is fluorescent labeled. An automated immune analyzer provides a numeric result	≈35 min
Semiquantitative rapid ELISA	This test uses an antibody to D-d that is tagged to a color-producing agent. Plasma D-d concentration is quantified from the degree of color intensity produced	< 10 min
Qualitative rapid ELISA	This test involves the presence of D-d detected by an anti-D-d monoclonal antibody. Activity is revealed by the addition of a substrate that causes a color change. The intensity of color is read virtually by comparison with a positive and negative control	10 min
Quantitative latex agglutination assay	This test uses monoclonal antibodies to D-d that are coated onto latex particles. In the presence of D-d, the particles aggregate to form larger aggregates. Quantification of D-d concentration is done with an analyzer that detects agglutination and precipitation turbidimetrically	7–15 min
Semiquantitative latex agglutination assay	This test relies on the use of monoclonal antibodies to D-d that are coated onto latex particles. Macroscopic agglutinations are seen when elevated D-d levels are present in plasma	3–4 min
Whole-blood agglutination assay	These assays use a freshly collected drop of a capillary or venous whole blood mixed with a conjugate of monoclonal antibody to D-d. visible agglutination takes place in the presence of elevated D-d levels. It can be performed at bedside. It is difficult to discriminate between weak-positive and normal results and observer dependent	2 min

ELISA enzyme-linked immunosorbent assay; *VTE* venous thromboembolism (From Stein et al. 2004, modified cited in S. Siragusa 2006)

Medical Conditions Affecting D-Dimer (DD) Levels

The level of DD is seldom high in healthy people, but it may be used as a negative predictor to rule out clinically suspected intravascular thrombotic coagulation processes in more than 97% of instances.

Furthermore, how long after the beginning of clot formation DD levels become increased or remain elevated in circulation is not well understood, and it may differ between patients (Haapaniemi and Tatlisumak 2009) (Table 3).

Although D-dimer testing has been known for lacking specificity, yet an extremely elevated D-dimer level > 5000 ng/mL is uniquely correlated with severe

Table 3 Medical conditions affecting D-dimer (DD) levels (S. Siragusa 2006 modified)

Increasing DD level	
Pathological	Physiological
Severe infections[a]	Psychological stress (von Känel and Dimsdale 2003)
Traumas	Pregnancy
Surgery	Delivery
Burns (King et al. 2010)	Aging
Tissue infarction	Race (black population)
Advanced cancer[a]	Cigarette smoking
Disseminated intravascular coagulation	Functional disability
Pre-eclampsia	Neonatal period (Hudson et al. 1990)
Sickle cell anemia (Dar et al. 2010)	
Renal failure (Lindner et al. 2014)	
Liver impairment (Violl et al. 1996)	
Acute hemorrhage	
Arterial or venous thrombosis[a]	
COVID-19 infection (Soni et al. 2021)	
Autoimmune disorders (Xue et al. 2021)	
Heart failure (Alehagen et al. 2004)	
Acute aortic dissection (Suzuki et al. 2009)	
Atrial fibrillation	
Acute respiratory distress syndrome (Weber et al. 2003)	

[a]Conditions with ultrahigh D-dimer levels >500 ng/mL (Yao et al. 2020; Soni et al. 2021). N.B normal D-dimer levels in non-pregnant adult <0.5 mg/L or μg/mL

diseases, mainly VTE, cancer, and/or sepsis (Schutte et al. 2016). Also, in a pre-COVID era, a recent study suggested that VTE, cancer, and pneumonia were the frequent causes of ultrahigh plasma D-dimer levels. Ultrahigh D-dimer was defined as levels >5000 ng/mL, and mortality was high when the levels were > 15,000 ng/mL (Kristin Schafer et al. 2021).

N.B vitamin K antagonist, heparin, decreases D-dimer levels (Lip et al. 1995; Couturaud et al. 2002). Dabigatran and rivaroxaban decrease D-dimer levels, yet they do not interfere with the D-dimer assay (Baglin et al. 2012).

The Clinical Implications of D-Dimer Measurement

In general, a D-dimer test may be conducted to determine the amount to which fibrin production has begun, as well as to determine if this process has changed throughout the course of a certain therapeutic or disease pathology (Lowe 2005) (Rathbun et al. 2004).

D-Dimer measurement has been thoroughly established in practice for (1) excluding VTE in specific patient groups and (2) diagnosing and monitoring coagulation activation in disseminated intravascular coagulation (DIC) (Adam et al. 2009).

Epidemiological studies have demonstrated that D-dimer is an independent predictor for future coronary heart disease in the general population (Danesh et al. 2001). However, it is unlikely that D-dimer will play a role in cardiovascular risk stratification in asymptomatic individuals. More promising is a role for D-dimer in optimizing risk stratification in acute settings such as patients presenting with chest pain due to acute coronary syndromes (Bayes-Genis et al. (2000), Menown et al. (2003), and Shitrit et al. (2006)) or patients with stroke (Ageno et al. (2002), Kelly et al. (2004), Barber et al. (2006), and Delgado et al. (2006)). D-Dimer may also have a prognostic role in atrial fibrillation (Nozawa et al. 2006) and heart failure (Alehagen et al. (2004) and Marcucci et al. (2006)).

Relation Between D-Dimer and Different Phases and Subtypes of IS

The molecules involved in hemostasis might be useful biomarkers for IS. For example, fibrinopeptide A and prothrombin fragment, which reflect thrombin activity, and D-dimer, a product of fibrin degradation, appear in the circulation when the coagulation system has been activated and red fibrin-rich thrombi have been formed. Those red clots typically originate in diseased cardiac chambers (Xu et al. 2008), being related to CE stroke etiology (Becker (2005) and Montaner et al. (2008)).

Several studies have demonstrated differences in coagulation and fibrinolysis markers between various ischemic stroke subtypes. One difference between atherothrombotic and cardioembolic stroke is the duration from thrombus formation to symptom onset, which is longer in the latter. Therefore, we hypothesized that differences in the levels of fibrin formation and fibrinolytic markers might be detectable in acute stroke (Isenegger et al. 2010).

In the acute period of ischemic stroke, DD levels are higher than in the healthy control population. All patients with ischemic stroke, patients with cardioembolic infarction (CEI), and patients with transient ischemic attack (TIA) or ischemic stroke due to thrombotic cause were divided into subgroups (Takano et al. 1991).

Subsequent studies of DD in acute ischemic stroke patients have confirmed these findings (Altès et al. 1995; Ince et al. 1999; Kataoka et al. 2000; Tombul et al. 2005).

In some studies (Tohgi et al. 1993, and Haapaniemi et al. 2004), DD levels did not differ between ischemic stroke patients and healthy controls many months after stroke onset, although some exhibited increased levels even 6 months after ischemic stroke (Takano et al. 1992), while others showed increased levels even 6 months after ischemic stroke (Takano et al. 1992) (Reganon et al. 2003).

DD levels rise progressively after an ischemic stroke, peaking about 2 weeks (Feinberg et al. 1989), staying high for many weeks (Tohgi et al. 1990), and then gradually declining. CEI patients had substantially greater plasma DD levels than other ischemic stroke patients or healthy controls (Kataoka et al. (2000), Tombul

et al. (2005), Takano et al. (1992), Yamazaki et al. (1993), Giroud et al. (1998), Feinberg et al. (1996), Dahl et al. (2000), and Ageno et al. (2002)).

The measurement of D-dimer levels has clinical utility, in that the absence of circulating D-dimer correlates with the absence of massive thrombosis (Bounameaux et al. 1994).

DD levels rise dramatically in IS patients undergoing thrombolytic treatment, with full recanalization being considerably greater than partial or no recanalization (Jin et al. 2000).

The hemorrhagic transformation group had greater DD levels after intra-arterial thrombolytic treatment than the non-hemorrhagic transformation group (Ueda et al. 1995).

These findings show that fibrinolytic medicines increase the development of DD (Haapaniemi and Tatlisumak 2009). The association between plasma hemostatic indicators and acute cerebral infarction (CI) subtypes has been studied in several researches.

D-Dimer is a marker of the fibrinolytic state, and the level of this marker is typically higher in cases with cardioembolism than in cases with other CI subtypes (i.e., atherothrombosis and lacunar infarction). An elevated D-dimer level was useful for distinguishing cardioembolic infarction (CEI) from other CI subtypes in the emergency room (Dougu et al. 2008).

It is important to remember that the reference ranges vary with the test used and there is no international D-dimer standard. Many laboratories (rightly or wrongly) use the manufacturer's reference range. The best agreement between assays has been obtained using reference plasma preparations prepared from pools of clinical patient samples (Keeling et al. 2004).

A DD cutoff point of 300 ng/ml for distinguishing CEI from atherothrombotic infarction (ATI) and lacunar infarction (LI), yielding a sensitivity of 80% and a specificity of 77%, has been reported (Takano et al. 1992). Another study proposed that the optimal DD cutoff point for discriminating between the presence and absence of a cardioembolic source is 2.00 μg/ml to yield a specificity of 93% and a sensitivity of 59%. The optimal cutoff for discriminating lacunar infarctions was determined to be 0.54 μg/ml, with a specificity of 96% and a sensitivity of 61% (Ageno et al. 2002).

D-Dimers in Critically Ill COVID-19 Patients

D-Dimer levels increase in COVID-19 patients and predict mortality. Patients with SARS-CoV-2 pneumonia who died had significantly higher D-dimer levels on admission, and levels remained elevated in the late stages of the disease and in all deaths (Tang et al. 2020). Zhou F et al. reported that a D-dimer > 1 μg/mL at admission was associated with increased odds of in-hospital mortality (Zhou et al. 2020). A later study reported that D-dimer cutoff level ≥ 2.0 upon admission was an independent predictor of all-cause in-hospital mortality. It was noted that patients with elevated D-dimer had a higher incidence of comorbidities such as diabetes,

hypertension, ischemic heart diseases, and stroke. However, high D-dimer levels >2 μg/mL remained a significant predictor of mortality with or without underlying disease when data was adjusted for age and gender (Zhou et al. 2020).

Importance of D-Dimer in Different Diseases

D-Dimer and Venous Thromboembolism

Although the number of research studies is limited, the evidence suggests that POCT for D-dimer can be used in the primary care setting as a low-cost, easily accessible emergency tool to reliably guide diagnostic and management strategies for patients with suspected VTE, reducing the time to diagnosis and appropriate treatment.

DVT and PE referrals to the hospital may be safely reduced if a good D-dimer POCT equipment was used to rule out a diagnosis. Some of the savings might be used into POCT technology and infrastructure (CP Price et al. 2021).

Pulmonary Embolism and D-Dimer (PE)

D-Dimer levels are a useful indicator, but they are insufficient to establish the diagnosis of PE on their own.

In individuals with a low clinical suspicion of PE and a normal D-dimer level of less than 500 ng/mL, however, PE can be ruled out, and no additional testing is required (Stamm 2012).

Elevated values of D-dimer (> 500 ng/mL) should suggest additional testing with a computed tomography (CT) pulmonary angiography in individuals with a low clinical suspicion of PE.

Without D-dimer testing, patients with a moderate or high suspicion of PE should have imaging tests.

Silva BV et al. looked at the best approach to rule out PE.

They compared YEARS algorithm [Three elements clinical picture of deep vein thrombosis, haemoptysis, and if PE is the most likely diagnosis], and PEGed (Pulmonary embolism graded study), age-adjusted levels and fixed ddimer cut-off values. When compared to fixed D-dimer values, All methods were linked to improved pulmonary embolism diagnostic specificity. however, age-adjusted cut-off levels were the only ones that did not affect sensitivity, allowing CTPA to be safely reduced (Silva et al. 2021).

D-Dimer Significance in Critically Ill Patients

Due to its connection with thrombosis, D-dimer elevation has gained interest as a predictive biomarker for COVID-19 pneumonia (Wang et al. 2020; Phelan et al. 2020; Klok et al. 2020).

In critically ill patients, it has also been identified as a high-risk factor.

Infectious disease, trauma, heart failure, thrombotic disease such as cerebral thrombosis, acute myocardial infarction (AMI), VTE, intracranial hemorrhage, and other diseases are all examples of critically sick disease. The plasma D-dimer levels in non-survivors of critically ill diseases were considerably greater than in survivors.

Disseminated intravascular coagulation (DIC) score, D-dimer value, and prothrombin time-international normalized ratio (PT-INR) were all found to be significant predictors of death by Ichkawa et al. The following were the best cutoff values for indicating a bad outcome: DIC score, 3 points; D-dimer, 4.2 mg/L; and PT-INR, 1.08. D-Dimer, a thrombosis biomarker, is elevated in a variety of underlying illnesses and predicts a bad prognosis (Ichkawa et al. 2020).

Stroke Biomarkers' Limitations

Despite their rising importance, biomarkers' implications in the management of AIS now face certain constraints.

First, due to the existence of the blood-brain barrier, ischemic alterations in the brain are not adequately reflected by blood biomarkers, unlike acute myocardial infarction (low sensitivity and underpowered).

Second, the presence of a number of concomitant illnesses, as well as brain injury itself, might cause biomarkers to alter (confounders and lack of specificities).

Asymptomatic coronary atherosclerosis, for example, was shown to be present in one-fifth of non-disabling non-cardioembolic ischemic strokes (Calvet et al. 2010), which might cause biomarker levels to be muddled.

Furthermore, it may be difficult to determine the direct function of biomarkers in the illness process. For example, despite the fact that matrix metalloproteinase 9 (MMP-9) is established as a measure of hemorrhagic transition following thrombolysis (Castellanos et al. 2007), the magnitude of the cerebral infarction is substantially related with it (Bang et al. 2005).

As a result, establishing a causal link between biomarkers and ischemic stroke in a true clinical context is challenging (Kim et al. 2013).

Third, there is no one biomarker for stroke that is sufficiently reliable.

Ischemic stroke is a complex condition caused by a multitude of factors.

Furthermore, the brain is made up of numerous different cells, each with its own anatomical features.

Terms in Mini-Dictionary

D-Dimer: It is a fibrin breakdown product, a tiny protein fragment formed after fibrinolysis breaks down a blood clot.

Because it includes two D segments of the fibrin protein linked by a cross-link, it is the source of nomenclature.

AIS: It is when blood supply to a part of the brain suddenly stops, resulting in a loss of neurological function.

VTE: The term venous thromboembolism (VTE) encompasses the situations of DVT alone, PE alone, and DVT plus PE (VTE).

Key Facts of D-Dimers

1. D-Dimer has an important role as a biomarker in stroke diagnosis, etiological classification, final infarct volume and outcome, and hemorrhagic transformation.
2. Elevated D-dimer levels in general ICU population predict poor outcome.
3. Ultrahigh D-dimer levels (levels >500 ng/ mL) correlated with severe diseases, mainly VTE, COVID-19, cancer, and/or sepsis and predict poor outcome.

Summary of D-Dimer

1. D-Dimer is an important diagnostic and prognostic biomarker in several medical and surgical diseases.
2. D-Dimer shows relevance in stroke diagnosis, etiology, and prediction of thrombolytic effectiveness.
3. The major drawback of D-dimer assays is the high variability observed between immunoassays. This variability is explained by the reality that D-dimers comprise a wide mixture of cross-linked fibrin degradation products, by the use of different monoclonal antibodies, by the shortage of international certified internal controls or calibrators, and by the use of different units and clinical cutoffs. Further studies are needed to achieve synchronization of D-dimer measurements which can be essentially achieved by ongoing discussion among manufacturers, scientists, and clinicians (Favresse et al. 2018).

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Intraoperative Management and Its Influence on Postoperative Biomarker Release

24

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Abstract

Perioperative organ injury frequently complicates noncardiac surgery – occurring in over one in six patients. Initially clinically silent, its diagnosis hinges on postoperative biomarker release. However, many patients with perioperative organ injury will proceed to sustain further major adverse cardiovascular events in the days, weeks, and months following their operation. A wealth of data supports the notion that the intraoperative course may be a mediator of perioperative organ injury. Importantly, prognostically relevant intraoperative factors include hemodynamic disturbances and major bleeding, both associated with perioperative organ injury. Due to the paucity of interventional data, it remains unclear as to what extent intraoperative factors may cause postoperative morbidity and mortality. However, questions of causality aside, there is strong physiological rationale for avoiding intraoperative hypotension and minimizing intraoperative bleeding. This chapter summarizes the relevance and pathophysiology of perioperative organ injury. Therapeutic strategies to avoid or mitigate prognostically relevant intraoperative disturbances are discussed, and in conclusion, an outlook on future research perspectives is given.

Keywords

Intraoperative management · Noncardiac surgery · Hemodynamics · Blood pressure · Biomarker release · Perioperative organ injury

Abbreviations

AKI	Acute kidney injury
BP	Blood pressure
CaO_2	Blood oxygen content
CO	Cardiac output
DO_2	Oxygen delivery
Hb	Blood hemoglobin concentration
HR	Heart rate
hs-cTnT	High-sensitivity cardiac troponin T
MAP	Mean arterial blood pressure
MINS	Myocardial injury after noncardiac surgery
miRNA	MicroRNA
MRI	Magnetic resonance imaging
ncRNA	Non-coding RNA
PaO_2	Blood oxygen partial pressure

PBM	Patient blood management
PMI	Perioperative myocardial injury
PPV	Pulse pressure variation
RCT	Randomized controlled trial
RNA	Ribonucleic acid
SaO_2	Blood oxygen saturation
SPV	Systolic pressure variation
SV	Stroke volume
SVV	Stroke volume variation
TIA	Transient ischemic attack

Introduction

The advent of sensitive and organ-specific biomarker assays has revolutionized perioperative medicine – organ injury can now be detected at an earlier point than ever before. The discovery that perioperative organ injury – affecting upward of one in six high-risk patients – is not the exception, but the rule has set a paradigm change in motion. Whereas, in the past, patients sustaining organ injury would go undetected until the occurrence of a secondary sometimes devastating cardiovascular event, nowadays perioperative biomarker surveillance permits early detection of these high-risk patients and the timely institution of secondary preventive measures. The ability to detect subclinical organ injury has also facilitated research of the perioperative phase, with mounting evidence that the intraoperative course may be an important mediator of perioperative organ injury and major adverse cardiovascular events. This chapter will explore intraoperative factors during noncardiac surgery potentially contributing to organ injury and the role of biomarker measurement in the perioperative setting (Fig. 1).

The Importance of Perioperative Organ Injury Following Noncardiac Surgery

Organ Injury Versus Biomarker Release

It is all too easy to play down the significance of perioperative biomarker rises; however, it is paramount to recognize that biomarker release is often a sign of ongoing organ injury, as underlined by the guidelines on acute myocardial injury (Thygesen et al. 2018; Puelacher et al. 2021) and acute kidney injury (Mehta et al. 2007). In this chapter, we will emphasize this fact by primarily using the umbrella term *perioperative organ injury* to refer to prognostically relevant perioperative biomarker release.

Fig. 1 Chapter summary: intraoperative management and perioperative organ injury. This figure summarizes the relevance of perioperative biomarker release, the intraoperative factors implicated in perioperative organ injury, as well as their clinical and research implications

Perioperative Organ Injury Is Common

Noncardiac surgery is a major risk factor for perioperative organ injury, with the heart, kidneys, and brain at particularly high risk. Depending on the used definition and patient population, myocardial injury occurs in 16% (Puelacher et al. 2018) to 17.9% (Devereaux et al. 2017) of patients at elevated cardiovascular risk undergoing noncardiac surgery. The incidence of postoperative acute kidney injury is heavily dependent on the performed procedure, with incidences in vascular surgery above 50% depending on the performed procedure (Hobson et al. 2018). Finally,

although stroke has long been assumed to be a rare perioperative complication following noncardiac surgery, new evidence indicates that *covert*, MRI-confirmed stroke is a strikingly common perioperative complication affecting 7% of patients (Mrkobrada et al. 2019).

Perioperative Organ Injury Is Clinically Relevant

A wealth of evidence supports the notion that perioperative organ injury is not just prognostically relevant but in many instances a harbinger of further adverse outcomes to come.

Concerning perioperative acute myocardial injury, there is strong evidence supporting its prognostic relevance. However, due to heterogeneous definitions and terminologies, it can be challenging to gain an overview over the data. Recent consensus statements nicely summarize the various terminologies and their respective supporting evidence (Puelacher et al. 2021; Ruetzler et al. 2021). A central take-home message is that *all* perioperative troponin rises are prognostically relevant and indicative of acute myocardial injury; however, not all perioperative troponin rises are ischemic in nature.

The diagnosis of both myocardial injury after noncardiac surgery [MINS] and perioperative myocardial injury [PMI] is based on the 99th percentile of the upper reference limit of the used assay; however, MINS excludes presumed non-ischemic events (e.g., sepsis, pulmonary embolism, or arrhythmia). In contrast, PMI encompasses both presumed ischemic (cardiac PMI, e.g., myocardial ischemia, tachyarrhythmia, or decompensated heart failure) and non-ischemic etiologies (extra-cardiac PMI, e.g., sepsis, pulmonary embolism, stroke). Prognostic thresholds associated with moderate to long-term adverse cardiovascular events have been defined for both MINS and PMI.

The question of the acuity of the myocardial injury (chronic vs. acute) is solved by considering the perioperative increase in hs-cTnT (Devereaux et al. 2017; Puelacher et al. 2018). When considering their prognostic relevance, both MINS and PMI show similar signals with MINS independently associated with 30-day mortality (Devereaux et al. 2017) and PMI associated with increased 30-day and 1-year mortality (Puelacher et al. 2018). Importantly, many prognostically relevant episodes of MINS/PMI occur without ischemic symptoms (Devereaux et al. 2017; Puelacher et al. 2018). The clinical relevance of perioperative acute myocardial injury is underlined by the integration of postoperative troponin surveillance in high-risk patients into the *Canadian Cardiovascular Society Guidelines on Perioperative Cardiac Risk Assessment and Management for Patients Who Undergo Noncardiac Surgery [strong recommendation]* (Duceppe et al. 2017)*, the Updated Guideline from the European Society of Anaesthesiology on Preoperative Evaluation of Adults Undergoing Elective Noncardiac Surgery [grade 2B]* (De Hert et al. 2018), and the *Fourth Universal Definition of Myocardial Infarction* (Thygesen et al. 2018).

Similar to perioperative acute myocardial injury, perioperative acute kidney injury is of high prognostic relevance: Acute kidney injury has been shown to be independently associated with 1-year mortality following major noncardiac surgery, regardless of AKI stage (O'Connor et al. 2017). Long considered irrelevant, postoperative AKI is gaining acceptance as not just a prognostically relevant event but as a potential initiator of a multi-organ inflammatory response (Gumbert et al. 2020). In light of the importance of perioperative kidney injury and the unique aspects of the perioperative setting, recent consensus statements have proposed the terms postoperative acute kidney injury (PO-AKI) and postoperative acute kidney disease (PO-AKD) to characterize kidney injury occurring within 7 days respectively persisting after 7 days following an operative intervention (Prowle et al. 2021).

Finally, perioperative neurologic injury is a potentially devastating complication following noncardiac surgery. Due to the low incidence of *overt* stroke in noncardiac surgery – occurring in under 1% of patients (Devereaux et al. 2008, 2014) – perioperative neurological injury has generally not been considered a pressing issue in noncardiac surgery. However, the NeuroVISION study has questioned this assumption, finding that 7% of patients undergoing noncardiac surgery sustained a *covert*, MRI-confirmed stroke. Furthermore, perioperative covert stroke was associated with transient ischemic attack (TIA) or overt stroke up to 1 year postoperatively.

The Link Between Intraoperative Management and Perioperative Organ Injury Following Noncardiac Surgery

Physiologic Primer

Oxygen Supply/Demand Mismatch as an Initiator of Organ Injury

When discussing perioperative organ injury with subsequent biomarker release, it is vital to understand the pathophysiologic mechanisms underlying these complications. Any intraoperative condition causing an imbalance between oxygen delivery and demand can predispose patients to postoperative organ injury.

Oxygen delivery is defined as:

$$DO_2 = CO \cdot CaO_2 = SV \cdot HR \cdot CaO_2$$

with stroke volume (SV) and oxygen content (CaO_2) in turn being dependent on the following physiologic parameters:

$$SV = f(\text{preload}, \text{inotropy}, \text{afterload})$$

$$CaO_2 = f(Hb, SaO_2, PaO2)$$

Abbreviations

DO_2	oxygen delivery;
CO	cardiac output;
CaO_2	blood oxygen content;
SV	stroke volume;
HR	heart rate;
Hb	blood hemoglobin concentration;
SaO_2	blood oxygen saturation;
PaO2	blood oxygen partial pressure

The Importance of Pressure and Flow

Many vital organs (e.g., brain, heart, kidneys) exhibit flow autoregulation (Lassen 1959; Rubio and Berne 1975; Carlström et al. 2015), i.e., they may maintain sufficient blood flow and hence oxygen delivery within certain blood pressure (BP) limits (the *autoregulatory range*).

However, it is important to recognize that although blood pressure is the most frequently measured hemodynamic parameter in the perioperative setting, it only delivers part of the picture. The function of the circulation is to provide sufficient end-organ perfusion, matching supply to demand in all tissue beds. Ensuring adequate perfusion pressure to guarantee all organs are in their individual autoregulatory ranges is an important therapeutic goal; however, equally as important is ensuring sufficient systemic (*macro*circulatory) and end-organ (*micro*circulatory) blood flows.

On the one hand, the importance of not just pressure but also *macro*circulatory flow is illustrated by the dependence of the renal vasculature on both sufficient perfusion pressure and cardiac output, with renal blood flow compromising already early, "compensated" phases of hemorrhagic shock (Rhee et al. 2012). On the other hand, the central role of the microcirculation is underlined by the concept of hemodynamic incoherence, whereby, even with normal *macro*circulatory parameters, relevant *micro*circulatory compromise can be present, with ensuing organ dysfunction and failure (Ince 2015).

Myocardial Oxygen Demand in the Perioperative Setting

Particularly for the myocardium, the supply/demand relationship can be modified and optimized perioperatively. The primary determinants of myocardial oxygen demand are contractility, heart rate, and ventricular wall stress (primarily influenced by intracavitary pressure perioperatively).

Fig. 2 Chapter summary: intraoperative risk factors for perioperative organ injury. This figure summarizes the pathophysiological underpinnings of and relevant risk factors for perioperative organ injury. Abbreviations: *BP*, blood pressure

Risk Factors for Postoperative Organ Injury

Knowledge of the physiologic parameters determining end-organ oxygen delivery enables delineation of three key conditions predisposing to postoperative organ injury (Fig. 2):

- Major bleeding (compromising oxygen-carrying capacity and potentially cardiac output).
- Hemodynamic derangements (compromising end-organ perfusion pressure or increasing oxygen demand).
- Severe hypoxemia (compromising blood oxygen content).

Major Bleeding

Despite progress in operative technique and pharmacological adjuncts to facilitate hemostasis, major bleeding is still a primary risk factor for organ injury (Park et al. 2021) and mortality (Roshanov et al. 2021) following major noncardiac surgery. In a sub-analysis of the VISION 2 trial (Devereaux et al. 2017), major bleeding was found to have the highest attributable fraction for 30-day mortality post-noncardiac surgery, followed by MINS and sepsis (Spence et al. 2019).

Hemodynamic Derangements

Arterial Hypotension as the Common Denominator of Intraoperative Circulatory Insufficiency

Primary hemodynamic derangements predisposing to organ injury include inadequate perfusion pressure (due to arterial hypotension and/or venous congestion) and insufficient organ blood flow, due to low cardiac output.

Due to the infrequent use of stroke volume monitoring and the general lack of real-time microcirculatory monitoring in the noncardiac perioperative setting, a mixture of less sophisticated parameters such as blood pressure, urine output, and metabolic state is typically used to guide intraoperative hemodynamic management.

Despite its limitations, blood pressure monitoring remains a central hemodynamic monitoring modality, considering arterial hypotension is the common denominator where all forms and etiologies of circulatory insufficiency converge. A "normal" blood pressure may not guarantee an adequate circulation; however, an abnormal blood pressure generally indicates circulatory insufficiency. The central question revolves around the definition of abnormality.

Prognostic Importance of Intraoperative Hypotension.

A considerable body of research has been devoted to precisely the question of what constitutes a "normal" blood pressure intraoperatively. Historically, a physiological approach was used, based on experimental data of the postulated limits of autoregulation (Drummond 1997; Lassen 1959). Interest in the research of intraoperative BP targets only surged when it was found that intraoperative hypotension is associated with 1-year mortality (Monk et al. 2005), giving rise to a plethora of studies investigating the prognostic implications of varying degrees of hypotension.

The evidence supporting an association between intraoperative hypotension and postoperative organ injury with biomarker release is overwhelming (Gu et al. 2018; Wesselink et al. 2018; Wijnberge et al. 2021) (Table 1). Three important conclusions may be drawn from these data. First, the two organ systems most vulnerable to hypotension-associated organ injury are the kidney and heart. Second, increasing durations and extents of hypotension are associated with rising incidences of organ injury (Wesselink et al. 2018), potentially reflecting a dose-response relationship. Third, the mean arterial blood pressure (MAP) threshold where the incidence of organ injury

Table 1 Associations of varying degrees and durations of intraoperative arterial hypotension with postoperative organ injury. This table shows how increasing durations and extents of intraoperative arterial hypotension are associated with increasing risks of postoperative organ injury. The two organs showing the sensitivity in these analyses are the kidney and heart, with an inflection point around a mean arterial pressure of 65 mmHg

depth	duration	mortality		acute kidney injury		myocardial injury		stroke		delirium		overall organ injury	
MAPMAP	Minutes	based on quality score ≥ 80%	based on quality score ≥ 80% and significant result	based on quality score ≥ 80%	based on quality score ≥ 80% and significant result	based on quality score ≥ 80%	based on quality score ≥ 80% and significant result	based on quality score ≥ 80%	based on quality score ≥ 80% and significant result	based on quality score ≥ 80%	based on quality score ≥ 80% and significant result	based on quality score ≥ 80%	based on quality score ≥ 80% and significant result
< 80 mmHg	≥ 1												
	≥ 5												
	≥ 10	1.02	1.02									Low	Low
	≥ 20	1.04	1.04									Low	Low
< 75 mmHg	≥ 1												
	≥ 5												
	≥ 10	1.02	1.02									Low	Low
	≥ 20	1.09	1.09									Low	Low
< 70 mmHg	≥ 1	1.002 *						1.003*				Low	
	≥ 5	1.01 *						1.015*				Low	
	≥ 10	1.04	1.04					1.030*				Low	Low
	≥ 20	1.09	1.09					1.062*				Low	Low
< 65 mmHg	≥ 1	1.002 *		1.3 *		1.01 *		1.003*				Low	
	≥ 5	1.01 *		1.6 *		1.2 *		1.015*				Moderate	
	≥ 10	1.04	1.04	1.6 *		1.3	1.3	1.030*				Moderate	Low
	≥ 20	1.09	1.09	2.3 *		1.8	1.8	1.062*				High	Moderate
< 60 mmHg	≥ 1	1.1 *		1.3 *		1.1 *		1.003*				Low	
	≥ 5	1.1 *		1.6 *		1.2 *		1.015*				Moderate	
	≥ 10	1.1 *	1.09	1.8	1.8	1.5	1.5	1.030*				Moderate	Moderate
	≥ 20	1.2	1.2	2.3	2.3	2.5	1.8	1.062*				High	High
< 55 mmHg	≥ 1	1.2 *	1.04	1.4*	1.2	1.3	1.3	1.003*				Moderate	Low
	≥ 5	1.2	1.2	1.6 *	1.2	1.5	1.5	1.015*				Moderate	Moderate
	≥ 10	1.4	1.4	2.3	2.3	1.8	1.8	1.030*				High	High
	≥ 20	2.0	2.0	3.5	3.5	2.5	2.5	1.062*				High	High
< 50 mmHg	≥ 1	1.2 *	1.04	1.6 *	1.2	1.3	1.3	1.004*		• p = 0.409 *		Moderate	Low
	≥ 5	2.4	2.4	1.6 *	1.2	4.4	4.4	1.020*		• p = 0.409 *		High	High
	≥ 10	2.4	2.4	2.3	2.3	4.4	4.4	1.041*		• p = 0.409 *		High	High
	≥ 20	2.4	2.4	3.5	3.5	4.4	4.4	1.083*		• p = 0.409 *		High	High
< 45 mmHg	≥ 1	1.2 *	1.04	1.6 *	1.2	1.3	1.3	1.013*		• p = 0.409 *		Moderate	Low
	≥ 5	2.4	2.4	1.6 *	1.2	4.4	4.4	1.067*		• p = 0.409 *		High	High
	≥ 10	2.4	2.4	2.3	2.3	4.4	4.4	1.138*		• p = 0.409 *		High	High
	≥ 20	2.4	2.4	3.5	3.5	4.4	4.4	1.295*		• p = 0.409 *		High	High
< 40 mmHg	≥ 1	1.2 *	1.04	3.8	3.8	1.3	1.3	1.013*		• p = 0.409 *		High	High
	≥ 5	2.4	2.4	3.8	3.8	4.4	4.4	1.067*		• p = 0.409 *		High	High
	≥ 10	2.4	2.4	5.1	5.1	4.4	4.4	1.138*		• p = 0.409 *		High	High
	≥ 20	2.4	2.4	5.1	5.1	4.4	4.4	1.295*		• p = 0.409 *		High	High

Low	1.0 < OR, RR or HR < 1.4
Moderate	1.4 ≤ OR, RR or HR < 2.0
High	OR, RR or HR ≥ 2.0
	evidence with quality score < 80%
	no evidence available

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begins to rapidly rise in the population lies around 65 mmHg, giving rise to current consensus statements (Sessler et al. 2019). Readers may be surprised by the weak association of hypotension with postoperative stroke (Wesselink et al. 2018). A plausible explanation could be that overt stroke is an outcome far too insensitive to estimate the *true* extent of postoperative brain injury, as suggested by the much higher incidence of covert compared to overt stroke (Mrkobrada et al. 2019). Furthermore, postoperative delirium is an outcome far too unspecific to solely enable investigation of postoperative brain injury.

When discussing the association of hypotension with organ injury, two important points must be considered. First, due to the paucity of interventional trials to date, the predominance of the evidence is of an observational nature and is hence subject to bias. A central question is to what extent the association between hypotension and organ injury may be of a causal nature (Myles 2014). Important confounders certainly include patient comorbidities, surgical complexity, and intraoperative course. However, considering that half of prognostically relevant hypotensive episodes takes place prior to skin incision (Maheshwari et al. 2018), surgical complexity cannot fully explain the association between hypotension and postoperative adverse events. Furthermore, even when adjusted for intraoperative bleeding, hypotension remains a predictor of postoperative myocardial and acute kidney injury (Walsh et al. 2013). Second, the blood pressure thresholds in these systematic reviews are all population-derived. As autoregulatory boundaries are exquisitely individual and organ-related parameters – intimately related to patient comorbidities – translation of these findings to individual patients must be approached with caution (Brady et al. 2020; Kamenetsky and Hogue 2021), as illustrated by the failure of the few RCTs to date to demonstrate convincing reductions in the incidence of postoperative organ injury (Futier et al. 2017; Wanner et al. 2021).

Myocardial Injury: Other Relevant Hemodynamic Disturbances.

In the context of myocardial injury, the perioperative phase differs quite markedly from nonoperative settings. The classical etiology of myocardial infarction (MI) in the nonoperative setting is the type 1 MI, i.e., due to plaque rupture and vessel occlusion. In the perioperative setting, there is a much higher incidence of type 2 MI, i.e., due to an oxygen supply/demand mismatch (Thygesen et al. 2018). This distinction is of paramount importance since type 1 and 2 MIs have greatly differing treatments, with prevention and treatment of type 2 MI revolving around optimizing the balance between myocardial oxygen supply and demand. Unlike in type 1 MI, intensive platelet inhibition and anticoagulation are not just low priorities; they can be detrimental – especially considering many type 2 MIs occur due to bleeding.

Two common culprits increasing perioperative myocardial oxygen demand and potentially predisposing to a supply/demand mismatch are:

- Arterial hypertension (increasing demand via increased ventricular wall stress, decreasing primarily subendocardial perfusion), e.g., due to high sympathetic tone.
- Tachycardia (increasing demand and decreasing diastolic coronary perfusion time), e.g., sinus tachycardia or rapid atrial fibrillation due to hypovolemia or high sympathetic tone (due to pain, shivering, or other stressors).

Severe Hypoxemia

As an overall rare cause of organ injury in the perioperative setting, severe hypoxemia will not be discussed in detail in this chapter. However, the clinician should not forget that in the shocked patient with severely reduced cardiac output, augmentation of oxygen-carrying capacity by increasing the PaO_2 and if necessary the hemoglobin concentration can be a valuable therapeutic adjunct until hemodynamics may be normalized.

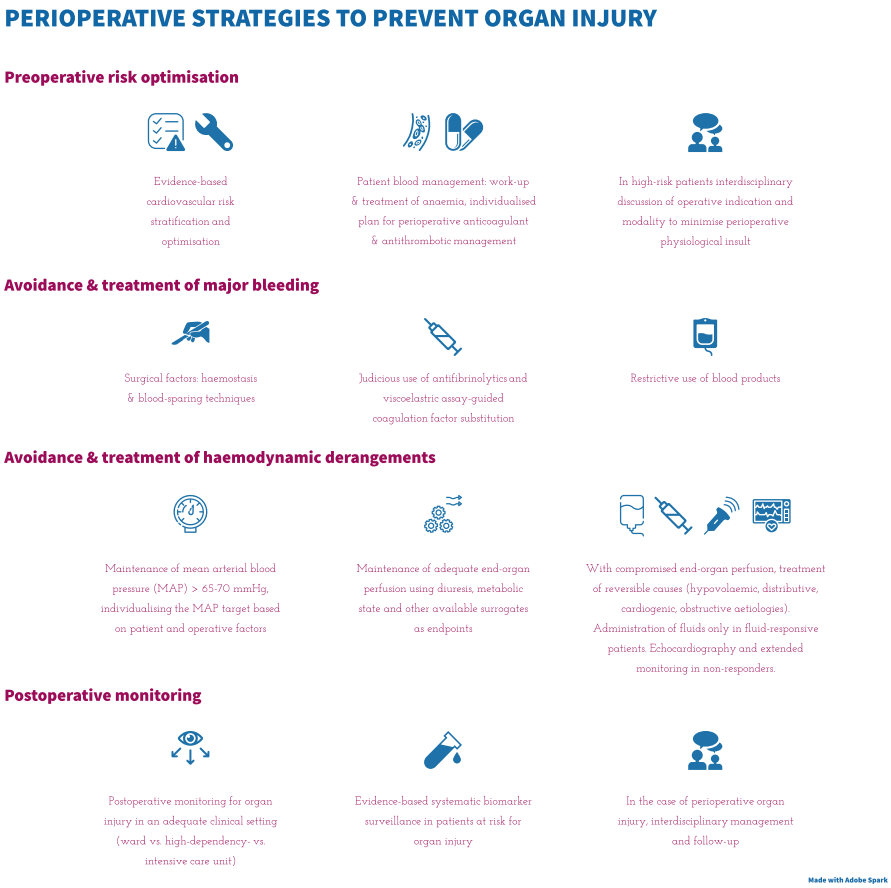

Fig. 3 Perioperative strategies to prevent organ injury. This figure summarizes important pre-, intra-, and postoperative strategies to prevent perioperative organ injury. Abbreviations: *MAP*, mean arterial blood pressure

Perioperative Strategies to Avoid Postoperative Organ Injury Following Noncardiac Surgery

See Fig. 3.

Risk Stratification

As the old saying goes, *an ounce of prevention is worth a pound of cure.* Although the focus of this chapter is the impact of the intraoperative phase on perioperative organ injury and biomarker release, one must not forget that through proper preoperative risk stratification, the occurrence of organ injury can in many cases be attenuated or even prevented altogether. Preoperative optimization of cardiovascular disease, institution of extended intraoperative monitoring when indicated, postoperative care in an intensive care or high-dependency unit setting in select patients, and critical appraisal of the operative modality and indication can all contribute to a reduction in the incidence of postoperative adverse events. Finally, a central pillar of perioperative risk optimization is postoperative risk re-stratification with biomarker surveillance. Currently, a paradigm change is underway with postoperative troponin surveillance becoming a standard of care in high-risk patients following noncardiac surgery (Duceppe et al. 2017), laying the foundation for similar trends in other areas.

Major Bleeding

Considering the prognostic implications of perioperative major bleeding and its association with organ injury, reduction of bleeding-associated complications is a major perioperative goal. In this context, one proposed paradigm is patient blood management (PBM), which stands on three pillars: preoperative optimization of hematopoiesis, minimization of blood loss, and harnessing resp. optimization of anemia tolerance (Shander et al. 2012). In a network meta-analysis, PBM interventions were shown to reduce bleeding and transfusion requirements, however, with a high risk of publication bias (Roman et al. 2020).

The first pillar of PBM is anchored in the preoperative phase and includes work-up and treatment of anemia, as well as formulation of a perioperative plan for continuation/discontinuation of anticoagulants and antiplatelet agents considering the patient's comorbidities and the risk of perioperative bleeding (Yurttas et al. 2017). Two important intraoperative elements of PBM to be highlighted are the use of meticulous hemostasis and blood-sparing surgical techniques and of pharmacologic/hemostatic agents. Whenever feasible, endovascular or minimally invasive approaches should be favored over more invasive procedures. Furthermore, the judicious intraoperative use of the antifibrinolytic tranexamic acid and when necessary viscoelastic assay-guided factor substitution can facilitate hemostasis and reduce transfusion requirements (American Society of Anesthesiologists Task Force on Perioperative Blood Management 2015), compatible with findings from

the trauma literature (Spahn et al. 2019). This is highlighted by the POISE-3 trial (Perioperative Ischemic Evaluation-3), a multinational, 2 × 2 factorial RCT in patients undergoing noncardiac surgery on the prophylactic use of tranexamic acid to prevent bleeding complications. Compared to placebo, intraoperative administration of tranexamic acid was shown to reduce the incidence of the composite outcome of life-threatening bleeding, major bleeding, and bleeding into a critical organ (hazard ratio 0.76 [95% CI 0.67–0.87]). However, non-inferiority with regards to cardiovascular complications could not be demonstrated (hazard ratio 1.02 [95% CI 0.92–1.14]) (Devereaux et al. 2022). Hence, despite the clear efficacy of tranexamic acid in preventing bleeding complications, these findings underline the continued importance of an individualized perioperative risk/benefit analysis to balance the risk of bleeding and of cardiovascular complications.

Intraoperative Hemodynamic Derangements

Blood Pressure Goals

As outlined above, the paucity of RCTs on the association between intraoperative hypotension and perioperative organ injury precludes the formulation of strong/high-grade evidence-based recommendations. One of the largest and most recent RCTs on intraoperative BP targets to date examined the effect of targeting higher intraoperative blood pressures on the incidence of acute myocardial injury and/or 30-day MACE in patients at cardiovascular risk undergoing major noncardiac surgery (Wanner et al. 2021). Despite a 60% reduction in intraoperative hypotension with MAP <65 mmHg achieved by targeting a MAP ≥75 mmHg (compared to standard BP management per the *2014 ESC/ESA Guidelines on Noncardiac Surgery* (Kristensen et al. 2014)), no significant differences could be found between the study groups with regard to acute myocardial injury and/or 30-day MACE. These findings are supported by the preliminary results of the blood pressure arm of the POISE-3 trial (The Effects of a Hypotension-Avoidance Strategy vs. a Hypertension-Avoidance Strategy in Patients Undergoing Noncardiac Surgery) presented at the 2022 American College of Cardiology yearly conference (https://www.acc.org/Latest-in-Cardiology/Clinical-Trials/2022/04/01/03/11/POISE-3, accessed 31 May 2022). This multinational RCT in patients at cardiovascular risk undergoing noncardiac surgery compared a *hypotension* avoidance strategy – entailing targeting an intraoperative MAP ≥ 80 mmHg and algorithm-based perioperative administration of antihypertensives based on perioperative BPs – with a *hypertension* avoidance strategy – entailing targeting an intraoperative MAP ≥ 60 mmHg and perioperative continuation of chronic antihypertensives in 7490 patients. The hypotension avoidance strategy was not associated with any significant differences in the incidence of major vascular complications (hazard ratio 0.99 [95% CI 0.88–1.12]). Finally, the INPRESS trial (Intraoperative Norepinephrine to Control Arterial Pressure) – a multicenter RCT in patients undergoing major abdominal surgery – compared a regime of individualized BP management (defined as systolic BP within 10% of the preoperative reference) to standard BP management (defined as targeting a systolic BP > 80 mmHg or within 40% of the preoperative reference), finding a significant

reduction in the composite outcome of postoperative organ dysfunction (Futier et al. 2017). Methodological limitations, including the use of different vasopressors in the two study groups, prohibit drawing definitive conclusions on the use of individualized BP targets based on preoperative values in noncardiac surgery. Hence, the current data suggest that targeting higher intraoperative BPs in all patients or using preoperative BP values to guide perioperative hemodynamic management are strategies *not* associated with relevant reductions in the incidence of postoperative major adverse cardiovascular events. This underlines the need for further studies on the relationship between hypotension and perioperative organ injury.

The *2014 ESC/ESA Guidelines on Noncardiac Surgery* (Kristensen et al. 2014) and the *Perioperative Quality Initiative consensus statement on intraoperative blood pressure, risk and outcomes for elective surgery* (Sessler et al. 2019) acknowledge these points, pragmatically recommending avoidance of hypotensive episodes with MAP <60–65 mmHg. Considering the disparity between pooled observational data indicating increasing risk of organ injury beginning at MAPs as high as 80 mmHg and the disappointing results of RCTs to date, the authors' conclusion is that an intraoperative MAP of 65 mmHg should be considered an absolute minimum in most patients and that certain patients likely benefit from targeting higher intraoperative MAPs. At the moment, this remains a clinical decision call based on patient comorbidities, preoperative blood pressure (acknowledging the shortcomings of using preoperative BP measurements as a baseline (Joshi et al. 2012)), and gestalt. The central question in clinical practice and future research is how we may determine the optimal perioperative blood pressure in individual patients.

Etiologies of Intraoperative Hemodynamic Derangements

Putting the issue of the optimal target BP threshold aside, another central question is *how* to go about avoiding or correcting intraoperative hypotension. As the common denominator of circulatory insufficiency, arterial hypotension can be caused by a myriad of diverse etiologies. An important tenet in the intraoperative setting is to use an inductive approach, using the response to treatment as a diagnostic tool. Considering the unique etiologies of intraoperative circulatory compromise, it can be helpful to use a systematic approach to rule out the common causes of hypotension:

- Hypovolemic: bleeding, third spacing, other fluid losses, excessive pneumoperitoneum.
- Distributive: deep anesthesia, anaphylaxis, sepsis.
- Cardiogenic: deep anesthesia, acute cardiac pathology.
- Obstructive: tension pneumothorax/severe breath stacking, pulmonary embolism spectrum, pericardial tamponade.

Treatment of Intraoperative Hemodynamic Derangements

Fluid Therapy

Hypovolemia is one of the most common causes of intraoperative circulatory insufficiency (usually multifactorial due to venous pooling and intraoperative fluid losses, exacerbated by positive pressure ventilation). In patients with hemodynamic

derangements, the current fluid administration paradigm is to give the *optimal* amount of fluids, balancing the risks of under-resuscitation (organ hypoperfusion) with the those of over-resuscitation (secondary organ dysfunction due to volume overload), as both extremes are associated with an increased incidence of AKI (Brandstrup 2018; Myles et al. 2018).

The central question when considering administering fluids is whether the primary hemodynamic disturbance is hypovolemia, i.e., whether the patient shows signs of hypoperfusion *and* is presumed to be volume-responsive (i.e., a fluid bolus is likely to augment stroke volume). It is important to recognize that volume responsiveness per se does not indicate hypovolemia – the diagnosis of hypovolemia is a clinical assessment and an integration of multiple parameters. For an excellent evidence-based consensus statement on perioperative fluid therapy, please refer to the statement of the International Fluid Optimization Group (Navarro et al. 2015).

Volume responsiveness may be evaluated in a multitude of ways. In patients with invasive arterial BP monitoring, the respiratory variability of the pulse pressure (pulse pressure variability, PPV) or the systolic BP (systolic BP variability, SPV) may be used. In patients with pulse contour analysis monitoring (e.g., PiCCO), the stroke volume variability (SVV) may be used to predict volume responsiveness (Navarro et al. 2015). In patients with cardiac output monitoring in place (pulse contour analysis, transpulmonary thermodilution, or pulmonary artery catheter), volume responsiveness may be evaluated using a passive leg raise (PLR) or administration of a mini-fluid bolus of 250 ml while observing the change in CO. In critically ill patients, the central venous pressure (CVP) should be monitored continuously, as an incipient rise in CVP can be a harbinger of right ventricular volume overload and failure and should raise questions as to the possibility of over-resuscitation. Regardless of the monitoring modalities used, it is imperative to be aware of their respective limitations (e.g., irregular heart rhythms when using SVV, PPV, and SPV, rapid changes in vascular tone/acute hemodynamic instability when using pulse contour analysis, etc.).

The fluid of choice in most patients will consist initially of balanced crystalloids. In select patients, colloids (e.g., albumin 5%) may be administered as an adjunct. Our practice is to avoid starch-based colloid solutions due to the black box warnings in effect in Europe/the USA and the concerns regarding their nephrotoxicity in septic shock (Wiedermann and Eisendle 2017), even if data in the perioperative setting are not as clear-cut (Gillies et al. 2014). Blood products should be administered with restraint, with a transfusion threshold of 7–8 g/dl in the absence of contraindications for a restrictive transfusion threshold (Shander et al. 2012).

Vasopressors

In patients with disturbed vasomotor tone fitting the distributive hemodynamic profile (e.g., as a side effect of anesthesia or in sepsis), vasopressors are the therapy of choice. Noradrenaline, ephedrine, and phenylephrine are typical frontline vasopressors. In hypovolemic patients, the primary therapy remains fluid administration; however, vasopressors are frequently administered as a temporizing measure until euvolemia is reached. Furthermore, in critically ill patients or the elderly, one must

anticipate their diminished adrenergic reserves and hence the need for higher vasopressor doses. Finally, care should be given to avoid normalizing BP at the cost of cardiac output. This can particularly be a concern with pure alpha-agonists such as phenylephrine.

Inotropes

In some patients, hemodynamic derangements will be due to a cardiogenic or obstructive cause. Often the diagnosis will have been made by transthoracic/transesophageal echocardiography or by means of extended hemodynamic monitoring. Depending on the acuity of the situation and the hemodynamic profile, adrenaline or dobutamine/noradrenaline can be indicated. In cases of suspected anaphylaxis, adrenaline should be administered as a first-line inopressor without delay. Patients requiring administration of inotropes should – if not done already – have extended hemodynamic monitoring instituted (e.g., transpulmonary thermodilution).

Avoidance of Venous Congestion

In the context of hemodynamic derangements, one should not forget that for the kidneys and brain, perfusion pressure is normally the difference between mean arterial and central venous pressures (CVP). High CVPs can be the result of excessive fluid administration, of backward heart failure, or of excessive intraabdominal or intrathoracic pressures during laparoscopy resp. thoracoscopy. In the context of organ dysfunction and high CVPs, consideration should be given to offloading of the venous circulation by improving ventricular function and/or, primarily postoperatively in the case of volume overload, cautiously diuresing. Echocardiography is central to establishing the primary hemodynamic problem.

Future Directions

Research Priorities in Perioperative Medicine

In our opinion, two central questions in perioperative medicine demand our attention: What is the pathophysiology of perioperative organ injury and how could effective interventions be designed to reduce the burden of postoperative complications?

Understanding Perioperative Organ Injury: Moving from Populations to Individuals

Large case-control and cohort studies are important first steps in identifying risk factors for adverse outcomes, like perioperative organ injury. However, basing interventional trials on such data is problematic. When investigating complex, multifactorial clinical entities, an understanding of the involved pathophysiology is of paramount importance. The various patient phenotypes in play may only become

apparent with deeper insight into the underlying biological mechanisms, with potentially far-reaching implications for the design of subsequent RCTs (Girbes and Grooth 2019). Hence, what is needed is much more granular characterization of perioperative organ injury trajectories. To accomplish this, biomarkers and technologies both old and new will need to be applied in novel ways.

Biomarkers Poised to Transform Perioperative Medicine

A group of biomarkers that has the potential to revolutionize our understanding of perioperative organ injury are the non-coding ribonucleic acids (ncRNA), which include microRNAs (miRNAs). The perioperative implications of miRNAs have been excellently reviewed in other articles (Neudecker et al. 2016); however, in summary, they are short, non-coding RNA molecules whose primary role is post-transcriptional regulation of gene expression. Involved in the regulation of a myriad of cellular processes, miRNAs are important cellular regulators in both states of health and disease. Importantly, with their upstream location in disease processes, they are highly attractive biomarkers with the potential to detect cellular stress before organ injury sets in, as manifested by rises in classical biomarkers. This has been demonstrated for perioperative myocardial injury (May et al. 2020). Furthermore, with growing understanding of the involved cellular cascades and the optimization of miRNA delivery, modulation of miRNAs has the potential to one day become a therapeutic modality (Neudecker et al. 2016; Rupaimoole and Slack 2017; Brandenburger and Lorenzen 2020).

Preventing Perioperative Organ Injury: Leveraging Not Fearing Heterogeneity Through Individualization

Finally, with a deeper understanding of the putative mechanisms of perioperative organ injury, interventional trials directing the right individualized interventions to the right patients will finally be possible. Hot areas of research will include developing strategies for personalized perioperative hemodynamic management and hemostatic optimization, investigation of novel postoperative biomarker surveillance paradigms, and elucidation of possible therapies for patients at risk for or sustaining postoperative organ injury.

Conclusion

Postoperative biomarker release is much more than just a laboratory abnormality – it reflects organ injury and can be a harbinger of potentially devastating complications to come. Mounting evidence indicates that what we do perioperatively matters. Although ridden with risk, let us not forget that the perioperative phase is also a chance to improve the outcomes of our patients. Avoiding intraoperative hemodynamic derangements and major bleeding while ensuring oxygen delivery are strategies likely to help reduce the burden of perioperative organ injury. The adoption of biomarkers in the perioperative setting has unmasked a problem of a scope we could never have imagined. However, only through critical appraisal of our practices will

we succeed in making perioperative care safer. In the words of Albert Einstein, "*In the middle of difficulty lies opportunity.*"

Applications to Prognosis

In this chapter, we've reviewed the pathophysiology of perioperative organ injury following noncardiac surgery and the intraoperative factors associated with it. It is likely that the many of the same principles and strategies discussed have applicability in the postoperative phase and other clinical settings.

Mini-Dictionary of Terms

- Acute kidney injury: Acute and usually reversible drop in the glomerular filtration rate, diagnosed as a rise in serum creatinine and/or drop in urine output.
- Autoregulation: The capacity of an organ to maintain a (nearly) constant blood flow with varying perfusion pressures.
- Mean arterial blood pressure (MAP): The average blood pressure in the arterial system throughout the cardiac cycle. In contrast to the systolic and diastolic components of the blood pressure, the MAP is one of the primary determinants of organ perfusion (= MAP – post-capillary pressure).
- Myocardial injury: A high-sensitivity troponin value above the 99th percentile of the upper reference limit (URL) for the used assay. The presence or absence of a troponin rise/fall determines whether myocardial injury is acute or chronic in nature.
- Organ injury: The biochemical and functional manifestations in an organ system following an injurious stimulus.

Key Facts of Perioperative Organ Injury

- Biomarker release is central to the diagnosis of perioperative organ injury.
- Perioperative organ injury complicates noncardiac surgery frequently. The two organs most commonly affected are the heart and kidney.
- Myocardial injury is estimated to occur in 15–20% of patients at cardiovascular risk undergoing major noncardiac surgery.
- Acute kidney injury has a variable incidence depending on the type of surgery performed, ranging from 7% to over 50%.
- Both myocardial and kidney injuries have prognostic relevance, being associated with short- and long-term morbidity and mortality.
- The most important intraoperative contributors to perioperative organ injury are major bleeding and hemodynamic derangements.
- A strategy of prevention, ensuring adequate preoperative risk stratification and optimization as well as proactive intraoperative managementtargeted at avoiding

bleeding and hemodynamic disturbances, may help in reducing the incidence of perioperative organ injury.

Summary Points

- Postoperative biomarker release signifies organ injury and should be taken seriously, as such events are predictive of future major adverse cardiovascular events to come.
- Perioperative organ injury occurs in over one in six patients following major noncardiac surgery, most commonly affecting the kidneys and heart.
- A central mechanism involved in perioperative organ injury is an imbalance in oxygen supply and demand.
- Intraoperative factors predisposing to oxygen supply and demand mismatch include major bleeding, extremes of blood pressure (hypo- and hypertension), and tachycardia.
- Particularly major bleeding and arterial hypotension are strongly associated with postoperative adverse outcomes. However, due to the observational nature of much of the data, to what extent perioperative outcomes can be improved by prevention of these events remains unclear.
- Nonetheless, based on the current data, it is considered standard of care to minimize the risk of perioperative bleeding, e.g., by instituting patient blood management in qualifying patients, as well as avoiding intraoperative hypotension.

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Biomarkers in Neonatal Brain Injury: Interpreting Research into Clinical Practice

25

Nikolaos Efstathiou

Contents

Abstract

This chapter synopsizes all clinically relevant information found in the literature regarding brain injury in preterm and full-term neonates for the two most studied biomarkers, S100B and NSE. Biomarker's prognostic ability and levels in cord blood, serum, urine, and saliva, in healthy and diseased infants, that are reported

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among different studies are brought together and correlated with each other, so that similarities will lead to practical conclusions for the bedside clinician and the future researcher. S100B and NSE seem to be reliable biomarkers for neonatal brain injury in most studies. They correlate well with neuroimaging and long-term neurodevelopmental outcome. S100B is the most studied and appears superior to NSE. Serum is probably the most suitable sample for clinical use. Pitfalls can be avoided when S100B and NSE are used in clinical practice by having control measurements in healthy neonates comparable with ones in bibliography and skipping hemolyzed samples. These two biomarkers are able to help distinguishing neonates of higher risk of adverse long-term outcome in an early phase, so additional neuroprotective strategies and therapeutic protocols can be applied.

Keywords

Neonate · Brain · Injury · Preterm · Full-term · Encephalopathy · Asphyxia · Biomarker · S100B · NSE

Abbreviations

aEEG	Amplitude-integrated electroencephalography
BBB	Blood–brain barrier
CNS	Central nervous system
CP	Cerebral palsy
CS	Cesarean section
CSF	Cerebrospinal fluid
CT	Computed tomography scan
EoP	Encephalopathy of prematurity
GA	Gestational age
H/HT	Therapeutic hypothermia
HIE	Hypoxic ischemic encephalopathy
IVH	Intraventricular hemorrhage
MRI	Magnetic resonance imaging
NICU	Neonatal intensive care unit
PA	Perinatal asphyxia
PET	Positron emission tomography scan
PVL	Periventricular leukomalacia
RDS	Respiratory distress syndrome
SHC	Selective head cooling
WBC	Whole-body cooling

Introduction

Neonatal brain injury is always a severe clinical situation that requires hospitalization in the NICU and advanced therapeutic approach. It has major long-term consequences for the patient, the family, and the society. Therefore, early diagnosis, close follow-up, and appropriate therapeutic strategies are of great value.

Most centers rely on clinical examination and neuroimaging to identify newborns of high risk. However, clinical examination is subjective, changes over time, and is often confounded by medications and medical support devices. On the other hand, MRI – although remains the gold standard for diagnosis of brain damage – has limited value in the first 24 h of life, a critical period when key therapeutic decisions must be made. Additionally, it usually presupposes the transport of a critically ill neonate and therefore it takes place after the immediate postnatal period, leaving crucial time period without objective way to distinguish infants of higher risk. aEEG is also used for prognosis but requires special equipment and its predictive value is modest. Also, its interpretation is subjective, requires expertise, and several confounding factors are involved (medical interventions, sedatives, therapeutic hypothermia, etc.). Especially regarding preterms, neuroimaging diagnosis of periventricular lesions are often nonspecific or delayed even weeks, whereas clinical examination is not a criterion for latter neurodevelopmental outcome. Consequently, brain injury can exist subclinically, when imaging is still silent and symptoms are being hidden by sedation. Therefore, there is an urge to find new markers to early prognose brain lesions and outcomes, to individualize assessment of treatment efficacy and identify newborns who are failing to respond to therapeutic measures (e.g., cooling), newborns who might benefit from adjuvant therapy in the near future (as neurotrophic factors or stem cells). Criteria of ideal biomarkers are (a) capability to provide objective quantitative measures, (b) good reproducibility, (c) measurement in an easily accessible biological fluid in a noninvasive way, (d) having a relatively small half-life, allowing repeated measurement for close follow-up, and (e) measured by an assay that is easy to perform, rapid, inexpensive, and commercially available (Gazzolo et al. 2009; Massaro et al. 2012, 2018).

Although brain injury biomarkers have proven their value in adults and are already in clinical use, the diversity of reported results in neonatology during the last three decades hampers clinical utility. In this chapter, we reviewed the literature for the two most studied biomarkers, S100B and NSE, aiming to synopsize all useful clinical information and assist the interpretation of research data into clinical practice.

S100B

S100B is mainly concentrated in astrocytes and other glial cell types (such as oligodendrocytes, Schwann cells, ependymal cells, retinal Muller cells, enteric glial cells). S100B intervenes in glial proliferation, neuronal differentiation, maturation, as well as other neuroprotective actions at very low (nanomolar) extracellular concentrations through calcium homeostasis and signal transduction. At higher (micromolar) levels, S100B has apoptotic and neurotoxic effects (Gazzolo et al. 2005a; Costantine et al. 2011). It is detectable in several biological fluids (CSF, serum, urine, saliva, amniotic fluid, breast milk) since its small molecular weight allows passage through BBB, especially in cases of CNS injury. It is increased in both acute (traumatic brain injury, ischemic encephalopathy) or chronic brain

damage (degenerative diseases, congenital or intrauterine disorders, psychiatric disorders). Sources outside CNS have also been reported (muscle, kidney, heart, and adipose tissue), that could theoretically affect reliability of results in infants with multiorgan systemic disease, but their clinical significance is questioned. S100B has a half-life of 30–100 min and is eliminated mainly by the kidneys (Serpero et al. 2013; Satriano et al. 2017; Michetti et al. 2019).

S100B in Neonatal Brain Injury

S100B protein is extensively used in research since the late 1990s, and it is considered as one of the most reliable biomarkers. Despite the plethora of published studies, the diversity among them (regarding specimen, timing, and clinical definitions) and especially the small size of the cohorts have not allowed definite conclusions to be drawn, that would allow clinical utility for the bedside neonatologist.

Summarized results of different studies in the literature are presented in the following sections. Tables 1 and 2 present S100B levels in preterm and full-term newborns, respectively, in both healthy controls and neonates with brain injury, in different specimens and time points, whereas Table 3 presents prognostic ability of the biomarker. Figures 1 and 2 illustrate the results, showing the serum kinetics of S100B.

S100B in CSF

Lumbar puncture is considered an invasive or even contraindicated procedure in unstable patients, as neonates in NICU. Therefore, it is not a routine procedure. It is not easily collected and not appropriate if repeated measurements for continuous follow-up are required.

HIE: Blennow et al. (2001) and Sun et al. (2012) indicated possible prognostic usefulness for S100B: CSF levels in the first 4 days of life were significantly higher in neonates with HIE (vs. controls), in ones that were not receiving hypothermia or had adverse neurological long-term outcome (Table 2).

EoP: Likewise, Whitelaw et al. (2001) reported discrete higher levels in preterms with IVH/grade 3 and even higher in IVH/grade 4, as well as in ones with adverse long-term outcome (Table 1), but no correlation with shunt dependence.

S100B in Cord Blood

HIE: Although it is highly intriguing to have a reliable prognostic marker at the time a baby is born, this is usually not the case in clinical practice. Encephalopathy is a phenotype of heterogenous origin and timing at the perinatal period. It is obvious that if asphyxia (of any cause) begins at the latter stages of labor and delivery or even immediate after that, brain injury markers would not be elevated in the cord blood,

Table 1 S100B levels (μg/l) in different biological fluids and time points in encephalopathy of prematurity

GA	Specimen	Collection time	Controls (Group A)	PVL (Group B)	IVH (no disability) (Group C)	IVH 3 (Group D)	IVH 4 (Group E)	Other (Group F)	Group comparison[a] (p)	Reference
24–28 w	Serum	2–6 h						**Various or no disability** 4.24 (1.7–8)*[$n = 8$]		(Ruetzler et al. 2006)
	Urine	1–12 h						**Various or no disability** 8.02 (0.24–44.9)*[$n = 8$]		(Ruetzler et al. 2006)
27	Serum	1 d		5.51 ± 6.43[b][$n = 11$]					**B/C/D/E vs. A**(30w)$^{(0.06)}$	(Efstathiou et al. 2012, 2015)
				No-D/CP:1.86 (1.81)*[$n = 4$]				**D/CP:**7.63 (18.5)*[$n = 7$]		
		3 d		1.69 ± 1.48[b][$n = 10$]						
				No-D/CP:0.99 (0.62)*[$n = 5$]				**D/CP:**2.02 (4.26)*[$n = 5$]		
				1.53 ± 0.69[b][$n = 8$]					**B vs. A (30w)**$^{(<0.05)}$	(Chiang et al. 2015)
		7 d		2.68 ± 0.90[b][$n = 8$]					**B vs. A (30w)**$^{(<0.05)}$	
		9 d		1.5 ± 1.27[b][$n = 9$]						(Efstathiou et al. 2012, 2015)
				No-D/CP:0.99 (0.74)*[$n = 4$]				**D/CP:**1.3 (3.94)*[$n = 5$]		
		14 d		3.79 ± 1.24[b][$n = 8$]					**B vs. A (30w)**$^{(<0.05)}$	(Chiang et al. 2015)
		18 d		1.25 ± 0.93[b][$n = 8$]						(Efstathiou et al. 2012, 2015)
				No-D/CP:1.19 (1.09)*[$n = 4$]				**D/CP:**1.23 (2.73)*[$n = 4$]		
		21 d		3.18 ± 1.26[b][$n = 8$]					**B vs. A (30w)**$^{(<0.05)}$	(Chiang et al. 2015)

(continued)

Table 1 (continued)

GA	Specimen	Collection time	Controls (Group A)	PVL (Group B)	IVH (no disability) (Group C)	IVH 3 (Group D)	IVH 4 (Group E)	Other (Group F)	Group comparison[a] (p)	Reference
		45 d		1.39 ± 0.28[b] [$n = 3$]						(Efstathiou et al. 2012, 2015)
28 w	CB							**D:**0.47 (0.31–0.93)**[$n =$ 25]	**CP vs. controls (30w)**$^{(<0.05)}$	(Costantine et al. 2011)
								CP:0.85 (0.53–1.33)**[$n =$ 16]		
29	CSF		0.82 (0.39–1.09)*[$n = 4$]			3.56 (2.19–9.73)*[$n =$ 18]	9.85 (2.19–36)*[$n =$ 18]	**N(LT):**9.85 (2.19–36)*[$n =$ 18]	**A vs. E, A vs. E,** and **D vs. E**$^{(<0.05)}$	(Whitelaw et al. 2001)
30	CB		0.5 (0.31–0.85)**[$n =$ 82]							(Costantine et al. 2011)
	Serum	1 d	1.41 ± 0.8[b] [$n = 27$]							(Efstathiou et al. 2012, 2015)
			4.48 (6.69)**[$n =$ 29]	5.33 (9.29)**[$n = 29$]					**A vs. B/C/D/E**$^{(<0.05)}$	(Metallinou et al. 2020)
			4.5 (3.67)**[$n = 50$]			**D/IVH2-4:**20.66 (10.6)**[$n = 8$]			**A/B/C vs. D/E/F**$^{(<0.001)}$	
		2 d	1.88 (1.55)**[$n =$ 29]	3.44 (4.68)**[$n = 29$]					**A vs. B/C/D/E**$^{(<0.05)}$	
			2.14 (2.02)1.49**[$n = 50$]			**D/IVH2-4:**11.57 (16.56)**[$n = 8$]			**A/B/C vs. D/E/F**$^{(<0.001)}$	
		3 d	1.29 (0.91)**[$n =$ 29]	1.96 (2.64)**[$n = 29$]					**A vs. B/C/D/E**$^{(<0.005)}$	
			1.49 (0.86)**[$n = 50$]			**D/IVH2-4:**9.53 (11.85)**[$n = 8$]			**A/B/C vs. D/E/F**$^{(<0.001)}$	
			1.11 ± 0.59[b] [$n =$ 27]							(Efstathiou et al. 2012, 2015)

			0.70 ± 0.28[b][n = 14]							(Chiang et al. 2015)
		7 d	0.93 ± 0.44[b][n = 14]							
		9 d	1.42 ± 0.44 [b][n = 25]							(Efstathiou et al. 2012, 2015)
		14 d	0.70 ± 0.29[b][n = 14]							(Chiang et al. 2015)
		18 d	1.45 ± 0.51[b][n = 21]							(Efstathiou et al. 2012, 2015)
		21 d	0.52 ± 0.20[b][n = 14]							(Chiang et al. 2015)
		45 d	1.23 ± 0.36[b][n = 14]							(Efstathiou et al. 2012, 2015)
	Urine	1st	0.67(0.1–2)[cd][n = 121]		9.5 (1.7–15.6)[cd][n = 33]			**D:**20.3 (13.4–33)[cd][n = 11]	**F vs. A**, **C vs. A**$^{(<0.001)}$ **F vs. C**$^{(<0.01)}$	(Gazzolo et al. 2005a)
		24 h	0.9 (0.11–2.7)[cd][n = 121]		18.1 (1.44–39.3)[cd][n = 33]			**D:**35.3 (5.6–84)[cd][n = 11]	**F vs. A**, **B vs. A**$^{(<0.001)}$ **F vs. C**$^{(<0.01)}$	
		48 h	0.92 (0.12–3.79)[cd][n = 121]		25.5 (1.3–68)[cd][n = 33]			**D:**59.4 (21–113.7)[cd][n = 11]	**F vs. A**, **C vs. A**$^{(<0.001)}$ **F vs. C**$^{(<0.01)}$	
		96 h	0.9 (0.14–2.7)[cd][n = 121]		33.1 (1.8–96.5)[cd][n = 33]			**D:**81.1 (22–220)[cd][n = 11]	**F vs. A**, **C vs. A**$^{(<0.001)}$ **F vs. C**$^{(<0.01)}$	
31	Serum	24 h	5.24 ± 1.89[b][n = 204]	12.52 ± 4.60[b][n = 39]	8.93 ± 2.47[b][n = 69]				**C/D vs. A**, **B/C vs. A**, and **B/C vs. C/D**$^{(<0.05)}$	(Zhou et al. 2015)
		3 d	5.48 ± 0.97[b][n = 204]	11.64 ± 3.59[b][n = 39]	7.66 ± 1.85[b][n = 69]				**C/D vs. A**, **B/C vs. A**, and **B/C vs. C/D**$^{(<0.05)}$	

(continued)

Table 1 (continued)

GA	Specimen	Collection time	Controls (Group A)	PVL (Group B)	IVH (no disability) (Group C)	IVH 3 (Group D)	IVH 4 (Group E)	Other (Group F)	Group comparison[a] (p)	Reference
		7 d	5.57 ± 0.59[b][n = 204]	12.73 ± 4.82[b][n = 39]	6.25 ± 0.71[b][n = 69]				**B/C vs. A**$^{(<0.05)}$ **B/C vs. C/D**$^{(<0.05)}$	
		14 d	4.81 ± 0.78[b][n = 204]	10.91 ± 4.32[b][n = 39]	5.13 ± 1.43[b][n = 69]				**B/C vs. A, B/C vs. C/D**$^{(<0.05)}$	
32	CBv		0.92 (0.6–2.64)[***][n = 87]	2.07 (1.13–4.27)[***][n = 43]					**A vs. B/C/D/E**$^{(<0.0005)}$	(Lu et al. 2018)
	AF	Labor	1.22 (0.72–1.76)[***][n = 87]	1.24 (0.76–2.45)[***][n = 43]					A vs. B/C/D/E$^{(N/S)}$	
	Serum	24 h	0.6 ± 0.3[b][n = 10]	**PA (88%PVL):** 5.7 ± 2.9[b][n = 32]					**A vs. B**$^{(<0.05)}$	(Distefano et al. 2002)
		7 d	0.7 ± 0.2[b][n = 10]	**PA (88%PVL):** 3.3 ± 2.4[b][n = 32]					**A vs. B**$^{(<0.05)}$	
		21 d	0.6 ± 0.4[b][n = 10]	**PA (88%PVL):** 2.2 ± 1.3[b][n = 32]					**A vs. B**$^{(<0.05)}$	
33	Urine	1st			**noGC:**1.69 ± 0.29[n = 35],**GCx1:**2.91 ± 1.04[n = 19], **GCx2:**0.78 ± 0.29[b][n = 16]				**GCx2 vs. GCx1**$^{(<0.05)}$ **GCx2 vs. noCG**$^{(<0.05)}$ GCx1 vs. noGC$^{(NS)}$	(Sannia et al. 2010)
		24 h			**noGC:**1.68 ± 0.31[n = 35],**GCx1:**3.56 ± 1.27[n = 19], **GCx2:**1.03 ± 0.56[b][n = 16]					
		48 h			**noGC:**2.38 ± 0.57[n = 35], **GCx1:**3.77 ± 1.49[n = 19], **GCx2:**1.4 ± 0.89[b][n = 16]					
		72 h			**noGC:**2.13 ± 0.63[n = 35], **GCx1:**3.57 ± 1.55[n = 19], **GCx2:**1.46 ± 0.9[b][n = 16]					
<34	Serum	1 d	2.46 ± 0.77[b][n = 43]	3.82 ± 0.68[b][n = 78]					**A vs. B**$^{(<0.05)}$	(Huang et al. 2015)
		3 d	2.61 ± 0.91[b][n = 43]	4.41 ± 0.91[b][n = 78]					**A vs. B**$^{(<0.01)}$	

		7 d	2.93 ± 1.20[b][n = 43]	5.78 ± 1.54[b][n = 78]					**A vs. B**[(<0.001)]	
		14 d	2.38 ± 0.87[b][n = 43]	3.27 ± 0.86[b][n = 78]					A vs. B[(NS)]	
34	CB		**noGC:**1.21 (0.24)[b] [n = 15]		**noGC:**3.67 ± 1.20[b][n = 24]				n**oGS(A) vs. C**[(<0.001)] noGC (A/C) vs. GC[(NS)] noGS(A) vs. GC[(NS)]	(Gazzolo et al. 2003a)
			GC:1.11 (0.24)[b] [n = 39]							
	Urine	0–6 h	♂**:** 0.2 (0.03–0.2)[**][n = 141]						**Male vs. female**[(<0.01)]	(Sannia et al. 2013)
			♀**:**0.75 (0.2–0.85)[**][n = 136]							
		1st	**noGC:**0.59 (0.23)[b] [n = 39](controls and IVH)						**noGC (controls only) vs. GS**[(<0.01)] **noGS (control/ IVH) vs. GS**[(<0.001)]	(Gazzolo et al. 2003a)
			GC:0.11 (0.08)[b] [n = 39]							
		5 d	**noGC:**0.28 (0.19)[b] [n = 39](controls and IVH)						**noGC (controls only) vs. GS**[(<0.01)] **noGS (control/ IVH) vs. GS**[(<0.001)]	
			GC:0.27 (0.11)[b][n = 39]							

(continued)

Table 1 (continued)

GA	Specimen	Collection time	Controls (Group A)	PVL (Group B)	IVH (no disability) (Group C)	IVH 3 (Group D)	IVH 4 (Group E)	Other (Group F)	Group comparison[a] (p)	Reference
35	Serum	3 d	0.55 ± 0.09[b] [n = 17]							(Chiang et al. 2015)
	Urine	0–6 h	♂: 0.02 (0.02–0.2)** [n = 141]						**Male(♂) vs. female (♀)**$^{(<0.01)}$	(Sannia et al. 2013)
			♀:0.21 (0.11–1.35)** [n = 136]							
36	Urine	0–6 h	♂: 0.19 (0.15–0.2)** [n = 141]						**Male(♂) vs. female (♀)**$^{(<0.01)}$	
			♀:0.3 (0.21–0.7)** [n = 136]							
34–37	CB		0.42 (0.32–0.62)** [n = 183] 95th percentile: 1.36							(Bouvier et al. 2020)

CB cord blood, *D* death, *GC* antenatal glucocorticoid supplementation, *GCx1* half-course antenatal glucocorticoid supplementation, *GCx2* complete-course antenatal glucocorticoid supplementation, *noGC* no antenatal glucocorticoid supplementation, *N* neurologic abnormalities, *NS* not statistically significant, *LT* long-term, *n* number of infants (in cohort)

*Median (range)

**Median (quartiles)

***Median (lower-upper CI95%)

[a]Bold font (in "Groups comparison" column) = statistical significance is met

[b]Mean ±SD

[c]Multiples of median

[d]Mean (CI: 5–95)

Table 2 S100B levels (μg/l) in different biological fluids and time points in full-term neonates with HIE

Specimen	Collection time:	Detail	Controls (Group A)	PA, no Disability (Group B)	HIE 1 (Group C)	HIE 2 (Group D)	HIE 3 (Group E)	PA + (Group F)	Group comparison[a] (p)	Reference
CB			0.32 (0.18–5.27)*[n = 21]			0.52 (0.33–2.15)*[n = 6]	0.58 (0.23–3.22)*[n = 7]		**A vs. D/E**$^{(0.056)}$	(Zaigham et al. 2017)
		Art	0.31 (0.24–0.45)**[n = 113]	0.33 (0.19–0.63)**[n = 27]					A vs. B/C/D/E$^{(NS)}$	(Summanen et al. 2017)
		Art	1.05 (0.95–1.15)**[n = 25]		1.72 (1.48–2.08)**[n = 25]	3.61 (1.32–5.04)**[n = 15]			**C vs. D/E**$^{(<0.05)}$	(Qian et al. 2009)
					1.98 (1.48–2.99)**[n = 40]				**A vs. C/D/E**$^{(<0.05)}$	
		Art and ven	1.37 (0.2–3.02)* [n = 32]							(Thorngren-Jerneck et al. 2004)
		Art	0.34 (0.26–0.45)** 95th perc: 0.96[n = 200]							(Bouvier et al. 2020)
		Art	1.62 (1.25–2.09)**[n = 81]						art vs. ven$^{(N/S)}$	(Wirds et al. 2003)
		Ven	1.36 (1.01–1.81)**[n = 81]							
		Art	**nHem:** 1.1 (0.38–5.5)*[n = 58] **Hem:** 1.9 (0.75–16)*[n = 52]						**nHem vs. Hem**$^{(<0.001)}$	(Amer-Wåhlin et al. 2001)
		Ven	**nHem:** 0.98 (0.43–2.7)*[n = 65] **Hem:**1.4 (0.47–15)*[n = 47]						**nHem vs. Hem**$^{(<0.001)}$	

(continued)

Table 2 (continued)

Specimen	Collection time:	Detail	Controls (Group A)	PA, no Disability (Group B)	HIE 1 (Group C)	HIE 2 (Group D)	HIE 3 (Group E)	PA + (Group F)	Group comparison[a] (p)	Reference
			0.8 (0.7–1.0)**[n = 20]	1.5 (1.1–1.9)**[n = 22]		2.5 (1.5–3.7)**[n = 7]			**B/C/D/E vs. A**$^{(<0.0001)}$ **B/C vs. D/E**$^{(NS)}$	(Nagdyman et al. 2001)
			NL(8 h): 0.4 ± 0.05[b][n = 20]						**NL vs. PL/VD**$^{(<0.0001)}$ **NL vs. sCS**$^{(<0.05)}$ **PL/VD vs. sCS**$^{(<0.001)}$	(Schulpis et al. 2006)
			PL and **VD**: 0.67 ± 0.18[b][n = 18]							
			sCS: 0.31 ± 0.04[b][n = 22]							
PB	**0 h**	nH				7.37 (3.19–17.61)**[n = 84]			nH vs. WBC$^{(NS)}$	(Catherine et al. 2020)
		WBC				6.04 (1.61–17.04)*[n = 78]				
		nH and WBC				**no-N(LT):** 6.03 (1.75–17.8)**		**N(LT):** 8.64 (4.11–17.17)**	D/E vs. F$^{(NS)}$	
	1 h						9.5, 53.3[n = 2]			(Ruetzler et al. 2006)
	2 h	nH	1.6 (1.4–2.5)**[n = 20]	3.3 (2.2–8.4)**[n = 16]				**D/N(LT):** 3.6 (1.7–17)**[n = 1]	**B/F vs. A**$^{(<0.0001)}$ B vs. F$^{(NS)}$	(Nagdyman et al. 2003)
		nH		2.9 (1.8–4.7)** (n = 22)		17.0 (3.2–34.1)**[n = 7]			**B/C/D/E vs. A**$^{(<0.0001)}$ **B/C vs. D/E**$^{(<0.01)}$	(Nagdyman et al. 2001)
	6 h	nH	1.5 (1.2–2.3)**[n = 20]	2.5 (1.5–4.6)** [n = 16]				**D/N(LT):** 3.8 (1.9–27.6)**[n = 1]	**B/F vs. A**$^{(<0.001)}$ B vs. F$^{(NS)}$	(Nagdyman et al. 2003)
		nH		2.5 (1.6–3.8)**[n = 22]		27.6 (2.6–52.3)**[n = 7]			**B/C/D/E vs. A**$^{(<0.001)}$ **B/C vs. D/E**$^{(<0.05)}$	(Nagdyman et al. 2001)
		SHC	***S100^{c}(ng/l)*** *1.9 (0.7–4.7)**[n = 9]*		***S100^{c}(ng/l)*** *1.7 (0.8–25.6)**[n = 7]*	***S100^{c}(ng/l)*** *17.5 (5.6–60.4)**[n = 14]*			SHC vs. WBC$^{(N/S)}$ **SHC/ WBC vs. A**$^{(<0.05)}$	(Çelik et al. 2015)
		WBC				***S100^{c}(ng/l)*** *15.3 (7.7–86.9)**[n = 10]*				

		SHC/ WBC			***S100[c](ng/l) noN(LT):*** *22.4 (1.3–136.4)[**/f][n = 13]*			***D/N(LT)[c]:*** *13.5 (5.8–31)[**][n = 18]*	NS	
		H and nH				**No-severeN(LT):** 1.01 (0.39–14.30)[*][n = 18]		**severeN(LT)/D:** 18.22 (6.16–64.74)[*][n = 6]	**D/E vs. F**[(<0.001)]	(Roka et al. 2012)
		WBC				1.03 (0.52–52.90)[*][n = 13]			H vs. nH[(NS)]	
		nH				4.58 (0.39–64.74)[*][n = 11]				
			2.10 ± 0.12[b][n = 16]	3.47 ± 2.19[b][n = 19]	22.35 ± 26.12[b][n = 8]				**C/D/E vs. B**[(<0.0005)] **A vs. MS**[(<0.005)]	(Murabayashi et al. 2008)
			MS: 3.53 ± 0.36 [n = 6]							
	12 h		0.66 ± 0.31[n = 80]	**No-IVH:** 0.72 ± 0.39				**IVH:** 1.87 ± 0.60	**F vs. A** and **F vs. B**[(<0.05)]	(Gazzolo et al. 2002)
		nH	1.2 (1.1–1.5)[**][n = 20]	1.8 (1.5–2.9)[**][n = 16]				**D/N(LT):** 1.6 (1.3–2.4)[**][n = 11]	**B/F vs. A**[(<0.001)] B vs. F (NS)	(Nagdyman et al. 2003)
		nH		1.8 (1.5–2.3)[**][n = 22]		3.1 (1.3–23.1)[**][n = 7]			**B/C/D/E vs. A**[(0.001)] B/C vs. D/E[(NS)]	(Nagdyman et al. 2001)
		H and nH				**No-severe N(LT):** 0.7 (0.0–7.68)[*][n = 18]		**severeN(LT)/D:** 20.06 (3.0–31.82)[*][n = 6]	**D/E vs. F**[(<0.005)]	(Roka et al. 2012)
		WBC				0.77 (0.0–20.06)[*][n = 13]			H vs. nH[(NS)]	
		nH				1.04 (0.31–31.82)[*][n = 11]				
	1 d	nH	1.30 ± 0.21[b][n = 20]		1.91 ± 0.81[b][n = 28]				**A vs. C/D/E**[(<0.05)]	(Liu et al. 2013)
					1.50 ± 0.53[b][n = 16]	2.26 ± 1.45[b][n = 12]			**C vs. D/E**[(<0.05)]	
		nH		1.8 (1.0–4.5)[*][n = 5]	5.95 (1.4–118)[*][n = 16]	26 (0.2–120)[*][n = 19]	10.2 (0.5–64)[*][n = 10]			(Thorngren-Jerneck et al. 2004)
					No-N(LT): 5.5 (0.7–120)[*][n = 34]			**D:** 14.0 (0.5–60)[*][n = 7]		
								CP: 20.7 (0.2–64)[*][n = 9]		

(continued)

Table 2 (continued)

Specimen	Collection time:	Detail	Controls (Group A)	PA, no Disability (Group B)	HIE 1 (Group C)	HIE 2 (Group D)	HIE 3 (Group E)	PA + (Group F)	Group comparison[a] (p)	Reference
		nH			3.7 (1.9–8)**[n = 7]	3.7 (2.1–6.2)**[n = 6]	4.6 (2.6–7.6)**[n = 8]		NS	(Martins et al. 2006)
		H	1.03 ± 0.28^{b}[n = 11]		1.24 ± 0.62^{b}[n = 10]	1.13 ± 0.52^{b}[n = 5]	12.66 ± 11.39^{b}[n = 8]		**E vs. A, E vs. D**$^{(<0.05)}$	(Efstathiou et al. 2021)
						8.23 ± 10.48^{b}[n = 13]			**D/E vs. A**$^{(<0.05)}$	
						No-D/CP: 1.36 ± 1.21^{b}[n = 28]		**D/CP:** 15.42 ± 11.9^{b}[n = 6]	**D/E vs. F**$^{(0.001)}$	
		H				**No-N(ST):** 1.77 ± 2.01^{b}[n = 13]		**D/N(ST):** 3.71 ± 5.21^{b}[n = 18]	D/E vs. F$^{(NS)}$	(Alshweki et al. 2017)
						No-D: 1.97 ± 1.99^{b}[n = 25]		**D**: 6.75 ± 8.14^{b}[n = 6]	**D/E vs. F**$^{(<0.05)}$	
		H				0.5 (0.07–19)*[n = 50]			**Correlated** with MRI	(Massaro et al. 2018)
	24 h	nH			**No-N(LT):** 2.3 (0.4–110.0)*[n = 34]			**CP:** 3.8 (0.8–36.0)*[n = 9]		(Thorngren-Jerneck et al. 2004)
								D: 11.0 (0.4–71.3)*[n = 7]		
		nH	1.0 (0.9–1.4)**[n = 20]	1.6 (1.0–2.6)**[n = 22]		3.9 (1.2–9.0)**[n = 7]			**B/C/D/E vs. A**$^{(<0.05)}$ B/C vs. D/E$^{(NS)}$	(Nagdyman et al. 2001)
		nH		1.5 (0.9–2.6)**[n = 16]				**D/N(LT):** 1.9 (1.2–5.0)**[n = 11]	**B/F vs. A**$^{(<0.05)}$ B vs. F$^{(NS)}$	(Nagdyman et al. 2003)
		H and nH				**No-severeN(LT):** 0.48 (0.29–3.86)*[n = 18]		**severeN(LT)/D:** 4.12 (1.82–20.32)*[n = 6]	**D/E vs. F**$^{(<0.005)}$	(Roka et al. 2012)
		WBC				0.46 (0.29–4.66)*[n = 13]			H vs. nH$^{(NS)}$	
		nH				0.78 (0.37–20.3)*[n = 11]				
		nH and WBC				**No-N(LT):** 2.45 (1.31–9.37)**		**N(LT):** 4.51 (1.51–8.41)**	D/E vs. F$^{(NS)}$	(Catherine et al. 2020)

	2 d					**No-N(ST):** 0.86 ± 1.31[b][$n = 13$]	**D/N(ST):** 1.84 ± 3.88[b][$n = 16$]	D/E vs. F[(NS)]	(Alshweki et al. 2017)
						No-D: 1.34 ± 3.20[b][$n = 25$]	**D:** 1.77 ± 1.65[b][$n = 4$]	D/E vs. F[(NS)]	
				2.26 ± 1.25[b][$n = 19$]	9.00 ± 8.40[b][$n = 8$]			**C/D/E vs. B**[(<0.05)]	(Murabayashi et al. 2008)
		H and nH				**No-severeN(LT):** 0.45 (0.23–1.24)[*][$n = 18$]	**severeN(LT)/D:** 1.2 (1.04–1.91)[*][$n = 6$]	**D/E vs. F**[(<0.01)]	(Roka et al. 2012)
		WBC				0.36 (0.23–1.2)[*][$n = 13$]		**H vs. nH**[(<0.05)]	
		nH				0.61 (0.42–1.91)[*][$n = 11$]			
	3 d	H	1.06 ± 0.22[b][$n = 11$]		1.35 ± 1.17[b][$n = 9$]	1.04 ± 0.75[b][$n = 4$] / 3.98 ± 3.97[b][$n = 8$]		NS	(Efstathiou et al. 2021)
						3 ± 3.51[b][$n = 12$]		NS	
						No-D/CP: 1.13 ± 0.54[b][$n = 26$]	**D/CP:** 5.04 ± 4.08[b][$n = 6$]	D/E vs. F[(NS)]	
		nH			1.68 ± 0.62[b] [$n = 16$]	1.88 ± 0.49[b][$n = 12$]		C vs. D/E[(NS)]	(Liu et al. 2013)
		H and nH				**No-severeN(LT):** 0.52 (0.29–0.77)[*][$n = 18$]	**severeN(LT)/D:** 0.77 (0.67–1.2)[*][$n = 6$]	**D/E vs. F**[(<0.05)]	(Roka et al. 2012)
		WBC				0.43 (0.29–1.19)[*][$n = 13$]		H vs. nH[(NS)]	
		nH				0.70 (0.52–0.77)[*][$n = 11$]			
		H				**No-N(ST):** 0.48 ± 0.38[b][$n = 13$]	**D/N(ST):** 1.21 ± 2.14[b][$n = 14$]	**D/E vs. F**[(NS)]	(Alshweki et al. 2017)
						no-D: 0.81 ± 1.61[b][$n = 25$]	**D:** 1.49 ± 1.41[b][$n = 2$]	**D/E vs. F**[(NS)]	
		SHC	***S100[c] (ng/l)*** *1.5 (0.4–3.8)[*][$n = 9$]*		***S100[c] (ng/l)*** *1.6 (0.8–3.6)[**][$n = 7$]*	***S100[c] (ng/l)*** *2.9 (0.5–15.5)[**][$n = 14$]*		SHC vs. WBC vs. A vs. C[(NS)]	(Çelik et al. 2015)
		WBC				***S100[c] (ng/l)*** *9.5 (0.3–29.8)[**][$n = 10$]*			
		SHC/ WBC			***S100[c] (ng/l) No-N(LT):*** *3.5 (0.7–20.1)[**][$n = 13$]*		***D/N(LT)[c]:*** *1.9 (0.4–15.8)[**][$n = 18$]*	NS	
			nHem: 1.6 (0.48–9.7)[*][$n = 12$]						(Amer-Wåhlin et al. 2001)

(continued)

Table 2 (continued)

Specimen	Collection time:	Detail	Controls (Group A)	PA, no Disability (Group B)	HIE 1 (Group C)	HIE 2 (Group D)	HIE 3 (Group E)	PA + (Group F)	Group comparison[a] (p)	Reference
		nH				2.64 (0.87–5.97)**[$n = 84$]			nH vs. WBC$^{(NS)}$	(Catherine et al. 2020)
		WBC				2.19 (0.87–6.5)**[$n = 78$]				
		nH/ WBC				**No-N(LT):** 2.3 (0.54–6.5)**		**N(LT):** 1.75 (0.87–5.33)**	D/E vs. F$^{(NS)}$	
	4 d	nH			2.8 (1.5–4.8)**[$n = 7$]	2.0 (1.4–2.4)**[$n = 6$]	1.3 (1.2–2.9)**[$n = 8$]		NS	(Martins et al. 2006)
	5 d	H				0.41 (0.001–7.1)*[$n = 47$]			NS with MRI/N(LT)	(Massaro et al. 2018)
	6 d		2.28 ± 0.11^{b}[$n = 16$]	2.18 ± 0.97^{b}[$n = 19$]	3.11 ± 1.59^{b}[$n = 8$]				**A vs. MS**$^{(<0.05)}$	(Murabayashi et al. 2008)
			MS: 3.17 ± 0.37 [$n = 6$]							
	7 d	nH			1.77 ± 0.50^{b}[$n = 16$]	2.45 ± 0.99^{b}[$n = 7$]			**C vs. D/E**$^{(<0.05)}$	(Liu et al. 2013)
	1–7 d		(Stable over time) (0.66–3.33)^{e}[$n = 66$]							(Maschmann et al. 2000)
	9 d	H	0.97 ± 0.36^{b}[$n = 8$]		1.45 ± 1.01^{b}[$n = 8$]	0.92 ± 0.13^{b}[$n = 4$]	2.34 ± 3.31^{b}[$n = 6$]		NS	(Efstathiou et al. 2021)
						1.77 ± 2.58^{b}[$n = 10$]			NS	
						No-D/CP: 1.13 ± 0.66^{b}[$n = 22$]		**D/CP:** 3.01 ± 4^{b}[$n = 4$]	D/E vs. F$^{(NS)}$	
	18 d	H	1.31 ± 0.38^{b}[$n = 4$]		1.15 ± 0.19^{b}[n = 4]	0.82 ± 0.05^{b}[n = 4]	0.91 ± 0.17^{b}[$n = 5$]		NS	
						0.87 ± 0.13^{b}[$n = 9$]			NS	
						No-D/CP: 1.09 ± 0.28^{b}[$n = 14$]		**D/CP:** 0.79 (0.18)*[$n = 3$]	**D/E vs. F**$^{(<0.05)}$	

CSF	**1–4 d**	nH	0.5 (0.4–0.8)*[n = 8]		2.0 (0.25–66.3)*[n = 22]				**A vs. C/D/E**$^{(<0.005)}$	(Blennow et al. 2001)
	78 h	nH			1.92 ± 0.39^{b}[n = 28]				**nH vs. SHC**$^{(<0.05)}$	(Sun et al. 2012)
		SHC			0.81 ± 0.32^{b}[n = 23]					
					No-N(LT): 0.62 ± 0.15^{b} [n = 16]			**N(LT):** 1.01 ± 0.51^{b}[n = 7]	**C/D/E vs. F**$^{(<0.05)}$	
U	**2 h**	nH	0.11 ± 0.01^{b}[n = 96]	0.24 ± 0.06^{b}[n = 20]				**D/N(LT):** 1.92 ± 0.33^{b}[n = 18]	**F vs. B**$^{(<0.001)}$ **F vs. A**$^{(<0.001)}$	(Gazzolo et al. 2003b)
								D: 3.1 (3–4)*[n = 3]		
		nH	0.1 ± 0.09^{b}[n = 68]	1.09 ± 0.36^{b}[n = 44]				**D:** 3 (2.7–3.9)*[n = 3]	**B/C/D/E vs. A**$^{(<0.001)}$	(Gazzolo et al. 2004)
	3 h	nH	0.1 (0.15–0.23)***[n = 72]			**No-D:** 0.23 (0.17–0.31)^{d}[n = 48]		**D:** 3 (2.94–3.63)***[n = 12]	**F vs. A**$^{(<0.001)}$ **F vs. D/E**$^{(<0.001)}$	(Gazzolo et al. 2009)
	4 h	nH	0.11 ± 0.08^{b}[n = 68]	1.41 ± 0.54^{b}[n = 44]					**B/C/D/E vs. A**$^{(<0.001)}$	(Gazzolo et al. 2004)
	6 h	H	0.07 (0.06–0.08)**[n = 9]		0.11 (0.08–0.15)**[n = 7]	**SCH:** 0.08 (0.07–0.09)**[n = 14] **WBC:** 0.12 (0.07–0.15)*[n = 10]			NS	(Çelik et al. 2015)
					No-N(LT): 0.08 (0.07–0.15)**[n = 13]			**N(LT):** 0.08 (0.06–0.13)**[n = 18]	NS	
	8 h	nH	0.15 ± 0.02^{b}[n = 25]		0.41 ± 0.05^{b}[n = 31]	3.63 ± 1.04^{b}[n = 30]	4.18 ± 0.76^{b}[n = 17]		**C vs. A, D vs. A, and E vs. A**$^{(<0.01)}$ **D vs. C and E vs. C**$^{(<0.001)}$	(Liu et al. 2010)
					2.97 ± 0.30^{b}[n = 78]				**C/D/E vs. A**$^{(<0.01)}$	
		nH	0.11 ± 0.09^{b}[n = 68]	2.27 ± 1.06^{b}[n = 44]					**B/C/D/E vs. A**$^{(<0.001)}$	(Gazzolo et al. 2004)
	1–12 h	nH	0.4 (0.03–0.84)*[n = 8]				28.1, 28.4[n = 2]			(Ruetzler et al. 2006)

(continued)

Table 2 (continued)

Specimen	Collection time:	Detail	Controls (Group A)	PA, no Disability (Group B)	HIE 1 (Group C)	HIE 2 (Group D)	HIE 3 (Group E)	PA + (Group F)	Group comparison[a] (p)	Reference
	12 h	nH	$0.12 \pm 0.03^{b}[n = 96]$	$0.13 \pm 0.06^{b}[n = 20]$				**D/N(LT):** $2.78 \pm 1.71^{b}[n = 18]$	**F vs. B**$^{(<0.001)}$ **F vs. A**$^{(<0.001)}$	(Gazzolo et al. 2003b)
		nH	$0.11 \pm 0.11^{b}[n = 68]$	$2.78 \pm 1.45^{b}[n = 44]$					**B/C/D/E vs. A**$^{(<0.001)}$	(Gazzolo et al. 2004)
	16 h		$0.19 \pm 0.12^{b}[n = 68]$	$2.27 \pm 1.05^{b}[n = 44]$					**B/C/D/E vs. A**$^{(<0.001)}$	
	20 h		$0.2 \pm 0.11^{b}[n = 68]$	$2.78 \pm 1.46^{b}[n = 44]$					**B/C/D/E vs. A**$^{(<0.001)}$	
	24 h		$0.23 \pm 0.08^{b}[n = 68]$	$2.27 \pm 3.47^{b}[n = 44]$					**B/C/D/E vs. A**$^{(<0.001)}$	
		nH	$0.12 \pm 0.02^{b}[n = 96]$	$0.21 \pm 0.07^{b}[n = 20]$				**D/N(LT):** 4.75 ± 4.08 $[n = 18]$	**F vs. B**$^{(<0.001)}$ **F vs. A**$^{(<0.001)}$	(Gazzolo et al. 2003b)
		nH	0.05 $(0.12–0.19)^{***}(n = 72)$			**No-D:** 0.12 $(0.12–0.2)^{***}[n = 48]$		**D:** 4.25 $(3.52–6.27)^{***}[n = 12]$	**F vs. A**$^{(<0.001)}$ **F vs. D/E**$^{(<0.001)}$	(Gazzolo et al. 2009)
	1 d (0–24 h)	H				**No-N(ST):** $4.65 \pm 9.16^{b}[n = 13]$		**D/N(ST):**$10.58 \pm 14.82^{b}[n = 18]$	**D/E vs. F**$^{(<0.05)}$	(Alshweki et al. 2017)
						No-D: $5.85 \pm 8.73^{b}[n = 25]$		**D:** $17.44 \pm 22.49^{b}[n = 6]$	**D/E vs. F**$^{(<0.05)}$	
						No-MRIab: $4.49 \pm 9.14^{b}[n = 15]$		**MRIab:** $7.89 \pm 8.09^{b}[n = 10]$	**D/E vs. F**$^{(<0.05)}$	
	2 d	H				**No-N(ST):** $0.88 \pm 2.53^{b}[n = 13]$		**D/N(ST):** $5.16 \pm 7.63^{b}[n = 16]$	**D/E vs. F**$^{(<0.005)}$	
						No-D: $2.48 \pm 5.73^{b}[n = 25]$		**D:** $8.07 \pm 7.91^{b}[n = 4]$	D/E vs. F$^{(NS)}$	
						No-MRIab: $0.85 \pm 2.35^{b}[n = 15]$		**MRIab:** $4.92 \pm 8.23^{b}[n = 10]$	**D/E vs. F**$^{(<0.01)}$	
		nH	0.12 $(0.15–0.23)^{***}[n = 72]$			**No-D:** 0.21 $(0.17–0.29)^{***}[n = 48]$		**D:** 6 $(4.4–9.26)^{***}[n = 12]$	**F vs. A**$^{(<0.001)}$ **F vs. D/E**$^{(<0.001)}$	(Gazzolo et al. 2009)

		nH	$0.27 \pm 0.05^{b}[n = 68]$	$2.62 \pm 1.07^{b}[n = 44]$				**B/C/D/E vs. A**$^{(<0.001)}$	(Gazzolo et al. 2004)
		nH	$0.15 \pm 0.02^{b}[n = 25]$		$3.21 \pm 0.29^{b}[n = 78]$			**C/D/E vs. A**$^{(<0.01)}$	(Liu et al. 2010)
					$0.54 \pm 0.16^{b}[n = 31]$	$3.96 \pm 0.24^{b}[n = 30]$	$3.55 \pm 0.62^{b}[n = 17]$	**C vs. A, D vs. A,** and **E vs. A**$^{(<0.01)}$ **D vs. C** and **E vs. C**$^{(<0.001)}$	
	3 d	H				**No-N(ST):** $0.46 \pm 0.93^{b}[n = 13]$		**D/N(ST):** $1.81 \pm 3.60^{b}[n = 14]$	**D/E vs. F**$^{(<0.05)}$	(Alshweki et al. 2017)
						No-D: $0.80 \pm 2.35^{b}[n = 25]$		**D:** $5.63 \pm 3.96^{b}[n = 2]$	**D/E vs. F**$^{(<0.05)}$	
						No-MRIab: 0.41 ± 0.87^{b} $[n = 15]$		**MRIab:** $1.38 \pm 3.59^{b}[n = 10]$	NS	
		nH	0.12 ± 0.02^{b} $(n = 96)$	0.12 ± 0.04^{b} $(n = 20)$				**D/N(LT):** $5.93 \pm 1.63[n = 18]$	**F vs. B and F vs. A**$^{(<0.001)}$	(Gazzolo et al. 2003b)
		H	0.08 $(0.07–0.12)^{**}[n = 9]$		0.12 $(0.07–0.21)^{**}[n = 7]$	**SHC:** 0.08 $(0.07–0.15)^{**}[n = 14]$ **WBC:** 0.1 $(0.06–0.15)^{**}[n = 10]$			NS	(Çelik et al. 2015)
					No-N(LT): 0.11 $(0.07–0.21)^{**}[n = 13]$			**N(LT):** 0.09 $(0.06–0.12)^{**}[n = 18]$	NS	
		nH	$0.29 \pm 0.07^{b}[n = 68]$	$3.2 \pm 1.13^{b}[n = 44]$						(Gazzolo et al. 2004)
					4.61 ± 0.40^{b} $[n = 78]$				**C/D/E vs. A**$^{(<0.01)}$	(Liu et al. 2010)
		nH	$0.30 \pm 0.04^{b}[n = 25]$		$0.97 \pm 0.39^{b}[n = 31]$	$5.78 \pm 0.31^{b}[n = 30]$	$6.69 \pm 0.86^{b}[n = 17]$		**C vs. A, D vs. A,** and **E vs. A**$^{(<0.01)}$ **D vs. C and E vs. C**$^{(<0.001)}$	
	4 d	nH	0.2 $(0.16–0.24)^{***}[n = 72]$			**No-D:** 0.25 $(0.2–0.31)^{***}[n = 48]$		**D:** 4.45 $(4.22–11.64)^{***}[n = 12]$	**F vs. A**$^{(<0.001)}$ **F vs. D/E**$^{(<0.001)}$	(Gazzolo et al. 2009)

(continued)

Table 2 (continued)

Specimen	Collection time:	Detail	Controls (Group A)	PA, no Disability (Group B)	HIE 1 (Group C)	HIE 2 (Group D)	HIE 3 (Group E)	PA + (Group F)	Group comparison[a] (p)	Reference
Saliva	**Birth**							**D:** 40 (19.8–79.6)[f*][$n = 4$]		(Gazzolo et al. 2015)
	Birth–24 h		1.0 (0.9–1.2)[f***][$n = 244$]		**No-N(LT):** 1.0 (0.5–1.2)[f***][$n = 15$]			**N(LT):**23.8 (4.2–39.6)[f***][$n = 33$]	**F vs. A**$^{(<0.001)}$ **F vs. C/D/E**$^{(<0.001)}$ A vs. C/D/E$^{(NS)}$	
	48–96 h		1.1 (1.02–1.2)[f***][$n = 244$]		**No-N(LT):** 1.0 (0.9–1.1)[f***][$n = 15$]			**N(LT):** 1.0 (0.9–1.1)[f***][$n = 33$]	F vs. A, F vs. C/D/E, and A vs. C/D/E$^{(NS)}$	

AF amniotic fluid, *art* arterial, *BD* brain damage, *D* death, *Hem* hemolyzed specimen, *LT* long-term, *MS* meconium stain, *MRIab* abnormal brain MRI, *N* neurologic abnormalities, *nH* normothermia (no therapeutic hypothermia), *nHem* non-hemolyzed specimen, *NL* normal labor, *NS* not statistically significant, *PL* prolonged labor (16 h), *Sa* saliva, *sCS* scheduled cesarean section, *ST* short-term, *VD* vaginal delivery, *ven* venous, *n* number of infants (in cohort)

[*]Median (range)

[**]Median (quartiles)

[***]Median (lower-upper CI95%)

[a]Bold font (in "Groups comparison" column) = statistical significance is met

[b]Mean, SD

[c]Note that S100 is measured (in ng/l), not S100B

[d]Mean (CI: 5–95)

[e]2.5–97.5 percentiles

[f]Multiples of Median

Table 3 Prognostic ability of S100b (μg/l) in preterm and full-term neonates with encephalopathy

Full/ Pre term	Specimen	GA/ other	Collection time	*Controls*[a]	Disease	Cut-off	Sensitivity %	Specificity %	PPV %	NPV %	AUC	Reference
P	CB	32 w		*0.92 (0.6–2.64)*[***]	Brain injury	**1.07**	95.3	52.9			0.756	(Lu et al. 2018)
	Serum	27 w	1 d	1.41 ± 0.8[b]	IVH3/4	**2.51**	71.4	90.3	62.5	93.3	0.756	(Efstathiou et al. 2012, 2015)
					<1SD: LS (Rec)	**1.32**	80	64.7	40	91.7	0.835	
						2.19	60	94.1	75	88.9		
		27 w	3–21 d	*3 d: 0.70 ± 0.28[b] 7 d: 0.93 ± 0.44[b] 14 d: 0.70 ± 0.29[b] 12 d: 0.52 ± 0.20[b]*	PVL	**1**	93.8	90.5			0.985	(Chiang et al. 2015)
						1.5	84.4	98.3				
		30 w	1 d	*4.48 (6.69)*[**]	IVH2–4/ D	**10.51**	100	93.9			0.985	(Metallinou et al. 2020)
						17.74	75	100				
	U		0–72 h		IVH	**0.7**	100	100				(Gazzolo et al. 2001b)
		33 w	1st	*0.67 (0.1–2)*[cd]	D	**12.93**[c]	100	97.8	78.6	100	0.995	(Gazzolo et al. 2005a)
			24 h	*0.9 (0.11–2.7)*[cd]		**15.86**[c]	100	90.9	40	99	0.938	
			48 h	*0.92 (0.12–3.79)*[cd]		**38**[c]	90.9	94.8	62.5	99.1	0.967	
			96 h	*0.9 (0.14–2.7)*[cd]		**10**[c]	100	85.3	36.7	100	0.954	

(continued)

Table 3 (continued)

Full/ Pre term	Specimen	GA/ other	Collection time	*Controls*[a]	Disease	Cut-off	Sensitivity %	Specificity %	PPV %	NPV %	AUC	Reference
10F	CBa			*1.05 (0.95–1.15)***	HIE2/3	**2.02**	86.7	88				(Qian et al. 2009)
	Serum	nH	2 h	*1.6 (1.4–2.5)***	HIE2/3	**8.5**	71	90	71	90	0.832	(Nagdyman et al. 2001)
			6 h	*1.5 (1.2–2.3)***		**4.6**	71	86	63	90	0.805	
		nH	1 d/2 d	*[PA-no HIE: 1.8 (1.0–4.5)*]*	HIE2/3	**12**	50	85	82	55		(Thorngren-Jerneck et al. 2004)
					CP or D	**12**	58	75	50	80		
		nH	1–2 d	*2.10 ± 0.12*[b]	CP	**10** [$n = 2$]	100	92				(Murabayashi et al. 2008)
		H	1 d	*1.03 ± 0.28*[b]	HIE3	**1.6**	87.5	88.5	70	95.8	0.937	(Efstathiou et al. 2021)
					D/CP	**11.4**	66.7	100	100	93.3	0.937	
					Severe BGI	**1.6**	100	84.6	50	100	0.942	
					Severe WPI	**13.9**	100	100	100	100	1	
					<2SD: CS,LS	**2.03**	100	84.6	60	100	0.949	
					<1SD: CS,MoS	**1.5**	100	75	57.1	100	0.896	
					<1SD: LS	**1.5**	85.7	88.9	85.7	88.9	0.921	

		WBC	4.5 h		D/severe MRIab	**1.6**	40	91			0.786	(Massaro et al. 2012)
						0.7	64	84				
			77 h		D/N(ST)	**0.2**	71	82			0.814	
		WBC	4.5 h		D/ Severe N(LT)	**0.6**	67	69			0.681	(Massaro et al. 2014)
						1	52	81				
			16.5 h			**0.4**	73	75			0.788	
						0.8	59	89				
			28.5 h			**0.3**	65	66			0.715	
						0.8	49	98				
			77 h			**0.2**	68	69			0.732	
						0.3	53	95				
	U	nH	2 h	0.11 ± 0.01^b	N(LT)	**0.28**	100	87.3	46.2	100	0.978	(Gazzolo et al. 2003b)
			12 h	0.12 ± 0.03^b		**0.8**	100	98.2	85.7	100	0.999	
			24 h	0.12 ± 0.02^b		**0.52**	100	95.5	70.6	100	0.997	
			72 h	0.12 ± 0.02^b		**0.8**	100	98.2	85.7	100	0.998	
		nH	2 h	0.1 ± 0.09^b	HIE2/3	**0.41**	91.3	94.6	80.8	97.8	0.971	(Gazzolo et al. 2004)
			4 h	0.11 ± 0.08^b		**0.8**	100	98.9	95.5	100	0.999	
			8 h	0.11 ± 0.09^b		**0.52**	100	95.5	84	100	0.997	
			12 h	0.11 ± 0.11^b		**0.8**	100	98.9	95.5	100	0.998	
			16 h	0.19 ± 0.12^b		**0.56**	100	97.8	90.5	97.8	0.998	
			20 h	0.2 ± 0.11^b		**0.41**	100	98.6	91.3	100	0.998	

(continued)

Table 3 (continued)

Full/ Pre term	Specimen	GA/ other	Collection time	*Controls*[a]	Disease	Cut-off	Sensitivity %	Specificity %	PPV %	NPV %	AUC	Reference
			24 h	*0.23 ± 0.08*[b]		**0.52**	100	97.8	90.5	97.8	0.998	
			48 h	*0.27 ± 0.05*[b]		**0.51**	100	97.8	90.9	98.9	0.999	
			72 h	*0.29 ± 0.07*[b]		**0.5**	100	97.9	91	98.8	0.998	
		nH	3 h, 24 h, 48 h, 96 h	*0.05 (0.12–0.19)*[***]	D	**1.0**	100	100	100	100	1	(Gazzolo et al. 2009)
		WBC	1 d		D	**1.11**	100	60			0.767	(Alshweki et al. 2017)
					D/N(ST)	**0.66**	83	70			0.731	
		nH	8 h	*0.15 ± 0.02*[b]	HIE	**0.47**	77.2	74.1	81.1	87.4		(Liu et al. 2010)
			2 d	*0.15 ± 0.02*[b]			80.7	85.7	88.5	90.8		
			3 d	*0.30 ± 0.04*[b]			90.4	91.9	96.3	100		
			1–3 d		N (LT-6 m)	**3.49**	100					
		IUGR	1st		N(ST)	**7.37**[c]	95	99.1	91	99		(Florio et al. 2006)
	Saliva	nH	0 h	*1.0 (0.9–1.2)*[c***]	N(LT)	**3.25**[c]	100	100	100	100	1	(Gazzolo et al. 2015)

CBa arterial cord-blood, *D* death, *BGI* basal ganglia injury (MRI), *CS* cognitive scale (BayleyIII), *IUGR* intrauterine growth restriction, *MoS* motor scale (BayleyIII), *MRIab* abnormal brain MRI, *N* neurologic abnormalities, *LS* language scale (BayleyIII), *LT* long-term, *Rec* receptive subscale (of language scale) (BayleyIII), *SD* standard deviation, *ST* short-term, *WPI* watershed pattern injury (MRI)

*Median (range)

**Median (quartiles)

***Median (Lower-Upper CI95%)

[a]Controls are reminded for adjusting cut-off values while comparing them among different studies

[b]Mean±SD

[c]Multiples of Median

[d]Mean (CI:5-95)

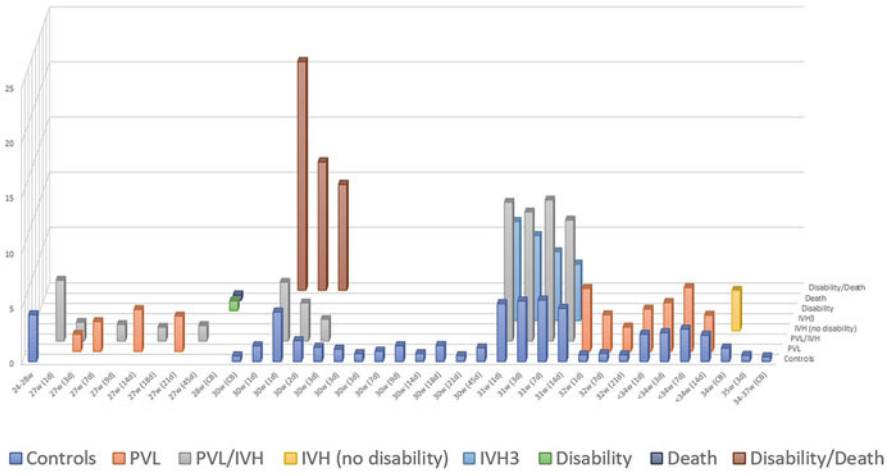

Fig. 1 Illustration of S100B (μg/l) kinetics in blood, in healthy and diseased preterm neonates of various gestational age. Mean or median S100B reported in different studies in literature are presented for illustration needs only. X-axis: weeks of GA (collection time of sample in parentheses)

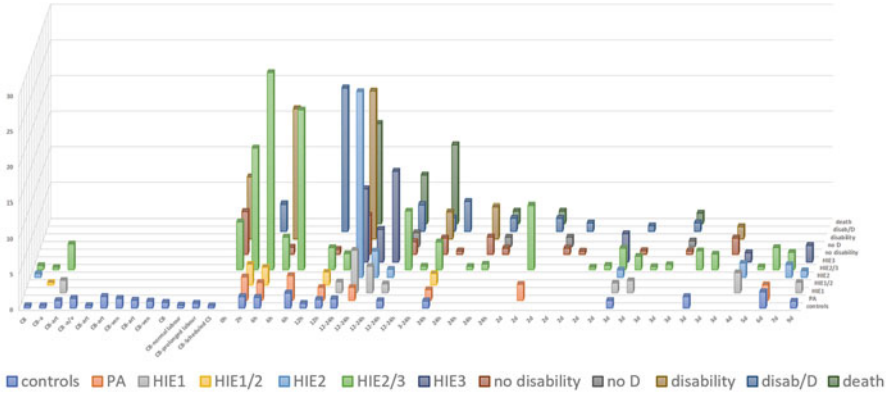

Fig. 2 Illustration of S100B (μg/l) kinetics in blood, from birth to ninth day of life, in healthy and diseased full-term neonates. Mean or median S100B reported in different studies in literature are presented for illustration needs only. X-axis: collection time of sample (same collection time in the axis indicates different study presented). **a/art:** arterial, **v/ven:** venous

since some time is required from the asphyxiated event to cell death and the resultant release of chemical markers outside CNS. Nevertheless, measuring cord blood levels could help to differentiate acute from antenatal or chronic hypoxic injury.

In accordance with the aforementioned theoretical approach, there is inconsistency among studies that are reporting either none, borderline, or good correlation of S100B levels with the grade of encephalopathy (Table 2). Good correlation with

cord blood pH, aEEG-pattern severity, long-term outcome (Zaigham et al. 2017), Apgar score, and CT findings (Qian et al. 2009; Summanen et al. 2017) are also reported. Likewise, some authors found increased S100B levels after vaginal delivery or prolonged labor (Wirds et al. 2003; Schulpis et al. 2006; Bouvier et al. 2020), where others mentioned no correlation with these two parameters or oxytocin infusion (Amer-Wåhlin et al. 2001; Summanen et al. 2017). Wirds et al. (2003) report no difference in S100B levels comparing elective with emergency CS, vacuum extraction with spontaneous vaginal delivery, as well as between CS under general, spinal, or epidural anesthesia.

Additionally, Amer-Wåhlin et al. (2001) found no correlation with gender or birth weight, and Bouvier et al. (2020) found no correlation with maternal smoking or the presence of neonatal jaundice. On the contrary, Summanen et al. (2017) mentioned higher levels in males. Two studies report a small increase in S100B levels in arterial cord blood versus venous cord blood, a significant finding indicating a fetus origin of this substance and not a placental/maternal one (Amer-Wåhlin et al. 2001; Wirds et al. 2003). Finally, hemolyzed samples seem to be an additional significant confounding factor, that falsely increases measured S100B levels (Amer-Wåhlin et al. 2001).

EoP: Unlike studies in full-term newborns, three studies in preterms correlated constantly S100B levels with brain injury (diagnosed by ultrasound scan), death, or later occurrence of CP (as seen in Table 1), indicating different pathophysiology and timing of brain damage between preterms and full-term newborns. Additionally, control preterms had higher cord blood levels than control full-term neonates (Gazzolo et al. 2000; Bouvier et al. 2020), possibly because of the more immature BBB. Among other parameters, treatment with magnesium sulfate did not alter S100B levels (Costantine et al. 2011), nor did maternal glucocorticoid supplementation (Gazzolo et al. 2003a).

S100B in Serum

For the bedside clinician repeated monitoring of brain injury especially in the first critical days of the asphyxiated event is very important for clinical decisions to be made and for efficacy of therapeutic measures to be evaluated. Similarly, close follow-up is imperative not only the first days but also until discharge, as comorbidity in the critical environment of the NICU can affect brain metabolism. S100B in serum fulfills the aforementioned criteria for an ideal biomarker. S100B has a small half-life allowing dynamic monitoring and serum sampling is easier than other fluids to be collected and can be carried out at exact time points. Additionally, a very small amount of blood is needed, received usually during routine blood laboratory testing. To be noted, since S100B is excreted in urine, renal failure must be considered as a confounding factor when persistent high levels of S100B are observed.

HIE: Table 2 shows serum S100B levels reported in 18 studies in full-term neonates during the first days of life. Most of them indicate increased levels in HIE the first day of life, decreasing thereafter. As it can be seen, healthy controls

maintain quite stable levels across all time points, usually about 1–1.5 μg/l, and in general not more than 2–2.5 μg/l. Neonates with meconium stain, asphyxia without HIE, and grade 1 HIE often show a small increase of S100B levels over 1.5 μg/l, usually around 2–3.5 μg/l, but sometimes their levels are similar with controls, depicting minimal or no cell injury in those clinical conditions. In the first 24 h of life, S100B levels in grade 2/3 HIE are substantially more increased in most studies, reaching levels even far above 6 μg/l and decreasing thereafter, delineating the timeframe of the acute devastating event in full-term neonates. Similarly, Efstathiou et al. (2021) report increase only in HIE3, where HIE1 and HIE2 presented comparable levels.

Discrimination between neonates with good outcome and the ones with disability or death is seen as early as the sixth hour of life and more constantly between 6 and 24 h of life. Neonates with HIE2/3 but eventually with good outcome usually presented levels similar to HIE1, in contrast to those with adverse outcome or death that had much higher levels, twice or even 28 times greater. This observation raises an important issue: S100B can distinguish which neonates with clinically the same presentation (grade 2/3 HIE) will die or have long-term severe neurological problems. It seems that a new categorization based on S100B levels might be more reliable (at least after the sixth hour of life) than Sarnat clinical categorization (Sarnat and Sarnat 1976), helping to individualize advanced therapeutic strategies to high-risk neonates. Additionally, Efstathiou et al. (2021) report that neonates with major adverse outcome (death or CP) had either extremely high S100B levels on day 1 or an abnormally increasing trend over time, a clinically significant and constant observation in several studies for different samples, biomarkers, or gestational age patients. This means that biomarkers as S100B are dynamic and might be able to distinguish even a patient with initially good clinical course that deteriorates after a specific time point. Finally, it will be interesting to be clarified in the future if identification of neonates with grade 1 HIE that later present mild developmental issues (an emerging theme in the literature) would be possible measuring brain biomarkers. These observations are crucial, could be a game-changer in neonatology, and require further research. The above notes are illustrated in Fig. 2, where the kinetics of S100B is shown. Discrimination between healthy and diseased neonates and optimum specimen time (6–24 h) is obvious. Inability of some studies to find correlation with outcome is important to be elucidated by further research (Nagdyman et al. 2003; Çelik et al. 2015; Alshweki et al. 2017; Catherine et al. 2020).

Among other parameters, S100B correlated with pH (Thorngren-Jerneck et al. 2004; Martins et al. 2006; Efstathiou et al. 2021), Apgar score (Murabayashi et al. 2008; Efstathiou et al. 2021), cardiotocography abnormalities (Thorngren-Jerneck et al. 2004), and MRI findings (Massaro et al. 2012, 2018; Alshweki et al. 2017; Efstathiou et al. 2021), whereas no correlation was reported with birth weight, gender, or mode of delivery (Murabayashi et al. 2008; Efstathiou et al. 2021). On the contrary, Alshweki et al. (2017) reported no correlation with aEEG, MRI, or PET, but in their study, S100B could not correlate with clinical outcome either. In the two studies comparing therapeutic hypothermia with normothermia, S100B levels were

decreased in infants with hypothermia, but barely reached statistical significance (Roka et al. 2012; Catherine et al. 2020). Çelik et al. (2015) report slightly smaller levels in selective head cooling versus total body hypothermia (not statistically significant).

Diversity among studies raises questions about clinical definitions, laboratory methods, and kits used. It seems substantial that any department that will use S100B in clinical practice as an assistant biomarker will need to have its own reference levels from healthy neonates as a baseline and compare them with the bibliography.

EoP: A constant finding in all eight studies found in the literature was an elevation of serum S100B levels in preterms with brain injury compared to preterms without obvious brain injury (controls), demonstrating also great increase in cases of adverse later neurological outcome (Table 1). It is very clear that serum S100B can serve as a reliable diagnostic and prognostic marker in encephalopathy of prematurity, even from the first day of life. Serum levels in controls were stable during the postnatal period in five out of six studies (Distefano et al. 2002; Chiang et al. 2015; Efstathiou et al. 2015; Huang et al. 2015; Zhou et al. 2015). S100B levels in neonates with IVH/PVL were reported to decrease over time (Distefano et al. 2002; Efstathiou et al. 2015; Zhou et al. 2015; Metallinou et al. 2020), while there were reports for newborns with PVL that S100B levels were stable (Zhou et al. 2015) or even increasing(Chiang et al. 2015; Huang et al. 2015). Giuseppe et al. (2009) report progressively increasing levels between the third and seventh day of life only in severely asphyxiated preterms, in contrast to mild asphyxiated ones that showed decreasing levels. In the study of Huang et al. (2015), clinical condition and long-term outcome of preterms were well prognosed based on their S100B levels: prolonged elevation (>7 days) was a bad prognostic factor, where clinical improvement was accompanied with decrease in serum S100B. Obviously, these discrepancies are due to different pathophysiology in IVH or PVL, as well as the degree of cell damage in different cohort studied and in different stages of those conditions. But the short half-life of S100B indicates that a persistently elevated concentration might imply an ongoing devastating event (cell death or apoptosis) for even several days rather than an acute brain injury situation, an observation of great clinical interest. It is reminded that renal function must be checked in cases of prolonged increased values. Kinetics of S100B in preterms as seen in different studies is illustrated in Fig. 1.

Furthermore, good correlation of S100B levels with middle cerebral artery pulsatility index (Gazzolo et al. 1999) or with brain echography findings of several days later (Chiang et al. 2015) indicates that early detection of brain damage using biomarkers is possible, in a time that there are no defined lesions in imaging and clinical symptoms may be silent. The above notes implicate the value of using brain biomarkers – and indeed S100B – in preterms and attenuate the need for close monitoring and individualized therapeutic approach to prevent further damage.

Most studies report an adverse correlation of S100B levels with gestational age and/or increased levels in control preterms when compared with control full-term neonates (Gazzolo et al. 2001a; Efstathiou et al. 2015; Zhou et al. 2015), possibly due to the more immature BBB that permits easier S100B passage into the blood

stream. Another additional explanation could be that the more immature brain uses higher extracellular concentrations of a trophic factor such S100B. Obviously, interpreting S100B levels in preterms presupposes consideration of gestational age, and reference values in the future should be referred to a specific gestational age. Finally, in the study of Efstathiou et al. (2012, 2015), S100B levels did not correlate with gender or antenatal corticosteroids and were higher in cases of cesarean section.

Additionally, in the latter study, S100B levels were lower in healthy controls than in neonates with severe respiratory distress syndrome, but even lower in preterms with mild RDS than in controls, indicating perhaps that a preconditioning model of major significance might be implicated: severe hypoxemia in severe RDS could have a neurotoxic effect, but there is a possibility of a neuroprotective role of mild hypercapnia (as in mild RDS), a well described model of endogenous response in the literature (Gidday 2006; Volpe et al. 2018). This observation needs further research. To be noted, hypothermia – the only neuroprotective strategy applied nowadays – is also an endogenous response, called anapyrexia. Delineating the pathways the human body reacts to deleterious situations and enhancing endogenous responses might be an easy and smart way to promote new therapeutic strategies in the future.

S100B in Urine

Urine samples are considered not invasive, especially in a critical ill neonate in the NICU. But the collection of samples in specific time points (that serve proper monitoring) is hampered by factors such as oliguria or anuria that follows asphyxia (especially in the first 24 h of life), renal damage, or the administration of sedative drugs. Additionally, having in mind that brain injury biomarkers are effused from cells into the extracellular space, thereinafter they cross the BBB and enter the blood circulation, and after that, they are filtered by the kidneys and discarded into the urine, their levels in urine samples are not accurate regarding the timing of the devastating event. They depict the mean relevant serum level of a quite wide range of time before the collection, in contrast to serum samples that depict levels far closer to the primary event. Therefore, urine samples may not be ideal for close monitoring of acute brain injury events.

HIE: All but one study showed very good correlation of urine S100B levels with the grade of HIE, with neurological long-term outcome and the outcome of death (Table 2). Healthy neonates, asphyxiated ones without HIE and infants with mild HIE present similar low levels, usually far less than 1 μg/l. Quite comparable levels are often seen in infants with HIE2/3 that proved to have good neurologic or imaging outcome, in contrast to infants with HIE 3 or adverse outcome, whose levels were much greater. As it is seen in these six studies, urine S100B is obviously a very reliable marker from the very first hours of life till the fourth day.

Moreover, Alshweki et al. (2017) report good correlation with brain MRI and PET findings for urine samples in days 1 and 2 (but not in the third day of life), but

no correlation with aEEG records. Gazzolo et al. (2003b, 2004) found no correlation with the mode of delivery.

EoP: Among six studies found in preterms (Table 1), Gazzolo et al. (2005a) report steady low levels in controls in the first 4 days of life, in contrast to higher and constantly increasing levels in neonates with IVH and even higher in those whose eventually died. Urine levels of S100B in controls (Gazzolo et al. 2003a) and in premature neonates with IVH (Sannia et al. 2010) were lower when complete course of antenatal glucocorticoid supplementation was given, but the interpretation of these findings are complicated and need further research, given the neurotrophic effect of S100B in low concentrations (especially in the immature brain), the possible effect of glucocorticoid supplementation in the BBB permeability, and the role of respiratory distress as mentioned above. Finally, correlation of urine S100B levels with gestational age and gender is reported (Gazzolo et al. 2001a; Sannia et al. 2013) (Table 1).

S100B in Saliva

Only Gazzolo et al. have studied S100B levels in saliva, finding adverse correlation with gestational age (Gazzolo et al. 2005b), and good correlation with MRI findings, neurological long-term outcome, and outcome of death (Gazzolo et al. 2015) (Table 2).

S100B in Amniotic Fluid

Lu et al. (2018) reported that S100B levels in amniotic fluid at labor were not correlated with brain injury or with levels in cord blood, indicating that amniotic fluid is not a verified sample for measuring S100B (Table 1).

Applications to Prognosis

In this chapter, prognostic ability of S100B in different fluids in full-term and preterm newborns is reviewed (Table 3). Several studies show that S100B is a good prognostic marker for short-term and long-term neurodevelopmental outcome, for neuroimaging abnormalities, and for the adverse outcome of early neonatal death. But discrepancies among them regarding outcomes studied and related cut-off points are large and impede definite conclusions to be drawn. Interpreting results after adjusting for associated control levels is imperative. Searching for similarities, levels above 10 μg/l in serum (Thorngren-Jerneck et al. 2004; Murabayashi et al. 2008; Efstathiou et al. 2021) and 1 μg/l in urine (Gazzolo et al. 2009; Alshweki et al. 2017) indicate full-term neonate which will possibly die or develop CP (Table 3).

NSE

NSE is found mainly in neurons and neuroendocrine cells, but also in erythrocytes. As S100B, its concentration after brain injury and impairment of BBB elevates in peripheral blood. It has a biological half-life of about 24 h. It increases in cases of tumors of neuroectodermal origin and brain injury of any cause (traumatic, asphyxia, cardiac surgery, stroke, subarachnoid hemorrhage, Guillian–Barre syndrome, meningitis, and encephalitis) (Çeltik et al. 2004; Roka et al. 2012). Its presence in erythrocytes implies elevation of NSE levels after hemolysis, an important confounding factor that always must be considered. Correction for this effect have been reported (Berger and Richichi 2009).

NSE in Cord Blood

HIE: Amer-Wåhlin et al. (2001) measured NSE levels in cord blood in healthy full-term neonates and found higher levels in umbilical arterial blood than in umbilical venous blood (Table 5). Even higher levels were detected in hemolyzed samples, indicating that these are unsuitable for clinical use. No correlation was found with mode of delivery, duration of labor, oxytocin infusion, gender, or birth weight. Nagdyman et al. (2001) found similar levels for controls with the previous study (23–30 μg/l), almost double levels for asphyxiated neonates with no or grade 1 HIE, and more than triple levels for ones with grade 2/3 HIE, but no statistical significance was met. Hemolysis was also reported as a confounding factor.

EoP: Costantine et al. (2011) could not find difference in cord blood NSE levels in preterms that developed CP or died than in prematures without obvious brain injury (Table 4). Additionally, treatment with magnesium sulfate did not alter NSE levels significantly.

NSE in Serum

Among 16 studies found, NSE levels correlated significantly with brain injury mostly after the 12th hour of life (Table 5), peaked around 24th hour (Kelen et al. 2017; Zhang et al. 2017; Alshweki et al. 2017) and were decreasing thereafter. Discrepancies between studies for NSE are greater than ones observed for S100B. Ezgü et al. (2002), Nagdyman et al. (2003), Massaro et al. (2018), Catherine et al. (2020), and Çelik et al. (2015) report that serum NSE is no useful marker for HIE. It is noteworthy that in the two latter studies, the levels of NSE (even in healthy controls) were almost 10–100 times lower than in most studies that did find significant correlation of NSE with encephalopathy. Moreover, Massaro reported good correlation of NSE levels with long-term outcome and MRI findings in his previous studies (Massaro et al. 2012, 2014), and so did Nagdyman for HIE grading (Nagdyman et al. 2001).

Table 4 NSE levels (μg/l) in different biological fluids and time points in preterm neonates

GA	Specimen	Collection Time	Controls (Group A)	PVL (Group B)	IVH (no disability) (Group C)	IVH 3 (Group D)	IVH 4 (Group E)	Other (Group F)	Group comparison[a] (p)	Reference
27	Serum	1 d		72.8 ± 67.46[b] [$n = 12$]					B/C/D/ E vs. A (30w)$^{(NS)}$	(Efstathiou et al. 2012, 2015)
				No-D/CP: 52.05 ± 35[b] [$n = 5$]				**D/CP:** 87.6 ± 83.1[b] [$n = 7$]	NS	
		3 d		38.12 ± 30.14[b] [$n = 10$]					B/C/D/ E vs. A (30w)$^{(NS)}$	
				No-D/CP: 34.7 ± 32.3[b] [$n = 5$]				**D/CP:** 41.5 ± 31.1[b] [$n = 5$]	NS	
		9 d		23.3 ± 22.25[b] [$n = 9$]					B/C/D/ E vs. A (30w)$^{(NS)}$	
				No-D/CP: 17.6 (18.4)[*] [$n = 4$]				**D/CP:** 19.7 (77)[*] [$n = 5$]	NS	
		18 d		34.91 ± 26.12[b] [$n = 8$]					B/C/D/ E vs. A (30w)$^{(NS)}$	
				No-D/CP: 38.4 (47.3)[*] [$n = 4$]				**D/CP:** 21.1 (71)[*] [$n = 4$]	NS	
		45 d		**No-D/CP:** 25.2 (17.2)[*] [$n = 3$]					**B/C/D/ E vs. A (30w)**$^{(<0.05)}$	

28w	CBv							**CP/D:** 14.87 (7.42–20.8)**[$n = 25$] **CP:** 18.25 (7.54–78.1)**[$n = 16$] **D:** 14.87 (7.42–20.8)**[$n = 25$]	CP/D vs. A (30w)$^{(NS)}$ CP vs. A (30w)$^{(NS)}$ D vs. A (30w)$^{(NS)}$	(Costantine et al. 2011)
30	CBv		13.67 (8.66–28.95)**[$n = 82$]							
	Serum	1 d	34.92 ± 19.13[b][$n = 26$]							(Efstathiou et al. 2012, 2015)
		3 d	25.45 ± 26.09[b][$n = 25$]							
		9 d	20.84 ± 20.97[b][$n = 25$]							
		18 d	16.64 ± 9.18[b][$n = 20$]							
		45 d	15.06 ± 5.68[b][$n = 14$]							

CBv venous cord blood, *D* death, *n* number of infants (in cohort)
[*]Median (range)
[**]Median (quartiles)
[a]Bold font (in "Groups comparison" column) = statistical significance is met
[b]Mean, SD

Table 5 NSE levels (μg/l) in different biological fluids and time points in full-term neonates with HIE

Specimen	Time	Details	Controls (Group A)	PA, no disability (Group B)	HIE 1 (Group C)	HIE 2 (Group D)	HIE 3 (Group E)	PA + disability (Group F)	Group comparison[a] (p)	Reference
CB			29.6 (17.8–55.9)**[n = 20]	48.9 (20.1–74.7)**[n = 22]		106.8 (60.5–108.1)**[n = 7]			B/C/D/E vs. A, B/C vs. D/E$^{(NS)}$	(Nagdyman et al. 2001)
		Art	**nHem:** 27(10–140)*[n = 52] **Hem**: 100(27–200)*[n = 60]						**nHem vs. hem**$^{(<0.001)}$	(Amer-Wåhlin et al. 2001)
		Ven	**nHem:** 23 (8.8–92)*[n = 58] **Hem**: 82 (18–≥200)*[n = 54]						**nHem vs. hem**$^{(<0.001)}$	
Serum	**0 h**	nH				5.25 (3.99–6.60)[n = 84]			nH vs. WBC$^{(NS)}$	(Catherine et al. 2020)
		WBC				5.64 (3.88–6.98)[n = 78]				
		nH/ WBC				**No-N(LT):** 5.3 (3.7–6.5)**		**N(LT):** 5.8 (4.2–7.3)**	D/E vs. F$^{(NS)}$	
	2 h	nH	30.3 (24.8–47.6)**[n = 20]	32.5 (19.9–53.8)**[n = 16]				**D/N(LT):**55.1 (32.5–89.0)**[n = 11]	B/F vs. A, B vs. F$^{(NS)}$	(Nagdyman et al. 2003)
		nH		32.5 (20.8–60.7)** [n = 22]		60.8 (49.3–89.0)**[n = 7]			B/C/D/E vs. A, B/C vs. D/E$^{(NS)}$	(Nagdyman et al. 2001)
	6 h	nH	37.1 (19–48.8)**[n = 20]	35.6 (30.1–70.3)** [n = 22]		52.7 (48.7–79.3)**[n = 7]			B/C/D/E vs. A, B/C vs. D/E$^{(NS)}$	(Nagdyman et al. 2001)
		nH		36.7(30.1–54.9)**[n = 16]				**D/N(LT):** 48.7 (33.7–95.8) **[n = 11]	B/F vs. A, B vs. F$^{(NS)}$	(Nagdyman et al. 2003)
		SCH	0.45 (0.36–0.51)**[n = 9]		0.49 (0.37–2.13)**[n = 7]	0.58 (0.44–1.62)**[n = 14]			SHC vs. WBC vs. A vs. C$^{(NS)}$	(Çelik et al. 2015)
		WBC				0.67 (0.48–2.65)**[n = 10]				
		SCH/ WBC			**No-N(LT):** 1.31 (0.46–2.15)**[n = 13]			**N(LT):** 0.55 (0.44–1.10)**[n = 18]	NS	
		H and nH				**No-sevN(LT):** 38.5 (23.36–114.7)*[n = 18]		**sevN(LT)/D:** 71.14 (32.86–132.5)*[n = 6]	D/E vs. F$^{(NS)}$	(Roka et al. 2012)
		WBC				43.86 (23.36–132.50)*[n = 13]			H vs. nH$^{(NS)}$	

		nH				46.96 (23.92–99.20)*[$n = 11$]			
		WBC				**No-sevN(LT):** 41.2(30.1–54.2)**[$n = 50$]	**N(LT)/D:** 66.8 (49.2–112.3)**[$n = 25$]	**D/E vs. F**$^{(<0.001)}$	(Kelen et al. 2017)
	12 h	nH	28.7 (19.8–39.4)**[$n =$ 20]	34.0 (24.6–48.7)**[$n = 22$]		54.3 (46.8–78.8)**[$n = 7$]		B/C/D/E vs. A$^{(NS)}$ **B/C vs. D/E**$^{(<0.05)}$	(Nagdyman et al. 2001)
		nH		38.5 (24.3–48.8)**[$n =$ 16]			**D/N(LT):** 47.8 (30.1–64.9)**[$n = 11$]	B/F vs. A, B vs. F$^{(NS)}$	(Nagdyman et al. 2003)
		H and nH				**No-sevN(LT):** 39 (27.34–166.1)*[$n = 18$]	**sevN(LT)/D:** 42.88 (26.98–141.8)*[$n = 6$]	D/E vs. F$^{(NS)}$	(Roka et al. 2012)
		WBC				43.12 (32.14–166.1)*[$n = 13$]		H vs. nH$^{(NS)}$	
		nH				38.58 (26.98–141.80)*[$n = 11$]			
		WBC				**No-sevN(LT):** 46.1 (31.3–55.7)**[$n = 50$]	**N(LT)/D:** 89.2 (53.7–115.6)**[$n = 25$]	**D/E vs. F**$^{(<0.0005)}$	(Kelen et al. 2017)
	24 h	nH	24.3 (17.2–39.5) **[$n = 20$]	34.8 (21.4–44.4)**[$n = 22$]		50.7 (41.8–70.9)**[$n = 7$]		B/C/D/E vs. A$^{(NS)}$ **B/C vs. D/E**$^{(<0.05)}$	(Nagdyman et al. 2001)
		nH		36.3(17.6–51.8)**[$n =$ 16]			**D/N(LT):** 39.1 (34.0–55.4)**[$n = 11$]	B/F vs. A, B vs. F$^{(NS)}$	(Nagdyman et al. 2003)
		WBC				**No-sevN(LT):** 33.82 (13.94–175.1)*[$n =$ 18]	**sevN(LT)/D:** 88.85 (43.78–129.2)*[$n = 6$]	**D/E vs. F**$^{(<0.05)}$	(Roka et al. 2012)
						36.13 (13.94–175.1)*[$n = 13$]		H vs. nH$^{(NS)}$	
		nH				38.76 (17.14–129.20)*[$n = 11$]			
		nH/ WBC				**No-N(LT):** 5.5 (3.2–6.9)**	**N(LT):** 5.4 (4.1–7.1) **	D/E vs. F$^{(NS)}$	(Catherine et al. 2020)
		WBC				49.2 (36.0–68.0)**[$n = 75$]			(Kelen et al. 2017)
						No-sevN(LT): 47.3 (28.3–57.4)**[$n = 50$]	**N(LT)/D:** 101 (47.6–123.7)**[$n =$ 25]	**D/E vs. F**$^{(<0.0005)}$	
	1 d	H				**no-N(ST):** 84.3 ± 28.8^{b}[$n = 13$]	**D/N(ST):** 109.1 ± 49.2^{b}[$n = 18$]	D/E vs. F$^{(NS)}$	(Alshweki et al. 2017)
						no-D: 86.6 ± 36.4^{b}[$n = 25$]	**D:** 149.1 ± 31.8^{b}[$n = 6$]	**D/E vs. F**$^{(0.01)}$	
		H	23.51 ± 10.76^{b}[$n = 12$]		30.57 ± 10.17^{b}[$n =$ 10]	66.1 ± 15.95^{b}[$n =$ 4] 89.9 ± 63.6^{b}[$n = 8$]		**D vs. A, E vs. A**$^{(<0.0005)}$	(Efstathiou et al. 2021)
						81.98 ± 52.75^{b} [$n = 13$]		**A vs. D/E**$^{(<0.0005)}$	
						No-D/CP: 34.4 ± 18.4^{b}[n = 27]	**D/CP:** 103.2 ± 69.4^{b}[$n = 6$]	**D/E vs. F**$^{(0.06)}$	

(continued)

Table 5 (continued)

Specimen	Time	Details	Controls (Group A)	PA, no disability (Group B)	HIE 1 (Group C)	HIE 2 (Group D)	HIE 3 (Group E)	PA + disability (Group F)	Group comparison[a] (p)	Reference
		H				98.8 (43.8–758)*[$n = 50$]			NS with MRI/N(LT)	(Massaro et al. 2018)
	4–48 h		21.0 ± 5.3[b][$n = 30$]	**MS:** 42 ± 24[b][$n = 18$]	65.3 ± 32.4[b][$n = 14$]	64.6 ± 32.9[b][$n = 19$]	115.7 ± 60.9[b][$n = 10$]		**C** vs. **E, D** vs. **E, B** vs. **C/D/E**$^{(<0.05)}$ **A** vs. **C/D/E**$^{(<0001)}$, A vs. B$^{(NS)}$	(Çeltik et al. 2004)
	2 d	H				**No-N(ST):** 68.6 ± 20.6[b][$n = 13$]		**D/N(ST):** 113.5 ± 59.4[b][$n = 16$]	**D/E vs. F**$^{(<0.05)}$	(Alshweki et al. 2017)
						No-D: 85.3 ± 40.9[b][$n = 25$]		**D:** 143.8 ± 82.7[b][$n = 4$]	D/E vs. F$^{(NS)}$	
		H and nH				**No-sevN(LT):** 30.2[13.82–110.3)*[$n = 18$]		**sevN(LT)/D:** 68.98 [35.52–94.42)*[$n = 6$]	D/E vs. F$^{(NS)}$	(Roka et al. 2012)
		WBC				29.44 (21.50–110.30)*[$n = 13$]			H vs. nH$^{(NS)}$	
		nH				38.38 (13.82–94.42)*[$n = 11$]				
		WBC				**No-sevN(LT):** 35.8 (25.6–49.7)**[$n = 50$]		**N(LT)/D:** 72.1 (38.1–127.2)**[$n = 25$]	**D/E vs. F**$^{(<0.001)}$	(Kelen et al. 2017)
	1–3 d	nH		30.81 (2.3–65.88)*[$n = 8$]	37.94 (19.77–43.88)*[$n = 5$]	16.93 (13.24–64.05)*[$n = 6$]	18.45 (13.59–88.01)*[$n = 7$]		NS	(Ezgü et al. 2002)
	2–3 d		7.6 (10.3–28.3)*[$n = 22$]		**No-D/N(LT):** 21.3 (7.4–40)*[$n = 19$]			**D/N(LT):** 116.4 (42–226)*[$n = 6$]	**C/D/E vs. F**$^{(<0.001)}$ A vs. C/D/E$^{(NS)}$	(Verdú Pérez et al. 2001)
	3 d	H				**No-N(ST):** 54.1 ± 16.3[b][$n = 13$]		**D/N(ST):** 97.3 ± 59.8[b][$n = 14$]	**D/E vs. F**$^{(<0.05)}$	(Alshweki et al. 2017)
						No-D: 66.7 ± 29.9[b][$n = 25$]		**D:** 199.0 ± 91.9[b][$n = 2$]	**D/E vs. F**$^{(<0.01)}$	
		SHC	0.38 (0.32–0.47)**[$n = 9$]		1.01 (0.4–1.93)**[$n = 7$]	0.51 (0.38–0.74)**[$n = 14$]			SHC vs. WBC vs. A vs. C$^{(NS)}$	(Çelik et al. 2015)
		WBC				0.57 (0.38–1.7)**[$n = 10$]				
		SHC/ WBC			**No-N(LT):** 0.98 (0.44–2.13)**[$n = 13$]			**N(LT):** 0.52 (0.38–0.74)**[$n = 18$]	NS	
		nH	62 (11–200)*[$n = 12$]							(Amer-Wåhlin et al. 2001)
		H and nH				**No-sevN(LT):** 28.56 (13.96–119.3)*[$n = 18$]		**sevN(LT)/D:** 45.16 (25.24–45.58)*[$n = 6$]	D/E vs. F$^{(NS)}$	(Roka et al. 2012)

		WBC				23.72 (13.96–119.3)*[$n = 13$]		H vs. nH$^{(NS)}$	
		nH				45.16 (22.20–62.18)*[$n = 11$]			
		nH				6 (2.25–8.30)**[$n = 84$]		nH vs. WBC$^{(NS)}$	(Catherine et al. 2020)
		WBC				5.07 (3.75–6.67)**[$n = 78$]			
		nH/ WBC				**No-N(LT):** 5.2 (3.2–6.9)**	**N(LT):** 6.1 (3.2–8.5)**	D/E vs. F$^{(NS)}$	
		WBC				**No-sevN(LT):** 34.4 (21.0–46.9)**[$n = 50$]	**N(LT)/D:** 60.4 (38.1–74.5)**[$n = 25$]	**D/E vs. F$^{(<0.005)}$**	(Kelen et al. 2017)
		H	16.91 ± 8.44^{b} [$n =$ 11]		20.65 ± 6.75^{b}[$n =$ 9]	41.3 ± 22.11^{b}[$n =$ 4]	42.57 ± 27.8^{b}[$n =$ 8]	**E vs. A$^{(<0.05)}$**	(Efstathiou et al. 2021)
						42.15 ± 15.02^{b} [$n = 12$]		**A vs. D/E$^{(<0.005)}$**	
						No-D/CP: 22.4 ± 13.2^{b}[$n = 26$]	**D/CP:** 49 ± 29.6^{b}[$n =$ 6]	**D/E vs. F$^{(<0.05)}$**	
	5 d	H				45.4 (12–236)* [$n = 47$]		NS with MRI	(Massaro et al. 2018)
	5–7 d	nH		**MS:** 22.1 ± 8.0^{b}[$n = 18$]	34.6 ± 13.9^{b}[$n =$ 14]	42 ± 32.7^{b}[$n = 19$]	70.8 ± 36.9^{b}[$n =$ 10]	**C vs. D, C vs. E$^{(<0.05)}$**	(Çeltik et al. 2004)
	7 d	WBC				30.4 (20.1–34.6)**[$n = 75$]			(Kelen et al. 2017)
	9 d	H	11.96 ± 9.75^{b}[$n =$ 8]		18.24 ± 6.75^{b}[$n =$ 8]	20.48 ± 6.68^{b}[$n =$ 4]	27.31 ± 18.21^{b}[$n =$ 6]	E vs. A$^{(0.068)}$	(Efstathiou et al. 2021)
						24.58 ± 14.54^{b} [$n = 10$]		**A vs. D/E$^{(<0.05)}$**	
						No-D/CP: 16.1 ± 8.2^{b}[$n = 22$]	**D/CP:** 33 ± 20.3^{b}[$n =$ 4]	D/E vs. F$^{(NS)}$	
	18 d		12.91 ± 1.57^{b}[$n =$ 4]		18.74 ± 5.83^{b}[$n =$ 4]	16.25 ± 1.84^{b}[$n =$ 4]	16.25 ± 8.99^{b}[$n =$ 5]	**D vs. A$^{(<0.05)}$**	
						16.25 ± 6.46^{b}[$n = 9$]		A vs. D/E$^{(NS)}$	
						No-D/CP: 15.5 ± 3.9^{b}[$n = 14$]	**D/CP:** 12.4 (21.3)*[$n =$ 3]	D/E vs. F$^{(NS)}$	
CSF	78 h	nH			36.6 ± 14.1^{b}[$n = 28$]			**nH vs. SHC$^{(<0.05)}$**	(Sun et al. 2012)
		SHC			27.2 ± 9.8^{b}[$n = 23$]				
					No-N(LT): 20.5 ± 8.2^{b}[$n = 16$]		**N(LT):** 39.4 ± 11.6^{b}[$n = 3$]	**C/D/E vs. F$^{(<0.05)}$**	
	2–32 h	nH			17.2 (9–43)* [$n = 9$]	34.5(12–114)*[$n =$ 8]	185(49–200)*[$n =$ 4]	**E vs. D$^{(<0.05)}$, E vs. C$^{(<0.01)}$ A vs. C/D/E$^{(<0.001)}$**	(Thornberg et al. 1995)

(continued)

Table 5 (continued)

Specimen	Time	Details	Controls (Group A)	PA, no disability (Group B)	HIE 1 (Group C)	HIE 2 (Group D)	HIE 3 (Group E)	PA + disability (Group F)	Group comparison[a] (p)	Reference
	0–72 h		10 (5.7–20)*[n = 9]							
	1–4 d	nH	5.8 (3.0–8.2)*[n = 8]		10.9 (4.6–460)*[n = 22]				**A vs. C/D/E**$^{(<0.005)}$	(Blennow et al. 2001)
	1–3 d	nH		3.22 (1.97–13.2)*[n = 8]	4.03 (3.63–8.37)* [n = 5]	20.63 (3.66–119.3)* [n = 6]	16.54 (4.75–127.8)* [n = 7]		**E vs. B**, **E vs. C**, **D/E vs. C**$^{(<0.01)}$ **D vs. B**$^{(<0.05)}$, B vs. C$^{(NS)}$ **D/E vs. B**$^{(<0.005)}$	(Ezgü et al. 2002)

Art arterial, *D* death, *Hem* hemolyzed specimen, *LT* long-term, *MS* meconium stain, *N* neurologic abnormalities, *nH* normothermia (no therapeutic hypothermia), *nHem* non-hemolyzed specimen, *NS* not statistically significant, *ST* short-term, *sev* severe, *ven* venous, *n* number of infants (in cohort)

*Median (range)

**Median (quartiles)

[a]Bold font (in "Groups comparison" column) = statistical significance is met

[b]Mean, SD

[c]Mean (CI:5–95)

[d]2.5–97.5 percentiles

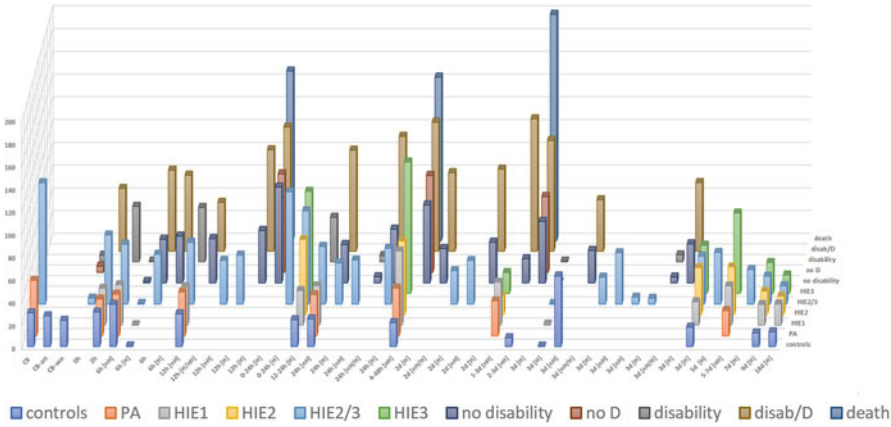

Fig. 3 Illustration of NSE (μg/l) kinetics in blood, from birth to 18th day of life, in healthy and diseased full-term neonates . Mean or median S100B reported in different studies in literature are presented for illustration needs only. X-axis: collection time of sample (same collection time in the axis indicates different study presented). **art:** arterial, **ven:** venous, **H:** hypothermia, **nH:** normothermia

For the remaining studies, NSE correlates well with the degree of encephalopathy and mostly with long-term outcome (Table 5). In an attempt to summarize results, NSE levels in healthy controls are generally around 21–37 μg/l on the first day of life, where infants with HIE2/3 and good or adverse outcome had levels around 40–84 μg/l and 88–150 μg/l, respectively. Similarly, NSE measured on days two and three of life proved to distinguish very well the infants with adverse outcome. Kinetics of NSE among different studies is illustrated in Fig. 3.

Extreme high levels or prolonged elevation of NSE were unfavorable prognostic factors (Zhang et al. 2017; Efstathiou et al. 2021). Some authors report good correlation of NSE with MRI findings (Massaro et al. 2012; Efstathiou et al. 2021), PET imaging (Alshweki et al. 2017), and aEEG (Thornberg et al. 1995; Zhang et al. 2017), in contrast to other studies that found no correlation with MRI (Alshweki et al. 2017; Massaro et al. 2018) or aEEG (Alshweki et al. 2017). Roka et al. (2012) and Catherine et al. (2020) report no difference of NSE in therapeutic hypothermia or normothermia. Efstathiou et al. (2021) report no difference for birth weight, gender, or mode of delivery, where Kelen et al. (2017) find that emergency CS had higher levels of NSE (vs. vaginal delivery) only when measuring 6 h after birth. Finally, hemolysis is seen to alter measurements of NSE levels (Nagdyman et al. 2001), a significant confounding factor that must always be taken into account.

EoP: Giuseppe et al. (2009) found significant elevated levels of serum NSE at 3 h, 24 h, 48 h, and on seventh day in prematures (of 33 week of GA) with severe asphyxia compared to ones with mild asphyxia or controls. Mild asphyxia differed from controls as well. NSE levels decreased constantly from birth to the seventh day. Efstathiou et al. (2012, 2015) also reported constantly decreasing levels but only for controls, where preterms (of 27 week GA) with encephalopathy maintain high levels

till the 45th day of life (Table 4). Higher levels on the first days could be attributed to the necrotic phase of neuronal damage, where prolonged high levels to either apoptotic phase or continuous necrotic damage (or both) in an extremely premature neonate in the NICU facing multiple comorbidities (lung immaturity, inflammation, etc.) that can affect brain metabolism. The clinical significance of continuous monitoring using biomarkers is obvious.

NSE in CSF

HIE: All six studies found in literature conclude that NSE measured in CSF is a very reliable marker for brain injury (Table 5). It correlates well with the degree of encephalopathy and neurodevelopmental outcome (Table 5, and Tekgul et al. 2004), with the outcome of death (Blennow et al. 2001), and with neuroimaging and aEEG (Ezgü et al. 2002). Garcia-Alix et al. (1994) reports no correlation with perinatal factors, and sampling at 12 h is considered more promising compared to that of 72 h. Thornberg et al. (1995) and Ezgü et al. (2002) report that simultaneous sampling from CSF and serum revealed higher levels in serum, mainly in healthy neonates or neonates without severe encephalopathy, raising significant questions about possible extra-neural sources of NSE or other confounding factors that must be elucidated in the future.

Applications to Prognosis

Prognostic ability of NSE as presented in eight studies for full-term neonates and in one study for preterms is presented in Table 6. Regarding HIE, serum levels over 40 μg/l on the first day of life seem to prognosticate adverse short- and long-term outcome as well as MRI findings. Higher levels indicate possibility for even more severe brain injury or for the outcome of death.

Conclusion

Most studies conclude that both S100B and NSE are capable to prognose neonatal brain damage and its consequences, but S100B seems to be more efficient in monitoring because of its smaller half-life. Extreme or persistently elevated levels indicate sustained and extensive cell injury and should alert the bedside clinician. NSE depicts neuronal damage and S100B glial damage, and repeated measurements can delineate necrotic or apoptotic phases of brain injury in an individualized way, providing significant information for the bedside neonatologist. Measurements in cord blood, urine, or saliva have revealed interesting results, but serum samples are far more widely studied and remain the most reasonable source for clinical use. Since S100B and NSE are dynamic biomarkers and change constantly over time, diversity of results among studies could be minimized by adopting uniform time points and

Table 6 Prognostic ability of NSE (μg/l) in preterm and full-term neonates with encephalopathy

Full-term/ preterm	Fluid	Details	Specimen time	*Controls*[a]	Disease	Cut-off	Sensitivity, %	Specificity, %	PPV, %	NPV, %	AUC	Reference
P	Serum		1 d	34.92 ± 19.13[b]	<1SD: LS	**57.8**	75	88.9			0.889	(Efstathiou et al. 2012, 2015)
					<1 SD: LS-rec		80	100			0.953	
F	Serum	nH	2 h	30.3 (24.8–47.6)[**]	HIE2/3	**44**	83	68	46	93	0.768	(Nagdyman et al. 2001)
			6 h	37.1 (19–48.8)[**]		**46**	83	65	42	93	0.763	
		nH	2 h	30.3 (24.8–47.6)[**]	N(LT)	**44**	60	69	60	69	0.712	(Nagdyman et al. 2003)
			6 h	37.1 (19–48.8)[**]		**43**	67	67	54	77	0.674	
		H	1 d	23.51 ± 10.76[b]	HIE2/3	**38.8**	100	95.2	92.3	95.2	0.976	(Efstathiou et al. 2021)
			1 d		Severe BGI	**41.9**	100	76	40	100	0.9	
			3 d	16.91 ± 8.44[b]		**28.1**	100	76	40	100	0.9	
			1 d	23.51 ± 10.76[b]	Severe WPI	**108.2**	100	100	100	100	1	
			3 d	16.91 ± 8.44[b]		**78**	100	100	100	100	1	
					<2SD: CS,LS	**29.9**	100	76.9	50	100	0.923	
					<2SD: MoS		75	75	50	90	0.833	

(continued)

Table 6 (continued)

Full-term/ preterm	Fluid	Details	Specimen time	*Controls*[a]	Disease	Cut-off	Sensitivity, %	Specificity, %	PPV, %	NPV, %	AUC	Reference
		nH	20 h(±14)	21.0 ± 5.3[b]	HIE2/3	**40**	79	70	51	89		(Çeltik et al. 2004)
					D/N (LT)	**45.4**	84	70	39	95		
		WBC	4.5 h		D/ severe MRIab	**81**	71	83			0.793	(Massaro et al. 2012)
						110	33	93				
			77 h		D/N (ST)	**67**	70	88			0.816	
		WBC	4.5 h		D/ severe N(LT)	**80**	63	74			0.684	(Massaro et al. 2014)
			16.5 h			**73**	85	68			0.825	
			28.5 h			**73**	65	72			0.714	
			77 h			**53**	67	71			0.771	
	CSF	nH	12 h		N(LT)	**25**	90	86				(Garcia-Alix et al. 1994)
		nH	1–3 d		N(LT)	**6**	90	88.9	90	88.9		(Ezgü et al. 2002)
					MRIab		77.8	70	70	77.8		
					EEGab		90.9	100	100	88.9		

BGI basal ganglia injury (MRI), *CS* cognitive scale (BayleyIII), *D* death, *EEGab* abnormal aEEG, *F* full-term neonates, *LS* language scale (BayleyIII), *LT* long-term, *MoS* motor scale (BayleyIII), *MRIab* abnormal brain MRI, *N* neurologic abnormalities, *nH* normothermia, *P* preterms, *Rec* receptive subscale (of language scale) (BayleyIII), *SD* standard deviation, *WPI* watershed pattern injury (MRI)

[a]Controls are reminded for adjusting cut-off values while comparing them among different studies

[**]Median (quartiles)

clinical definitions. Moreover, effect of technical variables in reproducibility must be elucidated, such as time to processing, temperature, and additional freeze-thaw cycles. Currently, these biomarkers can be useful for individualized monitoring of neonates, but in the near future, elimination of discrepancies among researchers could lead to a new categorization for newborns from the first hours of life based on prognosis of long-term outcome, a game-changer in neonatology.

Mini-Dictionary of Terms

- **EoP:** Multifaceted gray and white matter lesions in premature neonates mainly as a result of asphyxia and inflammation. PVL and IVH are its major appearances.
- **HIE:** Neonatal encephalopathy of hypoxic-ischemic etiology.
- **Hypoxemia:** Deficiency in oxygen within blood circulation or at the cellular level.
- **Ischemia:** Insufficient perfusion in the brain.
- **Neonatal encephalopathy:** Altered central nervous system functioning in the neonate.
- **NSE:** The neuronal form of intracytoplasmic enolase, a dimeric glycolytic enzyme of two gamma subunits of 78 kDa.
- **Perinatal asphyxia:** Impairment of respiratory gas exchange that is characterized by profound metabolic acidosis (pH $<$ 7.2 on arterial CB), Apgar score $<$ 3 at 5 min, and associated brain (or multiorgan) damage.
- **S100B:** An acidic calcium-binding protein identified in the mid-1960s, taking its name because of its solubility in a 100% saturated solution with ammonium. It is a homodimer (2 ββ subunits) of 9–14 kDa per monomer, belonging in the S100 protein family of the EF-hand superfamily.

Key Facts of Neonatal Brain Injury

Progress in therapeutic measures in NICU nowadays resulted in a decrease in the cystic (more severe) form of PVL. Nevertheless, for the same reason, smaller prematures are born alive now, facing more severe brain lesions.

Sarnat and Sarnat in 1976 presented the clinical stages of HIE, a categorization that is still in use.

Except from supportive measures in the NICU, therapeutic hypothermia in full-term neonates with moderate or severe HIE is the only worldwide adopted neuroprotective intervention nowadays. It is considered as a standard of care in the developed world.

Therapeutic hypothermia is a mild decrease of 3–4 °C in body temperature for 72 h. It is applied in the first 6 h of life, either as SHC or WBC.

About 14% of hypothermia-treated neonates develop CP. Even without CP, cognitive outcomes are often poor (9% of survivors without CP).

Other neuroprotective strategies under research are xenon, antioxidants, magnesium, erythropoietin, and stem cells.

Summary Points

- *S100B and NSE are proved to be reliable biomarkers for neonatal brain injury in most studies. They correlate well with neuroimaging and long-term neurodevelopmental outcome.*
- *Serum samples are most studied and probably the most suitable biological sample for clinical use.*
- *S100B seems superior to NSE regarding diagnosis and prognosis of brain damage.*
- *Consonance between results of the numerous studies can help interpretation of results into clinical practice.*
- *Measuring levels in control (healthy) neonates and skipping hemolyzed samples can help to avoid pitfalls when S100B or NSE is used in clinical practice.*
- *S100B and NSE can help distinguishing neonates of higher risk of adverse long-term outcome, so additional neuroprotective strategies and therapeutic protocols can be applied.*

Cross-References

► Biomarkers of Oxidative Stress in Neonatal Hypoxic-Ischemic Encephalopathy
► Nerve Injury and Biomarkers
► S100B As a Biomarker in Traumatic Brain Injury

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26 Biomarkers in Patients with Chronic Obstructive Pulmonary Disease (COPD) in Emergency Medicine and the Intensive Care Unit: A Review

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Abstract

Biomarkers have been playing a pivotal role in diagnosis and monitoring therapeutic strategies in modern medicine. COPD is one of the leading causes of mortality across the world. Biomarkers in COPD have been in research stage in the last few years and now have become existential. They still remain elusive for clinical practice across the globe. Recent research in COPD has shown promising results for applying these parameters for diagnosing, prognosticating, and monitoring purposes. Blood biomarkers like fibrinogen and CRP have been utilized as markers for inflammation, whereas serum procalcitonin has been used for etiological diagnosis for reasons of exacerbation episodes. Lung function markers like CC-16 and markers for worsening emphysema like reduced levels of sRAGE have been discussed as few upcoming biomarkers in COPD patients. Imaging modalities have been used as monitoring tool for disease severity, and assessment of airway thickness in CT scan predicts chances of worsening and decreasing FEV1 volumes. Sputum biomarkers like neutrophilia and leukotriene B4 have been of importance in assessment of airway inflammation. More invasive techniques involving molecular changes in lung tissue have been possible to study with invasive techniques like bronchoscopy and biopsy specimens. Future research looks promising, and acquiring the knowledge of use of biomarkers has become quintessential for medical professionals dealing with pulmonary diseases.

Keywords

COPD · Biomarker · CRP · Exhaled breath condensate · Sputum biomarkers · Radiological biomarkers

Abbreviations

BNP	Brain natriuretic peptide
CRP	C-reactive peptide
EBC	Exhaled breath condensate
HPN	Human neutrophil peptides
hS-CRP	High sensitive CRP
SP-D	Surfactant protein D
sRAGE	Soluble receptor for advanced glycation products

Introduction

Chronic obstructive disease is one of the leading causes of morbidity affecting the world population. By the year 2030, it is expected to reach the fourth leading cause of mortality across the globe (Mannino and Buist 2007). Some of the common causes of COPD are smoking, environmental pollution, and use of biomass fuel for cooking (Vestbo et al. 2013). COPD is characterized by airflow limitation during expiration due to inflamed airways causing patients to develop symptoms like breathlessness and fatigue. Emergency physicians and intensivists often treat these patients presenting with exacerbation episodes in ER and ICUs. COPD exacerbation can occur due to various causes. The most common causes are infections and non-cessation of smoking (Goh et al. 2013). Presentation of patient with COPD range from stable course to frequent exacerbation causing respiratory failure and death (Jones and Agusti 2006). This chapter will review various biomarkers in COPD which can be useful in indicating disease progression, predicting outcomes, and monitoring the therapeutic changes. COPD is traditionally diagnosed with clinical symptoms and lung function tests. According to GOLD criteria, FEV1/FVC ratio less than or equal to 0.7 is hallmark finding for diagnosing COPD. COPD biomarker qualification consortium is actively involved in developing new biomarkers in COPD (Casaburi et al. 2013).

In order to improve outcomes in COPD patients, it is prudent to diagnose, monitor the progression of the disease, and also prognosticate the likely outcome. Biomarkers play a promising role in achieving these objectives. A biomarker has been defined by the USFDA in 2016 as an indicator of normal biological processes or response to an ongoing intervention including therapeutic interventions. Biomarkers can be a clinical feature, radiological finding, or blood test that can play a significant role in depicting disease activity, monitoring therapeutic benefits, and helping in diagnosis. Precision medicine has now become an important therapeutic strategy in disease management. Personalized treatment aims to slow down progression in rapid worsening patients with the help of biomarkers (Koutsokera et al. 2013). There have been many studies published in the medical literature related with biomarkers in COPD, but none of these biomarkers have been proven conclusively. Lack of utilization of invasive procedures, reproducibility, and standardization of measurements resulted in poor knowledge about biomarkers of COPD exacerbations (Woodcock 2010). The initial studies of biomarkers in COPD were focused upon the lung specimens like sputum, BAL samples, breath condensate, and lung biopsies. But these biomarkers could not make an impression as the samples were difficult to collect and assess. Imaging biomarkers are expensive; therefore, their application as a screening tool for diagnosis has not been popular.

There has been a paradigm shift in understanding the pathophysiology of COPD in recent times. Blood-based biomarkers have been extensively studied in recent times. These include C-reactive protein, procalcitonin, fibrinogen, chemokines, and soluble receptor for advanced glycation products (sRAGE).

Blood Biomarkers

Biomarkers measured in plasma have few unique advantages. They are cost-effective, easy to measure, and reliable. On the other hand, they are vulnerable to be modulated by other systemic morbidities (Dahl and Nordestgaard 2009). The role of biomarkers of blood in COPD is restricted to identifying COPD exacerbation and predicting mortality. The existing literature does not have much conclusive evidence regarding their role in diagnosis (Sin et al. 2015).

C-Reactive Peptide

CRP has been studied by large number of trials conducted in COPD patients. It indicates the severity of inflammation in COPD exacerbations and helps in predicting mortality.

It is one of the leading biomarkers researched in COPD and easily measurable. A rise in baseline value of CRP occurs over time as disease progresses in COPD patients with subsequent decrease in FEV1 value (Shaaban et al. 2006). CRP has been shown to increase the risk of hospitalization in COPD patients (de Torres et al. 2008). hS-CRP (high sensitive CRP) has been shown to increase proportionally with disease severity in early COPD subjects (Broekhuizen et al. 2006). The ratio of fibronectin:CRP has been associated with mortality unlike isolated CRP levels.

Fibrinogen

Fibrinogen has been shown to have a role in predicting the rate of percentage decline in predicted FEV1. Fibrinogen capability in diagnosing COPD has not been proven. Higher fibrinogen with associated increase in neutrophil levels predicts faster drop in FEV1 values (Duvoix et al. 2013).

Procalcitonin

Procalcitonin is a precursor molecule of hormone calcitonin. Exacerbations of COPD have been a major cause of readmissions to ER. One of the main reasons for exacerbation has been infectious causes.

A rise in procalcitonin helps to differentiate infectious etiology from non-infectious etiology. A rise in procalcitonin signifies occurrence of bacterial infection which helps in determining appropriate use of antibiotic therapy as treatment option in COPD, thereby avoiding irrational use of antibiotics (Becker et al. 2008).

B-Natriuretic Peptide (BNP)

BNP has been a useful biomarker for functioning of the heart. It is secreted by the ventricles and released by cardiac chambers in response to the volume status. It causes natriuresis regulation and diuresis. It has a role in risk stratification and prognostication in community-acquired pneumonia as a cause for COPD exacerbations (Beishuizen et al. 2005).

Neopterin

Neopterin is synthesized by macrophages and monocytes. It has a role in cell-mediated immunity toward intracellular bacteria, viruses, and parasites (Weiss et al. 1999). It has a role in etiological diagnosis of COPD.

SP-d

This plasma biomarker is a measure of the leakiness of pulmonary capillaries. It has been seen that SP-D levels correspond to risk of exacerbations in COPD subjects. This biomarker is secreted from the pneumocytes and bronchiolar cells. Their levels are seen to be higher in smokers who have bronchitis when compared to smokers without bronchitis or nonsmokers (Ambade et al. 2015). It has been seen as one of the emerging biomarkers for COPD prognostication in recent times.

sRAGE (Serum Levels of Soluble Receptor for Advanced Glycation End Products)

Reduction of this biomarker in blood plays a potential role in diagnosing severe COPD. Marked reduction in sRAGE levels are seen in emphysema and rapid declining FEV1. TESRA and ECLIPSE studies showed the association of reduced sRAGE with FEV1 decline and emphysema (Cheng et al. 2013).

Biomarker Panels

Panel of blood biomarkers will increase the accuracy of diagnosing severe form of COPD in the future. The Copenhagen City Heart Study showed increased risk (OR 3.7) of exacerbation in patients with simultaneous increase in CRP, fibrinogen, and leucocyte count (Cockayne et al. 2012; Thomsen et al. 2013). ECLIPSE study also showed benefit of biomarker panel in mortality prediction and COPD exacerbation (Celli et al. 2012). Model panel and studies relating to biomarker panels have been published in literature signifying their potential benefits (Table 1).

Table 1 Blood biomarkers and application

Biomarker	Use in COPD
CRP	Monitor prognosis and identify etiology
Procalcitonin	Bacterial causes of exacerbation
Neopterin	Monitor therapeutic effect and determine etiological cause
BNP	Determine prognosis
SP-D	Determine prognosis

Table mentioning blood biomarkers and their significance

Exhaled Biomarkers

Exhaled biomarkers are an easy and reproducible method for assessment of exacerbation of COPD. Exhaled breath has been studied as marker for presence of inflammation in the peripheral airways as seen in COPD.

Fractional Excretion of Nitric Oxide (Fe No)

Nitric oxide has been seen to be increased in expired gas in patients with asthma and has been shown to be an indicator for responsiveness for bronchodilation therapy (Makris et al. 2008).

In COPD, the role of FeNo seems to be limited. There is no change or decrease in FeNo in stable COPD patients. But in patients with cor pulmonale, it has been shown to be an indicator for need for prolonged therapy with oxygen support (Croxton and Bailey 2006). Endothelial release has been shown to be impaired leading to this phenomenon in COPD (Table 2).

Exhaled Carbon Monoxide

Exhaled gases in humans have carbon monoxide as a content. These levels are increased in exhaled gases of subjects who have smoking history and due to environmental pollution. In acute exacerbations, the levels of expired CO are elevated at higher levels and help in diagnosing such occurrences (Montuschi et al. 2001).

Exhaled Breath Condensate (EBC)

Exhaled gases consist of volatile organic compounds which have been studied extensively as a biomarker for COPD. This technique involves collection of exhaled air, freezing them, and analyzing the component biomarkers (Borrill et al. 2005). Electronic nose is the equipment designed with sensors to adsorb volatile organic compounds in exhaled gases.

Table 2 Various Biomarkers studied in COPD

Biomarkers of COPD
A. Blood-based biomarkers
1. Harmokines
A. Procalcitonin C. Adrenomedullin D. Copeptin E. Endothelin(ET-1)
2. Natriuretic peptides.
A. Atrial natriuretic peptide B. Brain natriuretic peptide
3. Acute phase reactants.
A. C-reactive protein B. Neopterin C. Strem-1 D. SP-D
B. Imaging biomarkers
A. Airway wall thickness. B. Type of emphysema C. Coronary artery calcification
C. Exhaled breath and sputum biomarkers
A. PH of exhaled breath condensate B. Fractional excretion of nitric oxide C. Cell counts in sputum D. Leukotriene B4 levels
D. Broncho-alveolar lavage biomarkers
A. CD8/CD4 ratio

Table mentioning about various types of biomarkers in COPD

VOC patterns are helpful in differentiating some phenotypes like exacerbations and eosinophils in sputum. Association of cell counts in sputum and EBC have been shown in mild to moderate COPD (Incalzi et al. 2012). EBC pH has been shown to correlate with airway inflammation, exacerbation episodes, and FEV1. Validation of these newer technologies needs to be carried out before its widespread use. There have been major limitations in development of this methodology due to difficulty in sample collection and analyzing the contents which are in small amounts. Gas chromatography and mass spectrometry are being used to study various gas components and showing some promising results (MacNee et al. 2011).

8-Isoprostane levels have been shown to increase in COPD patients as a biomarker in exhaled gas. It has been to correlated well with degree of emphysema. The levels of 8-isopropane are seen to be increased in severe emphysema and indicate exacerbation episodes in COPD (Montuschi et al. 2000).

EBC leukotriene has been studied as an indicator for exacerbation episodes, and it has been shown to be an indicator for airway inflammation. It has not been seen to be raised in normal patients and smokers without COPD where there is no presence of inflammation (Kostikas et al. 2005).

Sputum Biomarkers

Sputum analysis for biomarkers has been an easier approach as it is noninvasive and reproducible. The contents are basically cell count analysis and qualitative analysis. Neutrophil counts in sputum signify the intensity of airway inflammation. The levels are indicative of severity of disease and decrease in FEV1 values (Stanescu et al. 1999). Increase in human neutrophil peptides (HPN), neutrophil elastase, IL-8, and MMP-9 over a 2-year period in a study indicated rapid decliners in COPD (Koutsokera et al. 2013). Sputum eosinophil levels are indicative of overlap of asthma in COPD, and presence of high eosinophil counts signifies response to bronchodilator therapy. Many other sputum biomarkers have been studied like sputum 8-isopropane, IL-18, apolipoprotein A1 and lipocalin-1, TIMP-1, MMP-8, etc. (Gao et al. 2013) IL-6, IL-8, and MMP are associated with exacerbation episodes. Leukotriene B4 levels coincided with the occurrence of exacerbation and perhaps could be seen as possible exacerbation biomarker. One of the main problems with sputum biomarkers are the difficulty in few patients to collect and thereby inducing sputum samples has been shown to alter cell counts leading to diagnosing errors. It has been proposed not to induce sputum within 48 hr period (Barnes et al. 2006).

Biomarkers in BAL and Bronchial Biopsies

Bronchoscopic alveolar lavage fluid sampling involves performing bronchoscopy in COPD patients, instilling saline, and collecting the lavage fluid for biomarker assessment. It is invasive, skill requiring and difficult to reproduce. The entire lavage fluid is difficult to collect due to outflow obstruction seen in COPD patients. The supernatant fluid can sometimes give erroneous findings in biomarker levels.

BAL fluid sampling helps in detecting alveolar macrophages. The lymphocyte counts especially the ratio of CD8+ and CD4+ T cells are much higher in COPD BAL fluids. The neutrophil count and eosinophil counts have been seen to be increased, signifying inflammatory pathology in the airways. This increase is not seen in mild emphysema (Fabbri et al. 1998).

Other biomarkers in BAL fluid analysis includes neutrophil chemokine IL-8, eosinophil cationic protein, myeloperoxidase, etc. (Paone et al. 2011).

Bronchial biopsies are obtained in the form of brush biopsies and endobronchial biopsies. Biopsies help to document the inflammatory changes in the airway. Biopsies can help to depict structural changes in the airway with help of immunostaining.

The procedure is invasive and cumbersome and plays a limiting factor in diagnosing COPD exacerbation. With the help of newer techniques like laser microdissection, components can be studied individually (Franciosi et al. 2006). Bronchial biopsy studies have shown increased macrophages and CD8+ in the lamina propria. Neutrophils are increased in biopsy specimen analysis of severe COPD patients. Other biomarkers like neutrophil attractants ENA-78 and IL-8 and receptors like CXCR-1 and CXCR-2 are seen to be increased in biopsy analysis (Qiu et al. 2003).

COPD patients also exhibit raised levels of immunoreactive cells with IL-22+ and IL-23+ in the epithelial layers (Di Stefano et al. 2009). Other biomarkers like YKL-40 are found in the submucosal layer of biopsy specimens in COPD patients which help in diagnosing COPD patients with inflammatory airway pattern and can play a role in diagnosing exacerbation episodes (Vlachaki et al. 2010).

Imaging Biomarkers

MRI

The application of radiological imaging in determining disease progression in early stages of COPD has been well documented. Airway anatomy, alveolar microstructure, and ventilation defects can be identified, and disease prognosis can be determined. Magnetic resonance imaging can provide detailed information regarding these changes in COPD (Litmanovich et al. 2014).

These measurement can be useful in diagnosing patients with airway and alveolar changes, who may progress in to terminal COPD stages in the initial stages.

Imaging in COPD lungs depicts changes like airway dilatation, enlargement of the right ventricle, and coronary calcification which can help in improving the clinical accuracy in prediction of hospitalization risk in COPD patients (Galbán et al. 2012). Kirby et al. showed that helium MRI measurements could predict hospitalization risk in mild to moderate COPD, which were superior in prognostication capabilities when compared to lung function tests. Radiological imaging can help in augmenting clinical prediction and early diagnosis but cannot replace clinical acumen (Kirby et al. 2012).

CT Scan

COPD patients need to be evaluated with CT imaging in order to know burden of disease, and plan medical attention for future period. CT scans can be used to study central airway morphologies which can predict exacerbation episodes. Parametric response mapping with the help of CT scans have shown be correlate with lung function tests and with MRI functional abnormalities added, they can predict the FEV1 longitudinal changes (Coxson et al. 2013). Apart from airway imaging, perfusion imaging of the COPD lung plays a pivotal role in prognostication.

Dual-energy CT ventilation-perfusion measurements have been shown to have a relationship with lung function in published literature. One of the limitations of perfusion imaging is its lack of functional information that can provide about lung ventilation or perfusion. CT findings of centrilobular and panlobular emphysema in MESA study was associated with increased respiratory distress and reduced exercise capacity (Smith et al. 2014). The COPDGene study showed correlation between CT emphysema index and increased airway wall thickness with COPD-related mortality (Han et al. 2011).

Conclusion

COPD management focuses on diagnosis, prediction of worsening of disease, occurrence of exacerbation episodes, and monitoring therapeutic measures. Each of these biomarkers mentioned above have been precisely studied with these specific goals of management. COPD biomarkers will always play an integral part in future development of the many upcoming therapeutic strategies. Blood biomarkers like fibrinogen have been approved as biomarker tool. Imaging methods like CT scan and MRI for assessment of functional parameters and airway thickness have been recognized as important modality in COPD management already.

Sputum biomarkers will be an interesting opportunity for clinicians to study the occurrence of exacerbation episodes and monitor airway inflammation. More invasive techniques like BAL biomarkers and biopsy techniques would be used in patients according to appropriate clinical requirements. Emerging techniques like exhaled breath concentrate, gas chromatography, environment gene modification, lung microbiome, and gene expression markers need further study and research, in order to be used in clinical practice.

Mini-Dictionary of Terms (5–15 Terms)

1. **Procalcitonin:** Procalcitonin is a precursor molecule of hormone calcitonin. A rise in procalcitonin signifies occurrence of bacterial infection.
2. **sRAGE (Serum Levels of Soluble Receptor for Advanced Glycation End Products):** Reduction of this biomarker in blood plays a potential role in diagnosing severe COPD.
3. **Electronic nose:** Electronic nose consists of sensors which detect volatile agents in expired gas.
4. **Laser microdissection:** Instrument used to procure subpopulation of tissue specimen.
5. **Gas chromatography and mass spectrometry:** Technique used to study various gas components.

Key Facts of Biomarkers in Patients with Chronic Obstructive Pulmonary Disease (COPD) in Intensive care unit and Emergency medicine.

Key Facts of COPD

COPD is the third leading cause of death.
COPD consists of emphysema and chronic bronchitis.
Symptoms include chronic cough and shortness of breath.
Smoking and air pollutants are major cause factors.

Worsening of airway inflammation causes exacerbation episodes.
Causes decline in lung function.

Key Facts of Ideal Biomarkers

Should be usable in primary health centers.
Easy to be collected and analyzed.
Should pick up disease in early stages.
Should be able to classify disease in subcategories.
Should be reproducible.

Summary Points

- COPD is characterized by airflow limitation during expiration due to inflamed airways.
- The most common causes are infections in the lung and non-cessation of smoking.
- Blood biomarkers like fibrinogen and CRP have been utilized as markers for inflammation.
- Airway thickness in CT scan can predict chances of worsening and decreasing FEV1 volumes.
- Sputum analysis for biomarkers has been an easier approach as it is noninvasive and reproducible.
- CT ventilation-perfusion measurements have been shown to have corelationship with lung function.
- Lung microbiome and gene expression markers need further study and research.

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Part V

Specific Components

Procalcitonin As a Biomarker and Mediator of Sepsis: Implications for Critical Care

27

Paul Knapstein, Antonia Donat, and Johannes Keller

Contents

Abstract

Sepsis represents the most common cause of death in noncardiac intensive care units (ICU). Procalcitonin (PCT), a peptide overexpressed ubiquitously during systemic inflammation, is considered the most sensitive and specific biomarker for bacterial sepsis. In this chapter, we provide an overview of the existing literature regarding the pathophysiology of PCT secretion, its diagnostic and

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prognostic accuracy, as well as its value in antibiotic stewardship. The impact of monitoring PCT levels in critically ill patients with COVID-19 infections is described. Finally, biologic functions of PCT and a potential mediator role in sepsis-related events are discussed. The use of PCT as a sepsis biomarker is a valuable aid for ICU physicians and may potentially improve the outcome of affected patients if interpretation of PCT measurements is performed adequately.

Keywords

Sepsis · Procalcitonin · Critical care · Biomarker · Infection · Antibiotic stewardship · COVID-19 · Immune system · Prognosis · *CALCA* gene

Abbreviations

ALA-PRO	Alanin-Proline
ARDS	Acute Respiratory Distress Syndrome
CALCA	Calcitonin-Related Polypeptide Alpha
CD11b	Integrin Alpha M
CGRP	Calcitonin Gene-Related Peptide
CGRP1	Calcitonin Gene-Related Peptide 1
CRP	C-Reactive Protein
CT	Calcitonin
CTR	Calcitonin Receptor
DDP IV	Dipeptidyl peptidase IV
E. Coli	Escherichia Coli
IgG	Immunoglobin G
IL	Interleukin
KO	Knockout
LPS	Lipopolysaccharide
MODS	Multiple Organ Dysfunction
N-PCT	N-Procalcitonin
PBMCs	Peripheral Blood Mononuclear Cells
PCT	Procalcitonin
SIRS	Systemic Inflammatory Response Syndrome
SOFA	Sequential Organ Failure Assessment Score
qSOFA	quickSOFA
TNFa	Tumor Necrosis Factor alpha

Introduction

Sepsis is a life-threatening complication of infection. Although most data are collected in high-income countries, sepsis is accountable for nearly 20% of all deaths worldwide (Rudd et al. 2020; Fleischmann-Struzek et al. 2020). As a result, the World Health Organization made sepsis a global health priority and adopted a resolution to improve the prevention, diagnosis, and management of sepsis

(*WHA70.7*). Over the past several decades, a substantial amount of research and improved clinical processes have increased the speed of recognition and treatment of sepsis. In 2016, a new definition was adopted (Sepsis-3) to further refine this process, with an increased focus on recognizing organ dysfunction in the context of a dysregulated host response to infection (Shankar-Hari et al. 2016). Sepsis is now defined as the presence of an infection combined with an acute change of ≥ 2 points in SOFA score, which estimates the overall functioning of six major organs (Table 1). Septic shock is defined as a subset of sepsis in which underlying circulatory, cellular, and metabolic abnormalities increase the overall risk of mortality.

Sepsis represents a heterogeneous condition in which numerous humoral and cellular systems are activated, with a subsequent release of various molecules that mediate the host's response to infection. Bacteremia is only observed in about 30–43% of patients with sepsis, depending on age, previous antibiotic treatment, and severity (Brun-Buisson et al. 1996; Bates et al. 1997; Flayhart et al. 2007; Girard and Ely 2007). Early clinical signs of sepsis, such as fever, tachycardia, or leukocytosis, are nonspecific and overlap with signs of systemic inflammatory response syndromes (SIRS) of noninfectious origin, especially in patients who have suffered from major trauma or major surgery. Other clinical signs including arterial hypotension, thrombocytopenia, or organ dysfunction often manifest too late to initiate life-saving treatment. Delayed diagnosis and treatment initiation prolongs length of hospitalization, increases mortality, and the overall socioeconomic burden of sepsis. As clinical signs carry the potential risk of misinterpretation, biomarkers are playing an increasingly important role for early and reliable detection of sepsis.

The early identification of patients at risk of developing infectious complications is critical to enable early and appropriate treatment of sepsis. It has been demonstrated that prompt management of sepsis significantly prevents multiple organ dysfunction (MOD), reduces mortality and improves clinical outcomes (Dellinger et al. 2013; Cecconi et al. 2018a). Hence, a quantifiable sepsis parameter that allows early diagnosis and warrants early and appropriate treatment would provide optimal patient care. Based on the heterogeneous nature of sepsis-related events, multiple studies have been carried out to identify such a parameter, which also enables the differentiation between focal bacterial infection, sepsis, and/or noninfectious SIRS. Although a broad array of potential bloodstream biomarkers was tested for their ability to diagnose bacterial sepsis and to correlate with disease severity and outcome, Procalcitonin (PCT) has shown the highest sensitivity and specificity among all candidates investigated to date.

Pathophysiology of PCT Secretion

For a biomarker to be both sensitive and specific, it must be secreted into the blood stream during specific cellular and molecular events characteristic to the respective condition. In the case of sepsis, these events assumedly are triggered by the presence of bacteria or some of their cellular components in the systemic circulation. Mechanistically, bacterial components, defined as "pathogen-associated molecular

Table 1 The (q)SOFA score

System Parameter Unit	Respiration PaO_2/ FiO2 mmHg	CNS GSC	Circulation MAP or Vasopressors mmHg or µg/kg/min	Liver Bilirubin mg/dl	Coagulation Thrombocytes $\times 10^3$/µl	Renal Creatinine mg/dl
0	≥ 400	15	≥ 70	< 1.2	≥ 150	< 1.2
1	< 400	13–14	< 70	1.2–1.9	< 150	1.2–1.9
2	< 300	10–12	Dopamin ≤ 5 or Dobutamine	2.0–5.9	< 100	2.0–3.4
3	< 200 + mechanical ventilation	6–9	Dopamin > 5 or Adrenaline ≤ 0.1 or Noradrenaline ≤ 0.1	6.0–11.9	< 50	3.5–4.9
4	< 100 + mechanical ventilation	< 6	Dopamin > 15 or Adrenaline > 0.1 or Noradrenaline > 0.1	> 12.0	< 20	> 5
qSOFA	RR ≥ 22/min	< 15	Systolic BP ≤ 100			

The (quick) sepsis-related organ failure assessment (SOFA) score, modified according to (Vincent et al. 1996; Cecconi et al. 2018b). *BP* blood pressure, GCS Glascow Coma Scale, *CNS* central nervous system, *MAP* mean arterial pressure, *RR* respiratory rate

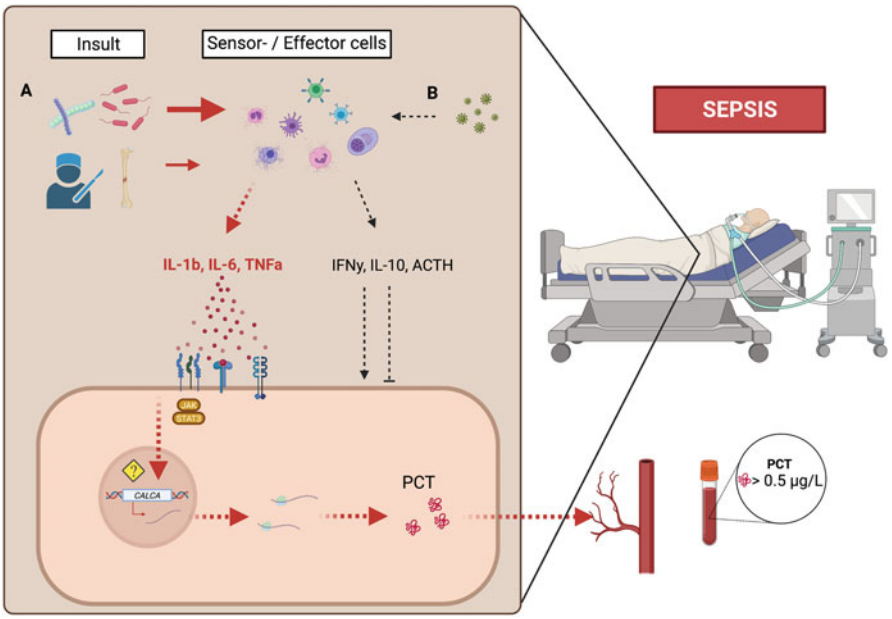

Fig. 1 Induction of PCT expression. **(A)** Bacterial infection, trauma, or major surgery may trigger immune responses, resulting in high concentration of specific circulating molecules and activated immune cells especially in the case of bacterial etiologies. By unknown mechanisms, these responses are associated with increased gene expression of *CALCA* ubiquitously, resulting in elevated PCT levels systemically. **(B)** Viral infections lead to altered levels of different sets of cytokines and chemokines with nonequivalent elevations of *PCT* levels. IL Interleukin, *TNFα* Tumor necrosis factor alpha, *IFNγ* Interferon gamma, PCT Procalcitonin.

patterns" (PAMP) including endotoxins or bacterial DNA, are recognized by the innate immune system through pattern-recognition receptors including toll-like receptors. This elicits an immediate immune response through activation of the intracellular core mediator nuclear factor κB, resulting in the release of numerous pro-inflammatory cytokines such as Interleukin-1, -6 (IL-1, IL-6), or tumor necrosis factor alpha (TNFa), among many others. Concurrently, anti-inflammatory molecules including IL-10, -11, -13, or hormones like adrenocorticotropic hormone (ACTH) raise in concentration to counteract the pro-inflammatory response (Opal and DePalo 2000; Cohen 2002) (Fig. 1). As a net result, the initially excessive pro-inflammatory state of the organism is shifted toward immune paralysis at later stages. On a functional level, the elevated concentrations of circulating inflammatory mediators result in vasodilation, increased vascular permeability, and reduced vascular resistance, leading to absolute volume depletion and hypoperfusion with ischemia in distant organs. Additionally, mitochondrial dysfunction may lead to impaired cellular oxygen utilization despite sufficient oxygen delivery, a phenomenon referred to as cytopathic hypoxia (Singer 2014). If the organism is unable to

counteract with compensatory increased cardiac output, cardiocirculatory failure occurs.

As high concentrations of calcitonin-like immunoreactivity have been reported in the blood of patients with various extrathyroidal diseases, Assicot et al. were the first to measure the calcitonin precursor PCT in patients with suspected infections. In their landmark publication in 1993, the authors demonstrated for the first time that high serum levels of PCT (6–53 µg/L) are found in patients with severe and invasive bacterial infection (Assicot et al. 1993), a finding which has been confirmed in countless clinical studies up to now. Structurally, the PCT peptide consists of 116 amino acids and is comprised of the amino terminus N-PCT, the calcitonin sequence, and the carboxy terminus sequence (Fig. 2). PCT is encoded by the *CALCA* gene, which through alternative splicing also gives rise to calcitonin gene-related peptide (CGRP), a neuropeptide known for its vasodilatory properties. While CGRP is synthesized in the central and peripheral nervous system in the healthy organism, PCT is primarily expressed in the thyroid gland and, after proteolytic cleavage, released as mature calcitonin into the circulation (Rosenfeld et al. 1983; Adema and Baas 1992). Although *CALCA* expression is comparatively strictly limited to thyroidal, neuronal, and neuroendocrine tissue, its expression becomes ubiquitous during sepsis and is detectable in nearly all tissues examined to date,

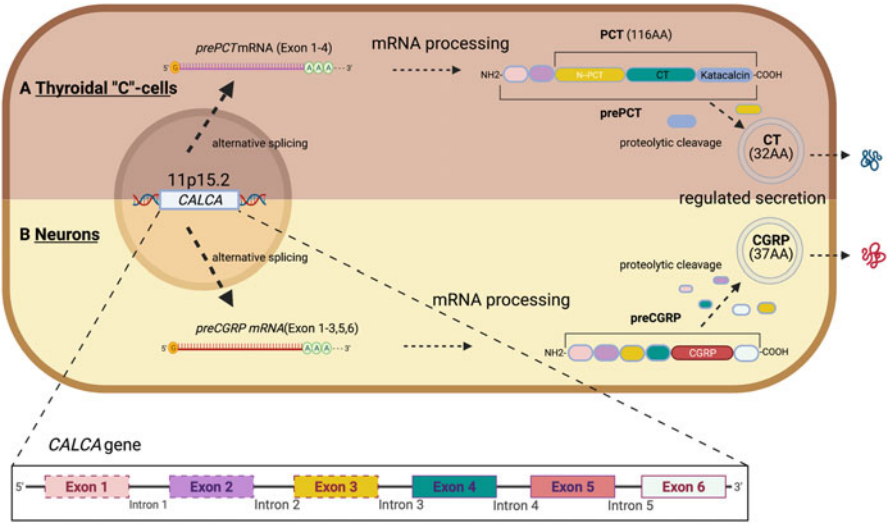

Fig. 2 Structure of the *CALCA* gene. **(a)** *CALCA* gene expression in thyroidal C-cells in the healthy organism. Once transcribed, PCT-mRNA strands are synthesized through alternative splicing. The PCT-mRNA is then translated into the PCT protein. After proteolytic cleavage of the N- and C-terminus, mature CT is stored in vesicles until release upon specific stimuli (e.g., serum calcium levels or other hormones). **(b)** In neurons, alternative splicing results in the synthesis of CGRP mRNA. After translation, CGRP protein is stored in vesicles and transported to nerve endings for release. *AA* Amino acid, *CT* calcitonin, *CGRP* Calcitonin-gene–related peptide, *CALCA* Calcitonin-related polypeptidealpha gene

including liver, lung, heart, muscle, fat, brain, and GI tract (Müller et al. 2001). Likewise, studies employing in situ hybridization showed that a broad array of different cell types within these tissues participate in the induction of *CALCA* expression (Linscheid et al. 2004). Although undetectable under normal conditions, considerate amounts of immunoreactive PCT are present in thyroidectomized individuals with sepsis (Silva et al. 1978).

Apart from PCT, the other *CALCA*-encoded peptide, CGRP, is also found at increased concentrations during sepsis. Based on its vasodilatory function, it was speculated that CGRP is involved in progressive hypotension during septic shock (Arnalich 1995; Arnalich et al. 1996; Beer et al. 2002; Shimizu et al. 2003). However, since the concentration of CGRP remains in the picomole range, it is not of great clinical value.

As global overexpression of a particular gene upon sepsis-related stimuli has not been reported for other peptides, this phenomenon seems to be unique for PCT. As noted by Becker at al. the entire organism functions as an endocrine gland during sepsis, secreting PCT in an ongoing and unregulated constitutive fashion (Burgess and Kelly 1987; Becker et al. 2010). It can be concluded that non-neuroendocrine tissue lacks the enzymatic potential to adequately process and activate the immature PCT peptide. It is currently not known why the calcitonin precursor protein is highly expressed in such pathologic settings, although it has been speculated that ubiquitous *CALCA* induction is mediated via stimulus-specific response elements within the promoter of the gene. Likewise, it is unclear why this mechanism has been evolutionarily conserved over a wide range of different species, and whether this phenomenon serves a particular purpose in the organism's response to invading bacteria or sepsis-related events.

To date, the key findings by Assicot et al. have been confirmed in hundreds of clinical studies, and serum PCT concentrations have been further correlated with severity of microbial invasion. PCT levels were shown to exhibit a short time of induction (within hours) after disease onset, a long half-life (approx. 24 h) and a wide biological range, with concentrations ranging from tens, to hundreds, to thousands of times the normal levels of less than 0.1 μg/L in bacterial sepsis (Meisner et al. 2001). In contrast, local bacterial, systemic viral and fungal infections are associated with only slightly increased PCT levels (0.1–1.5 μg/L) (Wacker et al. 2013). Additionally, PCT levels decrease rapidly upon appropriate antibiotic therapy (Cabral et al. 2018). Although a single biomarker is unlikely to provide a sufficiently comprehensive picture of sepsis progression, to date PCT is considered the most specific (55–94%) and sensitive (66–89%) biomarker for systemic bacterial infection (Müller et al. 2000; Tang et al. 2007; Hoeboer et al. 2015).

Diagnostic Accuracy

In the past several decades, substantial research has been conducted to precisely determine the diagnostic accuracy of PCT. However, heterogenic study approaches (sample timing, cut-off values, consideration of blood culture results) and alternating

definitions for sepsis have obscured a clear diagnostic significance. Therefore, it does not seem surprising that perspectives on PCT as a diagnostic and prognostic biomarker vary significantly among experts, and that PCT measurements have not been implicated in consensus guidelines for clinically suspected infection. As mentioned above, PCT was consistently found to be more specific in case of bacterial infections than any other commonly used inflammatory marker, including C-reactive protein, white blood cell count, or erythrocyte sedimentation rate. Importantly, the diagnostic accuracy of PCT was found to improve with worsening disease severity, demonstrating the highest accuracy (89% sensitivity and 94% specificity) in critically ill patients (Müller et al. 2000; Riedel et al. 2011; Wacker et al. 2013; Hoeboer et al. 2015). On the other hand, reported sensitivities ranging from 66% to 89% and specificities ranging from 55% to 79% illustrate the heterogeneity of clinical settings in which diagnostic accuracy was studied.

Observational studies showed that most patients with noninfectious SIRS have an inflammatory-mediated procalcitonin level between 0.3 μg/L and 0.8 μg/L, while patients with bacterial sepsis (from any source) in an ICU setting demonstrated levels ranging from 4.5 μg/L to 12.0 μg/L or higher (Castelli et al. 2004; Meynaar et al. 2011; Deng et al. 2013). In addition, rapid decline back to normal PCT levels most often indicated resolution of systemic inflammation. Therefore, the initial peak in PCT levels is regarded to reliably differentiate between bacterial sepsis and noninfectious SIRS in critically ill patients, which is not the case for alternative biomarkers including C-reactive protein (CRP) and IL-6. Furthermore, since the initial increase in serum levels usually precedes the onset of clinical symptoms, PCT allows an earlier detection of systemic infection compared to conventional standard methods.

The high prognostic value of PCT in sepsis was demonstrated in one large US study in which the kinetics of PCT levels within the first 72 h of ICU admission were strongly associated with mortality (Schuetz et al. 2017a). The authors concluded that mortality increases twofold if PCT levels do not drop by at least 80% from the initial maximum within this time frame, representing a significant independent predictor. Importantly, nonbacterial etiologies that moderately increase PCT include severe trauma, cardiac arrest, surgery, burns, pancreatitis, malaria, invasive *Candida* infections, or intracranial hemorrhage. However, in these settings PCT levels are consistently found to be significantly lower than in sepsis (Meisner et al. 1998; Charles et al. 2006; Deng et al. 2013; Engel et al. 2013; Bruneel et al. 2016; Cabral et al. 2018; He et al. 2018; Alrawahi et al. 2019; Shi et al. 2021) (Table 2).

Most studies investigating the diagnostic reliability of PCT in local infections relied on study cohorts with pulmonary infection, as pneumonia represents the most common site of infection leading to sepsis. The diagnostic accuracy of PCT (AUC=0.75) for pneumonia alone was found to be lower than IL-6 (AUC=0.80) or CRP (AUC=0.82) (Wussler et al. 2019), but it was still possible to distinguish pneumonia from a COPD exacerbation or asthma (Bafadhel et al. 2011). Interestingly, median procalcitonin levels were higher (2.5 μg/L) in patients with pneumonia caused by typical bacteria, such as *Streptococcus pneumoniae* or *Staphylococcus aureus,* compared to atypical bacteria (0.20 μg/L) or viruses (0.09 μg/L) (Self et al.

Table 2 PCT reference ranges in relevant clinical conditions

Infection	PCT range in μg/L	Citation
None	< 0.1	Assicot et al. (1993)
Local infection	0.1–1.5	Wacker et al. (2013)
Beginning sepsis	0.5–2.0	Vijayan at al. (2017)
Severe bacterial sepsis	6–53	Assicot et al. (1993)
Severe virai sepsis	0.05–0.54	Self et al. (2017)
Severe fungal sepsis	< 5.5	Charles et al. (2006)
Malaria	5.6–41.1	Bruneel et al. (2016)
Non-infectious SIRS	0.3–0.8	Castelli et al. (2004) and Meynaar et al. (2011)
Other causes	**PCT range in μg/L**	Citation
Minor/aseptic surgery	0.18–0.6	Meisner et al. (1998)
Major surgery	0.3–1.49	Meisner et al. (1998)
TBI	0.08–0.31	Deng et al. (2013)
Intracranial hemorrhage	0.035–0.078	He et al. (2018)
Burn > 15% of body surface	2.1–7.0	Cabral et al. (2018)
Cardiac arrest	1.08–3.07	Engel et al. (2013)

An overview of exemplary human PCT values related to infectious and non-infectious conditions. Overall, higher PCT levels can be observed in severe bacterial infection, while viral sepsis and noninfectious conditions increase PCT only slightly above the reference range

Table 3 PCT levels related to different pathogens

Pathogen	Mean PCT value in μg/L	Regression coefficient
S. aureus	7.2	0.00[1] (p = 0.1)
Streptococcus spp.	18.2	0.50 (p = <0.001)
Enterococcus spp.	6.8	0.06 (p = 0.6)
E. coli	26.8	0.50 (p = <0.001)
Other enterobacteriaceae	24.9	0.49 (p = <0.001)
P. aeruginosa	23	0.04[1] (p = 0.8)
Candida spp.	4.7	-

PCT values and regression coefficients associated with bacteremia of different pathogens. Modified after (Thomas-Rüddel et al. 2018) [1]values were calculated for Streptococcus spp. and Pseudomonas spp., respectively

2017). Among atypical bacteria, *Legionella* species were reported to cause only modest elevations in PCT, while *Mycoplasma* and *Chlamydia* species may not be associated with detectable elevations (Haeuptle et al. 2009; Bellmann-Weiler et al. 2010) (Table 3). Most importantly, PCT-guided antibiotic stewardship for pneumonia did not lead to improved outcomes (Huang et al. 2018). In other locally confined infections, such as urinary tract infections, PCT plays only a limited role in diagnosing or monitoring disease progression (van der Starre et al. 2014).

While PCT does not have a clinical use in diagnosing localized bacterial infections, it is useful for early diagnosis of sepsis and allows clinicians to estimate disease severity and outcome when measured on a regular basis. However, PCT levels must be interpreted carefully in the context of medical history, physical examination, and microbiological assessments.

Procalcitonin-Guided Antibiotic Stewardship

Besides early diagnosis, immediate and adequate antibiotic therapy is of vital importance in treating sepsis in critically ill patients. However, excessive and prolonged antimicrobial treatment is undesirable due to potential antibiotic resistance and damage to the physiological microbiological flora, harboring more than five trillion bacteria (Jernberg et al. 2010; Shin et al. 2015). Individualizing antibiotic treatment improves antibiotic stewardship efforts to encourage reasonable and indicated use of these agents, which mitigates the emergence of multidrug-resistant pathogens, one of the most imminent threats to global health directly linked to antibiotic overuse (WHO, 2020. Antimicrobial Resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance. Accessed: 30 Sep 2021). Therefore, specific markers for the resolution of infection may assist physicians in making adequate decisions regarding antibiotic therapy on a more personalized basis. However, physicians may be especially reluctant to shorten the duration of antimicrobial treatment in critically ill patients.

Much research has focused on the potential benefits of using serum PCT levels to guide antibiotic therapy (referred to as PCT-guided antibiotic stewardship), especially in patients suffering from proven or suspected bacterial infection in the ICU. As discussed above, PCT has been advocated as a biomarker with higher specificity and sensitivity than any other biomarker for monitoring the course of sepsis in critical ill patients. Mounting evidence indicates that PCT guidance is associated with a reduction in treatment duration and the daily antibiotic dose in critically ill patients with presumed bacterial infection, which may reduce overall mortality.

In this regard, Wirz et al. compared 2,252 PCT-guided ICU patients with 2,230 control group patients and found an earlier discontinuation of antibiotics with a reduction in treatment duration in PCT-guided patients (9.3 days vs 10.4 in control), with stronger reductions seen in patients with less severe sepsis and those with initial respiratory infections. This meta-analysis revealed that PCT use was found to result in significantly improved survival (21.1% mortality in PCT-guided vs 23.7% in control group, $p = 0.03$), which was consistent in subgroup analyses stratified by type of infection, Sepsis-3 definition, or severity of sepsis (Wirz et al. 2018). In contrast, in a randomized clinical trial, Bloos et al. found no significant differences in the frequency of diagnostic or therapeutic procedures, although there was still a reduction in overall antimicrobial exposure from 862 days in the conventional group to 823 days in the procalcitonin guidance group (4,5%, $p = 0.02$). However, the

recommendation to stop antimicrobial therapy, based on serial PCT measurements on day 0, 1, 4, 7, 10, and 14 after admission, was overruled by the treating physicians in 50.4% of cases due to the presence of fever, microbiologic findings, or changes in white blood cell count. In this study, PCT was measured only every 3 days, which could result in loss of potential antibiotic-free days (Bloos et al. 2016). In the PRORATA trial, daily PCT measurements in patients receiving antibiotics led to a decrease in treatment duration of nearly 3 days with no changes in overall mortality (Bouadma et al. 2010). Of note, this study included patients with less severe disease, which may have resulted in a more confident handling of antibiotic discontinuation. Similarly, a randomized, controlled, open label trial demonstrated reduced antibiotic exposure and mortality in the ICU when measuring PCT levels daily. In the PCT-guided group, the median consumption of antibiotics was 7.5 days versus 9.3 in the control group. Furthermore, mortality was found to be significantly higher in the control group (25% within 28 days and 43% after 1 year) than it was stated for the PCT-guided group (20% within 28 days and 36% after 1 year, $p=0.0122$). The advice to discontinue antibiotics was defined by 80% reduction of initial PCT levels or a level of 0.5μg/L or lower (de Jong et al. 2016). Additionally, a recently published meta-analysis demonstrated that patients with sepsis and confirmed bacteremia exhibit a lower antibiotic exposure by almost 3 days without an apparent increase in mortality when subjected to PCT-guided management (Meier et al. 2019). Infections with gram-positive bacteria or *Escherichia coli* tended to resolve with shorter antibiotic use compared to other bacteria.

In such complex settings, it remains unclear whether the reduction in antibiotic exposure fully explains the differences in mortality reported by some authors. Low adherence to PCT-guided algorithms was an issue in trials failing to demonstrate a benefit of PCT-guided care. Current evidence on other types of infections is sparse and few studies have included patients with immunosuppression, limiting the generalizability of respective conclusions to more vulnerable patient populations. Another important consideration is the quality of PCT assays, which varied considerably among the most important clinical studies discussed above. Only high-sensitivity PCT assays should be used in clinical practice on a daily basis, as semiquantitative assays may not detect changes in PCT levels at lower ranges. Since clinical judgment and monitoring of standard laboratory parameters plays an important role in most modern ICU settings, training and experience in the proper use of PCT is essential.

Overall, individualization of antibiotic treatment regiments based on biomarker levels is preferable to the use of rigid treatment regiments. As a marker with both diagnostic and prognostic implications, PCT-guided antibiotic stewardship has shown promising and consistent evidence in its ability to reduce antibiotic use and improve clinical outcomes in critically ill patients with sepsis, if measured daily. This could be an important step in promoting the judicious and correct use of antibiotics, and aid in mitigating the further evolution and spread of multidrug resistant pathogens (Fig. 3).

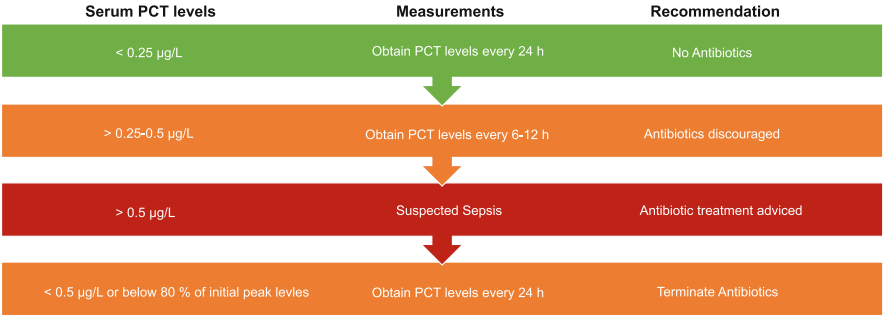

Fig. 3 PCT-guided treatment algorithm. Recommended schematic PCT-guided antibiotic stewardship. Initiation of empirical antibiotic treatment is recommended when PCT levels start to rise above 0.5 mg/L. Discontinuation of antibiotics is recommended when PCT serum levels drop below 80% of initial peak values or fall below 0.5 mg/L

COVID-19 and PCT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection or coronavirus disease 2019 (COVID-19) is a pandemic disease, resulting in high intensive care burden. Prior to the introduction of vaccine against SARS-CoV-2, about 80% of cases experienced mild symptoms such as fatigue, dry cough, or fever and recovered without hospitalization. Around 14% required ventilation in an ICU setting, and 5% were classified critical, as they are associated with acute respiratory distress syndrome or respiratory failure, sepsis, acute cardiac injury, and heart failure (Yang et al. 2020).

COVID-19 infection is initiated by viral spike protein S1 attaching to its complementary host cell receptor (ACE2R), which is highly expressed in the respiratory tract, leading to endocytosis of the virus. The virus replicates and spreads in lytic cycles while the host's immune response is triggered to contain the pathogen, resulting in considerably high cytokine concentrations (Hu et al. 2021). Subsequently, the lung tissue is damaged and thus more susceptible to secondary bacterial infection, as seen in 3.2–14.3% of COVID-19 patients, adding to the mortality in severe cases (Chen et al. 2020; Hughes et al. 2020; Langford et al. 2020; Rawson et al. 2020b; Wu and McGoogan 2020; Garcia-Vidal et al. 2021).

Since PCT is a biomarker for bacterial infections, the initial levels usually remain unaltered during the admission of viral COVID-19 patients exhibiting mild symptoms (Zeng et al. 2020; Gao et al. 2021). General inflammation parameters such as leukocyte count and CRP are frequently elevated at the time of diagnosis, but do not allow differentiation between viral and bacterial infection. Evidence has shown that PCT levels increase significantly in critical or fatal COVID-19 courses (Chen et al. 2020; Guan et al. 2020; Huang et al. 2020; Rello et al. 2020; Zhang et al. 2020; Yan et al. 2020). Interestingly, initial elevated PCT values have been associated with a nearly fivefold higher risk of severe SARS-CoV-2 infection (Hu et al. 2020; Gao et al. 2021). Furthermore, between 25% and 36% of deceased patients had PCT

levels of 0.5 μg/L or higher, whereas only 2 % of patients recovered from such high levels (Guan et al. 2020; Zhou et al. 2020). This observation suggests that PCT levels in COVID-19 patients may be important in dynamically predicting disease severity and provide information about subsequently occurring secondary infections.

Most patients with COVID-19 receive antibiotics (79 %) but only 14.3 % exhibit secondary bacterial infection (Langford et al. 2020; Rawson et al. 2020a; Garcia-Vidal et al. 2021). The adverse events caused by unnecessary use of antibiotics could potentially be reduced, as this widespread use is not supported by contemporary data (Langford et al. 2020). Interestingly, 93 % of all deceased and 89 % of all recovered patients received empirical antibacterial therapy (moxifloxacin, cefoperazone, or azithromycin), which leads to the conclusion of no advantage in terms of survival after rigid antibiotic treatment (Chen et al. 2020). Moreover, use of broad-spectrum empiric antibiotics were associated with higher mortality (Rhee et al. 2020). Although larger studies are needed, a prospective, single-center, cohort study suggests that the use of PCT as a guide for de-escalation of antibiotics significantly reduced antibiotic usage by 2 days in COVID-19 patients (Heesom et al. 2020).

Overall, currently available data show that serum levels of PCT increase significantly as COVID-19 disease deteriorates. Therefore, daily measurement of PCT levels may be important for the decision on whether additional antibiotics are indicated in severe and critical cases of COVID-19 infection.

Experimental Data on a Potential Mediator Role of PCT

Even though PCT represents a valuable biomarker in sepsis and sepsis-related events, it is unclear why the calcitonin precursor protein is highly expressed ubiquitously in such pathologic settings, and why this mechanism has been evolutionarily conserved over a wide range of species. While the biologic function of other biomarkers, for example, TNFα or IL-6, have been studied intensively, only limited information regarding a potential mediator role of PCT is available. Possible reasons include the limited availability of adequate gene deficiency models due the complex organization of the *CALCA* gene (Hoff et al. 2002). Furthermore, PCT levels are barely measurable in the healthy organism, contributing to the ongoing challenge to understand its function. Thus, a clearly defined role of PCT in health and disease is still missing. However, as PCT expression is increased in most vertebrates during sepsis (Redl et al. 2001; Bonelli et al. 2015; Perez-Ecija et al. 2021), experimental approaches employing laboratory animals and cell culture systems have brought forward our understanding of PCT action during systemic inflammation to some extent, as discussed in the following sections.

PCT Mediates Cytokine Release and Immune Cell Migration

Whereas thyroidal C cells are the principal source of the minute amounts of circulating PCT under physiological conditions, large quantities of PCT are released

from various peripheral tissues in sepsis. In hamsters with an experimentally induced, systemic *E. Coli* infection, PCT levels in the spleen, liver, adrenal glands, brain, and spine increased hundreds-fold over controls and reached more than tenfold over controls in six distinct major organs including lung, pancreas, and kidney (Müller et al. 2001). Moreover, in vitro experiments using a variety of different cell types showed that different pro-inflammatory signals are capable of inducing *CALCA* expression. For example, liver samples from healthy donors were found to produce PCT upon stimulation with TNFα or IL-6, even without addition of lipopolysaccharide (LPS) (Nijsten et al. 2000). In a different study, human peripheral mononuclear blood cells, in particular lymphocytes and monocytes, were shown to release PCT upon incubation with LPS only (Oberhoffer et al. 1999). In addition, Linscheid et al. reported a transient overexpression of *CALCA* mRNA in human monocytes after incubation with IL-1β and LPS, which eventually returned to baseline after 18 h (Linscheid et al. 2004). However, anti-inflammatory molecules such as IL-10 did not affect PCT expression in human mononuclear cells (Oberhoffer et al. 1999). Incubation of renal mesangial cells with PCT alone led to a time-dependent increase in IL-6 and TNFα (Araujo et al. 2013), pointing toward a feed-forward mechanism between bacterial stimuli and inflammatory signaling. In a different study, reduced levels of anti- and proinflammatory signals after pre-incubation of human PBMCs with PCT have been reported (Matera et al. 2012). These contrasting results might indicate a more complex regulation in a time-dependent manner in vivo, even though the exact same concentrations of PCT have been employed in the respective studies in vitro. In clinical trials, PCT reached its peak later than other inflammatory markers (Bloos 2015) and studies investigating the effects of preincubation with PCT should therefore be carefully interpreted.

From a functional perspective, evidence for pro-inflammatory effects of PCT has been found in different in vitro studies, where aberrant immune cell migration and endothelial function under incubation with PCT was observed. In particular, PCT inhibited LPS-induced expression of factor CD11b on neutrophiles and macrophages in a dose-dependent manner, thus decreasing the migration of immune cells toward the site of infection and presumably augmenting levels of inflammatory immune cells in the bloodstream (Monneret et al. 2003). Furthermore, PCT increased the intracellular calcium uptake of monocytes and neutrophiles derived from human whole blood samples with the same efficiency compared to LPS (Wei et al. 2008), substantiating further evidence on potential pro-inflammatory properties of PCT. In addition, PCT deactivated chemotaxis of monocytes and neutrophils in the presence of other chemotactic factors in different trials (Wiedermann et al. 2002; Liappis et al. 2011). However, no direct interference or toxic effect on bacteria has been observed to date, supporting the hypothesis that PCT acts as a crucial mediator of sepsis with a function similar to acute phase proteins and immunomodulatory cytokines.

In contrast to the above considerations, two different studies reported anti-inflammatory effects of PCT and raised further speculation about a beneficial and thus evolutionarily conserved role of PCT. First, addition of exogenous PCT to a

whole-blood culture model incubated with LPS diminished TNFα levels by up to 27 %, even though the applied dose of PCT (10^{-7} M) exceed pathophysiological PCT levels in sepsis, and results should therefore be subject to careful interpretation (Monneret et al. 2000). Second, PCT inhibited TNFα-dependent overexpression of inducible nitric oxide synthase in rat vascular muscle cells (Hoffmann et al. 2001), potentially representing compensatory mechanism for hypotension in patients with septic shock. In contrast, Wagner et al. reported PCT decreased vascular endothelial cadherin expression, and therefore disrupted the endothelial barrier, contributing to the capillary leakage clinically observed in sepsis (Wagner et al. 2017).

Neutralization of PCT Is Associated with Reduced Mortality and Morbidity in Experimental Sepsis

As most in vitro studies described above reported divergent functions of PCT and do not allow definite conclusions regarding its functions, a considerate amount of experimental in vivo studies have also been conducted. Human PCT levels rise multifold during sepsis, and a similar response has been observed in other vertebrates. A prospective study found PCT levels of septic horses to be increased 10-fold in comparison to baseline in healthy controls (Bonelli et al. 2015). In addition, experimental sepsis caused by injection of LPS or lethal total body irradiation raised levels of PCT in dogs up to 2.5-fold and in rodents up to 20-fold, respectively (Biju et al. 2012; Easley et al. 2020). In baboons, a similar PCT response toward an infusion of *E. Coli* was observed, and excessive levels of PCT were measured in lethal courses of disease (Redl et al. 2000).

Concurrently, trials investigating the effects of exogenous PCT administration as well as PCT blockage in experimental sepsis have been carried out (Fig. 4). Over two decades ago, Nylen et al. reported an increase in lethality from 43% to 94% after treating septic hamsters with human PCT. Within the same study, blockage of PCT via immunoreactive goat serum decreased rodent mortality by 50 % (Nylen et al. 1998). In a large animal study, rabbit serum with IgG targeting the aminoterminus of PCT was administered to pigs 3 hours after induction of sepsis and led to improved cardiac and renal function, as well as increased short-term survival (Martinez et al. 2001). Under the same experimental conditions, administration of PCT-reactive rabbit serum during the induction of sepsis had similar positive effects on porcine cardiac index, creatinine clearance, and short-term survival (Wagner et al. 2002), giving further evidence for a crucial role of PCT during early stages of infection. From a structural point of view, it is yet to be determined whether the ALA-PRO sequence, hence N-PCT, is responsible for the observed pro-inflammatory effects of PCT. Two distinct trials reported improved outcomes in sepsis after neutralization of N-PCT. In particular, septic rats displayed an inhibition of inflammatory cytokine production, as well as increased survival rates upon treatment with a highly specific N-PCT antibody (Tavares and Miñano 2010). Furthermore, the severity of acute lung injury following sepsis was significantly reduced by administration of anti-rat N-PCT after induction of sepis (Tavares et al. 2014).

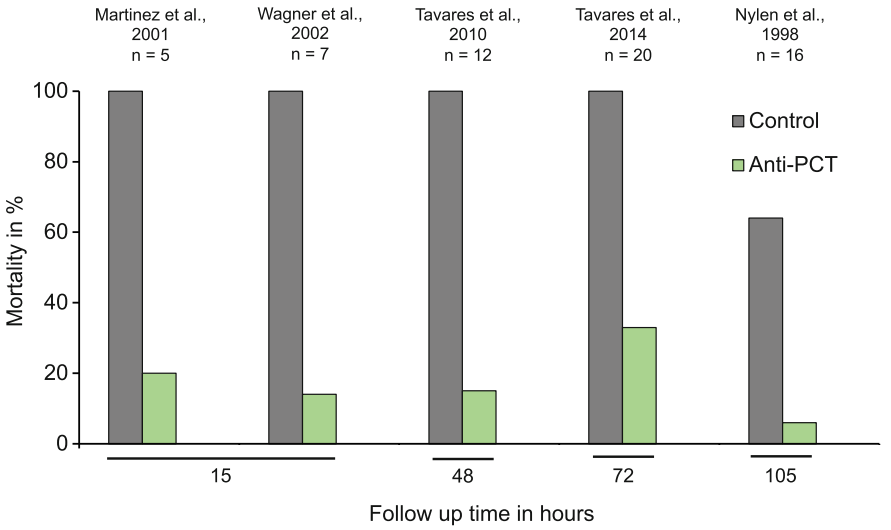

Fig. 4 PCT antibodies in experimental sepsis. Treatment with antibodies neutralizing PCT improves survival in various animal models of experimental sepsis within observation periods ranging from 15 up to 102 h

The role of mice as an adequate model to investigate PCT in sepsis has been critically examined, as a study employing models of severe bacterial infection did not observe extrathyroidal PCT expression or release in WT, nor a difference in survival in *CALCA*-deficient mice, despite the fact that exposure to respective bacteria in the respiratory tract or in the abdomen provoked lethal courses in 20–80% of all cases (Tuvim et al. 2013). In contrast, using more aggressive models of experimental sepsis including LPS injection and cecal ligation and puncture with mortality rates of 100%, Baranowsky et al. detected increased *CALCA* expression and elevated PCT levels in septic mice, even though the respective increases were rather small compared to humans with sepsis (Baranowsky et al. 2021). In these models, mice lacking *CALCA* demonstrated a moderate, yet significant survival benefit, indicating that overall PCT exerts a negative effect on disease outcomes in sepsis. Mechanistically, an immunomodulatory effect of PCT on innate immune cells including macrophages was observed, resulting in increased release of pro-inflammatory IL-17a from gamma delta (γδ) T cells (Baranowsky et al. 2021).

Limited information is available to date on the relevant PCT receptor. Sexton et al. investigated whether PCT has bioactivity at the calcitonin receptor family complexes. The authors showed that PCT had only moderate activity at the calcitonin receptor (CTR) but acted as a partial agonist at the calcitonin receptor-like receptor (CGRP receptor) in vitro (Sexton et al. 2008). In this regard, Baranowsky et al. found that mice lacking CTR globally did not display altered

survival curves during sepsis, ruling out CTR as the biologically relevant PCT receptor in vivo. However, application of the CGRP receptor antagonist olcegepant, initially developed for the treatment of migraine, resulted in improved survival in experimental sepsis in a PCT-dependent manner. Together, these results indicate that the harmful effects of PCT observed in experimental sepsis are mediated by the CGRP receptor, at least in mice (Baranowsky et al. 2021). However, blockage of the CGRP receptor did not improve survival in a porcine model of autologous polymicrobial sepsis, suggesting that therapeutic usage might be limited due to adverse effects on the cardiovascular system (Messerer et al. 2022).

In sum, experimental research provides comparatively strong evidence for a mediator role of PCT in sepsis. Beneficial effects of PCT-neutralization or CGRP receptor antagonism in different experimental settings (Nylen et al. 1998; Tavares et al. 2014; Baranowsky et al. 2021) further suggest PCT as a potential pharmacologic target to improve disease outcomes in patients with sepsis.

Applications to Prognosis

As a biomarker with both diagnostic and prognostic use in clinical practice (Riedel et al. 2011; Wacker et al. 2013; Hoeboer et al. 2015), stringent PCT-guided antibiotic stewardship consistently reduced antibiotic use and improved outcomes in critically ill patients with sepsis, if measured daily. The current recommendation to discontinue antibiotics was defined by an 80% reduction of initial PCT levels, or a serum concentration of 0.5 μg/L or lower (de Jong et al. 2016; Schuetz et al. 2017b). Since multidrug-resistant pathogens represent an increasing threat to global health (WHO, 2020 Antimicrobial Resistance https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance. Accessed: 30 Sep 2021), the use of individualized antibiotic treatment regimens guided by one or more biomarkers is considered advantageous over rigid treatment regiments.

Applications to Other Diseases or Conditions

Although a biomarker role of PCT was first reported in a pediatric population with sepsis (Assicot et al. 1993), most clinical studies investigated PCT in adults only. Concerning neonatal sepsis, a condition primarily affecting infants, which were delivered preterm and/or with a low birth weight, PCT was found to have a higher sensitivity and specificity for diagnosing early-onset (<72 h after birth) sepsis when compared to IL-6 and CRP, respectively (Chiesa et al. 2003). More recently, PCT was found to be superior for estimating outcome of late-onset (>72 h after birth) neonatal sepsis in comparison to CRP and correlated with 7-day mortality in a similar manner as IL-6 (Kurul et al. 2021). Overall, PCT might be a valuable diagnostic and prognostic biomarker independent of age.

Mini-Dictionary of Terms

- **Antibiotic Stewardship.** Rational and responsible use of antibiotics through the detection of a (bacterial) infection, the choice of appropriate antibiotic, adaptation of duration of therapy, dosage, and form of antibiotic administration.
- **C-reactive Protein.** An acute phase protein of hepatic origin. Clinically used as a biomarker to estimate the severity of overall infection.
- **Interleukin-6.** A pro-inflammatory cytokine which is, upon infectious stimuli, derived from various cells such as macrophages, monocytes, and endothelial cells.
- **Procalcitonin.** A 116-amino acid bioactive molecule, which is encoded by the *CALCA* gene. It is found to play a mediator role in sepsis and is clinically used to diagnose systemic bacterial infection.
- **Sepsis.** Severe organ dysfunction upon an infectious stimulus with increased risk of overall mortality.
- **Systemic inflammatory response syndrome.** Severe organ dysfunction due to a systemic inflammatory response to noninfectious stimuli.

Key Facts of Procalcitonin As a Biomarker and Mediator of Sepsis: Implications for Critical Care

Key Facts of PCT As Prognostic Marker of Sepsis

Patients with severe or systemic bacterial infections display significantly increased PCT values. Diagnosis of sepsis should be based on clinical features (e.g., qSOFA score) in combination with biomarkers (e.g., PCT, IL-6). Measuring PCT on a regular basis may help to predict severity and outcome of sepsis. In addition, rapidly decreasing PCT values can be used as a guide for discontinuation of antibiotic treatment.

Key Facts of PCT in COVID-19

In COVID-19 patients with mild symptoms, PCT levels are usually within the physiological reference range upon admission. In critically ill patients or lethal courses of the disease, PCT increases significantly. In addition, 14.3% of COVID-19 patients suffer from secondary bacterial infection. PCT therefore may be used to predict the clinical course and outcome of COVID-19 infections. Interactions of empirical antibiotic usage and potential adverse effects must be taken into consideration.

Key Facts of PCT in Experimental Sepsis

Most vertebrates display increased PCT levels in severe bacterial infection and sepsis. The addition of exogenous PCT increases mortality in experimental sepsis. In turn, antibodies against PCT improve survival in experimental sepsis in pigs, hamsters, rats, and mice. Harmful effects of PCT might result from its action on the CGRP receptor.

Summary Points (5–15)

- Sepsis accounts for nearly 20% of deaths worldwide, while bacteremia is only observed in 30–40% of patients.
- In sepsis, PCT is released ubiquitously from nearly all tissues and serum levels can rise hundreds-fold above the reference range (< 0.1 μg/L).
- PCT is the biomarker with the highest sensitivity and specificity for diagnosing bacterial sepsis.
- Higher levels of PCT are associated with higher risk of severe SARS-CoV-2 infection.
- Inhibition of PCT or its receptor decreases mortality in experimental sepsis.

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Serum Interleukin-6 Levels as a Biomarker in Trauma

28

Onder Kalenderer and Serkan Erkus

Contents

Abstract

The degree of injury that occurs after trauma is proportional to the exposure of trauma energy exceeding the threshold of physiological tolerance of the body or organs. Therefore, it is crucial for the evaluation of traumatized patients to analyze the physiological limits appropriate and not to ignore possible messengers, such as biomarkers in serum. Biomarkers secreted following trauma aim to defend and heal the organism through intercellular and/or intracellular communication pathways. IL-6 is a biomarker due to this purpose and is detected in large amounts in serum, especially after severe traumas. Nevertheless, if the organism

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cannot compensate for the effects of this biomarker, serious and devastating problems can develop.

Keywords

Biomarker · Trauma · Inflammatory process · SIRS · Interleukin-6

Abbreviations

APC	antigen presenting cells
ARDS	acute respiratory distress syndrome
CARS	compensatory anti-inflammatory response syndrome
CRP	C-reactive protein
IL	Interleukin
MODS	Multiple Organ Dysfunction Syndrome
MOF	multiple organ failure syndrome
PCT	Procalcitonin
PTX3	Pentraxin 3
SIRS	systemic inflammatory response syndrome
TGF-β	Transforming Growth Factor-Beta
TNF-α	Tumor Necrosis Factor-alpha
VEGF	vascular endothelial growth factor

The Effect of Trauma on the Organism

Trauma is one of the leading causes of death in the world. Especially the management and treatment of multiple trauma patients and the prevention of developing complications are of importance. When the cases resulting in death after trauma are examined, 45% of the cases die at the time of the trauma. It is also known that 10% of patients in this population die within the first 24 h. This is often due to traumatic brain injury or uncontrolled ongoing hemorrhage. The remaining deaths occur due to various systemic complications such as sepsis, acute respiratory distress syndrome (ARDS), and multiple organ failure syndrome (MOF) that develop following trauma (Baker et al. 1980).

The organism gives about some systemic and local responses in order to regulate its homeostasis and protect itself immediately after being exposed to trauma. In this protective process that occurs after trauma, biochemical markers are revealed together with the neuroendocrine response as intracellular and intercellular metabolic changes emerge. The duration of exposure to trauma and the severity of trauma are closely related to this process. While short-term or minor traumas are quickly repaired by the organism, in severe or long-term trauma, a systemic response may develop that can lead to devastating consequences such as multiple organ failures and death.

Response to trauma can be examined in separate categories as local and systemic responses that are intertwined with each other. *Local response*: Vasoconstriction,

local vasospasm and activation of the coagulation cascade in post-traumatic bleeding, regional inflammation that start with vascular and cellular stimulation constitutes this response. In this type of response, where vascular permeability and blood flow to the trauma area increase in the trauma area, tissue edema, increase in lymph flow, vascular stasis, fibroblast proliferation, and activation of the macrophage system occur. *Systemic response*: This process is actually a compensation mechanism. It happens when many systems work in harmony. Its main purpose is to ensure the survival of the organism and to maintain its homeostasis (Stone and Fabien 1993; Moore 1955).

The systemic response to trauma is examined in three phases as defined by Cuthbertson in terms of metabolic changes (Cuthbertson 1982; Sobotka and Soeters 2004; Jan and Lowry 2010). *Ebb phase*: It is the phase that starts immediately after injury and ends with the onset of the flow phase within the first 24–48 h. In this period, when energy consumption and nitrogen loss in the urine decrease, there is hemodynamic instability with a decrease in blood volume and flow. In this process, where oxygen consumption and body temperature decreases, which can cause shock, the sympathetic nervous system is stimulated by the stimulation of baroreceptors and tension receptors, and the body's first reaction occurs by catecholamine discharge. The main purpose is to slow down the metabolism and provide homeostasis. *Flow phase*: This period, which constitutes the catabolic process and lasts for an average of 6–7 days, can last for days depending on the severity and duration of the trauma. In this process, where the production of positive acute phase reactants increases, biochemical and hormonal markers and oxygen consumption increase, and catabolic events occur. While the body's energy stores are rapidly consumed, the immune system is also suppressed. *Anabolic phase*: The key point in this process, which can last for months, is the superiority of the compensation mechanism. If the organism shows an effective compensation dynamic, the anaerobic pathways that cause catabolism change to the aerobic process that will ensure wound and tissue healing.

The immune response starts within minutes after the systemic response, and the immunological markers that are released are examined in two different groups according to their effects. *Pro-inflammatory markers*: These markers play active roles in the flow phase, although they are in the post-traumatic reduction phase. Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 (IL-1), IL-6, IL-8 are the main markers in this group. IL-6 is a marker that has been shown to be closely related to the severity of trauma and its devastating consequences (multiple organ failure, acute respiratory distress syndrome, sepsis, systemic inflammatory response syndrome, death). It is mainly responsible for the synthesis of acute phase proteins in the liver (Martin et al. 1997; Pallister and Empson 2005). *Anti-inflammatory markers*: These markers suppress the pro-inflammatory phase in order to provide homeostasis after trauma. Transforming Growth Factor-Beta (TGF-β), IL-4, IL-10, IL-11, IL-12, and IL-13 are the main markers in this group. While the increase in IL-10 level causes more devastating consequences such as increased bacterial load and death, IL-12 plays a key role in the regulation of cellular immunity after trauma.

Skeletal system traumas can vary from a simple soft tissue injury to multiple fractures and even multiple organ injuries involving more than one organ system.

Table 1 Clinical parameters of systemic inflammatory response syndrome (SIRS)

Heart rate (/min)	> 90
Temperature (°C)	> 38 ***or*** < 36
Breathing rate (/min)	> 20
Number of leukocytes (/mm^3)	> 12,000 ***or*** < 4000 ***or*** ≥ 10% juvenile neutrophil granulocytes

After a simple fracture occurs, the fracture site is surrounded by various inflammatory cells along with the fracture hematoma. This is essentially the first step in fracture healing and is a local response inflammatory process. This process, which can be described as relatively simple, becomes more complex in more severe traumas as systemic response develops. In such cases, where a more severe response occurs, an increase in vascular and endothelial permeability becomes more serious after interstitial edema, vascular stasis, tissue hypoperfusion, and a vicious cycle may begin, which may lead to more devastating consequences such as organ failure or death (Lee et al. 2001). Apart from complex injuries, it should be kept in mind that multiple injuries (brain, chest, abdomen, and pelvic injury), hypovolemia and systemic hypoxia can create a serious and vital systemic inflammatory response cycle in the body. The variety of trauma severity causes systemic inflammatory response syndrome (SIRS) to occur at different levels in the body, depending on the variety and levels of biomarkers released due to trauma (Table 1). In severe traumas, many biomarkers such as serum lactate, PMN elastase, pro-inflammatory cytokines, and adhesion molecules are released into the circulation at excessive levels (Abramson et al. 1993; Pape et al. 2001). SIRS formation occurs with the release of pro-inflammatory biomarkers such as IL-6, CRP, TNF-α, and fibrinogen into the circulation (Fig. 1). Increased levels of these biomarkers are also closely associated with morbidity and mortality (Lenz et al. 2007; Wutzler et al. 2013; Mörs et al. 2019).

In order for the organism to continue its life against the systemic inflammatory response and to balance its internal system, homeostasis, the anti-inflammatory process called "compensatory anti-inflammatory response syndrome (CARS)" begins. In this CARS system, IL-10 plays a key role and various anti-inflammatory biomarkers are released into the blood circulation (Mörs et al. 2019). The inflammatory response, in which the SIRS and CARS pathways follow each other, is actually a "host defense" and is a crucial step in the healing process (Fig. 2). The cytokines involved in these pathways basically act as short-range intercellular messengers. In this way, cell activities and interaction are regulated.

SIRS Caused by Trauma

Pathophysiology

After traumatic injury, antigen presenting cells (APC) are activated by various endogenous signals released on account of cell necrosis in damaged tissues. Activated APC releases large amounts of proinflammatory mediators such as IL-1β, IL-6, IL-8, IL-18,

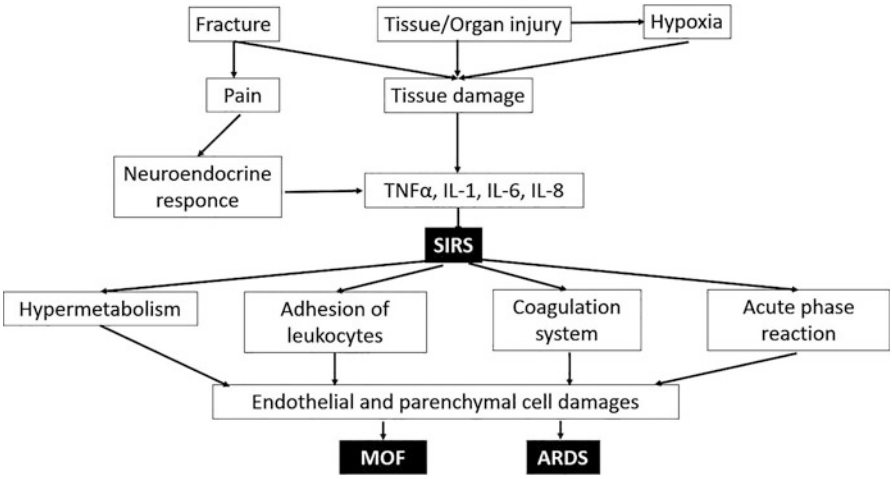

Fig. 1 Pathogenesis of SIRS resulted from trauma

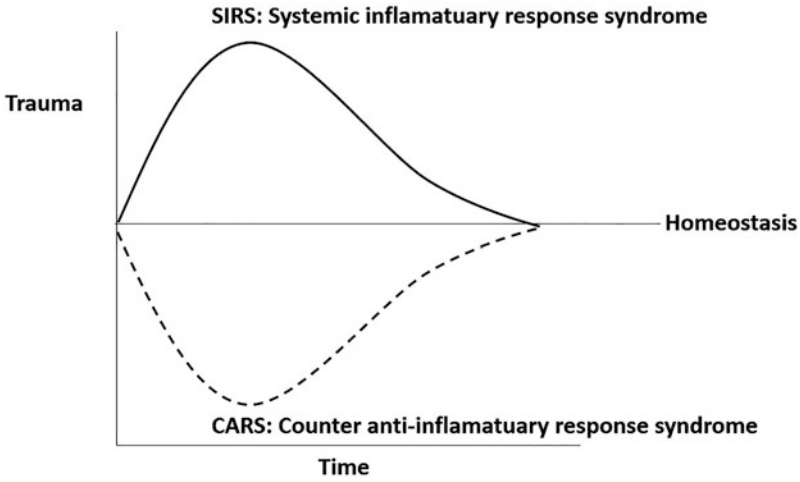

Fig. 2 Schematic representation of the inflammatory response

and TNF-α, which regulate the increase in neutrophil and macrophage activation and proliferation, and endothelial activation. Activated endothelial cells increase the expression of adhesive molecules, permeability, and pro-coagulatory activity. This proinflammatory cascade, together with the activation of the immune and neuroendocrine systems and changes in microcirculation and coagulation properties, suddenly transforms the local reaction into a systemic dynamic process (Giannoudis 2003; Tschoeke and Ertel 2007; Keel and Trentz 2005). That is, with the progression of metabolic and immunological changes caused by a traumatic injury, systemic

inflammatory response syndrome (SIRS) occurs (Ciriello et al. 2013; Bone et al. 1992; DeLong and Born 2004). Trauma severity, type of injury, age, comorbidities, and habits such as smoking, drug, and alcohol can affect the immunological response (Mörs et al. 2019).

Interleukin-6

The term interleukin derives from (inter-) "as a means of communication," and (−leukin) "deriving from the fact that many of these proteins are produced by leukocytes and act on leukocytes." The majority of interleukins are synthesized by helper CD4 T lymphocytes, as well as through macrophages, monocytes, and endothelial cells. They promote the development and differentiation of T and B lymphocytes, and hematopoietic cells. IL-6 is glycoproteins of about 170 to 180 amino acid residues with a molecular weight of 20 kilodaltons (Clogston et al. 1989). They have a compact, globular fold, stabilized by the two disulfide bonds. One half of the structure is dominated by a 4-alpha-helix bundle with a left-handed twist; the helices are anti-parallel, with two overhand connections, which fall into a double-stranded anti-parallel beta-sheet. The fourth alpha-helix is important to the biological activity of the molecule (Lütticken et al. 1991; Walter et al. 1992).

IL-6 binds to the receptor of IL-6, which is on the surface of the cell membrane or soluble, and intracellular signaling is provided through membrane-bound glycoprotein 130 (gp130), a signal-transducing element. Its essential effect is to stimulate the hepatocytes to provide the synthesis of acute phase proteins (Heinrich et al. 2003; Taga et al. 1989). It also has effects on T and B cells, neutrophils. Increment of the life span and activation of neutrophils are provided. IL-6 increases vascular endothelial growth factor (VEGF) synthesis by showing a synergistic effect with Tumor Necrosis Factor-alpha (TNF-α) and IL-1. In the presence of Transforming Growth Factor beta (TGF-β) in the extracellular matrix, naive helper T cell (Th0) transforms into T helper 17 (Th17). Likewise, they are also active in the differentiation of B cells and in this way cause the production of antibodies from plasma cells. And moreover, B cells can also synthesize IL-6 (Mangan et al. 2006; Bettelli et al. 2006).

The reference range for IL-6 is 0–8 pg/mL. The level of IL-6 released following trauma is important to evaluate the severity of the injury and to predict about the expected result (Bernard 1995; Borden and Chin 1994). IL-6 cutoff value is found to be 200 pg/ml in traumatized patients. It has been reported that the probability of SIRS is high if clinical findings are present in the early stages of traumatized patients above this value (83% sensitivity and 75% specificity) (Giannoudis et al. 2008). If the IL-6 value is above 500 pg/mL, the prognosis is poor and the risk of MOF is higher. It has been reported that IL-6 release has a prognostic significance especially in the first 48 h and decreases its prognostic significance from the third day (Giannoudis et al. 2008). Studies have shown an association between post-traumatic IL-6 concentrations and the incidence of MODS, infections, mortality, and severity of injury (Hensler et al. 2003; Jensen and Lundgren 2009; Meisner 2005; Sauerland et al. 2003). Nevertheless, the prognostic relevance is still controversial (Jensen and

Lundgren 2009; Giannoudis et al. 1998). Based on Sapan et al., threshold for severe inflammation response is IL-6 level 50 pg/mL. More severe inflammation response greater than this threshold will lead to death (Sapan et al. 2016).

The relationship between IL-6 and trauma, as far as can be determined in the literature, are based on previous data obtained from studies on adult traumatized patients. In children, a commonly used marker for systemic inflammation is IL-6 (Bian et al. 2017). Recently IL-6 has also been proposed to correlate with injury severity and the onset of organ dysfunction in pediatric patients (Andruszkow et al. 2014), but in another study, it failed to predict mortality after trauma (Ozturk et al. 2007). The authors have study on pediatric traumatized patients on this subject. It was determined that the normal threshold range was 8.6 ± 4.5 pg/mL for pediatric volunteers (Erkus et al. 2021). According to the results of the study, the levels significantly increased if trauma severity increases. IL-6 level of 14.3 pg/mL was found as a cutoff value suggesting that the pediatric patient may have multiple injuries (91% sensitivity and 84% specificity). IL-6 had decreased to almost normal value on the third day (Erkus et al. 2021). Likewise, according to previous data in adults (Bogner et al. 2009; Kleber et al. 2013; Maier et al. 2009), increment of IL-6 levels occurs within 24 h following trauma on account of SIRS. By day 3, serum IL-6 levels are significantly reduced.

The Other Biomarkers of the SIRS

Procalcitonin (PCT), the precursor of calcitonin hormone, is predominantly synthesized by the C-cells of the thyroid gland and to a lesser extent by the neuroendocrine tissue of other organs such as the lung and intestine. Moreover, overexpression of calcitonin genes (CALC-I) occurs after stimulation of parenchymal cells in almost every organ with inflammatory cytokines, especially during sepsis. IL-1β, tumor necrosis factor (TNF)-α, and IL-6, bacterial products containing lipopolysaccharide, and necrotic tissue cells synergistically stimulate the production of PCT, a product of the CALC-I gene.

Acting as an acute phase protein, PCT levels generally drive up immediately and peak 24–48 h after trauma. It decreases rapidly to the baseline value in non-complicated patients after 24–48 h due to its short half-life period (Sakran et al. 2012; Meisner et al. 2006; Wanner et al. 2000). Hence, high levels in serum or an increment over again appear to be a sufficient indicator for sepsis and MOF (Hensler et al. 2003; Billeter et al. 2009).

C-Reactive Protein (CRP) which has a pentameric structure, is an acute phase protein that rises in cases of inflammation, infection, and tissue damage. Despite peaking in the first 3 days, it takes a long term to return to the normal level (Gabay and Kushner 1999; Balci et al. 2009). According to previous data, the possibility of developing MOF and the risk of mortality were found to be higher in patients with high CRP values. Therefore, it is a fact that serial CRP measurement is also appropriate in critically ill patients with multiple injuries. In this respect, CRP

measurements between 2–7 days are very important in critical multiple trauma patients (Lausevic et al. 2008).

Tumor Necrosis Factor (TNF)-α produced as a trans-membrane precursor form, is expressed as a cell surface on many cell types. TNF-α is produced mainly by active macrophages but many other cell types such as lymphocytes, NK cells, neutrophils, mast cells, eosinophils, and neurons. It plays a role in systemic inflammation by regulating immune cells. TNF-α levels ascent earlier with the development of systemic inflammation owing to trauma, peak within a few hours, and then gradually decline (Giamarellos-Bourboulis et al. 2008; Menges et al. 1999).

Pentraxin 3 (PTX3) is a novel extrahepatic acute phase reactant similar to CRP in structure and function. PTX-3 can been produced in all trauma-relevant cells such as endothelial cells, fibroblasts, etc., in response to primary inflammatory signals including IL-1β and TNF-α. This biomarker of inflammation is involved in ischemia, sepsis, and tissue injury (Kleber et al. 2013; Libby et al. 2009). PTC3, which is a rapid marker for local response, increase rapidly and peak at 6–8 h, correlating with the severity of the disease. Considering the change in blood levels, IL-6 and PTX3 seem to be early markers for post-traumatic inflammation, while CRP and PCT are late markers.

Conclusion

The first stage of healing after any trauma is the inflammatory response that occurs at the injury site. Although it does not lead a major problem in simple fractures and injuries, it could be very harmful in patients with multiple fractures or multiple injuries. Systemic inflammatory response syndrome (SIRS) is a host defense, while the organism activates the compensatory anti-inflammatory response syndrome (CARS). The balance between SIRS and CARS mediated by mediators called Cytokines provides information about the patient's condition. The most important and earliest indicator of SIRS is IL-6. The higher the IL-6 level, the higher the mortality and complication rates due to multiple injuries such as ARDS and MOF. Although IL-6 is not of predictive importance, it is an important biomarker in the follow-up of patients with multiple injuries and in predicting the complications that may develop.

Applications to Prognosis, Other Diseases, or Conditions

In this chapter, a general approach of the inflammatory process, especially IL-6 levels, is handled with traumatized patients. It should not be forgotten that destructive and devastating results may develop with the extent of exceeding the threshold

of IL-6 levels. It should not be forgotten that similar increases can be seen after trauma in the pediatric age group, as in many studies conducted for adult patients, but may be associated with a more innocent clinical course compared to adults.

IL-6, a pro-inflammatory biomarker, is also encountered in diseases in which inflammation plays a fundamental role in the pathogenesis. The IL-6 process, which causes the increase of other acute phase reactants, especially CRP, brings with it some systemic effects. In addition, it causes effects such as anemia, weakness, deterioration in the balance of bone formation, and destruction, insulin resistance, hypertension (Nemeth et al. 2004; Yoshitake et al. 2008; Späth-Schwalbe et al. 1998).

Mini-Dictionary of Terms

Compensatory Anti-Inflammatory Response Syndrome The deactivation of the immune system against the pro-inflammatory response to severe infection or injury.

Ebb Phase The stage that develops in the first hours following injury is characterized by the body's reactions to restructure normal tissue perfusion and maintain homeostasis.

Flow Phase It is the period following the ebb phase after days. It reflects the convalescence period. Catabolic and anabolic processes work in the period, respectively.

Multiple Organ Dysfunction Syndrome MODS can be defined as a reversible physiological disorder in two or more organ systems that are not involved in the disease, occurring after a life-threatening situation.

Systemic Inflammatory Response Syndrome It is an abnormal generalized inflammatory reaction of the organism to any infectious or noninfectious stimulus.

Key Facts of Serum IL-6 Levels

IL-6 is a crucial biomarker for homeostasis induced with inflammatory response.
The higher the IL-6 level, the greater the likelihood of devastating consequences.
In pediatric traumatized patients, elevated IL-6 levels may not be frightening. Prospective studies with large samples are definitely needed.
The effects of IL-6 should be well understood, and their use should be increased for follow-up purposes.
Treatment options and results against elevated IL-6 due to the response occurring following trauma, maintain a mystery.

Summary Points

Biomarkers secreted following trauma aim to defend and heal the organism.
Interleukin-6 is a biomarker that acts as a role in the pro-inflammatory process.
The effect increases proportionally with the extent of release.
IL-6 appears to be less likely to trigger the development of inflammation in children.
IL-6 may cause systemic consequences such as anemia in the chronic period.

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The Role of Vitamin D As a Biomarker in Trauma

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Erick E. Pons, Lauren S. Kelly, Jennifer A. Munley, and Alicia M. Mohr

Contents

Abstract

Vitamin D is a collective term for two main forms of hormonal sterols that form a shared active metabolite, calcitriol. While it is essential for regulation of calcium and phosphate homeostasis, it also exerts widespread immunomodulatory effects, with vitamin D receptors found throughout the body. This chapter will provide an overview of the connection between vitamin D and immune system function, including innate immune functions, antigen presentation, B and T cell functionality, and cytokine release, with particular attention to critical illness and trauma.

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Vitamin D and its neuroprotective characteristics will then be discussed, followed by its potential in accelerating wound healing and muscle recovery after injury. The role of vitamin D status will be reviewed with regard to outcomes following traumatic injury and critical illness, including acute lung injury, acute kidney injury, and mortality. Throughout this chapter, the potential benefits of vitamin D supplementation will be addressed.

Keywords

Vitamin D · Trauma · Inflammation · Sepsis · Bone · Kidney · Immune system · Antigen presenting cells · B cells · T cells · Infection · Mortality · Cytokines · Critical illness · Traumatic brain injury

Abbreviations

$1,25(OH)_2D$	1,25-dihydroxy vitamin D
25(OH)D	25-hydroxy vitamin D
ACL	Anterior cruciate ligament
AKI	Acute kidney injury
ARDS	Acute respiratory distress syndrome
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
GCS	Glasgow Coma Scale
HIV	Human immunodeficiency virus
HSP	Heat shock protein
IBD	Inflammatory bowel disease
ICU	Intensive care unit
IFNγ	Interferon gamma
IL-10	Interleukin-10
IL-17	Interleukin-17
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
IL-22	Interleukin-22
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-7	Interleukin-7
IU	International units
LPS	Lipopolysaccharide
MHC II	Major histocompatibility complex II
MPK1	Mitogen-activated protein kinase 1
MVSSS	Modified Vancouver Scar Scale Score
NFκB	Nuclear factor kappa B
PD1	Programmed cell death protein 1
TBI	Traumatic brain injury
TBSA	Total body surface area
TGF-β	Transforming growth factor-beta
TNFα	Tumor necrosis factor alpha

UVB	Ultraviolet B
VDR	Vitamin D receptor
VDRE1	Vitamin D response element

Introduction

Vitamin D, or calciferol, collectively refers to a group of fat-soluble seco-sterols that has gained status as both a vitamin and a prohormone (Cesari et al. 2011). Vitamin D is found in two major forms: D_2 and D_3. Vitamin D_2, also known as ergocalciferol, is synthetic and often used for dietary fortification. Vitamin D_3, also known as cholecalciferol, is synthesized by the skin after exposure to sunlight and found naturally occurring in some animal products (Ross et al. 2011). Despite their unique side chains, both forms are nearly biologically identical (Ross et al. 2011). Both vitamin D_2 and D_3 are converted into 25-hydroxy vitamin D (25(OH)D) in the liver. This is the form maintained in the circulation and best reflects total body vitamin D reserves, and as such is used to measure vitamin D levels in humans (Alshahrani and Aljohani 2013). In the liver, 25(OH)D is then converted into 1,25-dihydroxy vitamin D (1,25 $(OH)_2D$), better known as calcitriol, which is the active form of vitamin D and the ligand to the vitamin D receptors (VDRs) (Alshahrani and Aljohani 2013; Charoenngam and Holick 2020; Olliver et al. 2013). Calcitriol then exerts influence not only on cells essential to calcium and phosphate homeostasis, but also on regulation of cell proliferation, differentiation, and apoptosis, and immunomodulation and inflammation, all critical elements in the care for injured patients (Charoenngam and Holick 2020; Dewar et al. 2009; Sauaia et al. 2017).

Production and Absorption

The two main sources of vitamin D are from the diet or cutaneous synthesis from sunlight exposure, and relative proportions vary across population groups and food practices (Fig. 1) (Prentice et al. 2008). It is difficult to absorb sufficient quantities of vitamin D from dietary sources alone (Holick 2004). Instead, over 90% of most humans' daily vitamin D requirement is produced by casual exposure to sunlight (Holick 2004). As ultraviolet B (UVB) radiation is absorbed by 7-dehydrocholesterol in skin cells, the compound is converted to previtamin D_3 through photolysis (Holick 2004). Previtamin D_3 then undergoes thermal isomerization into its more stable form, vitamin D_3 (Holick 2004).

Many factors affect vitamin D_3 synthesis, including age, skin pigmentation, surface area and duration of sun exposure, and seasonal variation. Vitamin D_3 synthesis declines with age due to a decrease in 7-dehydrocholesterol in the stratum basale layer of the skin (MacLaughlin and Holick 1985). Intuitively, the degree of melanin pigmentation shares an inverse correlation with vitamin D_3 synthesis, with increased melanin leading to longer exposure times to produce adequate vitamin D_3

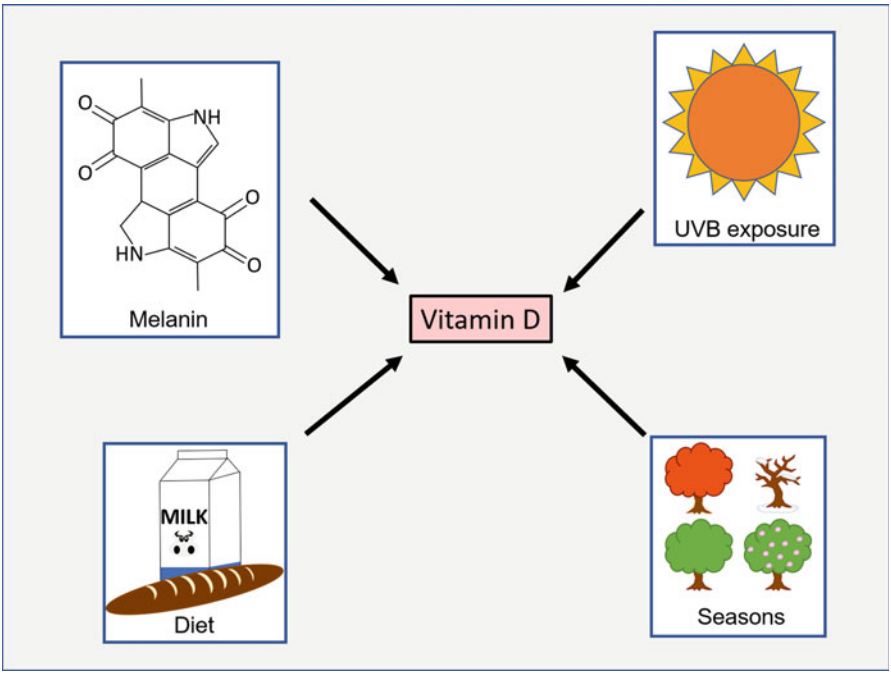

Fig. 1 Factors affecting vitamin D synthesis. This figure shows common sources that affect an individual's vitamin D levels. (UVB: ultraviolet B)

levels (Clemens et al. 1982; Holick 2004; Ross et al. 2011). Similarly, application of sunscreen can decrease vitamin D_3 synthesis because of its ability to absorb UVB radiation (Misra et al. 2008). Reductions in UVB exposure during winter months leads to less vitamin D_3 synthesis compared to the summer months because of increased clothing coverage, less time spent outdoors, and a reduction in daylight hours (Kift et al. 2018).

Despite public health efforts in dietary fortification, vitamin D deficiency is still one of the most common chronic medical conditions in the world across all age groups, with an estimated 1 billion individuals worldwide meeting deficiency criteria (Holick 2007). Although definitions vary, a serum vitamin D level of over 50 nmol/L is widely considered sufficient; insufficiency has been defined by levels ranging from 50–30 nmol/L, and levels below 30 nmol/L are considered a severe deficiency (Table 1) (Ross et al. 2011). In order for the average person to maintain a normal range of vitamin D levels, the United States Institute of Medicine recommended that all individuals up to the age of 50 take a supplement of 200 international units (IU) vitamin D daily and that those over the age of 50 take 600 IU vitamin D daily (Institute of Medicine,(Ross et al. 1997). This dose is variable depending on sun exposure, skin pigmentation, and other factors that affect UVB exposure (Bischoff-Ferrari 2009).

Table 1 Categorization of circulating vitamin D levels

	Sufficient	Insufficient	Deficient
American Endocrine Society task force	>50 nmol/L (>20 ng/L)	---	<50 nmol/L (<20 ng/L)
US Institute of medicine	>50 nmol/L (>20 ng/L)	30–50 nmol/L (12–20 ng/L)	<30 nmol/L (< 12 ng/L)
Central European guidelines (polish Society of Endocrinology)	75–125 nmol/L (30–50 ng/L)	50–75 nmol/L (20–30 ng/L)	<50 nmol/L (<20 ng/L)
European Society for Clinical and Economic Aspects of osteoporosis and osteoarthritis	>50 nmol/L (>20 ng/L)	---	---

This table shows the different ways different health organizations define vitamin D sufficiency, insufficiency, and deficiency (Holick et al. 2011; Pludowski et al. 2013; Rizzoli et al. 2013; Ross et al. 2011)

Immune Function

While classically associated with calcium-phosphate homeostasis, many studies have identified a multitude of other physiological roles for vitamin D, including cardiovascular regulation, muscular contraction, organ function, tissue healing, and immune function (Battault et al. 2013; Makariou et al. 2011). Traumatic injury brings about a robust pro-inflammatory response that initiates the release of pro-inflammatory cytokines including tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6), and interferon gamma (Lenz et al. 2007). Many types of immune cells express VDRs on their membranes, including $CD4^+$ and $CD8^+$ T cells, B cells, neutrophils, and antigen-presenting cells like macrophages and dendritic cells (Fig. 2) (Baeke et al. 2010). Interactions between vitamin D and VDRs lead to negative regulation of nuclear factor kappa B (NFκB) and signal transducers and activators of transcription 1- and 5-mediated signaling, which downregulates transcription of inflammatory cytokines such as TNFα, IL-6, and monocyte chemoattractant protein 1 (Fig. 3) (Alharbi 2021; Calton et al. 2015; Shojaei et al. 2019). This reduces expression of adhesion molecules, which reduces platelet leukocyte aggregation, and initiation of antibacterial responses of the innate immune system (Alharbi 2021; Calton et al. 2015; Shojaei et al. 2019). An in vitro study found that introducing calcitriol decreases production of TNFα and tissue factor by human monocytes that have been stimulated with lipopolysaccharide (Kempker et al. 2012). Another study looking at human immunodeficiency virus (HIV) patients found that HIV patients with deficient levels of vitamin D were found to have significantly higher levels of IL-6 and TNFα compared to HIV patients with normal vitamin D levels (Manion et al. 2017).

Vitamin D may also help regulate innate immune responses to bacterial endotoxins. Following intraperitoneal lipopolysaccharide (LPS) administration, mice who received an oral vitamin D analogue saw reduced mortality rates and a return to normal levels of certain eicosanide metabolites known to mediate endotoxemia

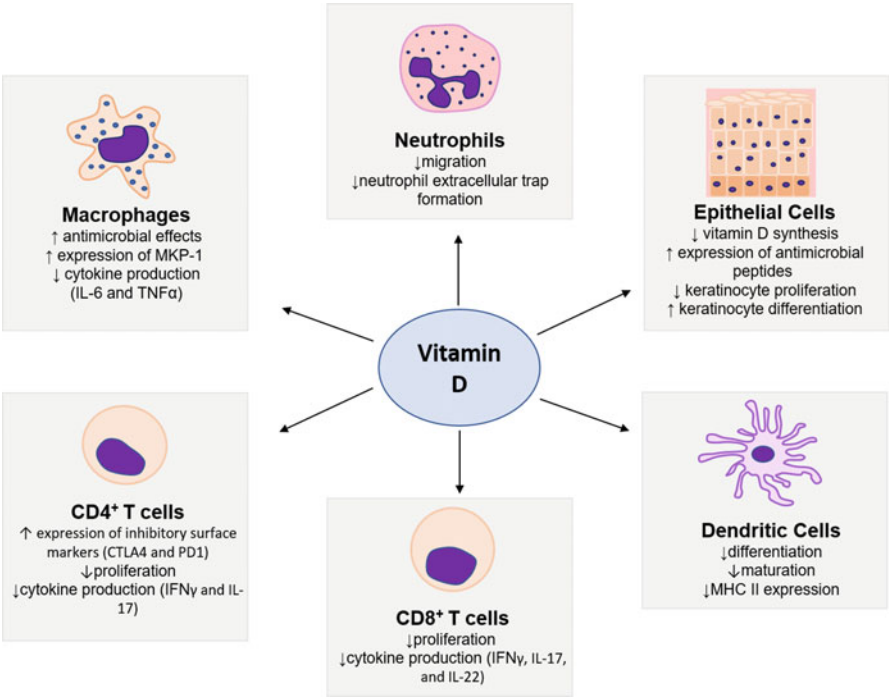

Fig. 2 How vitamin D influences the immune system. This figure shows how vitamin D influences different components of the immune system. (CTLA4: cytotoxic T-lymphocyte-associated protein 4, IFNγ: interferon gamma, IL-6: interleukin-6, IL-17: interleukin-17, IL-22: interleukin-22, MHC II: major histocompatibility complex II, MKP-1: mitogen-activated protein kinase 1, PD1: programmed cell death protein 1, TNFα: tumor necrosis factor alpha)

(Horiuchi et al. 1991). In a model of dextran sulfate sodium (DSS)-induced colitis, VDR knockout mice exhibited substantially higher levels of inflammatory cytokines such as TNFα, IL-10, IL-1α, and IL-1β compared to wild type counterparts, which persisted up to 10 days later, even after complete resolution of inflammatory response in wild type mice (Froicu and Cantorna 2007). VDR knockout mice exhibited dramatically different 7-day mortality rates than wild-type mice (80% vs 30%, respectively) (Froicu and Cantorna 2007).

Through its stimulation of cytokines, traumatic injury leads to proliferation and activation of B and T cells (Lenz et al. 2007). In addition to vitamin D's ability to inhibit production of those pro-inflammatory cytokines, it has also been shown to affect multiple steps in the inflammatory response pathways after trauma. In the adaptive system, vitamin D affects proliferation and differentiation of B and T cells and modulates antigen presentation and antibody production by inhibiting the surface expression of major histocompatibility complex II-bound antigens (Baeke et al. 2010; Kempker et al. 2012; Yin and Agrawal 2014). In vitro, vitamin D enhanced the maturation response of dendritic cells stimulated with pneumococcal

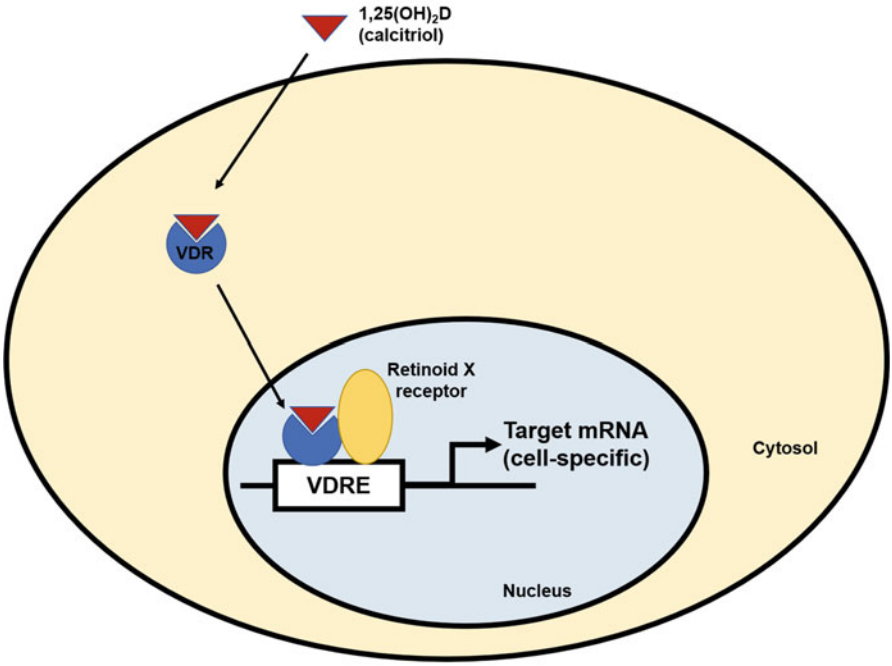

Fig. 3 Interaction between circulating vitamin D ($1,25(OH)_2D$) and a VDR. This figure shows how calcitriol (circulating vitamin D) binds to a VDR and initiates transcription regulation. (VDR: vitamin D receptor, VDRE: vitamin D response element)

peptidoglycan (Olliver et al. 2013). Conversely, another study using apolipoprotein E knockout mice found that 3 months of oral calcitriol supplementation was able to decrease the number of mature dendritic cells and reduce T cell proliferation in atherosclerotic mice, as well as increased expression of immunoregulatory cytokines IL-10 and transforming growth factor-beta (TGF-β) (Takeda et al. 2010). Vitamin D supplementation was associated with reduced amounts of atherosclerotic plaque formation in the same mice (Takeda et al. 2010).

Traumatic Brain Injury

Vitamin D plays a neuroprotective role and upregulates neurotrophic growth factors, such as nerve growth factor and brain-derived neurotrophic factor, and other essential markers in neuronal function and neuronal survival (Fig. 4) (Buell and Dawson-Hughes 2008; Farghali et al. 2020). In an in vitro study, rat cortical neurons were cultured with and without $1,25(OH)_2D$ doses ranging from 0 to 1 μg/ml. After incubation, the cells were subjected to oxidative stress by adding hydrogen peroxide to the culture medium for 2 h. When comparing cell viability, it was shown that 0.25 μg/ml of $1,25(OH)_2D$ resulted in the greatest neuroprotection when compared

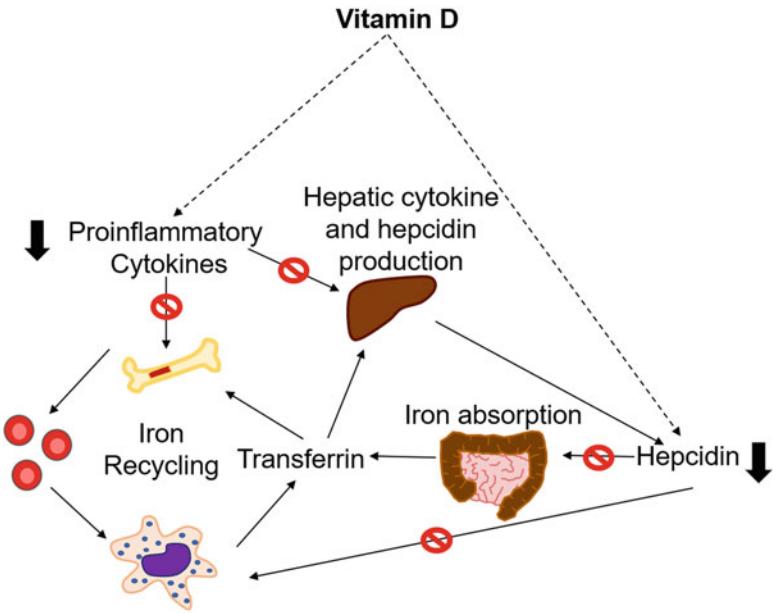

Fig. 4 Vitamin D and iron recycling in the body. This figure shows the effect of vitamin D on hepcidin levels and in turn iron regulation in the body

to cells that were not incubated with $1,25(OH)_2D$ (AlJohri et al. 2019). Mechanistically, it was found that vitamin D treatment resulted in upregulation of the antioxidant enzyme defense system, as demonstrated by an increase in the reduced form of glutathione (AlJohri et al. 2019).

Preclinical studies on the role of vitamin D in recovery from traumatic brain injury (TBI) have linked the vitamin to neuroinflammation. Known for their neuroprotective and neurorestorative effects on the brain, female sex hormones are a commonly used benchmark in traumatic brain injury research (Suzuki et al. 2006). Rats subjected to photothrombotic lesions of the cerebral cortex treated with a single injection of $1,25(OH)_2D$ in conjunction with 17β-estradiol after injury showed that combination treatment worked synergistically to reduce levels of heat-shock protein 27 (HSP-27), a well-known marker of oxidative stress in the central nervous system and is an inhibitor of apoptosis (Losem-Heinrichs et al. 2005). Another study measured the effects of two intraperitoneal injections of either vitamin D, progesterone, or both, within the first 6 h after a cortical contusion (Tang et al. 2015). Rats receiving combination therapy displayed significantly reduced cortical levels of toll-like receptor 4 and phosphorylated NFκB, less neuronal losses, and decreased astrocyte activation at 24 h after injury (Tang et al. 2015). The combination therapy also showed to create a more robust inhibition of pro-inflammatory markers, such as inducible nitric oxide synthase, when compared to either treatment alone.

Systemically, the addition of vitamin D to the progesterone regimen resulted in significant reduction of circulating inflammatory markers IL-1β and TNFα (Tang et al. 2015).

In a comparison of two groups of moderate to severe TBI patients randomized to receive either a single oral dose of 120,000 IU of vitamin D or a placebo immediately after admission, the vitamin D-treated patients showed significant improvement in Glasgow Coma Score at hospital day 7 (Sharma et al. 2020). The vitamin D supplemented patients also showed significantly reduced levels of IL-6 and TNFα, reinforcing the role vitamin D has in altering release of inflammatory cytokines and overall immunomodulation (Sharma et al. 2020).

Burns

Burn patients with a median total body surface area (TBSA) of 4–5% had a higher rate of both vitamin D insufficiency and deficiency compared to the general population. Among these patients, lower vitamin D levels also correlated with higher incidence of complications, including bacteremia, UTI, graft loss, and wound infection, and a longer hospital length of stay (Blay et al. 2017). The authors proposed that this was due to the lack of ability to fight infections in the vitamin D deficient patients (Blay et al. 2017). In a study of young adult male patients who had sustained at least 30% TBSA burns within the past year, the authors identified a correlation between lower vitamin D levels and worsened markers of wound healing and scar formation, defined by a higher Modified Vancouver Scar Scale Score (MVSSS) (Terzi and Guven 2016). An elevated MVSSS was linked to poorer long-term quality of life measures (Finlay et al. 2017). In a pilot study, a single high dose (50,000, 100,000, or 200,000 IU) of vitamin D significantly upregulated skin repair genes such as arginase-1 in patients that received an experimentally induced sunburn (Scott et al. 2017). Patients that received a dose of vitamin D also exhibited significantly less erythema compared to those who received a placebo dose (Scott et al. 2017). Although these patients had sustained only a minor sunburn, this study demonstrated that vitamin D supplementation in burn patients could be effective for rapid recovery.

Musculoskeletal Injury

Orthopedic injury accounts for up to 47% percent of trauma center admissions (Clement et al. 2013). As such, the impact of vitamin D on recovery from musculoskeletal injury is of particular relevance. Human articular chondrocytes treated with IL-1β in vitro exhibited reduced inflammatory effects after adding $1,25(OH)_2D$ (Boyan et al. 2016). Treatment with vitamin D was also able to reverse IL-1β's inhibitory effect on cartilage matrix synthesis (Boyan et al. 2016). In the same study, rats who had sustained a transection of the anterior cruciate ligament (ACL) and were treated with intra-articular vitamin D demonstrated reduced levels of multiple

inflammatory markers in the serum including IL-1α, IL-1β, IL-1α, IL-7, and TNFα along with increased levels of serum anti-inflammatory markers like erythropoietin, IL-4, and IL-10 (Boyan et al. 2016). Development of osteoarthritis after ACL injury was associated with increased inflammatory markers like IL-1β and decreased anti-inflammatory factors such as IL-10 in rats, but intra-articular treatment with a vitamin D analogue resulted in systemic anti-inflammatory effect with reduced serum IL-1α and TNFα and increased IL-10 and erythropoietin (Boyan et al. 2016). The authors went on to suggest that this reduction in inflammatory markers with vitamin D treatment might prevent or reduce development of postinjury osteoarthritis (Boyan et al. 2016). Another study found that 2 weeks after rotator cuff transection and repair, vitamin D-deficient rats exhibited worse collagen fiber organization and decreased bone formation and muscle alignment at the site of injury (Angeline et al. 2014).

Vitamin D has also been shown to have an influence on muscle recovery after injury. After blunt soleus muscle injury in rats, immediate vitamin D supplementation accelerated the return of muscle strength by upregulating genes believed to be involved in structural restoration, increasing cellular proliferation, and reducing the overall number of apoptotic cells within the muscle (Stratos et al. 2013). When vitamin D supplementation was delayed until 4 days after a tibialis anterior injury, no muscle regeneration was observed (Srikuea and Hirunsai 2016). This suggests that timing of vitamin D supplementation may be crucial to optimize muscle regeneration and healing after injury (Latham et al. 2021). In humans, a double-blinded placebo-controlled study looking at vitamin D supplementation in 24 endurance runners found that the cohort that received oral vitamin D supplementation at 1000 IU twice a day for 3 weeks had significantly decreased level of markers signaling muscle dysfunction and fatigue such as troponin, myoglobin, and creatine kinase suggesting vitamin D supplementation plays a role in recovery from muscle injury and regeneration (Zebrowska et al. 2020). Another study found that pre-exercise vitamin D levels were able to predict immediate and persistent muscle weakness measured by peak isometric force after intense exercise (Barker et al. 2013). A randomized, placebo-controlled study consisting of 20 vitamin D insufficient males found that after 6 weeks of oral vitamin D supplementation of 4000 IU daily, participants exhibited a significant improvement in maximum voluntary contraction force following muscle exhaustion compared to the participants who received a placebo (Owens et al. 2015). Muscle biopsy specimens were then taken from the same patients and subjected to damage by a mechanical scrape and either received no treatment or were treated with 10 nmol $1,25(OH)_2D$ or 100 nmol $1,25(OH)_2D$. Both high and low doses of vitamin D caused a significant increase in satellite cell migration, in terms of velocity and distance, to the wound site compared to those cells that did not receive vitamin D (Owens et al. 2015). However, the cells treated with 100 nmol had decreased migration directionality suggesting that more vitamin D is not necessarily more beneficial when it comes to muscle regeneration after injury (Owens et al. 2015).

Lung Injury and Acute Respiratory Distress Syndrome

Following trauma, up to 16% of injured patients develop some form of acute lung injury, including acute respiratory distress syndrome (ARDS) (Daher et al. 2018). The role of vitamin D in inflammation, immune activity, and epithelial function make it an enticing target to investigate as a biomarker in lung injury after trauma (Charoenngam and Holick 2020; Kim et al. 2020). Vitamin D supplementation was associated with an improved rate of injury repair in human lung resection specimens (Dancer et al. 2015). Vitamin D-deficient mice receiving intratracheal LPS exhibited increased alveolar epithelial permeability, bronchioalveolar neutrophil apoptosis, and circulating levels of proinflammatory cytokines, as well as worsened hypoxia at 48 h (Dancer et al. 2015). In a similar hamster model of acute lung injury from LPS inhalation, a calcitriol analogue inhibited neutrophil recruitment in a dose-dependent manner, and although intratracheal therapy led to more pronounced effects, improvements were also significant among animals receiving oral treatment (Takano et al. 2011).

Data on the role of vitamin D in lung injury in humans is conflicting. In one UK study, out of 52 enrolled ARDS patients, 100% were vitamin D deficient (Dancer et al. 2015). Survivors of ARDS had significantly higher levels of vitamin D versus non-survivors, and plasma vitamin D levels were lower among those who developed ARDS compared to healthy controls. Furthermore, worsening deficiency was associated with worsened epithelial integrity and increased alveolar capillary permeability (Dancer et al. 2015). On the other hand, a case-control study of 478 patients found no relationship between vitamin D deficiency and lung injury after severe trauma (Barnett et al. 2014). A randomized controlled trial of patients undergoing elective esophagectomy found that those assigned to the vitamin D supplementation cohort (3–14 days of 300,000 IU total vitamin D) saw reductions in pulmonary vascular permeability indices but no differences in clinical outcomes (Parekh et al. 2018). Additionally, surgery was noted to reduce vitamin D levels through postoperative day 3 regardless of supplementation status (Parekh et al. 2018).

Critical Illness

Approximately 25% of all hospitalized trauma patients spend at least part of their admission in the intensive care unit (ICU) (Bowman et al. 2020; Nathens et al. 2006). Among those critically ill trauma patients, approximately one-third develop sepsis and 10% develop septic shock (Michetti et al. 2019). A retrospective study of 3386 patients found that preadmission vitamin D levels were a strong predictor of developing sepsis later during admission, even after adjustments for age, gender, and race (Moromizato et al. 2014). In addition, vitamin D deficient patients had a 1.5-fold higher chance of developing sepsis (Moromizato et al. 2014). A retrospective cohort study of 121 patients found that sepsis caused by bacteremia occurred significantly more often in vitamin D deficient patients, with 19% of vitamin D deficient patients developing sepsis caused by bacteremia compared to 0% of

nondeficient patients (Rech et al. 2014). ICU patients with low 25(OH)D concentrations saw greater incidence of acute respiratory failure, acute liver failure, and infections (Gomes et al. 2019). A systematic review identified ICU patients with vitamin D levels under 50 nmol/L as having increased rates of infection, sepsis, in-hospital, and 30-day mortality (Table 2) (de Haan et al. 2014).

A pilot study looking at 81 patients admitted to the emergency department found that vitamin D insufficiency was also associated with sepsis severity and a worsened clinical course suggesting that vitamin D supplementation may have the potential to lower risk of incident infection such as sepsis (Ginde et al. 2011). Patients who were vitamin D insufficient were significantly more likely to develop severe sepsis, with 61% of those patients developing severe sepsis, compared to only 29% of vitamin D sufficient patients. Among critically ill patients, vitamin D deficiency had a significant correlation with ICU length of stay as well as treatment costs (Matthews et al. 2012). Plasma vitamin D levels were significantly decreased in patients that suffered severe traumatic injury compared to controls (Apple et al. 2020). With this decreased level of vitamin D came an increased concentration of IL-6 and TNFα, again reaffirming an inverse relationship between vitamin D levels and inflammation (Apple et al. 2020).

Among critically ill patients with vitamin D deficiency, a single dose of vitamin D was enough to correct the deficiency and reduce pro-inflammatory cytokines like IL-6 (Nair et al. 2015). When comparing a vitamin D treatment to a placebo treatment in patients suffering a TBI and in the ICU, the vitamin D-treated group had a shorter ICU stay and shorter mechanical ventilation time (Sharma et al. 2020). In contrast, a randomized double-blinded, placebo-controlled study by Amrein et al. (2014a) did not find a significant difference in ICU length of stay or hospital mortality between critically ill ICU patients treated with high doses of oral or enteral vitamin D (540,000 IU loading dose followed by 90,000 IU maintenance dose for 5 months) compared to placebo, although authors reported a trend in lower mortality among those with severe deficiency (<12 ng/mL).

Table 2 Trends among vitamin D deficient patients and the effects of vitamin D supplementation

	Vitamin D deficiency	Vitamin D supplementation
Hospital length of stay	↑	↓
Intensive care unit length of stay	↑	↓
Mortality	↑	?
Pneumonia	↑	?
Wound healing	↓	↑
Post-injury arthritis	↑	↓

This figure shows the effects of vitamin D deficiency and its supplementation on common characteristics in traumatic injury patients

Acute Kidney Injury

There is evidence to suggest that low vitamin D levels exert a negative impact on the kidney, either by endothelial or immune dysfunction, or impairment of tissue healing after injury. Some of these effects on the kidney seem to be mediated through suppression of the renin-angiotensin activating system (Wang et al. 2011; Zhang et al. 2010). VDR-knockout mice develop more severe histologic renal damage after unilateral ureteral obstruction, marked by tubular atrophy, interstitial fibrosis, and local fibrogenic and inflammatory factors like TGF-β, which are reversed with administration of the angiotensin II receptor blocker losartan (Zhang et al. 2010). Administration of a vitamin D receptor agonist was able to improve proteinuria, podocyte injury, and other markers of renal injury in obese mice (Wang et al. 2011). Vitamin D deficiency alone was sufficient to reduce renal capillary density in rats (de Braganca et al. 2016). After renal ischemia/reperfusion injury, vitamin D-deficient rats demonstrated worsened glomerular filtration rate, increased tubular necrosis, and urinary protein excretion, and worsened fibrotic damage, tubular recovery, and local inflammation compared to non-deficient rats (de Braganca et al. 2015; de Braganca et al. 2016). Reintroduction of vitamin D back into the diets led to improvements in interstitial expansion, renal fibrosis, and inflammatory cell infiltrate (Graidis et al. 2020).

Vitamin D has been linked as a risk factor in the development of acute kidney injury (AKI) in ICU patients (Braun and Christopher 2013; Braun et al. 2012). Zapatero et al. (2018) found that in addition to increased mortality, severely deficient vitamin D levels were also associated with a rate of AKI more than double that of ICU patients with higher vitamin D levels (29% vs. 13%, respectively). Lower levels of vitamin D have also been linked to increased risk for more rapid losses in glomerular filtration rate, suggesting that vitamin D status may play a role in development of chronic kidney disease (de Boer et al. 2011).

Anemia of Critical Illness

One common characteristic in patients with critical illness is anemia (Hayden et al. 2012; Shander 2004). A double-blinded, placebo-controlled pilot study of 28 healthy patients found that a single dose of 250,000 IU oral vitamin D was sufficient to cause a significant reduction in plasma hepcidin levels (Smith et al. 2017). In addition, an observational cohort study of 67 patients by Apple et al. (Apple et al. 2020) found that in trauma patients, vitamin D deficiency was associated with elevated hepcidin levels. Given hepcidin's inverse correlations with hemoglobin concentration, these findings suggest that vitamin D might be useful as a potential treatment for anemia in critically ill patients (Fig. 5) (Atkinson et al. 2015; Smith et al. 2017). The same group later published findings that among critically ill ventilated patients, vitamin D administration led to a significant increase in both 25(OH)D and hemoglobin and a

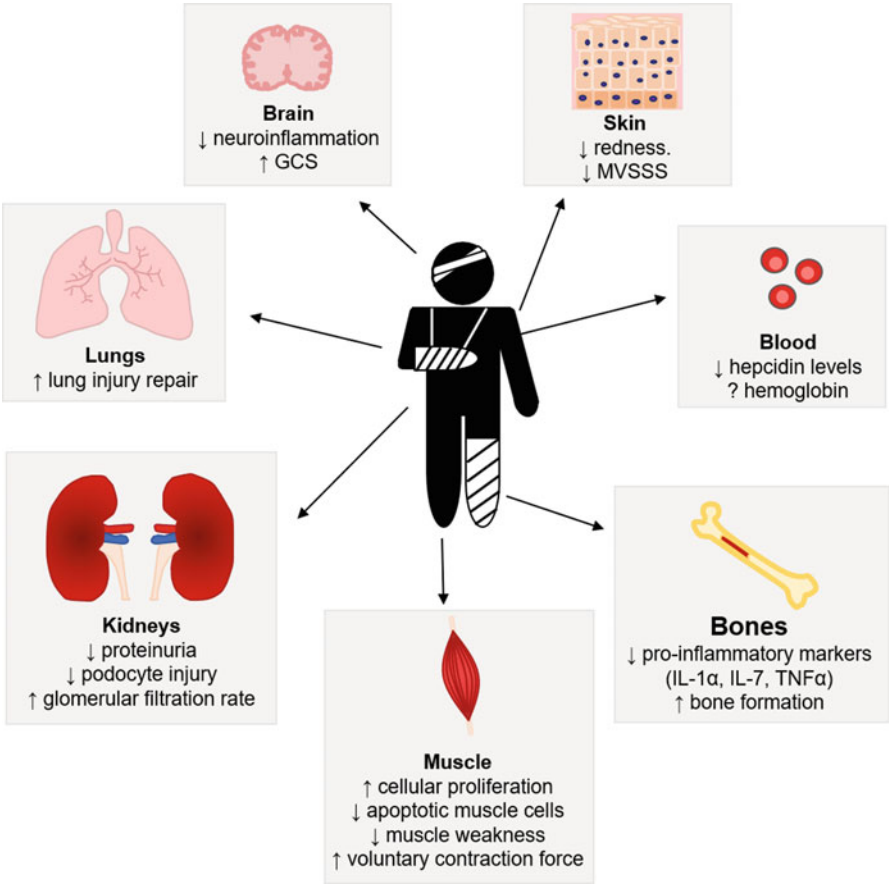

Fig. 5 The effects of vitamin D supplementation on a trauma patient. This figure shows the effects vitamin D supplementation may have on a trauma patient. (GCS: Glasgow Coma Scale, IL-1α: interleukin-1 alpha, IL-7: interleukin-7, MVSSS: Modified Vancouver Scar Scale Score, TNFα: tumor necrosis factor alpha)

reduction in hepcidin (Smith et al. 2018). The authors concluded that vitamin D could potentially be used as a therapeutic option for anemia in critical illness.

Another study in children with inflammatory bowel disease (IBD) found that although a low plasma 25(OH)D concentration was associated with elevated hepcidin levels and decreased hemoglobin levels, there was no association between vitamin D deficiency and anemia (Syed et al. 2017). They believed this is because vitamin D has been found to be correlated to anemia of inflammation and not necessarily to anemia due to iron deficiency (Syed et al. 2017). Pediatric patients with IBD, like the ones in this study, usually have anemia due to iron deficiency (Goodhand et al. 2012).

Mortality

A number of studies have been published regarding the connection between vitamin D status and patient mortality after trauma, sepsis, and critical illness. Levels of serum vitamin D_3 have been shown to have a significant inverse correlation with 30-day mortality in patients admitted to the emergency department with suspected sepsis (Shojaei et al. 2019). One study looked at 136 veterans admitted to either the medical or surgical ICU and found that vitamin D-deficient patients had a 44% survival rate, compared to 69% survival rate among vitamin D sufficient patients (McKinney et al. 2011). A study by Amrein et al. (2014b) took this a step further and found that among those patients who were vitamin D deficient, those with severe deficiency had the highest ICU and overall hospital mortality rates. They found that in the ICU the mortality rate among the severely deficient, low normal, and high normal were 18.7%, 6.0%, and 9.1%, respectively (Amrein et al. 2014b). Low vitamin D levels were also associated with a twofold increased risk for ICU stay longer than 3 days (McKinney et al. 2011). Among sepsis patients, although hospital mortality did not differ significantly between vitamin D deficient and sufficient, the 90-day overall mortality was significantly higher in vitamin D deficient patients (51%) when compared to the vitamin D sufficient group (25%) (Rech et al. 2014).

A multicenter observational study found that hospital preadmission vitamin D levels may be a strong predictor of mortality in patients treated in the medical and surgical intensive care units (Braun et al. 2011). Patients who were vitamin D deficient on admission saw up to a 1.7-fold higher mortality rate compared to those with sufficient vitamin D levels (Braun et al. 2011). When extended out to 1 year, preadmission vitamin D remained a strong predictor of post-ICU mortality at 30, 60, and 365 days (Braun et al. 2011). Among severely injured trauma patients, lower admission vitamin D was associated with higher 1-year mortality rates (Barnett et al. 2014). A systematic review looked into the effects of vitamin D supplementation among 50 randomized trials that included 94,148 participants with hospitalization due to a variety of reasons from hip fractures to heart failures. The study found that compared to a placebo treatment, supplementing vitamin D for 2 years significantly decreased mortality in patients with vitamin D insufficiency at the cost of increased risk of nephrolithiasis (Bjelakovic et al. 2011).

Applications to Prognosis

In this chapter, we reviewed the role of vitamin D in regulation of the immune system and its clinical implications as a biomarker in the settings of trauma and critical illness. A multitude of studies suggest low vitamin D levels are linked to worse outcomes after burn, traumatic brain injury, and orthopedic injuries, as well as among the critically ill (Christopher 2016; Putzu et al. 2017). As described previously, vitamin D levels may also be applicable to uninjured patients with sepsis or critical illness. Routine measurement of vitamin D levels may one day serve as a

useful adjunct to help guide discussions about overall prognosis with patients and their families.

Consumption of vitamin D supplements might be helpful in decreasing the prevalence of infection, sepsis, and mortality after trauma, but data remains conflicting (Shojaei et al. 2019). One study found that the vitamin D levels in deficient critically ill patients were able to be normalized with high doses of vitamin D treatment, but the study did not assess improvement in other outcomes (Mata-Granados et al. 2010). A 2019 randomized, double-blinded, phase 3 clinical trial administering 540,000 IU enteral vitamin D supplementation among deficient critically ill patients found that although they were able to successfully replete vitamin D deficiencies, they failed to find any benefit over placebo in any of the outcomes tested, and the study was ended prematurely out of futility (National Heart et al. 2019). On the other hand, a promising pilot study of ICU patients was able to identify an increase in hemoglobin following high-dose enteral vitamin D supplementation (Smith et al. 2018). This is clinically relevant for physicians treating anemia of critical illness. Vitamin D may serve as a safe and low-cost alternative to transfusions, which carry inherent risks to the patient including immunosuppression, nosocomial infections, prolonged ICU stay, and in-hospital mortality (Athar et al. 2012; Charles et al. 2007; Greenburg 1996; Malone et al. 2003; Shapiro et al. 2003; Vincent et al. 2002). Deciding which patients may benefit from vitamin D supplementation, how much to supplement, and for how long, remains a topic of ongoing study.

Mini-Dictionary of Terms

25-hydroxy vitamin D. The major circulating form of vitamin D found in the body. Used in laboratory testing as a surrogate marker for total vitamin D levels.

7-dehydrocholesterol. An unsaturated sterol found in the epidermis that is converted into vitamin D_3 upon exposure to UVB radiation.

Acute respiratory distress syndrome. Severe pulmonary edema caused by capillary leakage after diffuse alveolar damage, leading to an inability to oxygenate the blood. A common cause of respiratory failure and ICU admission, with a high mortality rate.

Antigen presenting cell. Cells that recognize and process antigens, either host or foreign, and present them to T cells. Aid in messaging between the innate and adaptive immune systems. Examples include dendritic cells, macrophages, and B cells.

Apolipoprotein. A class of proteins that can bind to lipid molecules. A common structural feature of lipoproteins, many can bind to specialized receptors or serve as cofactors.

Calcitriol. The active form of vitamin D, also known as 1,25-dihydroxy vitamin D.

Cholecalciferol. Also known as vitamin D3. Found in animal products and some fortified foods.

Creatine kinase. Enzyme in found in muscle cells that catalyzes conversion of ATP to creatine phosphate and ADP. Used as a biomarker of muscle injury when it is released into the circulation.

Endotoxin. Components of bacterial cell membranes (e.g., lipopolysaccharide) that produce massive and life-threatening immune reactions when recognized by the host.

Ergocalciferol. Also known as vitamin D2. Derived from plant-based products and commonly used in fortified foods.

Glasgow Coma Score. A common scale used to measure level of consciousness by measuring movement, eye opening, and vocalization in response to certain stimuli (e.g., voice or pain). Scores range from 3–15.

Heat shock protein. A family of chaperone proteins involved in folding, re-folding, and degradation of developing proteins in response to stressful conditions including thermal shock, harmful exposures, hypoxia, and injury.

Lipopolysaccharide. Surface molecule found in high quantities on the cell membranes of Gram-negative bacteria (such as *E. coli*). Induces a profound immune reaction that is commonly utilized in animal sepsis models.

Metabolic Syndrome. A cluster of vascular risk factors such as obesity, hypertension, and hyperglycemia that are associated with an increased risk for cardiovascular disease and type 2 diabetes mellitus.

Modified Vancouver Scar Scale Score. A common scar assessment scale that considers vascularity, pigmentation, pliability, and height of affected area. Scores range from 0 to 15.

Troponin. Calcium-binding protein found in skeletal and cardiac muscle fibers that causes interactions between actin and myosin when bound to calcium.

Key Facts About Vitamin D

- Vitamin D is a fat-soluble vitamin best absorbed alongside oils.
- Natural sources of vitamin D include foods such as oily fish (salmon, mackerel, and sardines) and cod liver oil, as are commonly fortified foods like milk, juices, breads, and cereals (Holick 2004).
- During winter months there is less vitamin D_3 synthesis compared to the summer months because of decreased sun exposure due to several factors such as less skin exposed to sunlight due to clothing worn, less time spent outdoors, and the decreased amount of daylight hours.
- Overdose of vitamin D supplements causes disruption of calcium balance, leading to high calcium levels, which can cause kidney stones and weakened bones but some highly sun-exposed professions like lifeguards can have very high levels of vitamin D with no evidence of adverse effects.
- Vitamin D deficiency is linked to loss of bone density and osteoporosis.

Summary Points

- Vitamin D is a collective term for two main forms of hormonal sterols that converge upon an active metabolite, calcitriol.
- While vitamin D is well known for regulation of calcium and phosphate homeostasis, it also exerts widespread immunomodulatory effects on immunomodulation, endothelial integrity.
- Vitamin D has been shown to have a neuroprotective role in traumatic brain injury patients and supplementation may lead to reduced neuroinflammation.
- Normal scar tissue formation and upregulated skin repair genes in burn patients have unveiled vitamin D's potential as a supplement for faster wound healing.
- In muscle injuries, vitamin D supplementation has led to decreased inflammatory markers and improved recovery after injury.
- Vitamin D's role in epithelial integrity and immune cell recruitment is believed to be responsible for the increased susceptibility to lung injury seen in vitamin D-deficient humans and animal models.
- Among ICU patients, low vitamin D levels may be a risk factor in development of infection, sepsis, acute respiratory failure, and acute kidney injury.
- Vitamin D deficiency, particularly severe deficiency, is an independent risk factor for in-hospital, ICU, 30-day, and even 1 year mortality.

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Selenium Concentrations and Multiple Trauma/Trace Elements in Trauma: A Focus on Selenium

30

Ji Young Jang and Jae Gil Lee

Contents

Abstract

Systematic inflammatory response and compensatory anti-inflammatory response caused by hemorrhage, ischemia, and reperfusion injury occur simultaneously in the acute phase of an injury, creating a sepsis-like state by releasing endogenous damage-associated molecular patterns into the systemic circulation in injured patients. Oxidative stress is increased in this immune-inflammatory activation status, and when it is exacerbated, multi-organ dysfunction is induced accordingly. Defense mechanisms exist in the body to protect cells (especially mitochondria). Antioxidants and antioxidant enzymes reduce oxidative stress. Vitamins and trace elements are required as cofactors for the functioning of

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antioxidants, and reductions in micronutrients have been reported in several critically ill conditions. In this chapter, we will focus on studying the effects of selenium deficiency and trace element supplementation in trauma patients.

Keywords

Trauma · Selenium · Trace elements · Micronutrients · Antioxidants · Postinjury inflammation · Critically ill · Selenoprotein P · Selenium deficiency · Selenium supplementation

Abbreviations

ATP	Adenosine triphosphate
CARS	Compensatory anti-inflammatory immune response
CI	Confidence intervals
DAMPs	Endogenous damage-associated molecular patterns
HMGB1	High mobility group box protein-1
ICU	Intensive care unit
NOS	Nitric oxide species
PAMPs	Pathogen-associated molecular patterns
RCT	Randomized control trial
ROS	Reactive oxygen species
RRs	Risk ratios
SIRS	Systemic inflammatory response syndrome
SOD	Superoxide dismutase

Introduction

Vitamins and trace elements are micronutrients, which are essential components of nutrition. In 2007, Heyland et al. conducted a large randomized controlled trial (RCT) of glutamine and antioxidant supplementation in critically ill patients, but contrary to expectations, this trial showed that antioxidants did not significantly affect clinical outcomes (Heyland et al. 2013). According to what is known until recently, the definition of appropriate amounts and specific composition of micronutrients remains unclear, however, it is known that the supply of adequate amounts of micronutrients prevents clinical deficiency (Berger and Ben-Hamouda 2020). Patients with critically ill conditions, including those with trauma, have states of hyperinflammation, oxidative stress, and mitochondrial and cellular immune dysfunction, and micronutrients are known to play an important role as antioxidants in these conditions. In addition, in the most recent guidelines by the European Society for Clinical Nutrition and Metabolism (ESPEN), "To enable substrate metabolism, micronutrients should be provided daily with parenteral nutrition." (Grade of recommendation: B – strong consensus) whereas "Antioxidants as high-dose monotherapy should not be administered without proven deficiency" (Grade of recommendation: B: Strong consensus) (Singer et al. 2019). Indeed, interest in the

supply of micronutrients among clinicians is still low. A recently published VITA-TRACE survey, an international survey by the European Society of Intensive Care Medicine, showed that only 24.3% of actual intensivists monitor micronutrient deficiencies, with parenteral micronutrient administration beginning 3 days after intensive care unit admission (Vankrunkelsven et al. 2021). In this chapter, we will review the pathophysiology of reactions in the body that occur in injured patients, changes in micronutrients, and micronutrient supplementation, with a special focus on selenium.

Physiological Changes in Patients after Trauma

In patients with major trauma, controlling acute hemorrhage and coagulopathy are important for treatment. However, systemic inflammatory response syndrome (SIRS) is common in patients surviving massive hemorrhage. In severe cases, it progresses to multiple organ failure. This mechanism is somewhat different from SIRS caused by an infection and it is known to affect post-traumatic survival (Rj et al. 2014). Previously, it was theorized that post-traumatic immune reaction begins with hyperinflammation (SIRS) followed by a compensatory anti-inflammatory immune response (CARS). Recently, it has been proposed that SIRS and CARS occur simultaneously in the initial phase after injury (Xiao et al. 2011). Severe SIRS, which is pro-inflammation by activation of the innate immune system, causes early organ dysfunction, whereas early anti-inflammation through inhibition of the adaptive immune system suppresses pro-inflammation and creates a pre-conditioned status that responds to the second hit and accelerates healing. Persistent anti-inflammation as opposed to disproportionate pro-inflammation leads to severe CARS, leading to conditions of immune-paralysis, impaired healing, infection, and late organ dysfunction (Fig. 1). Injured cells release endogenous damage-associated molecular patterns (DAMPs), similar to microbial pathogen-associated molecular patterns (PAMPs) released under sepsis conditions. DAMPs activate innate immunity, including HMGB1 (high mobility group box protein-1), heat-shock proteins, uric acid, and DNA (Levy et al. 2007). Injury releases mitochondrial DAMPs into the circulation that creates a sepsis-like state, which is known to be an important factor linking trauma, SIRS, and inflammation (Xiao et al. 2011; Zhang et al. 2010; Sauaia et al. 2017). In this state of immune-inflammatory activation, similar to sepsis, excessive reactive oxygen species (ROS) are generated, causing oxidative injury to cellular proteins and nucleic acids, and destruction of cell membranes by lipid peroxidation. Ischemia and reperfusion injury are induced in patients with severe trauma through the following mechanisms. (1) Hypoxemia reduces the consumption and production of adenosine triphosphate (ATP), which impairs cell membrane permeability, increases intracellular Na^{+}, and changes the concentration of cytosolic Ca^{2+}, leading to cell damage. (2) In the reperfusion stage, when molecular oxygen is re-supplied to the ischemic tissue, hypoxanthine accumulates in the tissue during the ischemia stage and reacts with oxygen, resulting in the production of superoxide anion ($\bullet O_2^-$). Superoxide anions are reduced to hydrogen

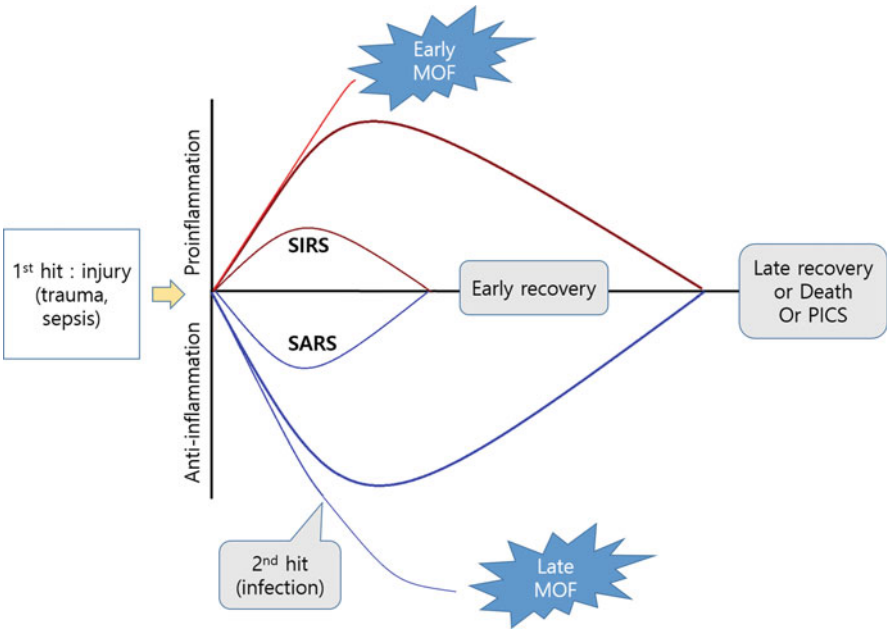

Fig. 1 Theoretic framework for postinjury multiple organ failure: The synchronous immunoinflammatory model From (Sauaia et al. 2017). (*PICS* persistent inflammation-immunosuppression catabolism syndrome, *SIRS* systemic inflammatory response syndrome, *CARS* compensatory anti-inflammatory response syndrome, *MOF* multiple organ failure)

peroxide (H_2O) by superoxide dismutase (SOD). These initial ROSs are relatively low, emerging radicals, and they do not have high cytotoxicity. However, the hydroxyl radical (•OH-) is the most dangerous ROS and it is produced by the Haber-Weiss reaction from superoxide anion or hydrogen peroxide, or by the Fenton reaction involving iron from hydrogen peroxide. ROS induce peroxidation of cellular membranes, aggravating the cellular necrosis process and initiate a biochemical reaction that can lead to cellular apoptosis (Tsukamoto et al. 2010).

Physiological Function of Trace Elements

Vitamins and trace elements are micronutrients that are an essential part of nutrition. Critical illness is characterized by hyperinflammation, mitochondrial dysfunction, cellular immune dysfunction, and oxidative stress. Under these conditions, micronutrients play a key role as antioxidants. In addition, micronutrients are essential for energy metabolism, implying ATP production and immune function in the mitochondria (Berger and Manzanares 2021). Antioxidants reduce the oxidative stress caused by ROS, and enzymes such as SOD, catalase, and glutathione are known (Reddell and Cotton 2012) in this regard. Cofactors for antioxidant function include selenium, zinc, manganese, iron, and vitamins C and E (Jones and Heyland 2008;

Santora and Kozar 2010). Zinc plays an important role in wound healing, immune function, glucose control, SOD, glutathione activity, and thiol pool stabilization (Luo et al. 2008; Taylor and Krenitsky 2010). Clinical manifestations of zinc deficiency include skin rash, glucose intolerance, abnormal homeostasis, diarrhea, and hair loss (Cander et al. 2011). Low levels of zinc are known to be associated with immune dysfunction, higher infection rates, and increased complications and mortality after infection (Rinaldi et al. 2009). Another observational study showed that serum levels in critically ill patients were negatively correlated with sequential organ failure assessment (SOFA) scores, which represented the patient's organ failure (Cander et al. 2011). In addition, since critical illness, sepsis, and inflammatory states are known to cause a decrease in serum zinc concentration, zinc supplementation is expected to be beneficial in critically ill patients. However, to date, no studies have shown that zinc supplementation in patients has significant differences in clinical outcomes, such as mortality or length of hospital stay (Heyland et al. 2008).

In the mitochondria, approximately 90% of intracellular oxygen is consumed for oxidative phosphorylation and ATP synthesis through electron transport. Antioxidants are essential for cell survival (1) to reduce the reactive species generated in this process. In particular, damaged and dysregulated mitochondria produce excessive superoxide, which damages intra-organelle components such as proteins, lipids, and DNA. This process eventually forms a vicious cycle that produces more ROS and nitric oxide species (NOS), which eventually triggers cell death. Vitamin C (ascorbic acid) is known to have various biological functions. First, vitamin C modulates the immune response by inhibiting the activation of nuclear factor kB, which is responsible for the progression of the pro-inflammatory cytokine storm, increasing leukocyte phagocytic activities, and decreasing macrophage superoxide production (Berger and Oudemans-Van Straaten 2015; Carr and Maggini 2017). In addition, vitamin C acts as a primary circulating antioxidant that neutralizes ROS and NOS (Wu et al. 2002; Wu et al. 2003). Vitamin C also inhibits bacterial replication and increases endogenous vasopressor synthesis and sensitivity. In addition, vitamin C is known to promote wound healing by acting as a cofactor in collagen synthesis (Mohammed et al. 2016).

Vitamin E is the most powerful lipid-soluble chain-breaking antioxidant that prevents lipid peroxidation and disrupts membrane integrity. To date, clinical studies on ischemia-reperfusion injury, such as revascularization of the lower extremities, kidney transplantation, liver surgery, and aortic aneurysm repair have shown positive effects of multivitamin antioxidant solutions, including vitamin E (Bartels et al. 2004; Cerwenka et al. 1998; Rabl et al. 1993; Wijnen et al. 2002).

Manganese is a part of the superoxide dismutase (SOD) enzyme along with copper and zinc, and it is known to promote the dismutation of superoxide anions to hydrogen peroxide and oxygen. However, studies on Mn levels and supplementation in specific clinical situations are limited (Żwierełło et al. 2020).

Selenium acts as a cofactor of glutathione, and it is known that a decrease in plasma glutathione peroxidase activity is directly related to a decrease in plasma selenium levels (Rinaldi et al. 2009; Todd et al. 2008).

Selenium Deficiency and Supplementation in Critically ill Patients

Selenium exists in the human body in organic forms such as selenocysteine, and selenomethionine. Inorganic forms of selenium, such as selenite and selenite, are mainly deposited in plants through the soil. Selenium is one of the most essential micronutrients in humans and animals. Plants accumulate inorganic selenium on their own through soil. Animals mainly consume selenium through vegetables and meats. Humans may also use dietary supplements to meet their selenium requirement. The dosage of selenium per day for adult men and women is 70 μg and 55 μg, respectively (Hariharan and Dharmaraj 2020). Selenium is an essential nutrient with regulatory, immunological, and antioxidant functions. It acts as an antioxidant in the glutathione peroxidase system and plays an important role as an anti-inflammatory substance (Huet et al. 2008). Selenium supplementation has been used as an adjuvant therapy for patients with SIRS, sepsis, and septic shock. A recent observational study in which micronutrient levels were serially measured from admission to 7 days in critically ill patients reported that selenium levels were consistently lower than in control patients (Koekkoek et al. 2021). Studies on the relationship between selenium deficiency and inflammatory status such as sepsis, postoperative state, trauma, and burn and critical illness have been reported, and several studies have been published to date on selenium deficiency, which is related to the clinical outcome of patients (Jang et al. 2014; Hariharan and Dharmaraj 2020; Choi et al. 2019; Braunstein et al. 2020). Recently, several meta-analyses on this topic have also been published. However, studies on high-dose selenium supplementation in critically ill patients have not shown strong and convincing results, as expected from previous observational studies (Berger and Manzanares 2021). Mahmoodpoor et al. performed an RCT supplying 3000 μg of selenium on the first day and 1500 μg on the following 9 days to critically ill patients receiving a mechanical ventilator. It was reported that serum selenium level and glutathione peroxidase-3 activity significantly increased in the study group but did not reduce the incidence of ventilator-associated pneumonia and death within 30 days of ICU admission (Mahmoodpoor et al. 2018). In the largest study involving 1000 specific patients using a 2×2 factorial design, 1000 μg selenium was supplied during their stay in the ICU, and the authors failed to show a significant difference. However, the 2×2 factorial design has statistical disadvantages, and intention-to-treat analysis showed a significant interaction between procalcitonin-guided antibiotic treatment and selenium supplementation. In addition, considering that a significant number of renal replacement therapy and emergency surgery patients were included in the selenium group, it seems that there was a problem in patient allocation. Therefore, caution is required when interpreting the results of this study (Bloos et al. 2016).

Recently, several meta-analyses have reported controversial results regarding the clinical outcomes of selenium supplementation. In 2015, Afshari et al. analyzed 16 RCTs and showed that they had the effect of significantly reducing overall mortality [risk ratios (RRs), 0.82; 95% confidence intervals (CI), 0.72–0.93]. Of these, 13 trials with sodium selenite showed a statistically significant reduction in overall mortality (RR, 0.82; 95% CI, 0.72–0.93), whereas three studies with

selenium-containing compounds showed no effect on mortality. However, no significant results were found in the results for deaths by time duration, number of infections, duration of mechanical ventilation, length of ICU stay, and length of hospital stay, excluding total deaths. This was due to difficulties in interpreting the results by the low quality of trial methodology and high risk of bias in the included trials (Allingstrup and Afshari 2015). In 2016, Manzanares et al. showed that intravenous selenite had no effect on mortality in a meta-analysis of 21 RCTs (RRs, 0.98; 95% CI, 0.90–1.08), and neither selenium monotherapy (RR, 0.91; 95% CI, 0.79–1.04), nor selenium combined therapy (RR, 1.08; 95% CI, 0.93–1.25), nor loading dose had any effect on mortality (Manzanares et al. 2016). However, in 2019, Zhao et al. controlled the risk of type I and type II errors using trial sequential analysis, and performed a meta-analysis of 19 studies in which selenium monotherapy was performed, and it was found that selenium supplementation significantly reduced overall mortality (RRs, 0.86; 95% CI, 0.78–0.95) and shortened the length of hospital stay (RRs, −2.30; 95% CI, −4.03 to −0.57) (Zhao et al. 2019).

Selenium in Trauma Patients

According to recent studies, severe trauma induces a complex systemic immune response, and SIRS and CARS can occur simultaneously in the initial phase after injury. Multiple organ dysfunction appears as a result of these opposing trauma-induced inflammatory immune reactions, which leads to high mortality rates. The detailed mechanism of this is described above. In addition, in hemorrhagic shock, ischemia, and reperfusion injury in severe trauma patients, ROS generation increases, which in turn induces mitochondrial dysfunction and eventually promotes cell death (Sauaia et al. 2017). Studies on the effect of selenoprotein (selenium-containing proteins) on suppressing oxidative stress are ongoing, including well-known glutathione peroxidase and thioredoxin reductase (Saito 2020; Hill et al. 2003; Hariharan and Dharmaraj 2020). In an observational study of patients with major trauma, selenium and selenoprotein P levels were significantly lower than normal within 1 h after injury, and this deficiency persisted for 72 h. In addition, selenium and selenoprotein P were maintained at a relatively constant level from 1 h to 72 h in the surviving patients compared to the dead patients (Braunstein et al. 2020). Recently, it has been found that selenoprotein P is a major selenium-containing protein, synthesized mainly in the liver, and it acts as a selenium transporter to maintain antioxidative selenoenzymes. This decrease in selenoprotein is known to cause various organ dysfunctions related to oxidative stress (Saito et al. 2003; Hill et al. 2003; Suzuki et al. 2008). Most selenium studies targeting trauma patients are observational studies, and large-scale studies on selenium supplementation are very limited (Wang et al. 2012; Berger et al. 1996). A recent retrospective observational study reported that multiple trauma patients with selenium deficiency within 48 h of admission had higher 30 day mortality, more frequent pneumonia, and infectious complications (Choi et al. 2019) (Table 1).

Selenium Supplementation in Trauma Patients

In critically ill patients, therapeutic intervention is aimed at alleviating organ failure. Since oxidative stress and immune depression occur in the early stage of disease, if micronutrients are considered, they should be supplied at an early stage. The goals of this micronutrient intervention are: 1) inflammation modulation, 2) antioxidant enforcement, and 3) mitochondrial stress attenuation (Berger and Manzanares 2021). In one prospective observational study, early selenium treatment for traumatic brain injury significantly reduced unfavorable functional outcomes at discharge (adjusted RR, 0.69; 95% CI, 0.51–0.92; $p = 0.002$) and at the months follow-up (adjusted RRs, 0.61; 95% CI, 0.44–0.83; $p = 0.002$) (Khalili et al. 2017). Berger et al. reported that trace element supplementation, including selenium, after major burn increased plasma and tissue antioxidants, decreased the incidence of infection and pneumonia, and improved wound healing (Berger et al. 2007). In an RCT of 411 patients undergoing elective cardiac surgery, administration of 4000 μg selenium after anesthesia induction and 1000 μg selenium during an ICU stay prevented postoperative falls in blood selenium levels and reduced the need for postoperative vasoactive support (Schmidt et al. 2018). In a similar cohort, the SodiUm SeleniTe Administration IN Cardiac Surgery (SUSTAIN trial, NCT 02002247), the largest phase III RCT aimed at investigating the effects of perioperative high-dose sodium selenite supplementation in patients undergoing open heart surgery is currently underway (Stoppe et al. 2014). As such, studies on patients with trauma and trauma-related clinical conditions are limited. Most successful selenium trials used medium doses of 300–800 μg/day, and considering that recent studies on high-dose single selenium did not show good results, interest in high-dose single selenium seems to be limited (Manzanares et al. 2016; Allingstrup and Afshari 2015). Until recently, several studies had used micronutrients to improve clinical outcomes; however, the results were generally poor (Table 2). It was similar to selenium, and this seemed to be due to several reasons. These included, firstly, a matter of intervention design in the study, whether it included combination or single selenium, the dose used, the timing of the supplementation, and the length of intervention. Second, the endpoints of the study were too diverse. Third, there were differences between studies in terms of the selection of biomarkers (Berger and Manzanares 2021). Since isolated selenium intervention does not support the entire antioxidant system, combination therapy with other micronutrients such as vitamin C, vitamin E, niacin (vitamin B3), copper, manganese, and zinc, which have important functions in endogenous antioxidant defense, should also be considered. In addition, recent data show that micronutrient status should be considered important in the development of organ failure, and that it is important to maintain the basal needs in patients with high oxidative stress (Wesselink et al. 2019). It is thought that the most rational strategy is to proceed with the purpose of checking the acute deficiency status of micronutrients according to the patient's condition and correcting it, as necessary. For tailored micronutrient supplementation according to the patient's injury characteristics, additional studies on trauma are needed (Wijnen et al. 2002; Berger et al. 1996; Reddell and Cotton 2012).

Table 1 Recent observational studies evaluating selenium deficiency in trauma patients

Population	Study design	Observation / intervention	Outcome	Reference
Patients admitted to trauma care $n = 4294$	Retrospective cohort study	Combination therapy (high-dose antioxidant) Before and after study	Significantly shorter length of hospital and ICU stays After adjusting for age, gender, and probability of survival, lower mortality (OR, 0.32; 95% CI, 0.22–0.46)	(Collier et al. 2008)
Traumatic brain injury $n = 307$	Prospective Quasi-randomization	Monotherapy (1000 μg for 5 days followed by 500 μg for additional 5 days)	Significant reduction of the risk of unfavorable functional outcomes during discharge and at 6 months follow-up Failed to improve survival	(Khalili et al. 2017)
Multiple trauma $n = 135$	Retrospective observational	Serum se level (within 48 h of admission)	30-day mortality, incidence rates of pneumonia, and infectious complications were higher in se deficiency group Se deficiency was an independent risk factor in cases with in-hospital infectious complications (OR, 3.995; 95% CI, 1.430–11.156; $p = 0.008$)	(Choi et al. 2019)
Major blunt trauma (ISS $\geq$ 16)	Prospective observational	Serum se and SELENOP levels (within 1 h of admission, 6 h, 12 h, 24 h, 48 h, and 72 h)	Serum se and SELENOP concentrations were significantly below the reference value Strong deficit of se and SELENOP at the first time point Inverse relation between health status and se biomarkers Significantly lower initial post-trauma se status in non-survivors than survivors	(Braunstein et al. 2020)

(continued)

Table 1 (continued)

Population	Study design	Observation / intervention	Outcome	Reference
Traumatic spinal cord injury $n = 52$	Prospective observational	Serum se, cu, SELENOP, and Ceruloplasmin (admission, and after 4, 9, 12, 24 h)	Significantly decreased levels of se, cu, SELENOP, and Ceruloplasmin within 24 h in patients with remission of neurological impairment	(Seelig et al. 2020)

ICU intensive care unit, *OR* odds ratio, *CI* confidence interval, *Se* selenium, *SELENOP* selenoprotein P, *ISS* injury severity score, *Cu* copper

Applications to Prognosis, Other Diseases or Conditions

Applications to Prognosis

In this chapter, we saw that oxidative stress caused by excessive reactive oxygen species occurs in trauma patients, and selenium, along with other micronutrients, plays an important role as an antioxidant that protects against oxidative stress (Sauaia et al. 2017*;* Lord et al. 2014). *In fact, previous studies have shown that selenium and selenoprotein P deficiency occurred immediately after injury, and that this was related to the patient's clinical outcomes (*Choi et al. 2019*;* Braunstein et al. 2020*). However, in practice, there is a gap in the improvement of clinical outcomes between micronutrient deficiency and supplementation. Previous studies on selenium supplementation have mostly been conducted on heterogeneous populations, including critically ill patients and sepsis, and studies on selenium monotherapy have failed to show improvement in clinical outcomes of patients (*Manzanares et al. 2016*). Therefore, further studies on combination therapy with reduced other micronutrients, rather than selenium alone, are needed.*

Applications to Other Diseases or Conditions

The trauma category includes burns, major torso injuries, brain injuries, and multiple skeletal injuries, which have various pathophysiologies such as hypovolemic shock, ischemia and reperfusion injury, and sepsis. To overcome this heterogeneity, selenium supplementation studies with various homogenous cohorts are needed.

Table 2 Randomized clinical trials evaluating selenium supplementation in trauma patients

Population	Intervention	Outcomes	Reference
Major burns (TBSA >30%), $n = 20$	Combination (se 159 μg, Zn 406 μmol, cu 40.4 μmol) + standard trace elements vs. standard trace elements	Reduction in nosocomial infections Decrease in number of infections Shorter hospital stays	(Berger et al. 1998)
Major burns (TBSA >20%), $n = 21$	Combination (se 375 μg, Zn 574 μmol, cu 59 μmol) for 14–21 days + multitrace element vs. multitrace element	Increase in se, Zn, and GSHPx Significant increase in concentration of se and Zn in the skin Lowered number of infections in the first 30 days Reduction in pulmonary infections Improvement of wound healing	(Berger et al. 2007)
Major trauma (mean ISS = 30) $n = 31$	Monotherapy (se 500 μg) for 5 days vs. control	Normalized plasma se level from day 1 More and faster increase of total T4 and T3 after day 2	(Berger et al. 2001)
Major trauma (cardiac surgery and major trauma) $n = 200$	Combination (se 270 μg, Zn 30 mg, vitamin C 1.1 g, thiamine 100 mg) for 5 days + multitrace element and vitamin	Shorter length of hospital stays in intervention group of trauma patients Significant increase in plasma concentration of se, Zn, and GSHPx, which was low on admission Faster decrease of C-reactive protein	(Berger et al. 2008)
Elective cardiac surgery $n = 411$	Monotherapy (4000 μg after induction of anesthesia +1000 μg in the ICU) vs. placebo	Failed in SOFA score reduction Significant prevention of decrease of blood and serum selenium levels Reduced need for postoperative inotropic and vasoactive support Greater increase in the postoperative procalcitonin and bilirubin serum level in intervention group	(Schmidt et al. 2018)

TBSA total body surface area, *Se* selenium, *Zn* zinc, *Cu* copper, *GSHPx* glutathione peroxidase, *ISS* injury severity score, *ICU* intensive care unit, *SOFA* Sequential Organ Failure Assessment

Mini-Dictionary of Terms

- ***Systematic inflammatory response syndrome (SIRS).*** *Inflammatory response to blood loss and tissue damage, rather than infection.*
- ***Compensatory anti-inflammatory response syndromes (CARS).*** *Inhibition of the adaptive immune system and apoptosis limits pro-inflammation and creates a preconditioned state to protect against second hits and hasten healing.*
- ***Antioxidants.*** *Enzymes that block or slow cell damage caused by reactive oxygen species are generated during mitochondrial electron transport, such as superoxide dismutase, catalase, and glutathione.*
- ***Selenoproteins.*** *Proteins containing selenocysteine (Se-Cys) amino acid residues. Twenty-five species have been identified, including five glutathione peroxidases and three thioredoxin reductases, which play important roles in antioxidative defense.*
- ***Selenoprotein P.*** *The most common selenoprotein in plasma, contains ten selenocysteins. It acts as an antioxidative defense and selenium transporter in the brain, kidney, and testis.*

Key Facts of Selenium in Trauma

Until recently, selenium deficiency was known to be associated with inflammatory status, such as sepsis, postoperative state, trauma, burn, and critical illness.

In patients with these clinical conditions, selenium deficiency is known to be associated with poor clinical outcomes (mortality, length of hospital stay, and infectious complications).

Similar results have recently been reported in patients with trauma.

Based on these expectations, clinical trials on selenium supplementation conducted to date have failed to show that high-dose single selenium therapy improves clinical outcomes.

In particular, there is very little evidence for selenium supplementation in the trauma area.

Considering the results to date, studies in the direction of selectively supplementing micronutrients with acute deficiency status as well as selenium are needed.

Summary Points

- *Systemic inflammation and compensatory anti-inflammation caused by hemorrhage, ischemia, and reperfusion injury occur simultaneously in the acute phase after injury, increasing oxidative stress through several processes.*
- *Antioxidants act as a defense mechanism against oxidative stress, and micronutrients such as selenium, zinc, manganese, iron, and vitamins C and E play important roles as cofactors.*

- *Selenium deficiency is associated with critical illness and inflammatory status such as sepsis, postoperative status, trauma, and burn, and several studies have reported that this is related to the patient's clinical outcomes.*
- *However, recent clinical trials of selenium single supplementation in critically ill patients have failed to show improvements in patients' clinical outcomes.*
- *In particular, studies on trauma-related patients are mostly small-scale studies on high-dose single selenium for patients with traumatic brain injury, burn, and elective cardiac surgery, and they did not show clinically satisfactory results.*
- *Additional clinical trials in a more homogenous patient population are needed.*

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Presepsin: Methods of Measure, Features and Biomarker Potential in Sepsis and Critical Care

31

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Contents

Abstract

sCD14-ST or presepsin is a circulating protein, originating after proteases cleavage from the soluble form of CD14, a member of Toll-Like Receptors family. Presepsin is significantly elevated in patients with bacterial infection,

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showing high sensitivity and specificity. Its kinetics, with a spike in concentration after 3 h and a half-life of 4–5 h, allow the physicians to confirm an infection suspicion in an early phase of disease. Plasma levels of presepsin could be indicators for the diagnosis and prognosis of sepsis and septic shock, even if the decline of renal function could alter presepsin concentrations. During the COVID-19 pandemia, presepsin has been used as a prognostic biomarker to stratify those patients who required prolonged hospitalization or recovery in ICU. Nevertheless, an increase of presepsin in patients who underwent major surgery has been reported, due to inflammatory host-immune response linked to the surgical operation itself. In conclusion, presepsin serum concentration indicates the innate immune system activation during a variety of conditions, but its role is not yet clear. The existence of several immunoenzymometic assays to measure presepsin in a simple, fast reliable way, makes it a promising biomarker in critical care.

Keywords

Presepsin · Biomarker · Sepsis · COVID-19 · ICU · Perioperative

List of abbreviations

AKI	acute kidney injury
ARDS	acute respiratory distress syndrome
AUC	Area under the curve
CLEIA	Chemiluminescence enzyme immunoassay
CLSI	Clinical and Laboratory Standard Institute
CRP	C reactive Protein
ELISA	Enzyme-linked immunosorbent assay
ESR	erythrocyte sedimentation rate
ICU	intensive care unit
IQR	InterQuartile Range
LBP	protein binding lipopolysaccharide
LPS	lipopolysaccharide
MACCE	major adverse cardiovascular and cerebrovascular event
MOF	Multi-Organ Failure
NPV	negative prognostic value
PCT	Procalcitonin
PJI	prosthetic joint infection
PMNC	peripheral mononuclear cell
PPV	positive prognostic value
ROC	Receiver Operating Characteristic
THP1	human cell line of monocyte cells
TLRs	Toll-like Receptors
WBC	white blood cells

Introduction

The discovery of presepsin was due, in 2005, to Yaegashi et al. who defined presepsin also as a biomarker to diagnose and predict the prognosis of sepsis (Yaegashi et al. 2005). Despite its biological activity, presepsin is not yet fully understood, it is described as a regulatory factor in immune system response. Also known as sCD14-ST, presepsin is the soluble form of CD14. By binding to the Toll-like Receptors (TLR) family, CD14 can recognize several gram-positive and -negative bacteria's ligands, like lipids, peptidoglycans, and others. The most known ligand of CD14 is the lipopolysaccharide (LPS) of gram-negative bacteria. The binding of the lipopolysaccharide to a specific protein (LBP) allows LPS to be recognized by CD14. CD14 is a co-receptor present on the surface of monocytes and macrophages; it plays a role in intracellular signaling and facilitates the activation of genes encoding for immunity response molecules, like cytokines and various types of cells with effector functions. The bond between TRLs and the protein complex CD14 – LPS – LPB trigger the immune response.

Furthermore, CD14 might have a role in diseases like cancer, atherosclerosis, metabolic diseases, because it is also expressed in non-hematopoietic cells (i.e., in epithelial cells of several organs, endothelial microglial, and vascular smooth muscle cells) after endotoxin stimulation.

CD14 subtypes can be detected in general blood circulation as membrane CD14 located on immune cells' surface and soluble form sCD14. sCD14 undergoes cleavage by proteases, like catepsin D, into phagolysosome of innate immune cells. The N terminal part of sCD14, weighting 13 kDa, is called sCD14-ST or presepsin (Chenevier-Gobeaux et al. 2015).

In this chapter, the main features and the applications of presepsin in different clinical conditions like sepsis, inflammatory diseases, perioperative medicine, and COVID-19 will be analyzed.

Presepsin: A Biomarker of Early Immune Response

The ability to fight infectious processes is affected by the ability of the immune function to identify germs and pathogens and to activate an effective response to them. This is possible thanks to the two components of the immune system: the innate and the adaptative immune system. The innate immune system is characterized by its immediate activation due to an easy and efficient signaling way: phagocytosis mechanism and activation of cells like monocytes and macrophages (Iwasaki and Medzhitov 2015). The increase of presepsin concentration in patients' blood is a likely proof of innate immune system activation in a variety of conditions. Monocytes are present in the general blood circulation, and they have a basal activity. Therefore, it is likely to find a basal level of presepsin in healthy individuals' plasma, while the increase of presepsin concentration is detectable early during pathological conditions, with a degree of elevation dependent on the intensity of innate immune response (Chenevier-Gobeaux et al. 2015).

Available data on presepsin kinetic are still scarce. In an experimental model on septic rabbits, presepsin was detected in animals' blood 2 h after the start of the experiment (Nakamura et al. 2008), and this elevation was faster than IL 6 and procalcitonin elevations, with a spike after 3 h since the start of the experiment. The half-life of presepsin has been observed to be 4–5 h. In a recent study, the concentrations of presepsin were studied after the addition of lipopolysaccharide in a culture of monocytes (THP1) and culture of monocytes from healthy volunteers (peripheral mononuclear cell (PMNC)). In both THP1 and PMCN, presepsin was detected after 1 h from the exposition to LPS, with a spike in concentration after 3 h (Chenevier-Gobeaux et al. 2016).

Presepsin in Sepsis Diagnosis and Prognosis

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al. 2016). In 2016, the task force of the Surviving Sepsis Campaign also changed the definition of septic shock, describing it as a subset of sepsis, in which the circulating and metabolic alterations are so severe to raise its mortality (Seymour et al. 2016).

Sepsis, despite its diffusion and high mortality and morbidity, remains difficult to diagnosticate. Up to now, the diagnosis is based on nonspecific signs, symptoms, medical tests, and biomarkers. Like many syndromes, sepsis diagnosis it is not well defined by a strict algorithm, but the use of scoring systems, as the SOFA score, together with biomarkers may improve the early diagnosis of sepsis and septic shock (Taeb et al. 2017). Sepsis is the first condition in which presepsin was valuated to allow an early diagnosis, which is crucial to start an adequate treatment and improve prognosis. Many prospective multicentric works have shown that the accuracy of presepsin depends on the cutoff values that are used (Fig. 1) (Carpio et al. 2015):

Godnic et al. demonstrated that presepsin is an early diagnostic and prognostic marker in septic patients. In their work, sensitivity and specificity of presepsin to identify infection in patients admitted to the ICU were found to be 84.6% and 62.5%, respectively (cutoff 864 pg/mL), and presepsin well correlated with APACHE II score (p-value = 0.016) (Godnic et al. 2015) and SOFA score when its value is two or more ($p < 0.01$).

Using a cutoff value of 466,5 pg/mL, Klouche et al. evaluated the usefulness of presepsin as a diagnostic and prognostic biomarker of sepsis, severe sepsis, and septic shock in patients admitted to ICU compared with PCT. For the diagnosis in the first 24 h from the admission in ICU, the percentages of sensibility, accuracy, positive and negative predictive values of presepsin appear to be 90%, 55%, 82%, and 71%, respectively. Meanwhile, the percentages of sensibility, accuracy, positive, and negative predictive values of PCT to detect in the first 24 h from the admission in ICU appear to be 80%, 59%, 82%, and 57%, respectively, allowing to assert that presepsin is not superior of PCT in sepsis diagnosis, but their combination could be useful to enhance diagnostic and prognostic accuracy (Klouche et al. 2016).

Presepsin (pg/ml)	Diagnosis
< 200	Exclusion of sepsis
< 300	Systemic infection not probable
< 500	Systemic infection (sepsis) probable
< 1000	Significant risk of the systemic infection progression (severe sepsis), increasing risk of unfavorable outcome
≥ 1000	High risk of the systemic infection progression (severe sepsis/septic shock). High risk for mortality after 30 day comparable with a SOFA score ≥ 8

Fig. 1 The figure shows different presepsin cutoffs used to distinguish from systemic infection, severe sepsis, or septic shock. Carpio et al. (2015) with permission

In their work, Dragoescu et al. compared presepsin with other inflammation or infection biomarkers (CRP, ESR, WBC, and SOFA score) to verify presepsin efficiency as mortality and severity biomarker in ICU. Patients with septic shock had a presepsin value of 2403 pg/mL, septic patients had a presepsin value of 1476 pg/mL. For a cutoff 1932 pg/mL, presepsin showed a sensitivity and sensibility of 79% and 63% and an AUC of 0.726 in predicting sepsis severity. For a cutoff value of 1365 pg/mL, presepsin revealed a sensitivity and sensibility of 74% and 88% and an AUC of 0.861 in predicting sepsis mortality. From the analysis of ROC curves, presepsin proved superior prognostic value to all other biomarkers (Drăgoescu et al. 2020).

In line with results of multiple prospective multicentric works, presepsin values are considerably high in patients who have bacterial infection, compared to patients with no bacterial infection. Cutoff value of 600 ng/mL is considered suitable to discriminate bacterial infection patients with a sensitivity and specificity of 87.8% and 81.4%, respectively (Endo et al. 2012). This value is not useful to distinguish infection by gram-positive or gram-negative bacteria, even if a value of presepsin around 946 ng/mL has been strongly associated with gram-negative infections (Masson et al. 2015).

Relevant use of the trend of variations in presepsin concentration is antibiotic therapy modulation. Presepsin values tend to decrease after 7 days in patients with positive blood culture and appropriate antibiotic therapy, while a high concentration of presepsin on the first day of hospitalization can be correlated to longer ICU hospitalization, prolonged mechanical ventilation, with a low grade of resolution of the infection and increased mortality (Kim et al. 2017).

Nevertheless, numerous factors could impair the prognostic meaning of presepsin, like renal function, drug administration (i.e., glucocorticoids, propofol), and genetic elements (Ackland and Prowle 2015). A retrospective study by Sousa et al. on the association of CD14 rs2569190 polymorphism and death related to septic shock in patients who underwent major cardiac or abdominal surgery shows that the effect of A to G polymorphism produces a downregulation of CD14 transcription and a lower expression of sCD14. The effect of A to G polymorphism produces a downregulation of CD14 transcription and a lower expression of sCD14. It is conceivable that the A allele generates a strongest pro-inflammatory response due to a higher CD14 expression. Patients who underwent cardiac surgery with G/G genotype were associated with higher mortality during the first 60 days ($p = 0.029$) compared to patients with A/A A/G genotype. Patients who underwent abdominal surgery with G/G genotype did not have a significant association with mortality at 28, 60, and 90 days after septic shock (Jiménez-Sousa et al. 2018). In 2017, Korpelainen and her group verified if presepsin could be a predictor of the progression of febrile neutropenia after intensive chemotherapy to sepsis, based on the idea of Urbonas et al. (Urbonas et al. 2013) that chemotherapy may influence the number and the function of leukocytes and other sources of presepsin. A high value of presepsin at days 1 and 2 of neutropenic fever was associated with the development of septic shock but it did not predict blood culture positivity or sepsis (Korpelainen et al. 2017).

Presepsin and Renal Function

Presepsin is a glycoprotein of 13 kDa; therefore it should be completely filtered from the glomerulus and re-adsorbed and metabolized by convoluted proximal tubes. For this reason, the decline of renal function could alter presepsin concentrations as during chronic kidney disease, sepsis, or in elderly patients. Chenevier-Gobeaux et al. enrolled 144 patients without infectious disease and 54 healthy patients to compare presepsin concentrations between the two groups. They showed a substantial difference between median presepsin concentration in young patients and aged patients (300 pg/mL vs 470 pg/mL) and higher values of presepsin in patients with renal dysfunction compared to healthy patients (470 pg/mL vs 386 pg/mL) (Chenevier-Gobeaux et al. 2014). Nakamura et al. enrolled 247 patients, dividing them into two groups: acute kidney injury (AKI) patients and non-AKI patients according to RIFLE criteria. Presepsin was an acceptable biomarker of sepsis if the renal damage was limited, but it got worse with severe or critical renal injury. However, the study presents several limitations, since it uses old criteria for sepsis (dated 1992) and they do not use other biomarkers to compare with (Nakamura et al. 2015).

Nagata et al. studied the relation between presepsin and chronic kidney failure, including 13 hemodialysis patients. They observed a linear correlation between presepsin and the stage of chronic kidney disease, classified according to KDIGO

2012 guidelines. In the hemodialysis group, presepsin concentration reached a value of 1160 pg/mL, as in septic patients. Probably, this rise was due to reduced or absent clearance of presepsin or due to inflammatory conditions in this kind of patients (Nagata et al. 2015).

Consequently, the use of normal cutoff values to detect sepsis in patients with chronic kidney disease is not correct. Miyoshi et al. calculated a reference range with a distribution-free method in healthy volunteers and in patients with CKD with G1 stage. The range was 59–153 pg/mL, lesser than the standard range. The new range did not fulfill the prerequisite for 120 cases suggested by CLSI guidelines so there is no proper information for determining a new reference scale (Miyoshi et al. 2019).

Pediatric Sepsis

Neonatal sepsis represents the main cause of morbidity and mortality in neonates. It is difficult to identify sepsis in pediatric patients for age-dependent vital signs. Sepsis in children is diagnosticated as in adults and the treatment follows adult guidelines (Goldstein et al. 2005).

In a meta-analysis, Bellos et al. (Bellos et al. 2018), identified 11 papers including 783 neonates; they calculated an AUC of 0.9751 for the use of presepsin to diagnosticating sepsis. This suggests a very high diagnostic performance, and presepsin has been mentioned as an available biomarker to diagnose neonatal sepsis while waiting for cutoff values for neonates. Measurement of presepsin in children and adolescents was considered in a systematic review of Yoon et al. (Kondo et al. 2019). The AUC for presepsin was 0,925, which is superior to the AUC of procalcitonin and reactive protein C (0,820 and 0.715, respectively), even if the authors recognized that the results should be interpreted with caution, due to the heterogeneity and the scarcity of studies included in their analysis.

Diagnosis of Sepsis in Emergency Department

Liu et al. showed that plasma levels of presepsin could be promising indicators for diagnosis and prognosis of sepsis and septic shock at 28 days after admission in the emergency department. With a cutoff value of 400 pg/mL, presepsin can predict sepsis severity with a sensibility of 82.4% and an accuracy of 72.4% (positive and negative predictive values of 71.3% and 83.2%, respectively; predictive accuracy of 77%). Septic shock can be predicted, with a cutoff value of 550 pg/mL of presepsin, with a sensibility, accuracy, positive, and negative predictive values of 85.7%, 63.6%, 28,5%, and 96.3%, respectively, and predictive precision of 66.8%. Moreover, presepsin may be a predictor of mortality at 28 days with a value of 556 pg/mL, with a sensitivity of 62.2%, specificity of 66.8%, (PPV 48.3%, NPV 78%, and predictive accuracy of 65.3%) (Liu et al. 2013).

Application in Perioperative Medicine

An adequate preoperative evaluation is the only available strategy to reduce and manage the onset of intra- and postoperative complications. The identification and management of high-risk patients are mandatory. In the last few years, presepsin has been identified as a reliable prognostic biomarker in surgical patients. Different trials have demonstrated a time-dependent increase of presepsin during and after major surgery, due to inflammatory host-immune response linked to the operation itself, as well as the possibility to quantity it, before procalcitonin and protein C reactive. Presepsin increases both in cardiac and noncardiac surgery. Popov et al., in a little group of patients who underwent cardiac surgery, described an elevation of presepsin after 7–12 days from surgery, especially in the group who developed infections. The patients with infectious complications needed extended ventilation support and longer hospitalization in ICU (Popov et al. 2015). Vodnik et al. used presepsin to identify abdominal sepsis during preoperative evaluation for elective surgery and to identify patients with high mortality risk (Vodnik et al. 2013). Bösch et al. also showed that presepsin is suitable to assess mortality risk after emergency surgery for abdominal sepsis (Bösch et al. 2020).

Koakutsu et al. examined the perioperative kinetic of presepsin during spinal surgery and have assessed the role of presepsin in the early diagnosis of surgical site infection. They enrolled 118 patients and measured presepsin before the surgery, right after and at 1- and 7-days distance from the operation. Presepsin concentrations in patients who did not have surgical site contamination ($n = 115$) were: 126, 171, 194, and 147 pg/mL, respectively; instead three patients with postoperative infection had higher presepsin (> 300 pg/mL) values 1 week after surgery (Koakutsu et al. 2018).

Besides the ability to recognize early infections, there is a hypothesis about the role of presepsin as a biomarker to estimate perioperative major adverse cardiovascular and cerebrovascular events (MACCEs). The rationale is in the pathophysiology of atherosclerosis, in which the immune system cells play a major role. The development and the destabilization of atheromatic plaque are associated with the increase of immune cells in the vessel wall. Different subsets of leukocytes have different roles in the pathogenesis of atherosclerosis disease: classical and intermediate monocytes have a protective function toward tissues, instead, nonclassical monocytes phagocytize oxidate LDL, turning into macrophages that enhance plaque weakening. Since presepsin is a biomarker of macrophages activation, it shall be considered that it can be a valid biomarker of adverse cardiovascular and cerebrovascular events during preoperative evaluation. It should not be forgotten that surgery itself is a trigger for the immune system, so it is interesting analyzing changes in leukocyte populations and possible correlations with adverse events post-surgery (Shive et al. 2015). Handke et al. studied if noncardiac surgery is related to changes in atherogenic leukocyte subsets and if these are linked to perioperative major adverse cardiovascular and cerebrovascular events in patients with high cardiovascular risk. They found that the noncardiac surgery is associated with a changing of monocytes subsets, and the atherogenic subtype baseline values

of patients who developed MACCEs were higher than patients without MACCEs. In the future, presepsin could be used as a biomarker to identify patients with a major cardiovascular risk, in the preoperative evaluation and like prognostic marker during post-surgery (Handke et al. 2020).

Clementi et al. assessed the prognostic utility of postoperative presepsin for adverse complications after elective cardiac surgery. Presepsin was measured 48 h after the procedure, the median presepsin levels were higher in patients who died during hospitalization and in patients with cardiovascular, respiratory, and renal complications despite patients without unfavorable outcome. In their study, presepsin showed a better predictive value for in-hospital, 30 days and 6 months mortality compared to procalcitonin (Clementi et al. 2019).

Presepsin in Orthopedic Surgery

Infections are one of the most common causes of morbidity, prolonged hospitalization, and mortality in orthopedic surgery. They represent serious events both for patients and for the health system, considering the necessary resources to treat them. Over the past 10 years, the definition of peri-prosthetic joint infection (PJI) changed many times because PJIs have large variability in their clinical presentation, from acute to the subacute manner, and the proposed diagnostic tests are not suitable for all the definitions. Presepsin could be a biomarker able to recognize the infected site early, so to improve the treatment. In a prospective trial made by Vicenti et al., presepsin was measured 24 h before surgery and 24, 48, 72, and 96 h after surgery in two groups: group A, patients undergoing total hip replacement, and group B, patients undergoing total knee replacement. The basal values overlap in both groups; instead, the post-surgery values were higher in group A than group B. Post-surgery trend described a gradual increase till 72 h when presepsin reached its peak, and a gradual decrease 96 h after surgery (Vicenti et al. 2017). Marazzi et al. enrolled 100 patients requiring hip prothesis revision in a prospective multicentric trial, they divided them into two groups: 48 patients with prosthesis infection confirmed by cultural tests and 52 patients with prosthesis disconnection that needed aseptic mobilization. Presepsin was measured before surgery, 1, 2, 3 months after surgery. The value of presepsin was higher in the first group due to prosthesis infection and this enables us to consider presepsin an evaluable biomarker for PJIs. The values of presepsin after surgery went down in the first group after the implant revision and were unchanged in the second group, making presepsin a good biomarker for prognosis and follow-up in hip surgery (Marazzi et al. 2018).

In an observational prospective single center study (Piazza et al., not published data), we enrolled 33 elderly patients scheduled for major orthopedics surgery (mostly hip fracture), divided into two groups: patients with preoperative higher value of presepsin ($n = 9$) and patients with a lower value of presepsin ($n = 24$) (cutoff value of 560 pg/mL). Our study aimed to establish if the preoperatory measure of presepsin is linked to mortality; cardiovascular, respiratory, neurological complications; duration of hospitalization, and admission to ICU after major

orthopedic surgery. The higher presepsin value group showed a median day of hospitalization of 11, versus 9 days in the low preoperative presepsin group. Thirty-three percent of patients with high presepsin value deceased at 28 days, versus 4% of low presepsin group. We observed that patients with a higher value of presepsin needed a longer hospitalization in ICU (33% of cases, compared to the 4% of the other group), with an increased incidence of neurological complications.

Application in Viral Infections

Presepsin concentrations reflect the activation of the innate immune system under both physiological and pathological conditions, so an increase of its values with bacterial and fungal infections, like pneumonia, is predictable. Several authors investigated the function of presepsin in diseases caused by viral infections. Demirpence et al. focused on presepsin as a biomarker in Crimea Congo hemorrhagic fever and reported median values of presepsin of 1499 pg/mL. This elevated value of presepsin concentration was explained by the reactive hemophagocytosis characteristic of this illness (Demirpençe et al. 2016). Jereb et al. studied 54 septic patients and 26 patients with aseptic meningitis. Their study aimed to evaluate presepsin as a biomarker in clinical practice to distinguish septic patients from patients with viral infection by measuring different concentrations between the two groups. The median value of presepsin in septic patients was 1614 pg/mL compared to a median value of 203 pg/mL in patients with aseptic meningitis, with a range of values from 53 to 987 pg/mL. The reason why some patients had high values of presepsin was not clear (Jereb et al. 2019).

Recently, it was reported that elevation in presepsin value could be prognostic for patients who develop COVID-19. The first clinical manifestation by SARS-CoV 2 infection was identified as a form of pneumonia that may induce ARDS and MOF. Hui et al. hypothesised that alveolar macrophages could limit immune response against SARS-CoV 2 in a mild stage of disease, while during the severe or critical stage of COVID-19, the alveolar-endothelial wall is damaged by both infection and immune response. To prevent the spread of infection, alveolar macrophages and/or the epithelial cells secrete many pro-inflammatory cytokines that invoke monocytes and neutrophils to remove the exudations with virus particles and infected cells. If the immune response is not well balanced between pro- and anti-inflammatory molecules, the viral infection blowout due to the reduction and dysfunction of lymphocytes, and macrophages action leads to an impairment of lung damage up to multiorgan failure (Li et al. 2020).

Among the few studies conducted on the role of presepsin during SARS-CoV 2 infection, the one by Zaninotto et al. examined the possibility to use presepsin as a prognostic biomarker for those who required a higher standard of care or prolonged hospitalization. The study was conducted on 75 patients, 21 ICU patients and 54 in the infective department, from January to March 2020. Presepsin concentrations were higher in patients who required a prolonged hospitalization or recovery in ICU.

According to ROC curve, the AUC to predict the mortality was 0,72 (p-value < 0,05). Patients who showed a value of presepsin higher than 250 pg/mL required prolonged hospitalization (median value 18 days) or had an unfavorable outcome, while patients with presepsin value < 250 pg/mL had shorter hospitalization (median value 10 days). The sensibility and specificity of presepsin >250 pg/mL was 85% and 43%, respectively. Elevation of presepsin, resulting from pathogen–hosting interaction, is present from the early phase and continues during the time according to the severity of illness (Zaninotto et al. 2020).

In a retrospective cohort study (Piazza et Al, not published data) presepsin was examined as severity and prognostic biomarker in 144 COVID-19 patients who required hospitalization in ICU. Patients were divided into three groups according to the P/F ratio:

- P/F ratio< 100: severe COVID-19
- 100< P/F ratio < 200: moderate COVID-19
- P/F ratio> 200: mild COVID-19.

We observed that patients with mild illness have a median value of presepsin of 223,5 pg/mL, while the median value of presepsin patients with severe COVID-19 was 413,7 pg/mL.(IQR 178,45 vs 494,4).This first result shows the possible role of presepsin as an illness severity biomarker and reflects the conclusion by Fukada et al., in which patients with severe stage of illness had higher values of presepsin compared to patients with milder stage (Fukada et al. 2021). Of 144 enrolled patients, 87 (60%) patients died, and 57 (40%) patients were discharged from the hospital. The presepsin median value of deceased patients was 588,9 pg/mL, versus 267,3 pg/mL of discharged patients (IQR 685,05 vs 260,4); presepsin concentrations had a linear correlation with SOFA score and SAPS score, proving the potential use of presepsin as a prognostic biomarker during COVID-19 (Table 1).

Considering P/F ratio as severity index of COVID-19, the observation of an inverse correlation between it and presepsin concentrations improve the latter role as severity biomarker. Presepsin elevation could be due to lung damage and/or to inappropriate immune response, characteristic of COVID-19 late phases (Table 2).

Methods of Measure

The ideal method of measure should be fast, highly sensitive, applicable in the clinical context, and must take standard, comparable, and clear data. The first method used to measure presepsin was ELISA (Enzyme-Linked Immunosorbent Assay), in sandwich variation, a technique that gives a qualitative and quantitative estimate of the molecule. It used a mix of antibodies that have an antigenic specificity for CD14, particularly, they were synthesized to bind exclusively high weight CD14 molecules, while other bound both high and low weight CD14

Table 1 Comparison of presepsin values between dead patients and discharged patients. Presepsin was measured on day 1 of hospitalization in both categories

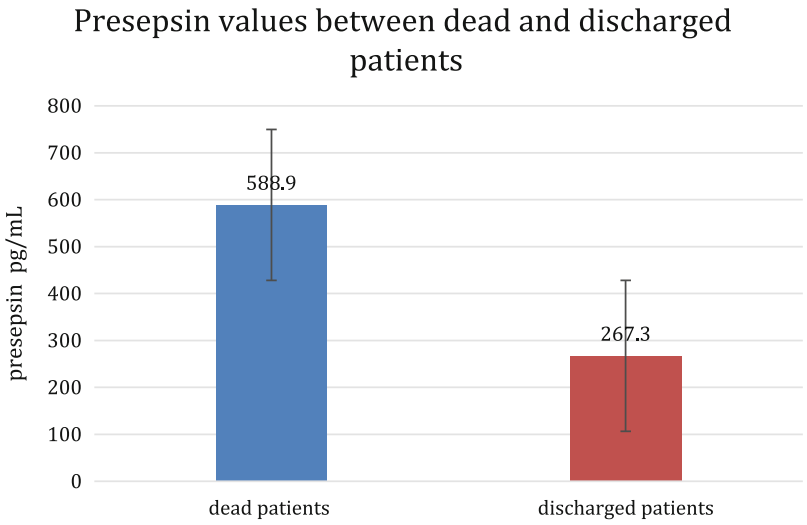
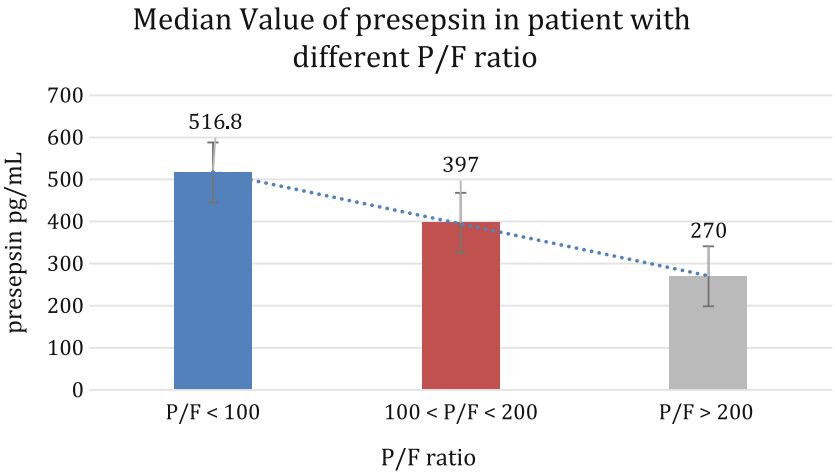


Table 2 Comparison of presepsin values and their correlation with P/F ratio. Presepsin was measured on day 1 of hospitalization and P/F ratio was calculated on the same day. The trendline shows an inverse correlation between them



molecules. So, there were not antibodies that bound only low weight CD14. The measurement was carried out directly for CD14, but it was indirectly for presepsin. This method gave the results in 4 h, but it was defined poorly fitting and not available

for clinical use due to poor precision and slowness. A few years later, the ELISA one step was developed, which used two antibodies anti-presepsin made on determining aminoacidic sequences (Shirakawa et al. 2011). This kind of test was faster than the previous one, the processing time was 1 h and half, and was more sensitive. In 2011, Yoshikazu and Hiroyuki developed an assay based on immune enzymatic method with indirect chemiluminescent with competitive enzymatic marker (CLEIA: ChemiLuminescent Enzyme ImmunoAssay), called PATHFAST system (Okamura and Yokoi 2011), a point of care test used for other biomarkers like troponin and myoglobin; the idea was to adapt the reagents to measure quickly and bedside the presepsin.

Tosoh Bioscience more recently developed an immunoenzymometic assay called ST AIA pack and a CLEIA test called CL AIA test pack to analyze presepsin concentrations.

The CL AIA-PACK Presepsin assay is a two-step chemiluminescence enzyme immunoassay (CLEIA) kit. The amount of enzyme-labeled antibodies that bind to the magnetic microparticles is directly proportional to the presepsin concentration in the test sample. A standard curve is constructed, unknown sample concentrations are calculated by using this curve.

Mini-Dictionary of Terms

Sepsis: It is a life-threatening organ dysfunction caused by a dysregulated host response to infection.

ICU: It is a unit in a hospital providing intensive care for critically ill or injured patients that is staffed by specially trained medical personnel and has equipment that allows continuous monitoring and life support.

Severe COVID-19: It is a form of COVID-19 we defined by a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <100 mm Hg, respiratory frequency >30 breaths/min, and lung infiltrates $>50\%$.

Key Facts of "Presepsin: Methods of Measure, Features and Biomarker Potential in Sepsis and Critical Care."

Key Facts of Sepsis

Sepsis is one of the most important causes of morbidity and mortality in the emergency department and ICU.

Presepsin value $>$ 500 pg/ml may be useful to identify and diagnose sepsis state.

Presepsin kinetics could allow to make an early diagnosis of sepsis compared to other inflammatory and infection biomarkers used in clinical practice.

The sensitivity and sensibility of presepsin change according to cutoff values used.

Key Facts of Severe COVID-19

Presepsin has been studied during SARS-CoV 2 outbreak as a potential prognostic biomarker for severe COVID-19 patients.

Presepsin reflects the activity level of immune system that, during severe COVID – 19, is overactive due to uncontrolled viral replication. So, it could be used to stratify patients who need a higher standard of care, like hospitalization in ICU.

Key Facts of Perioperative Medicine

Presepsin could be an appropriate biomarker both in preoperative and postoperative evaluation both in cardiac and noncardiac surgery.

Presepsin may be a predictor of adverse events, particularly cardiovascular and cerebrovascular, which occur postoperative because they are linked to inflammatory and immune response caused by surgery.

Summary Points

- Presepsin is significantly elevated in patients with bacterial infections even if presepsin serum concentration increase is not a specific biomarker of infection but indicates the innate immune system activation during a variety of conditions.
- Presepsin kinetics allows the physicians to confirm an infection suspicion in an early phase of the disease.
- Plasma levels of presepsin could be indicators for the diagnosis and prognosis of sepsis and septic shock. A cutoff value $>$ 500 pg/mL is suggestive of sepsis.
- Presepsin values could be affected by impaired renal function.

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Review of our Current Understanding of ADAMTS13 and Von Willebrand Factor in Sepsis and Other Critical Illnesses

32

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Contents

Abstract

Sepsis is defined as a life-threatening organ dysfunction caused by excessive host response to infection, and represents the most common cause of in-hospital deaths (V. Liu et al. JAMA 312:90, 2014). Sepsis accounts for 30% of all critically ill patients in the Intensive Care Unit (ICU), and has a global mortality rate of 20% ((Rudd et al. 2020; (Sakr et al. 2018)). Activation of blood coagulation during sepsis and septic shock can lead to disseminated intravascular coagulation (DIC), which is characterized by microvascular thrombosis. Von Willebrand Factor and

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ADAMTS13 are two important regulators of blood coagulation that may be important links between sepsis and mortality in the ICU. This chapter will review our current understanding of Von Willebrand Factor and ADAMTS13 in sepsis and other critical illnesses.

Keywords

ADAMTS13 · Von Willebrand factor · Sepsis · Disseminated intravascular coagulation · Hemostasis · Coagulation · Cardiovascular disease · Thrombotic microangiopathies · Thrombotic thrombocytopenia purpura · Von Willebrand disease · Inflammation · Neutrophil extracellular traps · Alpha-defensin

Abbreviations

ADAMTS13	A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
CLP	Cecal ligation and puncture
DIC	Disseminated intravascular coagulation
HNP	Human neutrophil peptides
ICU	Intensive care unit
IFN-γ	Interferon gamma
IL	Interleukin
IS	Ischemic stroke
MDTCS	Truncated form of ADAMTS13; lacks closed conformation
MI	Myocardial infraction
NETs	Neutrophil extracellular traps
OR	Odds ratio
PF4	Platelet factor 4
SNPs	Single nucleotide polymorphisms
TMA	Thrombotic microangiopathies
TNFα	Tumor necrosis factor
TTP	Thrombotic thrombocytopenic purpura
UL-VWF	Ultra large Von Willebrand factor
VWF	Von Willebrand factor

VWF and ADAMTS13 in Hemostasis

Hemostasis involves a coordinated response of circulating blood cells and soluble proteins to prevent blood loss from injured vessels. Circulating platelets are first recruited and then activated, while the coagulation system deposits a fibrin matrix to stabilize the platelet plug (Gale 2011; Stokol et al. 2013). This process is facilitated by endothelial cells that line the luminal face of blood vessels and rapidly switch from an anticoagulant to a procoagulant phenotype (Gale 2011; Stokol et al. 2013). Activation of the hemostatic response can occur during systemic inflammation, such as in sepsis, leading to disseminated intravascular coagulation (DIC) and increased

risk of mortality (Dempfle 2004). ADAMTS13 (A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) and Von Willebrand Factor are two key regulators of hemostasis that become dysregulated during critical illness, serving as biomarkers of disease progression and may contribute to the molecular events leading to DIC, thrombosis, tissue hypoperfusion, and death in the ICU.

Von Willebrand Factor (VWF) is a circulating multimeric protein that recruits platelets to sites of vascular injury and inflammation. A mature VWF monomer consists of 13 major domains/assemblies in the order D′-D3-A1-A2-A3-D4-C1-C2-C3-C4-C5-C6-CK, where each domain participates in a distinct function of VWF in circulation (Sadler 1998; Springer 2014). For example, the A1 and A3 domains are important for VWF to capture platelets to sites of vessel injury by binding to platelet receptor gpIb and subendothelial collagen, respectively (Sadler 1998). VWF is synthesized in endothelial cells and megakaryocytes where it is packaged into storage organelles: Weibel Palade Bodies and alpha granules, respectively (McGrath et al. 2010; Ruggeri 2003; Wagner 1990). Endothelial cell VWF is secreted through two distinct mechanisms. Basal secretion contributes to the majority of circulating VWF in healthy individuals, and does not involve Weibel-Palade body exocytosis (Lenting et al. 2015). Regulated secretion occurs in response to endothelial cell agonists during disease or acute injury, and can results in rapid Weibel-Palade Body release (Lenting et al. 2015). VWF secretion can be induced by agonists such as thrombin, histamine, fluid shear stress, and various cytokines (McGrath et al. 2010). These cytokines (such as IL-1, IL-6, IL-8, and TNFα) can be elevated during inflammation and contribute to the prothrombotic condition of critically ill patients in the ICU (Bernardo et al. 2004). Importantly, VWF released from endothelial cells through stimulated secretion consist of the greatest multimeric lengths, termed ultra-large VWF multimers, and possess the greatest thrombotic potential.

ADAMTS13 is a circulating metalloprotease that cleaves ultra-large VWF multimers, regulating its platelet-binding activity (Fig. 1). ADAMTS13 is predominantly expressed in the liver by hepatic stellate cells (Soejima et al. 2001; Uemura et al. 2005; Zhou et al. 2005), but is also found in platelets (L. Liu et al. 2005), endothelial cells (Turner et al. 2006), and astrocytes (Tauchi et al. 2012). ADAMTS13 is secreted into the circulation as an active enzyme and has a long circulating half-life of 2–4 days (Scully et al. 2017). VWF proteolysis is regulated by fluid shear stresses, which induces a conformational change in the VWF A2 domain that exposes the cleavage site to ADAMTS13 (Crawley et al. 2011). The shear dependency of VWF proteolysis serves to localize ADAMTS13 activity to sites of vessel injury, and limits the unregulated degradation of VWF in circulation. ADAMTS13 is comprised of various domains consisting of a metalloprotease domain (M), disintegrin-like domain (D), thrombospondin type 1 motif (T), cysteine-rich domain (C), spacer domain (S), seven thrombospondin repeats, and two CUB domains (South et al. 2014). ADAMTS13 binds to VWF in both a shear-dependent and shear-independent mechanism (Zhang et al. 2009). The disintigrin, cysteine-rich, and spacer domains bind to the unfolded VWF A2 domain, and are important for presenting the cleavage site to the metalloprotease domain for proteolysis (Crawley et al. 2011). The CUB domains of ADAMTS13 bind to the D4-CK interval of VWF

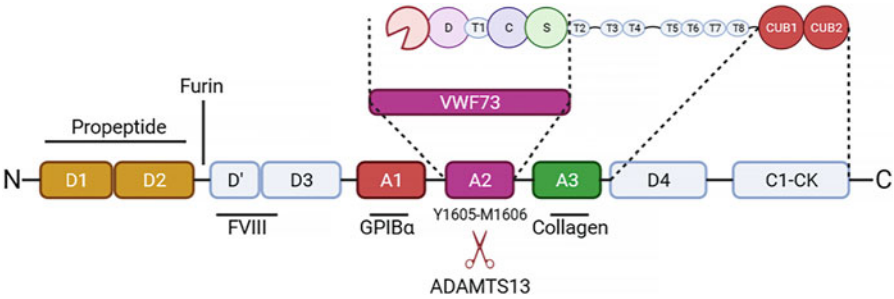

Fig. 1 VWF and ADAMTS13 interaction. The A1 domain captures platelets through GPIbα interactions, displays heparin binding, and serves as a minor binding site for collagen. The A2 domain harbors the Y1605-M1606 cleavage site for proteolysis by ADAMTS13. The Tyr1605-Met1606 is buried deep within the A2 domain structure. Both A1 and A2 domains require shear force to induce a conformational change and to capture platelets (A1 domain) and reveal cleavage site for ADAMTS13 proteolysis (A2 domain). The A3 domain facilitates the binding of VWF to the exposed subendothelial collagen at the site of vascular injury. The D'D3 domains bind and transport FVIII to the site of vascular injury. The domains of ADAMTS13 interact with D4-CK and anchor ADAMTS13 to VWF under flowing conditions. Domains – DTCS are important exosites that bind to the A2 domain of VWF and position the Y1605-M1606 scissle bond for proteolysis. VWF73 is a clinical substrate (with fluorogenic units added – FRETS-VWF73) taken from the A2 domain used to measure activity of ADAMTS13 under static conditions

in a shear-independent manner and facilitate ADAMTS13 localization to VWF strings under flowing conditions (Banno et al. 2009; Garland et al. 2017; Gogia and Neelamegham 2016). The CUB domain and the spacer domain of ADAMTS13 also interact with each other giving ADAMTS13 a compacted, or closed, conformation (Kim et al. 2021; Muia et al. 2014; South et al. 2014). Anti-ADAMTS13 antibodies from TTP patients have been shown to induce an open conformation in ADAMTS13 (Kangro et al. 2021); however, the importance of these conformational dynamics to the regulation of ADAMTS13 activity remains unclear.

Known Associations Between VWF and ADAMTS13 in Disease

ADAMTS13 and VWF must be tightly regulated in order to maintain balance in the hemostatic system. Insufficient ADAMTS13 activity can lead to an accumulation of high molecular weight multimers, which is a risk factor for thrombosis, whereas excessive ADAMTS13 activity leads to a loss of high molecular weight multimers and is a risk factor for bleeding. An imbalance between circulating levels of VWF and ADAMTS13 is associated with both congenital and acquired diseases, including TTP, Von Willebrand Disease, peripheral/coronary arterial disease, ischemic stroke, myocardial infarction (MI), preeclampsia, liver cirrhosis, and inflammatory bowel disease (Akyol et al. 2016; Moake 2002). Single nucleotide polymorphisms (SNPs) within ADAMTS13 have been associated with inherited TTP, cardiovascular

disease, and inflammation (Assink et al. 2003; Camilleri et al. 2012; Kokame et al. 2002; Lambers et al. 2013; Levy et al. 2001; Lotta et al. 2010; Matsumoto et al. 2003; Schneppenheim et al. 2003; Veyradier et al. 2004).

Thrombotic thrombocytopenic purpura (TTP) is a rare life-threatening disorder of the microvasculature caused by a deficiency in ADAMTS13, leading to an accumulation of high molecular weight VWF multimers that spontaneously bind to platelets in the microvasculature. The resultant microvascular thrombosis causes thrombocytopenia and hemolytic anemia and can lead to renal and cognitive dysfunction (Deford et al. 2013; Moake 2002). Other thrombotic microangiopathies (such as hemolytic uremic syndrome) are not associated with substantial reductions in ADAMTS13. Therefore, ADAMTS13 activity of $<10\%$, is used as a cutoff for diagnosing TTP (Franchini and Mannucci 2008). The cause of ADAMTS13 deficiency is often due to the development of autoinhibitory ADAMTS13 antibodies, but also caused by mutations in the ADAMTS13 gene (Cataland and Wu 2015; Kangro et al. 2021; Lancellotti et al. 2015; Romão De Souza et al. 2018; Roose et al. 2018). While TTP is not a common critical illness in the ICU, its pathophysiology serves as an archetype for microvascular thrombosis caused by an imbalance in ADAMTS13 and VWF.

Beyond TTP, ADAMTS13 and VWF have been associated with increased risk of arterial and venous thrombosis. The Rotterdam study evaluated 6,130 individuals to determine the association between ADAMTS13 activity, VWF levels, and all-cause and cardiovascular mortality (Sonneveld et al. 2016). Individuals with low ADAMTS13 activity had a 1.46-fold higher risk of mortality compared to those with high ADAMTS13 activity (Sonneveld et al. 2016). Factoring in the increase in VWF levels, individuals with low ADAMTS13 activity and high VWF levels had a 1.73-fold higher risk of mortality (Sonneveld et al. 2016). Several case-control studies have suggested an association between high plasma levels of VWF and arterial thrombosis (Ruggeri 2007), including myocardial infarction (odds ratios (OR) 95% CI: 1.24–2.28) (Chion et al. 2007; Crawley et al. 2008; Peyvandi et al. 2010) and ischemic stroke (95% CI: 1.13–2.20) (Bongers et al. 2006; Buchtele et al. 2018; Folsom et al. 1999; Hanson et al. 2011; Van Schie et al. 2010). Similarly, studies have suggested that low ADAMTS13 levels are associated with an increased risk of myocardial infarction, comparing the lowest quartile to the highest quartile of convalescent ADAMTS13 (95% CI: 1.4–5.1) and also for ischemic stroke (95% CI: 1.7–3.1) (Andersson et al. 2012; Bongers et al. 2009; Buchtele et al. 2018; Crawley et al. 2008). Patients with confirmed ST elevation myocardial infarction had lower total ADAMTS13 levels than healthy controls (95% CI: 0.5–0.8); however, VWF levels did not correlate with ADAMTS13 levels (Rutten et al. 2015), a pattern that has been observed in critical illnesses such as sepsis (Singh et al. 2021). Decreased ADAMTS13 activity and increased VWF levels may confer a higher combined risk of arterial thrombosis than either one alone. In young women who use oral contraceptives the combined OR for myocardial infarction was 11.3 (95% CI: 3.6–35.2), and the combined OR for ischemic stroke was 6.9 (95% CI: 2.0–23.0) (Andersson et al. 2012). On the contrary, some studies have also shown no significant correlation

between ADAMTS13 levels and risk of MI (He et al. 2001; Horii et al. 2008; Peyvandi et al. 2010). A recent meta-analysis found no significant association between ADAMTS13 and MI or coronary heart disease OR 1.92 (95% CI: 0.85–4.36) (Sonneveld et al. 2014), suggesting the risk is more related to changes in VWF levels. However, the same meta-analysis found a significant association between low ADAMTS13 levels and increased risk of ischemic stroke OR 2.72 (95% CI: 1.52–4.86) (Sonneveld et al. 2014). More prospective studies are needed to definitively assess the role for ADAMTS13 in myocardial infarction.

The mechanism by which VWF and ADAMTS13 are associated with MI and IS remains unclear. ADAMTS13 has been shown to downregulate the recruitment of platelets and leukocytes to sites of inflammation in animal models (Chauhan et al. 2008), contributing to atherosclerosis. In ApoE−/− hypercholesterolemic mice, ADAMTS13 reduced inflammatory atherosclerotic plaque progression via VWF-dependent mechanisms, including platelet adhesion and vascular inflammation (Gandhi et al. 2014). This activity may be impaired by high levels of LDL, a risk factor for coronary artery disease, which may bind and sequester ADAMTS13 through its apoB100 component (Cao et al. 2019). In ischemic stroke, ADAMTS13 may limit thrombotic occlusion of the microvasculature after endothelial damage. In a murine model of ischemic stroke, ADAMTS13$^{-/-}$ mice had greater infract volume than WT mice, and infarct volume was significantly reduced when WT mice were treated with recombinant ADAMTS13 (Zhao et al. 2009). Subsequent studies in other experimental models found that recombinant ADAMTS13 may be a useful therapy in stroke, exerting a thrombolytic effect that complements tPa-based therapies (Denorme et al. 2016). The contribution of ADAMTS13 and VWF to chronic and acute cardiovascular diseases illustrates their role as mediators of both vascular inflammation and thrombosis, which are hallmarks of sepsis in the ICU.

The clinical significance of secondary thrombotic microangiopathies (TMA) during infection has become increasingly apparent in patient outcome (Schwameis et al. 2015), and is clinically distinguishable from DIC (Wada et al. 2018). Reduction of ADAMTS13 is not unique to primary TMA such as TTP, but also in secondary TMA, particularly in infectious diseases such as sepsis-associated DIC and malaria (Schwameis et al. 2015). In these patients VWF levels are significantly elevated, usually correlating with the extent of systemic inflammation, and a negative correlation is often observed between ADAMTS13 and VWF (Furlan et al. 1999; Lerolle et al. 2009). Collectively, the dysfunction between ADAMTS13 and VWF increases the risk for secondary TMA (Lerolle et al. 2009; Schwameis et al. 2015). Patients at risk for secondary thrombosis may benefit from recombinant ADAMTS13 infusion or an agent that targets VWF production or release into the bloodstream (Schwameis et al. 2015).

Therefore, dysregulation of ADAMTS13 and VWF are common in chronic and acute thrombotic conditions and likely contributes to pathophysiology of these diseases. These patterns or associations are amplified in the ICU, especially in the setting of sepsis, septic shock, and DIC.

ADAMTS13 and VWF in Critically Ill Patients with Sepsis

Sepsis is defined as a life-threatening organ dysfunction caused by excessive host response to infection, and is a leading cause of mortality and critical illness worldwide (Fleischmann et al. 2016; Singer et al. 2016). Approximately 30% of ICU patients have a diagnosis of sepsis globally (Sakr et al. 2018). Activation of blood coagulation during sepsis and septic shock can lead to DIC, which is characterized by microvascular thrombosis, consumption of clotting factors and platelets, and bleeding (Dempfle 2004). Studies have suggested an inverse correlation between VWF and ADAMTS13 in sepsis (Kremer Hovinga et al. 2007), which may contribute to thrombosis and mortality in the ICU. Separate studies examining either activity or antigen levels have shown ADAMTS13 to be at least 50% lower in septic patients compared to healthy controls, and the magnitude of reduction correlates with severity (Azfar et al. 2017; Kremer Hovinga et al. 2007; Ono et al. 2006; Singh et al. 2021). Interestingly, specific activity of ADAMTS13 remains unchanged in septic patients, suggesting that changes to antigen level is the primary mechanism of reduced ADAMTS13 activity in patients (Singh et al. 2021). Measuring both ADAMTS13 activity and antigen has shown to be more accurate and should be preferred over measuring a single parameter (Feys et al. 2007; Singh et al. 2021). When comparing VWF antigen to ADAMTS13 [activity:antigen] in non-surviving septic patients, significant differences were observed compared to non-septic ICU patients and healthy controls (Singh et al. 2021). However, both VWF and ADAMTS13 levels are significantly altered in non-septic ICU patients compared to healthy controls, suggesting that their dysregulation may be an important parameter in critically ill patients (Singh et al. 2021).

In patients with sepsis-induced DIC, significant decreases in ADAMTS13 activity and antigen were observed with renal complications (Ono et al. 2006). These patients displayed ADAMTS13 activity below 20% compared to healthy individuals. Biochemical analysis revealed evidence of proteolytic degradation of ADAMTS13 in plasma, and an increase in thrombogenic UL-VWF multimers (Ono et al. 2006). In patients with ADAMTS13 activity $<20\%$, 41% of patients displayed renal injury and had levels of creatinine indicative of renal failure (1.81 ± 1.70 mg/dL) (Ono et al. 2006). In comparison, in patients with ADAMTS13 $>20\%$, only 15% of patients displayed renal injury and levels of creatinine of 0.95 ± 0.76 mg/dL (Ono et al. 2006). Another study found that sepsis severity is associated with DIC, ADAMTS13 levels, or both at admission to the ICU (Peigne et al. 2013). Interestingly, no correlation between ADAMTS13 and the International Society for Thrombosis and Hemostasis (ISTH) DIC score was found, suggesting that septic shock associated with functional ADAMTS13 deficiency and DIC were independent (Peigne et al. 2013). Therefore, deficiency in ADAMTS13 was independent of DIC and patients are at risk for renal complications.

Whether dysfunction between ADAMTS13 and VWF predicts mortality is yet to be definitively answered. However, recent studies suggest that there may be prognostic value in measuring VWF and ADAMTS13 levels. A clinical evaluation of

84 septic patients revealed that low ADAMTS13 level was associated with increased mortality OR 5.3 (95% CI: 1.73–16.17), and was comparable to APACHE II scores OR 4.13 (95% CI:1.28–13.25) in predicting morality (Azfar et al. 2017). Another study examining 72 septic patients observed ADAMTS13 activity levels below 30% to be prognostic value for mortality OR 11.86 (95% CI: 1.36–103.52) (Peigne et al. 2013). More specifically, there may be greater prognostic value in accessing VWF antigen:ADAMTS13 [activity:antigen] ratios as a measure of disease severity, rather than as separate entities because they share a substrate–protease relationship and correspond with the severity of sickness (Fukushima et al. 2013; Singh et al. 2021). Evaluating specific activity of ADAMTS13 in patient samples may provide a more accurate assessment of the capacity of ADAMTS13 to regulate VWF (Singh et al. 2021). Overall, assessing the dysfunction between ADAMTS13 and VWF may serve as a potential prognostic tool for predicting severity of sepsis.

Mechanistic Insights on the Dysfunction of ADAMTS13 and VWF in Sepsis

The cause of ADAMTS13 dysfunction in sepsis or other critical illnesses remains largely unknown, but is expected to be multifactorial. Recent animal studies have suggested the dysregulation of ADAMTS13 to be a consequence of reduced synthesis (Uemura et al. 2010), degradation by thrombin and/or plasmin (Garland et al. 2017; Levi et al. 2018), impaired proteolytic activity in the presence of inflammatory mediators (Bernardo et al. 2019; Reiter et al. 2005), or clearance by massive amounts VWF (Lerolle et al. 2009) (Fig. 2).

ADAMTS13 is predominately expressed in the liver and secreted from hepatic stellate cells (Uemura et al. 2005, 2010). Ono et al. studied 109 patients with sepsis-induced DIC and showed a slight decrease in serum albumin in patients with ADAMTS13 <20% compared to patients with ADAMTS13 >20% (2.3 ± 0.4 g/dL vs 2.9 ± 0.7 g/dL, respectively), suggestive of reduced liver function. However, overt liver damage was not significantly higher in the population of patients with low ADAMTS13 (Ono et al. 2006). ADAMTS13 dysfunction has been observed in patients with liver cirrhosis, alcoholic hepatitis, veno-occlusive disease, and adverse events following liver transplantation (Uemura et al. 2010). A murine study observed that inflammatory cytokines (INF-γ, IL-4, and TNF-α) inhibited synthesis of ADAMTS13 in hepatic stellate cells, which produce the majority of circulating ADAMTS13 (Cao et al. 2008). Therefore, severe liver injury during acute critical illness may contribute to reduced ADAMTS13 synthesis and production.

In patients with sepsis, particularly those with DIC, low molecular weight ADAMTS13 fragments are often observed (Levi et al. 2018; Ono et al. 2006). These degradation fragments of ADAMTS13 are suggestive of proteolytic cleavage. During DIC, excessive activation of the coagulation system combined with systemic release of neutrophil granule proteases overwhelms the natural protease inhibitors present in plasma leading to unregulated proteolytic activity in circulation. Many of these proteases have been shown to degrade ADAMTS13 (Crawley et al. 2005;

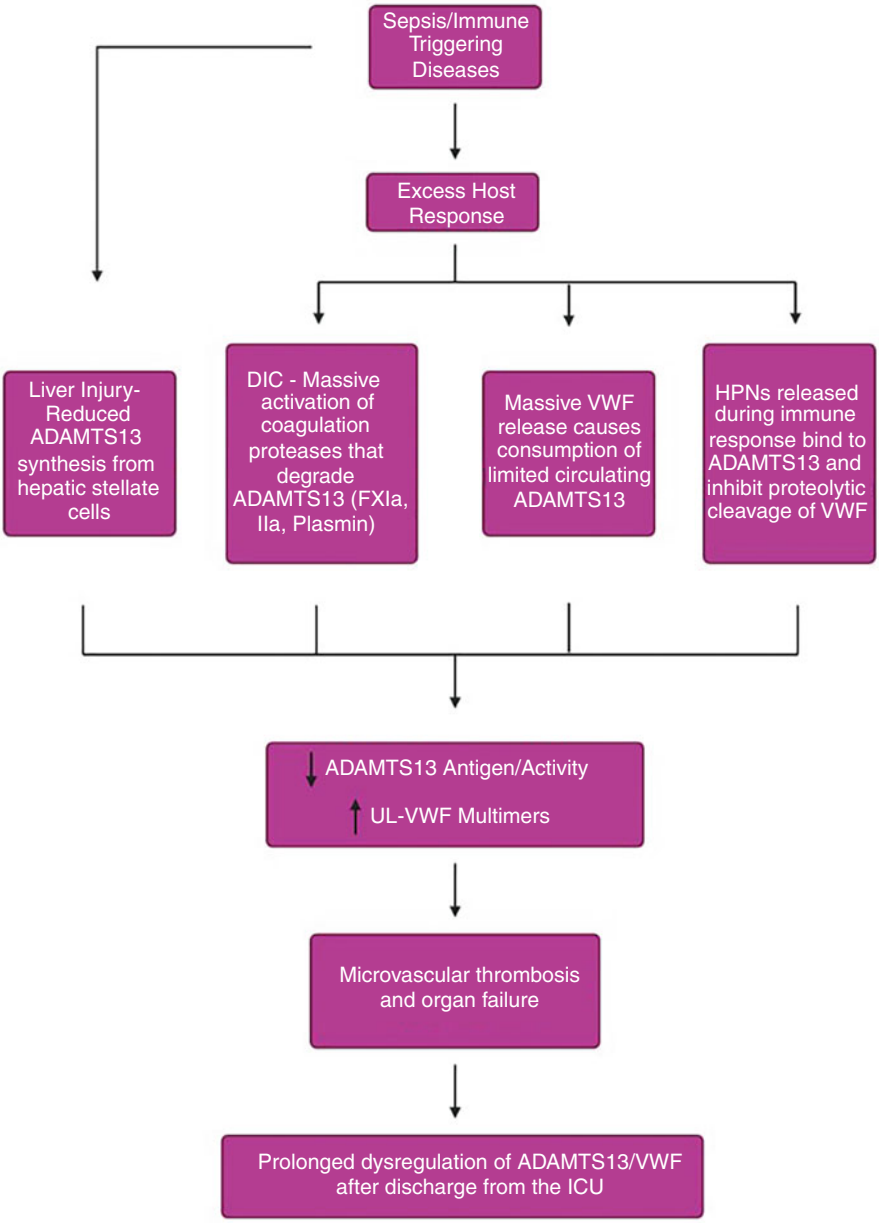

Fig. 2 Mechanistic Overview of ADAMTS13/VWF in Sepsis. An excessive immune response to an infection can cause dysfunction between the delicate ADAMTS13/VWF balance. Collectively, liver injury, DIC, immune-induced massive release of VWF into circulation, and HPNs results in the increase of UL-VWF, and decrease ADAMTS13 antigen and activity, to varying degree. As a result of this imbalance, microvascular thrombosis, organ failure, and death can occur. The levels of ADAMTS13 and VWF do not normalize immediately post infection

Garland et al. 2017; Okamoto et al. 2016). These proteolytic fragments of ADAMTS13 can possess normal enzymatic activity since the clinical ADAMTS13 activity assay (based on VWF73) only engages the MDTCS domains of ADAMTS13, which remains intact in these cleaved forms. However, removal of the C-terminal CUB domains from ADAMTS13 is known to limit proteolytic cleavage of VWF multimers under flow (Garland et al. 2017). In this regard, measuring ADAMTS13 using standard available activity assays may not accurately predict disease severity or outcome in ICU patients with DIC. Assessment of VWF multimer status may better reflect the dysfunction in ADAMTS13 and VWF in sepsis, but has not been systematically evaluated.

High levels of inflammatory cytokines have been shown to disrupt the ADAMTS13/VWF axis by increasing the circulating concentration of VWF (Bernardo et al. 2019; Pillai et al. 2016; Reiter et al. 2005; Remick et al. 2002). Human umbilical vein endothelial cells treated with IL-8 or TNFα can stimulate UL-VWF release from Weibel Palade Bodies (Bernardo et al. 2004). IL-6 can directly bind to VWF and inhibit its proteolysis by ADAMTS13 (Bernardo et al. 2004; Cao et al. 2008). Consistent with these findings, elevated IL-6 levels have been shown to correlate with reduced ADAMTS13 activity (Nguyen et al. 2007). Platelet counts have been shown to be reduced in septic patients with low ADAMTS13 activity, suggesting a connection between low ADAMTS13 and an accumulation of prothrombotic UL-VWF multimers (Nguyen et al. 2007). These findings coincide with higher incidence of thrombocytopenia and TMA in patients with severe ADAMTS13 deficiency (Nguyen et al. 2007).

The massive release of VWF during sepsis and other critical illnesses marked by a strong immune response, may directly contribute to reduced ADAMTS13 levels in circulation due to differences in their circulating half-lives (Singh et al. 2021). Normally, ADAMTS13 circulates at a concentration of 1 μg/mL with a half-life of 2–4 days (Catherine et al. 2015; Furlan et al. 1999; Scully et al. 2017), whereas VWF circulates at a concentration of 10 μg/mL with a half-life of 15 hours (Catherine et al. 2015; Pipe et al. 2016). Therefore, release of high concentrations of VWF may sequester and clear ADAMTS13 from circulation. This mechanism is supported by work in animal models. Cecal ligation and puncture (CLP)-induced sepsis in wild-type mice results in increased VWF and reduced ADAMTS13, similar to sepsis patients. However, ADAMTS13 levels remained unchanged in VWF−/− mice subjected to CLP-induced sepsis (Lerolle et al. 2009). No difference was found in ADAMTS13 levels between pre- and post-CLP surgery, and these VWF-deficient mice lived significantly longer than wild-type mice (Lerolle et al. 2009). Interestingly, infusion of recombinant ADAMTS13 into wild-type mice subjected to CLP did not impact survival. This observation may be explained by the rapid clearance of ADAMTS13 or its proteolytic degradation by systemic activation of coagulation and/or release of neutrophil-derived proteases (Lerolle et al. 2009). These preclinical data suggests that VWF release during critical illness is a major determinant of ADAMTS13 deficiency, and significantly contributes to mortality.

Neutrophil activation has been shown to be associated with sepsis prognosis in the ICU (Dwivedi et al. 2012), which may contribute to dysregulation of

ADAMTS13 and VWF. Activated neutrophils release neutrophil extracellular traps (NETs), proteases, and alpha-defensin peptides, which all contribute to microvascular thrombosis (Hickey and Kubes 2009; Ley et al. 2018; McDonald et al. 2017; Pillai et al. 2016; Sohrabipour et al. 2021). NETs comprised of genomic DNA, decorated with histones and other granular proteins and proteases. NETs have been shown to contribute to thrombosis by directly binding to platelets and VWF, and by activating the coagulation system via the contact pathway (Thålin et al. 2019). Alpha-defensins are small peptides that bind to bacteria, viruses, and fungi leading to microbial death (Lehrer and Lu 2012). Recently it has been observed that alpha-defensins can bind to the VWF A2 domain and block proteolysis by ADAMTS13 (Pillai et al. 2016). A similar mechanism has also been described for platelet factor 4 (PF4), which is released by activated platelets (Johnston et al. 2020; Nazy et al. 2020). These mechanisms, combined with the proteolytic degradation of ADAMTS13 by neutrophil elastase, suggest that substantial neutrophil activation during sepsis can directly contribute to dysregulation in ADAMTS13 and VWF, providing a direct link between the immune response and microvascular thrombosis.

Role of ADAMTS13 and VWF in Driving ICU Mortality

ICU mortality in patients with sepsis has been linked to a number of risk variables. In a prospective study designed to examine the risk variables associated with increase in mortality in the ICU, Mayr et al. examined 375 ICU cases across a university teaching hospital over 7 years and found that the most frequent cause of death in ICU was linked to multiple organ dysfunction syndrome (47%) as a consequence of failures in the central nervous system or the cardiovascular system (Mayr et al. 2006). Their analysis revealed that failure of the cardiovascular system increased the risk of mortality in the ICU 12-fold (Mayr et al. 2006), and ADAMTS13 and VWF appear to be important drivers of cardiovascular dysfunction that contribute to ICU sepsis mortality.

In a prospective study designed to assess the prognostic value of ADAMTS13 deficiency in septic patients, Peigne et al. examined 72 ICU patients enrolled over 1 year and found 26% of patients had DIC and 50% of patients had ADAMTS13 deficiency, both of which were determined to be important independent parameters of sepsis diagnosis (Peigne et al. 2013). The severity of ICU patient illness was associated with DIC or ADAMTS13 deficiency, or both (Peigne et al. 2013). Low ADAMTS13 levels were also associated with septic shock independent of overt DIC (Peigne et al. 2013). In addition, mortality was 33% in ICU sepsis patients with low ADAMTS13 activity (Peigne et al. 2013). In comparison of the non-survivor to the survivor group, 66% (19/29 patients) demonstrated low ADAMTS13 activity (<30%) compared to the 37% (17/43 patients) of survivors (Peigne et al. 2013). Azfar et al. examined 84 ICU patients enrolled over the course of 3 years, and noted that low ADAMTS13 (<365 ng/mL) was associated with an increase in mortality with an odds ratio of 4.5 (95% CI: 1.71–11.82) (Azfar et al. 2017). Unfortunately, VWF levels were not examined in this study to determine if ADAMTS13 was an

independent risk factor. According to both studies, ADAMTS13 deficiency plays a part in driving mortality in ICU patients, which emphasizes its role as prognosis markers.

Beyond ADAMTS13 levels, patients with septic shock exhibit elevated pro-inflammatory markers IL-6, PF4, and HNPs (Peigne et al. 2013; Maharaj et al., 2018; Kadir et al., 2020), which can directly attenuate VWF multimer cleavage by ADAMTS13 (Bernardo et al. 2004; Nazy et al. 2020; Pillai et al. 2016; Lehrer and Lu 2012). The presence of these inhibitors may exacerbate thrombosis risk already associated with reduced ADAMTS13 and increased VWF levels in sepsis patients. Whether these variables can be combined to improve our capacity to predict sepsis mortality in the ICU remains unknown.

In a prospective study of 392 septic patients enrolled in the ICU at 9 Canadian hospitals over the course of 27 months, Liaw et al. examined the utility of six time-varying biological indicators for predicating ICU mortality (Liaw et al. 2019). These variables include cell free DNA, protein C, platelet count, creatinine, Glasgow Coma Scale scores, and lactate. The study revealed how these indicators differentially and longitudinally account for the patients' overall mortality risk (Liaw et al. 2019). Whether ADAMTS13 and VWF can contribute to the predictive power of these biomarkers has not been addressed.

Unlike other coagulation markers of sepsis such as protein C, ADAMTS13 and VWF levels do not normalize in septic patients after discharge from the ICU (Singh et al. 2021). Moreover, the time to normalization of ADAMTS13 and VWF remains unknown (Singh et al. 2021). As a result, patients post discharge may remain at increased risk for microvascular thrombosis, cognitive impairment, and cardiovascular disease (Shankar-Hari and Rubenfeld 2016; Singh et al. 2021). Future research should focus on understanding the contribution of ADAMTS13 and VWF dysregulation and endothelial cell injury to post-sepsis syndrome.

Potential Therapies

Replenishing the levels of ADAMTS13 through plasma exchange has long been used as a therapeutic treatment for thrombotic thrombocytopenic purpura (TTP) patients (Tersteeg et al. 2015). Recombinant ADAMTS13 and Caplacizumab are in clinical development for the management and treatment of TTP (Scully et al. 2019; Tersteeg et al. 2015). Experimental models have shown that targeting VWF and ADAMTS13 may have potential benefit in ischemic stroke and acute coronary syndrome (De Meyer et al. 2012; Denorme et al. 2019). Indeed, infusion of recombinant ADAMTS13 averted TTP-like symptoms in a rat model of TTP (Tersteeg et al. 2015). The association of ADAMTS13 and VWF with sepsis mortality in the ICU invites questions about the potential for ADAMTS13 as a novel therapeutic. As observed in experimental stroke models, recombinant ADAMTS13 infusion was not associated with bleeding risk (De Meyer et al. 2012). This is in contrast to tPA treatment, which is associated with a 2–7% increase risk of bleeding (Yaghi et al. 2017). Whether restoring ADAMTS13 activity through plasma exchange or the

addition of recombinant ADAMTS13 has therapeutic value in sepsis by preventing TMA or mortality needs further investigation.

The use of Caplacizumab may also have a protective effect in septic patients. Caplacizumab is a drug intended for TTP patients, which inhibits the VWF/platelet interactions; thereby preventing platelet thrombi formation in circulation (Scully et al. 2019). ADAMTS13 may therefore be considered in addition to traditional anticoagulants such as heparin and its derivatives, which have already shown therapeutic benefit in clinical and preclinical studies of sepsis and DIC (Sohrabipour et al. 2021). Overall, therapies that reduce the size distribution of VWF-multimers or target platelet binding directly may prevent thrombi-induced organ dysfunction. Thus, therapeutic intervention aimed at ADAMTS13-VWF multimer dysfunction may be beneficial in decreasing the mortality rate and improve patient outcome in septic patients.

Conclusions

Sepsis mortality in the ICU remains a complex clinical problem. Patient management primarily focuses on supportive care, and ongoing research efforts seek to identify biomarkers of early sepsis in an effort to prevent progression to septic shock and ICU admission (Arora et al. 2021). Dysregulation of VWF and ADAMTS13 are important markers of sepsis mortality and their imbalance contributes to microvascular thrombosis and organ damage. Whether ADAMTS13 and VWF are simply a useful marker to aid in mortality prediction in sepsis patients, or are targets with therapeutic potential remains unknown. Future studies in experimental sepsis models should evaluate the therapeutic benefit of targeting VWF platelet-binding activity with antagonists and/or replenishing circulating ADAMTS13 levels with therapeutic plasma exchange or recombinant ADAMTS13 products.

Dictionary of Terms

ADAMTS13: A circulating metalloprotease that cleaves ultra-large VWF multimers, regulating its platelet-binding capacity. Plays an important role in primary hemostasis.

DIC: Characterized by microvascular thrombosis, consumption of clotting factors and platelets, thus increasing the risk for bleeding.

Sepsis: A life-threatening organ dysfunction caused by excessive host response to infection.

TMA: Pathologic description of thrombosis that occurs in the body's smallest blood vessels and especially inside vital organs. Characterized by microangiopathic hemolytic anemia, thrombocytopenia, and microthrombi causing ischemia.

TTP: A rare microvascular thrombotic disorder caused by impaired VWF cleavage by ADAMTS13, resulting in VWF/platelet-rich thrombi in microvasculature. Characterized by the pentad of symptoms: microangiopathic hemolytic anemia,

thrombocytopenia with purpura, acute renal insufficiency, neurological abnormalities, and fever.

VWD: A bleeding disorder that affects nearly 1% of the population. Characterized by dysfunction in VWF leading to defective platelet adhesion and aggregation.

VWF: A circulating multimeric protein that recruits platelets to sites of vascular injury. Plays an important role in primary hemostasis.

Key Facts of Review of our Current Understanding of ADAMTS13 and Von Willebrand Factor in Sepsis and Other Critical Illnesses

- In the ICU, sepsis accounts for 30% of all critically ill patients, and has a mortality rate of 20% globally.
- ADAMTS13 and VWF are two important regulators of hemostasis that ensure appropriate platelet capture at sites of vascular injury.
- The dysfunction between ADAMTS13 and VWF in sepsis and other critical illnesses is expected to be multifactorial. A combination of DIC, immune-induced massive release of VWF, consumption of limited ADAMTS13 by excessive circulating VWF, and degradation of ADAMTS13.
- In sepsis and other critical illness, significant decrease in ADAMTS13 activity and antigen, and increase in UL-VWF was observed.
- Evaluating specific activity of ADAMTS13 may provide a more accurate assessment of the capacity of ADAMTS13 to regulate VWF.
- Patients at post discharge may remain at risk for microvascular thrombosis, cognitive impairment, and cardiovascular diseases as ADAMTS13 and VWF do not normalize at time of discharge.
- Future studies should examine the therapeutic benefit of targeting VWF platelet-binding activity with antagonists and/or replenishing circulating ADAMTS13 levels.

Summary

- Sepsis and other critical diseases trigger an excessive immune response leading to a disruption in the ADAMTS13/VWF axis.
- ADAMTS13 antigen and activity is significantly decreased in septic and critically ill patients.
- UL-VWF multimers are significantly increased in septic and critically ill patients.
- A combination of DIC, massive release of VWF into circulation due to inflammatory mediators, consumption of limited circulating ADAMTS13 by excessive VWF, inhibition of ADAMTS13 by HNPs, degradation of ADAMTS13, and reduced synthesis contribute to the dysregulation between ADAMTS13 and VWF.
- Dysregulation between ADAMTS13 and VWF contribute to disease progression, microvascular thrombosis, and organ failure.

- Specific activity of ADAMTS13 may be more accurate in assessing the ability of ADAMTS13 to regulate VWF.
- Prolonged dysregulation between ADAMTS13 and VWF occurs after patient discharge from the ICU.

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Circulating TGF-β1 Levels: Linking Muscle and Trauma

33

La Li and Rocky S. Tuan

Contents

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Abstract

Transforming growth factor-beta 1 (TGF-β1) is a multifunctional growth factor that plays a key role in a variety of human tissues. Three TGF-β1 target cell types are of particular relevance to muscle trauma, involving TGF-β1 effects on (1) inflammatory cells, (2) myocytes, and (3) connective tissue cells. Normal wound healing process with proper immune system participation allows injured muscle to be restored to its original state, whereas abnormal wound healing leads to aberrant mesenchymal differentiation and resultant fibrosis. As the pleiotropic activities of TGF-β1 are highly context-dependent, its role in muscle regeneration following injury is complex, and its potential as a treatment option without unwanted side effects remains unsettled. Interestingly, recent research findings have suggested that circulating TGF-β1 levels are an indicator of disease status. This chapter provides an overview of these findings and proposes an avenue toward validating circulating TGF-β1 as prognostic biomarker following muscle trauma.

Keywords

Transforming growth factor-beta 1 · Muscle injury and regeneration · Trauma · Coagulation · Biomarker

Abbreviations

bHLH	Basic helix loop helix
BMP	Bone morphogenetic protein
DMD	Duchenne Muscular Dystrophy
ECM	Extracellular matrix
FAP	Fibro/adipogenic progenitor
HO	Heterotopic ossification
Id	Inhibitor of differentiation
LAP	Latency-associated peptide
LTBP	Latent TGF-β-binding protein
PDGFRα	Platelet-derived growth factor receptor α
TGF-β	Transforming growth factor-beta

Introduction

TGF-β was first discovered as a sarcoma growth factor, which was able to transform a normal cell to grow in soft agar as a malignant cell (Sporn 2006). Later, it was found that not only tumor cells produce TGF-β, but that its expression is present in

almost all normal cells. Platelets contain the most abundant level of TGF-β compared to other normal tissues, and tumor cells secrete more TGF-β compared to normal cells (Moses et al. 2016). Whether TGF-β functions to promote or inhibit cell proliferation is highly dependent on the responsive cell type and the presence of other growth factors in the environment (Morikawa et al. 2016). The three isoforms, TGF-β1, 2, 3, have overlapping functions as well as distinct functions (Bottinger et al. 1997). Knocking out TGF-β1 mostly affects the immune system, as TGF-β1 null mutation mice die with a phenotype of multifocal infiltration of lymphocytes and macrophages 2 weeks after birth (Christ et al. 1994). TGF-β2 deficiency results in perinatal mortality with multiple developmental defects, whereas TGF-β3 seems to affect mainly lung development and palatogenesis (Sanford et al. 1997; Kaartinen et al. 1995). TGF-β is secreted as a latent form associated with latency-associated peptide (LAP), which limits its activation. LAP is subsequently sequestered to the extracellular matrix (ECM) via binding to a latent TGF-β binding protein (LTBP) (Moses et al. 2016). TGF-β is activated primarily during injury, at a time when the ECM is perturbed or disrupted (Hinz 2015). Activated TGF-β acts on its surrounding cells in an autocrine or paracrine fashion to elicit its biological effects (Sporn 2006). Of note, TGF-β is also present in blood at ng/ml concentration, a level high enough to suggest potential systemic effect (Grainger et al. 1995b), which may differ in nature from its local effect (Wahl 1994). It is thus of interest to characterize and differentiate between the systemic and local functions of TGF-β. For example: Are they related? Do systemic effects due to circulating TGF-β also affect local tissue? Which effect(s) plays the dominant role in normal and abnormal tissue functions?

Our recent findings (Fig. 1) demonstrated that during muscle injury, the levels of phosphorylated Smad3, the key mediator of TGF-β signal transduction, correlate closely with total TGF-β1 levels in the muscle. In normal healing muscle, an early rise in phosphorylated Smad3 and TGF-β1 levels is followed by a quick decrease in their levels to the baseline. On the other hand, in abnormal healing muscle, such as

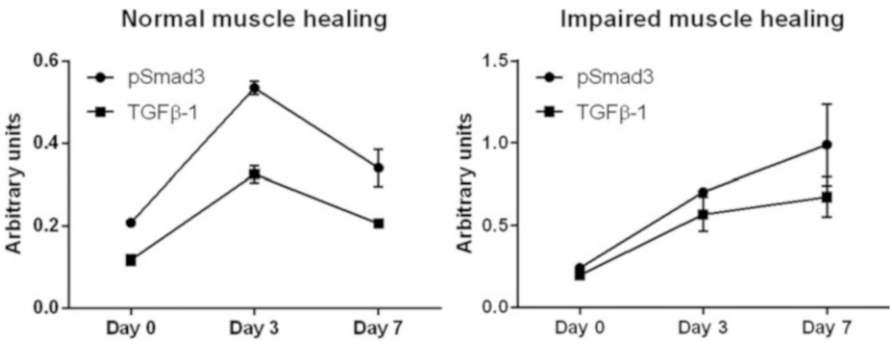

Fig. 1 Levels of phosphorylated Smad3 correlate closely with TGF-β1 levels in mouse muscle Phosphorylated Smad3 levels and TGF-β1 levels are measured at different time points in muscle by ELISA. Normal muscle healing is observed with only cardiotoxin intramuscular administration, whereas impaired muscle healing is found in cardiotoxin intramuscular administration together with systemic dexamethasone treatment

post-traumatic injury, a continued rise in phosphorylated Smad3 and TGF-β1 levels is found. These findings suggest that TGF-β1 is likely the key regulatory molecule among the TGF-β family proteins in the context of muscle trauma. Therefore, in addressing the topic of this chapter, we will focus mainly on the circulating levels of TGF-β1, and explore how changes in circulating TGF-β1 levels are functionally related to muscle regeneration.

Disease States with Altered Circulating TGF-β Levels

Cardiovascular Diseases

Patients with Marfan Syndrome exhibit elevated levels of circulating TGF-β1, thought to result from a failure in the sequestration of TGF-βs in the ECM by the mutated form of fibrillin-1 (Matt et al. 2009). The manifestations of Marfan Syndrome are mainly cardiovascular and musculoskeletal, with aortic aneurysm the leading cause of mortality in these patients (Goumans and Ten Dijke 2018). Treatment with Losartan, an angiotensin II type 1 blocker that blunts TGF-β activation, ameliorates aortic dilatation, which is correlated with decreased circulating TGF-β1 levels (Matt et al. 2009).

It has been previously reported that the serum concentration of TGF-β was severely depressed in advanced atherosclerosis, and that decreased TGF-β1 levels are a risk factor for atherosclerosis in end-stage renal disease (Grainger et al. 1995a; Stefoni et al. 2002). A protective role of TGF-β1 in stabilizing the plaque thus preventing it from rupture is proposed (Toma and McCaffrey 2012). However, excessive levels of TGF-βs are associated with cardiac hypertrophy and fibrosis with a poor disease prognosis (Goumans and Ten Dijke 2018; Ayca et al. 2015).

Fibrosis and Wound Healing

TGF-β1 is known to be the most potent fibrogenic factor by promoting cell ECM synthesis (Kim et al. 2018). Elevated circulating TGF-β1 levels contribute to local fibrosis (Kopp et al. 1996; Sanderson et al. 1995), and when tissue fibrosis is present, circulating TGF-β1 levels are elevated (Ishitobi et al. 2000; August and Suthanthiran 2003). Lowering TGF-β1 levels generally protects fibrosis in response to injury (Burks and Cohn 2011). Reducing TGF-β levels by Losartan treatment proves to be beneficial for kidney, cardiac, lung, liver, and skeletal muscle fibrosis (Kim et al. 2018).

Excessive scarring is a form of fibrosis, which is also correlated with high levels of TGF-β1 (Lichtman et al. 2016). Although scarring is more of a local event, elevated circulating levels of TGF-β1 have been shown to contribute to cutaneous fibrosis in certain fibrosing disorders, such as systemic sclerosis (Lafyatis 2014; Dantas et al. 2016). However, normal wound healing requires TGF-β1. An early increase in circulating TGF-β1 levels seems to prevent hypertrophic scarring in burn

patients (Rorison et al. 2010; Penn et al. 2012). Indeed, local application of TGF-β1 fully reverses glucocorticoid-induced impaired skin wound healing in rats by directly increasing the abundance of wound fibroblasts and procollagen type I production (Pierce et al. 1989). Also, a single intravenous injection of TGF-β1 is sufficient to enhance wound healing in diabetic rats (El Gazaerly et al. 2013; Beanes et al. 2003). Interestingly, diabetic rats already have increased circulating TGF-β1 levels, also seen in diabetic patients (Bollineni and Reddi 1993; Jakus et al. 2012; Qiao et al. 2017). It is still unknown whether this results from a positive feedback regulation between the impaired wound healing and elevated circulating TGF-β1 levels in diabetes mellitus.

Infection and Cancer

TGF-β1 acts as a strong immune suppressor by inhibiting inflammatory NF-kB signaling (Sanjabi et al. 2017). In general, circulating TGF-β1 levels increase in response to infection (Sanjabi et al. 2017; Wiercinska-Drapalo et al. 2004); for example, viral infections are associated with elevated TGF-β1 expression (Taniguchi et al. 2004; Mirzaei and Faghihloo 2018). TGF-β signaling activation is considered a major pathway used by parasites to successfully infect the host by damping the immune response (Reed 1999; Chen and Ten Dijke 2016). Oncogenic viruses-induced cancer patients have even higher circulating levels of TGF-β1 (Shirai et al. 1994). In advanced cancer, TGF-β can promote tumor invasiveness and metastasis by eliciting epithelial-to-mesenchymal transition (Seoane and Gomis 2017). Bone, with copious amount of TGF-β sequestered in its abundant ECM, is the most prevalent site for metastatic dissemination (Juarez and Guise 2011). Thus, high levels of circulating TGF-β in metastatic cancer are usually correlated with a poor clinical outcome (Panis et al. 2013; Ivanovic et al. 2006).

Trauma

Trauma often elicits a systemic inflammatory response, where a pro-inflammatory response is followed by a compensatory anti-inflammatory response with reduced resistance to infection (Lenz et al. 2007). TGF-β1, as a crucial immune regulator, also participates in this inflammatory response. Circulating levels of TGF-β1 have been observed to elevate shortly after trauma and gradually return to baseline at day 5 (Laun et al. 2003), and initial higher TGF-β1 levels are associated with injury severity and the development of sepsis (Windelov et al. 2015). It has also been noted that sustained low circulating levels of TGF-β1 are associated with liver and kidney dysfunction (Laun et al. 2003).

In orthopedic trauma, such as bone fractures, increased serum TGF-β1 concentration is found during the fracture healing period, and earlier decline in circulating TGF-β1 levels is observed in delayed or nonunion fracture healing (Zimmermann et al. 2005; Sarahrudi et al. 2011; Hara et al. 2017). However, in spinal cord injury,

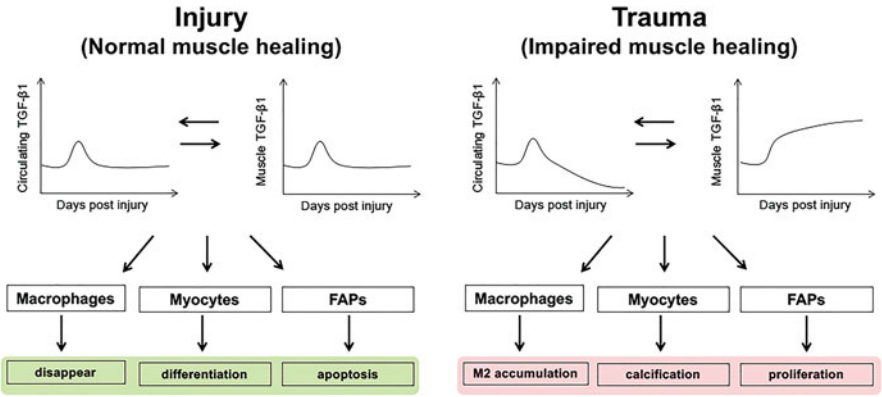

Fig. 2 Decreased circulating TGF-β1 levels are associated with impaired muscle healing Schematic showing changes in circulating and muscle TGF-β1 levels in a mouse model of muscle injury under normal muscle healing versus trauma-impaired muscle healing conditions, and their effects on different types of muscle cells

there is an initial decrease in serum TGF-β1 concentration followed by a significant increase thereafter, while improved neurological outcome is observed in patients with lower serum levels (Ferbert et al. 2017). In addition, we and others have reported a glucocorticoid-dependent mechanism in the regulation of circulating TGF-β1 levels, i.e., the stress hormone released during orthopedic or neurotrauma decreases circulating TGF-β1 levels (Li et al. 2020b; Li et al. 2017). We also found that decreased plasma TGF-β1 levels are associated with impaired muscle healing (Fig. 2) (Li et al. 2020a, b).

Effects of TGF-β1 on Different Cell Types During Muscle Regeneration

Skeletal muscle regeneration after injury consists of three steps: (1) myofiber necrosis allows infiltration of inflammatory cells into injury site; (2) activation of satellite cells, which differentiate into myoblasts to form new myofibers; and (3) apoptosis of mesenchymal progenitor cells to prevent fibrosis development. Apparently, TGF-β1 has effects on almost all cell types in the muscle, and almost every cell type is capable of producing TGF-β1.

Inflammatory Cells

The first wave of infiltrating inflammatory cells occurs around 24 h after muscle injury and is mainly comprised of neutrophils, while the second wave occurs by day 3 and is mainly comprised of macrophages (Yang and Hu 2018). TGF-β1 is a potent chemoattractant for neutrophils and macrophages (Reibman et al. 1991; Kim et al.

2006). Release of TGF-β1 from platelets as well as release of the ECM-bound TGF-β1 after muscle damage stimulate the migration of inflammatory cells from the blood into the muscle (Delaney et al. 2017). Our recent findings (Fig. 1) show that the levels of phosphorylated Smad3, the principal mediator of TGF-β signaling, and TGF-β1 both peaked at 2 days after cardiotoxin-induced muscle injury and dropped after 7 days, which correlated well with the second wave of macrophage infiltration. The third wave of immune cells, comprised mainly of macrophages, involves a proinflammatory to anti-inflammatory phenotype switch (Yang and Hu 2018). After removal of cell debris that results from the tissue injury, pro-inflammatory macrophages (CCR2+) switch to an anti-inflammatory (CX3CR1+) phenotype. The latter have been shown to produce a high level of TGF-β1 (Arnold et al. 2007). Overexpression of TGF-β1 in muscle causes muscle atrophy and fibrosis (Narola et al. 2013). However, in spite of the high level of TGF-β1, M2 macrophages promote myogenesis, likely because macrophages of this reparative phenotype are able to halt excessive inflammation (Arnold et al. 2007; Panci and Chazaud 2021). A study has implicated that failure in the macrophage phenotype transition is detrimental to muscle regeneration (Wang et al. 2014). However, another study has indicated that macrophage-specific TGF-β1 depletion improves muscle regeneration (Stepien et al. 2020). Interestingly, studies have shown that knocking out CCR2 in mice or ablation of monocytes generally impairs muscle regeneration (Chazaud 2020), whereas knocking out CX3CR1 may promote muscle repair (Arnold et al. 2015). These seemingly contradictory results will need further elucidation.

Similarly, in the case of chronic inflammation, such as in Duchenne Muscular Dystrophy (DMD), induced polarization of macrophages into an M2 phenotype by therapeutics that promote M1 to M2 macrophage transition, such as IL-10 and glucocorticoids, etc., appears to promote satellite cell activation and muscle strength (Villalta et al. 2009; Angelini 2007). However, M2 macrophage-derived TGF-β1 is also a key driver of muscle fibrosis (Mann et al. 2011). Macrophage-derived TGF-β1 prevents the apoptosis of fibro/adipogenic progenitors (FAPs) and stimulates these cells to produce and deposit collagens, thus leading to muscle fibrosis (Lemos et al. 2015). Reducing TGF-β1 levels has been proven to be beneficial in several myopathic conditions (Burks and Cohn 2011). It is still not understood how TGF-β1 regulates inflammation and fibrosis at the same time (Wynn and Barron 2010). Unfortunately, there is no M2 macrophage-specific TGF-β1 depletion model to study the above-mentioned processes.

Myogenic Cells

Satellite cells are activated after muscle damage and differentiate into myoblasts, which undergo subsequent fusion to generate multinucleated myofibers. A subset of the activated satellite cells also undergo asymmetric cell division to maintain the muscle stem cell pool (Knoblich 2008). TGF-β1 has been shown to inhibit myogenesis in the following ways. Firstly, TGF-β1 maintains and/or induces quiescence of satellite cells as a result of decreased MyoD expression (Rathbone et al.

2011). Secondly, in vitro studies performed on myoblast cell lines also show that TGF-β1 promotes myoblast proliferation (Mu and Li 2011), but prevents myoblast differentiation and myotube formation (Grafe et al. 2018; Melendez et al. 2021). These observed results are likely related to the known TGF-β1 induction of gene expression of inhibitor of differentiation (Id), which acts as a dominant negative regulator of basic helix loop helix (bHLH) transcription factors of myogenesis, such as MyoD and myogenin (Seoane and Gomis 2017). In addition, TGF-β1 treatment induces rapid muscle atrophy via expression of atrogin-1 and MuRF-1, two muscle-specific E3 ubiquitin ligases involved in muscle protein degradation (Mendias et al. 2012; Abrigo et al. 2016, 2018). TGF-β1 has also been shown to mediate denervation-induced muscle atrophy (Yang et al. 2018; Ismaeel et al. 2019).

Connective Tissue Cells

In addition to inflammatory cells and myogenic cells, connective tissue cells are also required for muscle regeneration by producing initially a temporary ECM scaffold to guide the migration of other cells into the wound site. A mesenchymal progenitor cell population, known as FAPs, which are positive for platelet-derived growth factor receptor α (PDGFRα), act as a source of myofibroblasts (Contreras et al. 2019b; Contreras et al. 2016). In acute injury, FAPs proliferate transiently to facilitate myogenesis (Joe et al. 2010; Murphy et al. 2011). However, in chronic injury, studies have demonstrated that macrophage-derived TGF-β1 prevents apoptosis of FAPs and promotes their ECM deposition (Lemos et al. 2015; Theret et al. 2021); progressive accumulation of FAPs leads to muscle fibrosis (Madaro et al. 2018). Indeed, it has been reported that both plasma and muscle TGF-β1 levels are elevated in DMD patients. For example, in mdx mice, a well characterized mouse DMD model, drugs that either induce FAP apoptosis or attenuate TGF-β1 levels can slow down fibrosis progression (Ismaeel et al. 2019).

Fibrosis is always accompanied by fatty infiltration and dystrophic calcification when efficient repair cannot be achieved (Mu et al. 2013; Mazala et al. 2020). This phenomenon impairs repair results via the skewed differentiation of FAPs into adipocytes and osteoblasts (Uezumi et al. 2014). Generally, TGF-β1 inhibits adipogenic differentiation of FAPs and promotes myofibroblast differentiation (Grafe et al. 2018; Contreras et al. 2019a). Therefore, in a TGF-β1-rich fibrotic environment, suppressed adipogenesis should be observed. However, the opposite is found, suggesting that in damaged muscle tissue, there is likely to be an uneven spatial distribution of TGF-β1. It has been reported that myogenic cells regulate FAP lineages (Theret et al. 2021), and they are also able to produce TGF-β1 (Li et al. 2004). We hypothesize that when myogenic cells are exhausted in continued cycles of degeneration and regeneration, their control over FAPs is compromised, such that FAPs can proceed to undergo adipogenic differentiation. The role of TGF-β1 on FAP osteogenesis is currently unclear. We have recently shown that muscle trauma-induced upregulation of local bone morphogenetic protein-7 (BMP-7) level, combined with glucocorticoid excess-induced downregulation of circulating TGF-β1,

could be an important causative mechanism of traumatic heterotopic ossification, i.e., the formation of ectopic bone in muscle (Li and Tuan 2020; Li et al. 2019). Thus, TGF-β1 acts antagonistically toward BMP-7 to inhibit osteogenesis in FAPs. However, it has also been suggested that TGF-β signaling enhances endochondral ossification through endothelial-mesenchymal transition during muscle injury (Medici et al. 2010; Medici and Olsen 2012).

Circulating TGF-β1 Levels: Linking Muscle and Trauma

Source of Circulating TGF-β1

It has been suggested that 45% of plasma TGF-β1s is derived from platelets, as platelet-specific depletion of TGF-β1 results in a 45% reduction of plasma TGF-β1 concentration (Meyer et al. 2012). The other half is likely to be derived primarily from inflammatory cells, especially macrophages (Grainger et al. 2000). A study has shown that macrophage-specific depletion of TGF-β1 in LysMCre-Tgfb1$^{fl/fl}$ mice reduces plasma TGF-β1 levels approximately by half (Sorkin et al. 2020). Importantly, it is critical to distinguish plasma or serum TGF-β1 levels as the measurement endpoint. We have actually found that serum and plasma TGF-β1 levels are always inversely correlated (data not shown). It thus appears that if platelets release TGF-β1 into plasma, serum TGF-β1 level will decrease. We also found that active TGF-β1 levels (detected without acid activation) are always proportional to total TGF-β1 levels (detected with acid activation) in both plasma and serum at about 1/5~1/10 ratio. It is noteworthy that these findings are different from previously published results (Grainger et al. 1995b).

Shortly following trauma, the initiation of the coagulation cascade is accompanied by platelet degranulation and the resultant release of TGF-β1 into the circulation, which may result in a high TGF-β1 plasma level (Karolczak and Watala 2021). On the other hand, clotting may exhaust platelets, thereby causing decreased circulating TGF-β1 levels post trauma, but an increased circulating TGF-β1 levels at later time points because dissolution of the clot further releases TGF-β1 into circulation (Grainger et al. 1995c). It is therefore also important to note the temporal changes in the circulating TGF-β1 levels following trauma.

Factors Affecting Circulating TGF-β1 Levels Following Trauma

Major trauma affects the coagulation system: a hypocoagulation state lasting up to 6 h after trauma is followed by a hypercoagulation state thereafter (Moore et al. 2021). Dysregulation of hemostasis alters platelet behavior, leading to platelet dysfunction (Vulliamy et al. 2021). Specifically, severe injury induces both platelet activation and function; in non-surviving patients, platelet activation is stimulated but not platelet function (Jacoby et al. 2001). Since TGF-β1 is a major component of platelet α-granules, we hypothesize that changes in the coagulation state will affect

circulating TGF-β1 levels. We have previously reported that hypercoagulation is correlated with lowered plasma TGF-β1 levels (Li et al. 2020b). However, it is still unknown whether it is a causal relationship, and whether it is mediated by platelets. As both thrombin and plasmin can activate platelets (Blakytny et al. 2004; Blockmans et al. 1996), it is possible that their exhaustion during trauma-induced coagulopathy affects platelet behavior, thus suppressing TGF-β1 release.

Prolonged immobilization is a phenomenon commonly seen in critically ill patients due to extended bed rest, use of orthopedic casts, as well as denervation (Parry and Puthucheary 2015). Immobilization rapidly induces muscle atrophy and muscle fibrosis (Wang et al. 2019), a phenotype similar to that associated with increased TGF-β1 signaling. Immobilization also contributes to a hypercoagulation state by slowing venous blood flow in lower extremities (Toker et al. 2011). Although the extent of changes in circulating TGF-β1 levels after prolonged immobilization is unknown, there is evidence suggesting that platelet activation is suppressed (Arinell et al. 2013). It is noteworthy that physical exercise is known to increase circulating TGF-β1 levels (Hering et al. 2002; Heinemeier et al. 2003). Therefore, it is plausible that a decrease in circulating TGF-β1 levels is associated with prolonged immobilization. However, whether circulating TGF-β1 levels could be used as a valid biomarker to indicate coagulation state and muscle degeneration remains to be further examined.

Circulating Versus Muscle TGF-β1

It is already known that TGF-β1 functions differently at the systemic level versus local level, with local administration promoting inflammation and systemic inoculation inhibiting inflammation (Wahl 1994). Under conditions of trauma, it is of interest to note, from both our studies and those of others, that a decrease in plasma TGF-β1 levels is always correlated with an increase in tissue TGF-β1 levels (Fig. 2) (Sorkin et al. 2020). Is there a causal relationship between the two? One possibility is that the traumatized tissue actively consumes circulating TGF-β1, thus depleting it from the systemic circulation, in a manner similar to our previous observation that fibrotic responses can consume circulating TGF-β1 (Li et al. 2020a). This phenomenon is likely related to fibrin function, as it has been reported that fibrin carries latent TGF-β1 in circulation (Schachtrup et al. 2010). Consequently, TGF-β1 may be brought to the sites where fibrin is deposited, further promoting the migration of macrophages to these sites for more TGF-β1 production (Vidal et al. 2008). In this manner, local consumption results in decreased circulating TGF-β1 levels. Another possible scenario is that the decrease in circulating TGF-β1 levels, related to platelet and monocyte dysfunction after severe trauma, is in fact the cause. In support, it is known that platelet function is suppressed after trauma, and diminished cytokine production in monocytes has been observed after trauma (Wutzler et al. 2009; Kirchhoff et al. 2009). On the other hand, increased tissue TGF-β1 levels may also be attributed to the proliferation of local M2 macrophages in an attempt to repair the damaged muscle tissue (Cote et al. 2013).

Function of Circulating TGF-β1 in Muscle

A major question that remains to be answered is: What are the consequences of reduced circulating TGF-β1 levels on muscle? Although there are no human studies on this subject to date, we have observed in a mouse model that decreased plasma TGF-β1 levels are associated with impaired muscle healing and dystrophic calcification (Fig. 2) (Li et al. 2020a, b). This muscle degenerative phenotype is different from that in muscle chronic inflammation, given that fibrosis is not evident (Fig. 3). Also, it is distinct from a heterotopic ossification phenotype usually found following trauma (Li and Tuan 2020). However, it is likely that this precursor stage may later progress into fibrosis or heterotopic ossification with uncorrected abnormal circulating TGF-β1 levels.

We have further shown that systemic treatment with TGF-β1 recombinant protein reduces dystrophic formation (Li et al. 2020b) (Fig. 4), whereas systemic treatment with TGF-β1 receptor inhibitor in normal mice promotes dystrophic formation

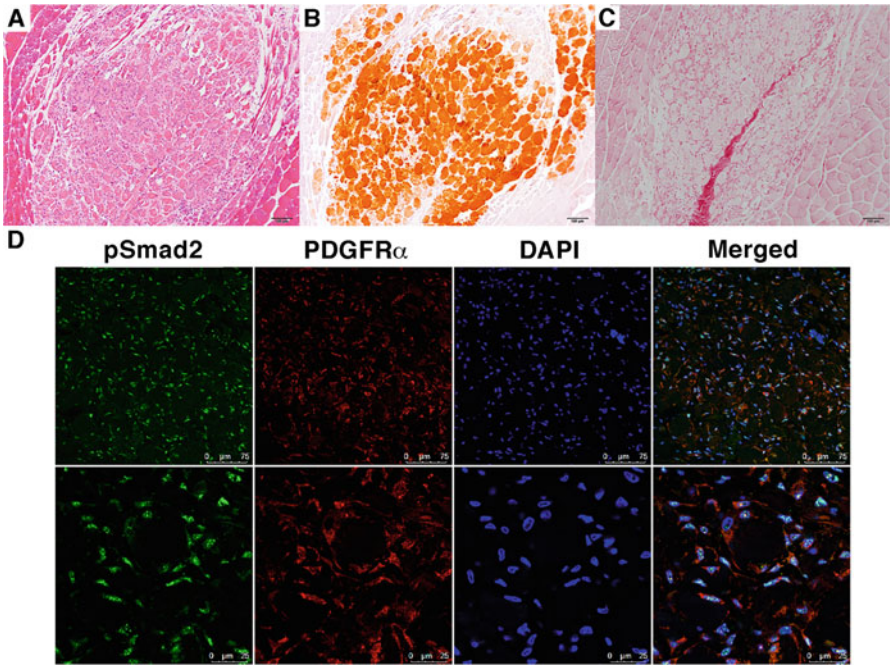

Fig. 3 Dystrophic calcification detected in mouse impaired healing muscle, with abundant presence of FAPs
(**a**) H and E staining showing the injured muscle. Bar = 100 μm. (**b**) Alizarin Red staining showing calcium deposition in degenerative muscle fibers. Bar = 100 μm. (**c**) Picosirius Red staining showing no collagen deposition in the injured muscle. Bar = 100 μm. (**d**) Immunofluorescence staining showing abundant presence of PDGFRα-positive FAPs in nonhealing calcified muscle tissue, which express phosphorylated Smad2 protein. Bar = 75 μm (top row, low magnification) or 25 μm (bottom row, high magnification)

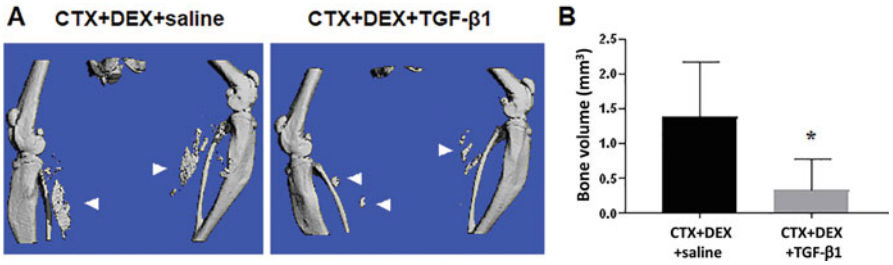

Fig. 4 TGF-β1 supplementation inhibited dystrophic calcification formation
Muscle injury was induced with the intramuscular administration of cardiotoxin with systemic co-administration of dexamethasone in a mouse model. (**a**) microCT imaging demonstrating reduced bone volume with TGF-β1 supplementation for 7 days. Arrowheads indicate ectopic mineralization. (**b**) microCT analysis of bone volume with and without TGF-β1 supplementation in addition to CTX + DEX co-treatments (*, $p < 0.05$; $n = 6$). Used with permission from Li et al. (2020b)

following muscle injury (Li et al. 2020a). Taken together, these findings confirm that circulating TGF-β1 does have a function on local muscle tissue, although the mechanism of action is still unknown. Therefore, a thorough analysis of the levels of phosphorylated Smad2/3 protein, the major mediator of TGF-β signaling, as well as TGF-β1 receptors in different cell types following muscle trauma should be performed to elucidate the regulation of TGF-β1 signaling in muscle cells. Our preliminary data showed abundant presence of PDGFRα-positive FAPs in non-healing calcified muscle tissue, which are also positive for phosphorylated Smad2 protein (Fig. 3). It is therefore important to assess how changes in circulating TGF-β1 affect FAPs fate. Further investigations are clearly needed.

Conclusion and Future Direction

In summary, altered circulating TGF-β1 levels can be found in various disease states, and the available evidence indicates that too little or too much circulating TGF-β1 can both be detrimental. In muscle, TGF-β1 affects different muscle cell types, and the overall effect of TGF-β1 on muscle in vivo is not a simple sum of known in vitro effects of TGF-β1 on each muscle cell type. In addition, the plasticity of macrophages and their ability to produce large amounts of TGF-β1 locally further complicates the biological picture. Based on currently available information, we hypothesize that following trauma, a decrease in circulating TGF-β1 levels results in impaired muscle healing and dystrophic calcification. However, more correlative and mechanistic studies on both experimental animal models as well as trauma patients are clearly needed to confirm if circulating TGF-β1 levels can be used as a valid prognostic biomarker to indicate muscle degeneration or regeneration state following trauma.

Applications to Prognosis, Other Diseases or Conditions.

In this chapter, we propose that circulating TGF-β1 has the potential to be used as prognostic biomarker following muscle trauma. This is based on the findings on our animal models (Li et al. 2020a, b) that a decrease in circulating TGF-β1 levels results in impaired muscle healing and dystrophic calcification. This phenomenon is possibly related to trauma-induced coagulopathy; however, few clinical studies have tested the possible use of TGF-β1 as a valid biomarker to indicate coagulation state and muscle regeneration state.

The muscle degeneration state resulting from decreased circulating TGF-β1 levels is accompanied by increased muscle TGF-β1 levels. Overexpression of TGF-β1 in muscle is generally correlated with a fibrosis phenotype. However, this dystrophic calcification phenotype is different from muscle fibrosis, in which both increased circulating and local TGF-β1 levels can always be found. This dystrophic calcification phenotype may relate to another human disease condition, i.e., heterotopic ossification (HO). HO happens most frequently in patients suffering from severe trauma, frequently associated with coagulopathy and prolonged immobilization. Therefore, we propose that decreased circulating TGF-β1 levels be used as a biomarker to indicate the likelihood of HO after muscle trauma. Further clinical studies are clearly needed.

Lastly, in using circulating TGF-β1 levels as a biomarker, it is important to distinguish between plasma and serum TGF-β1 levels. For most of the studies on this subject, plasma and serum TGF-β1 levels are used interchangeably, with a presumption that both of them indicate circulating TGF-β1 levels. However, we have observed that serum and plasma TGF-β1 levels are in fact inversely correlated. Therefore, we are of the opinion that plasma TGF-β1 levels be used as indicator of circulating TGF-β1 levels in order to rule out the influence of platelet degranulation.

Mini-Dictionary of Terms

- **Fibrosis**: Fibrosis refers to the collagen deposition that occurs as part of normal healing or to the excessive collagen deposition that occurs as a pathological process.
- **Dystrophic calcification**: Matrix calcification that occurs in degenerated or necrotic tissue.
- **Heterotopic ossification**: Abnormal formation of lamellar bone inside soft tissue structures where bone should not exist.
- **M1 macrophage**: Proinflammatory macrophage phenotype.
- **M2 macrophage**: Anti-inflammatory macrophage phenotype.
- **Fibro/adipogenic progenitors (FAPs)**: A mesenchymal progenitor cell population that can undergo aberrant differentiation, thus resulting in muscle fibrosis, fatty infiltration, and dystrophic calcification/heterotopic ossification.

Key Facts of TGF-β1

- Knocking out TGF-β1 affects the immune system.
- TGF-β1 is secreted as a latent form, which is activated primarily during injury.
- TGF-β1 is present in blood at a high concentration.
- Altered circulating TGF-β1 levels can be found in various disease states.
- Systemic effect of TGF-β1 differs from its local effect.
- Locally, TGF-β1 is a potent chemoattractant for neutrophils and macrophages.
- Paradoxically, TGF-β1 also acts as a strong immune suppressor.
- Little is known about the function of circulating TGF-β1.

Summary Points

- TGF-β1 is likely the key regulatory molecule among the TGF-β family proteins in the context of muscle trauma.
- TGF-β1 affects different muscle cell types.
- A decrease in circulating TGF-β1 levels and an increase in muscle TGF-β1 levels results in impaired muscle healing and dystrophic calcification.
- Circulating TGF-β1 levels can be influenced by platelet function and inflammatory cell function.
- Prolonged immobilization, independent of trauma-induced coagulopathy, can be the cause of decreased circulating TGF-β1 level.

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Neurofilament Light Chain in the Blood As Biochemical Markers in the Critically Ill

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Contents

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Abstract

Neurofilament light chain (NfL) is a protein of the neuronal cytoskeleton and a biochemical marker specific for neuroaxonal damage when released and detected in the blood of critically ill patients. As serum NfL (sNfL) is not disease-specific one must consider the natural history of diseases and the temporal evolution of sNfL concentrations when interpreting sNfL for diagnosis and prognostication in clinical practice. The interpretation is further challenged by many interfering and confounding factors that need to be considered, such as increasing age, pre-existing neurological comorbidities, altered renal function that decreases the clearance of sNfL from the blood, and non-neurological comorbidities that may damage the nervous system, including cardiovascular diseases and neurotoxic side effects of treatment measures. With increasing availability of sNfL test kits, analyses of sNfL concentrations have been on the forefront when it comes to new and promising diagnosis and prognosis. Their diagnostic and prognostic yields have been evaluated and validated in many (neuro-)critical illnesses and neurologic emergencies encountered and treated in intensive care units (ICU). Among these are ischemic and hemorrhagic strokes, subarachnoid hemorrhages, traumatic brain injuries, epilepsies, Guillain-Barré syndrome, hypoxic-ischemic encephalopathies, delirium, postoperative states, sepsis, and coronavirus infectious disease (COVID)-19. The several studies that have shown the great potential of sNfL as a promising diagnostic and prognostic marker that may also optimize current clinical risk scores call for a careful consideration and critical interpretation of the data.

The aim of this chapter is to elucidate the current evidence of the diagnostic and prognostic yield of sNfL and to discuss interfering factors and potential interferences and confounders in (neuro-)critically ill patients.

Keywords

Neurofilament light chain · Intensive care · Traumatic brain injury · Prognostic marker · Biomarkers · Cardiac arrest · Stroke · Sepsis · Delirium · COVID-19 · Hypoxic-ischemic encephalopathy · Guillain-Barré syndrome · Critical illness polyneuropathy and myopathy

Abbreviations

ADEM	Acute Disseminated Encephalomyelitis
ARDS	Acute Respiratory Distress Syndrome
BBB	Blood-Brain Barrier
BMI	Body Mass Index
CA	Cardiac Arrest

CAA	Cerebral Amyloidangiopathy
CAHP	Cardiac Arrest Hospital Prognosis (Score)
COVID-19	Coronavirus Infectious Disease
cMRI	Cerebral Magnetic Resonance Imaging
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
DAI	Diffuse Axonal Injury
DTI	Diffusion Tensor Imaging
ECMO	Extracorporeal Membrane Oxygenation
EEG	Electroencephalography
ICH	Intracerebral Hemorrhage
ICU	Intensive Care Unit
MRI	Magnetic Resonance Imaging
NfL	Neurofilament Light Chain
NIHSS	National Institute of Health Stroke Scale
NSE	Neuron-Specific Enolase
OHCA	Out-of-Hospital Cardiac Arrest (Score)
PNS	Peripheral Nervous System
ROSC	Return of Spontaneous Circulation
SAE	Sepsis-Associated Encephalopathy
SAH	Subarachnoid Hemorrhage
SARS-CoV	Severe Acute Respiratory Syndrome-associated CoronaVirus
SE	Status Epilepticus
sNfL	Serum Neurofilament Light chain
SSEP	Somatosensory Evoked Potentials
TBI	Traumatic Brain Injury
TIA	Transient Ischemic Attack

Introduction

Intensive care units are treatment areas where the sickest patients are treated and critical decisions must be made daily. Especially in scenarios where treatment is futile or prognosis is poor, delivering such bad news is challenging and can be supported by prognostically reliable biomarkers.

Neuronal damage in critical illness can result directly from diseases that alter the nervous system, such as with trauma, strokes, infections, and neoplasms or emerge by adverse effects from treatment measures or systemic reactions. Biomarkers that quantify the extent of neuronal damage may serve as prognostic tools for both direct and indirect (i.e., secondary) neuronal injury. Serum neurofilament light chain (sNfL) represents a main constituent of the neuronal cytoskeleton and plays an important role in axonal growth, stability, and intracellular transport mechanisms. Because of its specificity to neuroaxonal damage, it has been examined as a potential biochemical marker in many different clinical scenarios during critical care in recent years. Since numerous neuron-affecting circumstances can influence sNfL

concentrations to a greater or lesser extent, solid knowledge in this context is essential to avoid misinterpretations and to identify possible interferences and confounding factors.

The aim of this chapter is to elucidate the current evidence of the diagnostic and prognostic yield of sNfL and to discuss interfering factors and potential interferences and confounders in (neuro-)critically ill patients.

Biochemical and Biological Characteristics of Neurofilament Light Chain

Neurofilament light chain (NfL) is a protein of the cytoskeleton of neurons with a molecular wight of 70kD. It is solely located in the neuronal cytoplasm, especially in the axons of myelinated long neurons, as well as the dendrites and soma. It provides structural stability, mediates conduction velocity by expanding the myelinization, plays a part in the synaptic functional organization, and promotes radial axonal growth (Gaetani et al. 2019). The importance of NfL is reflected by the neuronal degeneration that can result from a defect in the NfL gene (NEFL), which can lead to specific forms of Charcot-Marie-Tooth disease. While NfL is an integral component of the peripheral and central neurons, it does not exist in other cells and is therefore a highly neuron-specific biochemical marker. There seems to be a constant turnover, degradation, and release of NfL from the neurons to the blood and the cerebrospinal fluid (CSF) even in the absence of neurodegenerative or neurotraumatic events, which leads to measurable levels in these compartments. However, the underlying mechanisms are poorly understood. The normal concentrations of NfL in the blood range approximately from 10 pg/ml to 100 pg/ml and from 1 ng/l to 10 ng/l in the CSF (Gafson et al. 2020). Due to its much higher levels in the CSF, early studies were restricted to measurements of NfL in CSF samples. This restriction to measurements following lumbar puncture, an invasive technique for sampling, has initially hampered its frequent routine clinical use. With technical diagnostic advances that have led to the development of ultrasensitive essays within the last years, NfL was finally measurable in the blood more easily enabling numerous studies in critically ill patients.

Potential Confounders and Interferences with Serum Neurofilament Light Chain Concentrations

Potential confounders of the association between serum NfL (sNfL) levels and specific outcomes in critically ill patients are conditions or factors that may influence sNfL levels and potentially have a direct causal link to outcome. Suspecting a cofounder in critically ill patients might lead to further testings, diagnostics, and evaluations to exclude such influences. Knowledge regarding potential confounders in daily clinical scenarios is important to understand the true diagnostic and

predictive value of sNfL levels. In the following, potential relevant confounders and their bidirectional effects on sNfL concentrations and outcomes are discussed.

Figure 1 presents factors that may promote or prevent increased NfL levels in the blood and the CSF and – if also having effects on outcome – may act as potential confounders regarding presumed associations.

Volume of Distribution

Conditions with increased volume of distribution in critically ill patients may be associated with specific outcomes, as well as with decrease of sNfL levels by dilution, as seen in patients with high body mass indexes (BMI), pregnancy, and in critically ill patients receiving mass transfusions. A high BMI and increased blood volume both seem to lower the levels of sNfL through an increased volume of

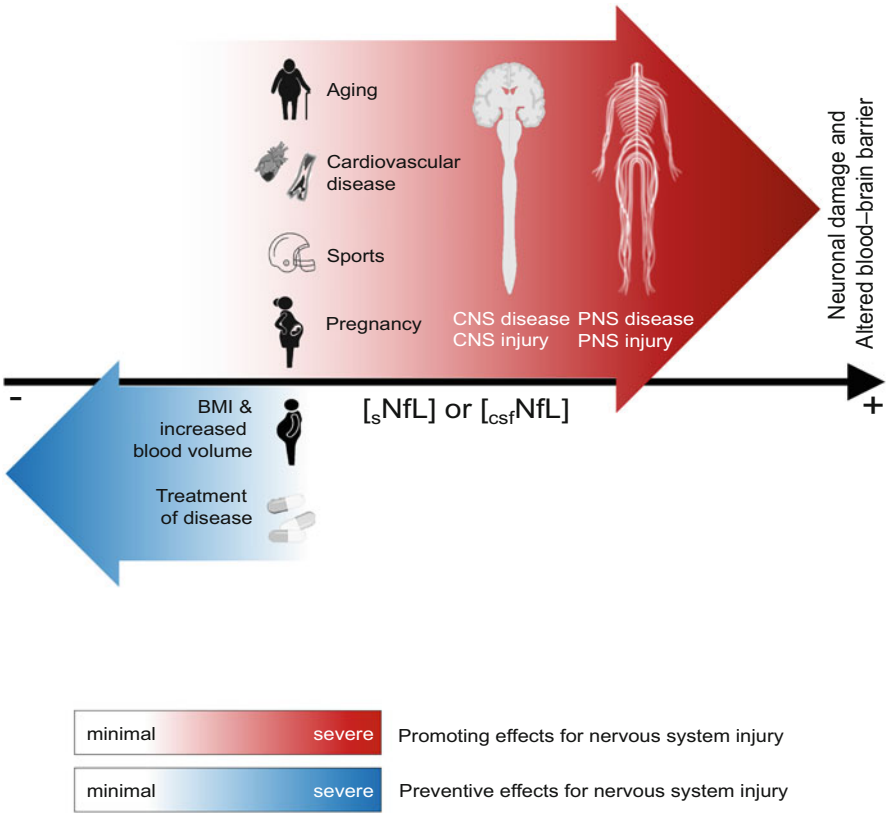

Fig. 1 Promotors and preventors of increase of neurofilament light chain concentration in the blood and the cerebrospinal fluid
NfL neurofilament light chain, *BMI* body mass index

distribution as shown in a study of 662 healthy controls and 2,586 multiple sclerosis patients (Manouchehrinia et al. 2020). However, sNfL levels are also described to be elevated in healthy pregnant women, as well as in women with preeclampsia (Andersson et al. 2021). The contribution of such confounding factors regarding absolute sNfL levels call for further studies to identify and define cutoff serum levels above which an increased sNfL concentration can be reliably used as a predictor of outcome in these contexts. In the meantime, clinicians should be careful when interpreting increased sNfL levels in patients with significant increased blood volume or BMI.

Increasing Age

Serum levels of NfL in healthy controls rise with every additional year of age by 2.2% in healthy adults (Barro et al. 2018; Disanto et al. 2017) up to the age of 60 years. Later in life, a more steep rise of sNfL levels is seen and interindividual variability appears to be more pronounced (Khalil et al. 2020). While interpreting sNfL levels in specific age groups the relative contribution of age to elevated serum levels has to be considered. In addition, the higher prevalence of potential interfering or confounding factors that may emerge with increasing age that may lead to a relative decline of the contribution by the primary disease to the absolute sNfL levels (Barro et al. 2020) must be recognized. To address this problem, measurements should be adjusted for specific age ranges prior to further interpretation, e.g., in the form of percentiles or age correlated z-score of log-normalized sNfL (Barro et al. 2018; Thebault et al. 2021), and study results must always be questioned in regard to such interfering or potentially confounding factors.

In a study of 385 inhabitants of Augsburg who were aged 65 years and older revealed an independent association between increased sNfL levels and all-cause mortality (Rübsamen et al. 2021). These findings suggest that increasing sNfL levels in the absence of an underlying overt neurologic disease reflect biological aging of the nervous system and identifies individuals with an increased risk of death.

Neurological Comorbidities

Another potential confounder of the associations between increased sNfL levels and specific outcomes are preexisting or developing neurological comorbidities. Such comorbidities can affect both the peripheral and the central nervous system. While they may be misleading in making diagnostic decisions about whether a particular acute disease is present after a suspicious clinical event, they may have a cumulative effect on prognosis and/or outcome. Large studies regarding such confounding effects in specific clinical scenarios are scarce and difficult to perform due to the fact that reliable detection or exclusion of neurologic comorbidities is time-consuming, labor-intensive, and demands detailed and extensive workups. Hence, a careful assessment of the medical history and repetitive clinical neurological

evaluation and monitoring are key to identify such potential cofounders and should be considered while interpreting sNfL levels in the context of an acute illness affecting the nervous system.

Renal Insufficiency

The Incidence of impaired renal function in ICU patients is high and therefore of great importance when it comes to the interpretation of sNfL measurements in the critically ill. In a study examining the association between sNfL levels and renal function among 43 healthy adult participants and 188 adult patients with diabetes mellitus (all being 60 years of age or older and none diagnosed with dementia) revealed that approximately 20% of sNfL level variability was well explained by impaired renal function (Akamine et al. 2020). Whether this effect is purely caused by impaired renal NfL clearance or also at least partially explained by decreased levels of neuroprotective agents, such as vitamin D and e*rythropoietin* (EPO), resulting from decreased renal function, could not be clarified. The authors suggested that renal function may have a stronger influence on sNfL levels in the elderly as compared to younger patients. This was supported by a prior study with patients with a mean age of 63 years that has shown similar results (Korley et al. 2019). Another study of younger patients diagnosed with HIV and a mean age of 41 years revealed no increase of sNfL levels with increasing age (Hermansson et al. 2019). Hence, these different findings are likely at least partially explained by the cumulative neuronal damage seen in the elderly (Barro et al. 2020).

Non-Neurological Comorbidities

Non-neurological diseases have the potential to damage the neurons as well. Increased glucose serum levels in patients with diabetes mellitus can lead to peripheral neurological damage or may promote cerebrovascular diseases that may lead to ischemic or hemorrhagic strokes. Although it seems more than plausible that diabetes mellitus and other cardiovascular risk factors, such as arterial hypertension, smoking, and chronic dyslipidemia are likely to have such destructive effects and thereby may act as potential confounders, formal large studies in this context are lacking.

Treatment-Related Influences on Serum Neurofilament Levels

In addition to acute critical illnesses, just being intensively treated in an ICU may put a strain on the body by numerous factors and influences. Apart from an unfamiliar environment and imposed diurnal rhythm, effects and neurotoxic side effects of treating agents, episodes of hypoxia, and hypotension, the impact of mechanical ventilation and organ substituting therapies must be considered. Hence, awareness in this context and close monitoring regarding such potential mediators of neuronal injury is required.

Furthermore, potential preventive measures resulting from disease-specific therapy targets, such as specific blood pressure or perfusion pressure limits, and disease modifying drug therapy may lower sNfL levels. However, to what degree such measures influence sNfL levels in the course of specific diseases is not well studied.

Advantage of Neurofilament Light Chains in Contrast to Neuron-Specific Enolase with Hemolysis

Several mechanisms may lead to hemolysis in critically ill patients. Aside from many disease-related effects, treatment-related hemolytic effects may come from mechanical devices for hemodynamic support, such as from extracorporeal membrane oxygenation (ECMO), intraaortic balloon pumps, or impellas. In such scenarios, hemolysis may alter the diagnostic and prognostic reliability of neurobiochemical markers that are also found in red blood cells. One such important and frequently used biomarker used for neurologic outcome prediction in resuscitated patients with persistent coma is the neuron-specific enolase (NSE), an enzyme located mainly within neurons and neuroectodermal cells that converts anaerobically glucose to metabolites suitable for oxidation (Luescher et al. 2019). Unfortunately NSE is also found in the red blood cells and is known to show significant high values with little hemolysis. In contrast to NSE, with sNfL no such influences of hemolysis have been demonstrated so far (Moseby-Knappe et al. 2019; Wihersaari et al. 2021).

Temporal Profile

Temporal leveling of NfL in the blood and the CSF depends on the natural course of disease. In general, this can be a slow beginning and chronically progressive disease (i.e., neurodegenerative syndromes, such as dementia, or slowly growing tumors) or an acute manifestation with abundant neuroaxonal trauma, as seen with ischemic or hemorrhagic strokes, or traumatic brain injuries. The latter may be followed by either full recovery or may lead to a progressive course with further neurological decline due to secondary neuronal injury.

The half-life of sNfL is a key factor when it comes to the question at which timepoint measurements should take place. Analyses in mice models point to a half-life in vivo in the brain of about 3 weeks, but can be months depending on the presence of an endogenous neurofilament network (Millecamps et al. 2007). In studies regarding the development of serum levels of NfL following acute neuronal injuries, studies point to a delayed rise in blood during the first 24 h compared to other biomarkers of neuronal damage. Due to its long half-life, levels remain steadily high even after 3 days and are sustained over weeks to months (Kanberg et al. 2020; Shahim et al. 2020a). While the prognostic use of NSE is currently restricted to post-cardiac arrest patients with repetitive measurements within the first 3 days after resuscitation, single sNfL levels may be reliably used in cases of initial missing measurements according to a single prospective case series of 14 patients initially

surviving cardiac arrest (Disanto et al. 2019). However, larger studies validating these findings are pending.

CSF/Serum Correlation and Blood–Brain Barrier

With the ability to quantify neuronal damage in the blood, NfL is being used to investigate and monitor severity of neurologic diseases or the concomitant neuronal damage with non-neurologic critical illnesses including the assessment of treatment efficacy. Although it is not clear to which degree the permeability of the blood–brain barrier (BBB) and the blood–CSF barrier influences the levels of sNfL, it seems to be widely accepted that NfL levels in the blood and the CSF seem to be strongly correlated (Barro et al. 2020). How NfL traffics between compartments and enters the blood is not fully understood. Hypothesized are drainages from the CSF via the lymphatic system, intramural periarterial drainage from the brain parenchyma, or direct drainage via lymphatic vessels to regional lymph nodes in the peripheral nervous system (Gafson et al. 2020).

The variability of NfL concentrations in the CSF, the blood, and in their ratio may be primarily caused by conditions affecting the blood levels independently from the CSF levels, as with increased blood volume or diseases affecting mainly the peripheral nervous system. The CSF/serum ratio and index of NfL have been used to discriminate the different origins, such as the central or the peripheral nervous system (Körtvelyessy et al. 2020). The BBB function can be measured by the albumin ratio (CSF/Serum), as albumin is synthesized in the liver and released to the blood, but not synthesized within the CNS. As the molecular size of albumin is similar to the one of NfL (66.5 vs 70 kDA) and NfL concentration is typically higher in the CSF, an efflux of NfL should be suspected with decreasing BBB stability. Some studies have shown an association of sNfL levels with the albumin quotient, mainly in diseases primarily affecting the CNS (Uher et al. 2021). This finding could, unfortunately, not be replicated in other studies where the albumin and NfL ratio did not correlate (Garland et al. 2021; Kalm et al. 2017). These discrepancies may indicate at least to some extent an independence of NfL concentrations from the integrity of the BBB or at least points to different kinetics than albumin. How the BBB permeability influences NfL blood levels is not entirely clear yet and deserves to be studied further.

Evidence Regarding Neurofilament Light Chain in Specific Critical Illnesses

Cerebrovascular Accidents

In contrast to patients with hemorrhagic strokes where there is limited evidence regarding the diagnostic and prognostic yield of sNfL, there are several studies investigating sNfL in patients with ischemic strokes.

Biomarkers in stroke could (1) help in the initial workup regarding the assumed time of onset of ischemia, complement the diagnostic workup and imaging at identifying stroke and separating it from stroke mimics, (2) serve as a marker of outcome, or (3) could monitor the course of disease and identify patients at risk for progressive or reoccurring disease and complications.

(1) After an acute event like ischemic stroke, sNfL levels seem to rise more slowly compared to other neuronal biomarkers: sNfL measurements within the first 24 h after onset of ischemic stroke revealed that NfL levels seem to be high enough to discriminate ischemic stroke and transient ischemic attacks (TIAs) from healthy controls and show an association with stroke severity, but seemed not to be able to predict 3 months outcome (De Marchis et al. 2018). However, there are conflicting findings when it comes to associations between sNfL levels and size of the infarct (De Marchis et al. 2018; Onatsu et al. 2019; Uphaus et al. 2019).
(2) In ischemic stroke, sNfL levels beyond 24 h after symptom onset seem to be a relevant predictor for functional outcome at 3 months (Tiedt et al. 2018; Uphaus et al. 2019). This reflects the important influence and awareness of the exact time of measurements, even if these observations need to be confirmed by further studies. The sNfL levels rise further within the first weeks and stay high for up to 3–6 months (Liu et al. 2020).
(3) In a case-control study of patients with diabetes mellitus without a history of ischemic stroke at inclusion, sNfL concentrations were able to predict the occurrence of new intracerebral ischemic lesions (Korley et al. 2019). Given the association of sNfL with the occurrence of new discrete lesions on neuroimaging without clinical symptoms in cerebral small vessel diseases (Gattringer et al. 2017) and the association with new recurrent ischemic strokes (Uphaus et al. 2019), it might serve as a marker for ongoing disease activity and therefore help identifying patients at risk of being in need for more strict and intensified primary or secondary preventive strategies.

    Aside from several studies on ischemic stroke, data regarding the use of sNfL in patients with hemorrhagic strokes are limited. Currently, one small Chinese prospective study of 68 patients with a first probable amyloid angiopathy-related intracerebral hemorrhage (ICH) revealed that increased sNfL levels were associated with the recurrence of ICH independent of the burden of small vessel disease on magnetic resonance imaging (MRI), with baseline ICH volume, the NIHSS, and with outcome at 6-month as quantified by the modified Rankin scale (Cheng et al. 2020). However, as these findings have not been confirmed in other studies and the sample size was small, clinicians should interpret these results with caution.

Nontraumatic Subarachnoid Hemorrhage (SAH)

In nontraumatic SAH, neurological deficits are not only caused by early brain injury resulting from the initial hemorrhage, classically following the rupture of aneurysms

of the brain base arteries. Additional neuronal damage can also be promoted by subsequent vasospasms with consecutive delayed cerebral ischemia, and increased intracerebral pressure resulting from CSF accumulation that finally leads to hydrocephalus occlusivus. Serum NfL can add value to the challenge of prognostication in this complex clinical scenarios. In a small prospective study of Denmark on the prognostic yield of sNfL measured at admission in 44 adult patients with spontaneous SAH revealed that increased sNfL levels were associated with increasing disease severity during the early brain injury phase (Hviid et al. 2020). Furthermore, higher sNfL levels were associated with poor functional outcome 30 days after ictus and with an increased mortality rate.

Another small prospective study from the UK of 42 adult nontraumatic SAH patients revealed that CSF and serum NfL levels were higher than the upper limit of a control population on days 1–3 following SAH and that such increases predicted unfavorable outcomes as quantified by the modified Rankin scale at 6 months independent of the World Federation of Neurosurgical Societies score (Garland et al. 2021). Since NfL correlates with scavengeable hemoglobin in the CSF with SAH, it could be used as a potential marker in clinical trials for therapy response to intrathecal hemoglobin-binding haptoglobin as a counter measurement of hemoglobin toxicitiy. However, studies in this context are pending.

Traumatic Brain Injury (TBI)

While TBI is a major contributor to morbidity and mortality, even in young people, the incidence of TBI in developed countries is increasing in the elderly population over 65 years of age due to falls. TBI is generally classified into three categories of severity: mild (with an initial GCS of 13–15), moderate (GCS 9–12), and severe (GCS 3–8). Even mild TBI and concussion, as especially encountered repetitively in contact sports, can lead to mild diffuse axonal injury (DAI) with an insufficient detection rate in routine neuroimaging and later to chronic traumatic encephalopathy. The latter being described in a Swedish cohort examining sNfL levels in 45 hockey players suffering concussions with long-lasting elevated sNfL levels for months (Shahim et al. 2020a).

Even the frequently used diffusion tensor imaging (DTI) has its limits in detecting DAI, including its availability and costs. In TBI and sports-related concussion, sNfl has been shown to be associated with atrophy and DTI estimates in two studies (Shahim et al. 2020a, b).

The limited data available for moderate or severe TBI demonstrates an increase in sNfL levels with the severity of TBI and a linear rise over the first 1–2 weeks after the initial impact. In addition, elevated 24 h serum levels were associated with a poor 12-month outcome in three prospective studies on adults and could discriminate between survivors and nonsurvivors with a sensitivity of 71% and specificity 88% in one study (Czeiter et al. 2020; Shahim et al. 2016; Thelin et al. 2019).

No further statement can be made about subgroups estimating the impact of focal versus diffuse lesions (Gao et al. 2020). A biomarker that can predict neurocognitive

impairment, especially in younger patients, would improve subsequent management and facilitate counseling regarding disease-related deficits and career decisions. However, further data in this regard are pending.

Although not relevant to ICU populations, it seems notable to mention that according to a recent systematic review of 24 studies, there is plenty of data suggesting sNfL to be a reliable marker in sports-related concussion and mild TBI. In this review, sNfL levels were found to be higher in all patients suffering from concussion compared to healthy controls (Karantali et al. 2021).

Hypoxic-Ischemic Brain Injury After Cardiac Arrest (CA)

There have been great advances in the treatment and management of patients with hypoxic-ischemic encephalopathy following CA, yet it remains one of the greatest challenges to adopt the patient's will to care and manage interactions with the patients' families in the ICU. Mortality and disability rates remain high. Prognostic assessment in particular remains a challenge, while there is a great risk of falling into a "self-fulfilling prophecy," in the event where the prognostic markers point to an unfavorable outcome and are assigned too great a value.

The distribution pattern in hypoxic-ischemic encephalopathy shows a more diffuse distribution pattern compared to ischemic strokes with emphasis on damage first in the cerebellar hemispheres, basal ganglia, and cerebral cortex (in particular, the peri-rolandic and occipital cortices). Furthermore, the hippocampi, thalami, and, finally, the brainstem may be affected. Alteration of the white matter, where the long NfL-rich axons are located, is associated with poor outcome (Luyt et al. 2012). Hence, sNfL levels show great potential in the prognostication of patients remaining in altered consciousness or coma after surviving CA. In a small prospective Swiss case series, examining sNfL levels in 14 adult CA survivors, nonsurvivors at 1 month after CA had higher median sNfL levels with a better prediction of death (with a 83% sensitivity and a 100% specificity for the prediction of death using a cutoff level of 3436.8 pg/ml) as compared to the extensively validated serum concentration of NSE (Disanto et al. 2019). Remarkably, other known prognostic tools and marker of neuronal damage, such as time to return of spontaneous circulation and the severity of the EEG pattern (especially the suppressed electrical activity) seemed to correlate with sNfL levels in this small pilot study. Time of blood sampling, however, was not standardized. In another larger study, examining the 717 adult resuscitated patients of the TTM trial, blood was taken at the same standard times as the established measurements of serum NSE (i.e., at 24 h, 48 h, and 72 h after cardiac arrest) (Moseby-Knappe et al. 2019). Analyses revealed that sNfL performed better than serum NSE levels as predictors of poor neurological outcome at 6 months as quantified by the Cerebral Performance Category Scale and showed a better sensitivity than electroencephalography (EEG), somatosensory evoked potentials (SSEPs), cerebral computed tomography (CT), and pupillary/corneal reflexes. In addition, this study revealed that sNfL levels increased over time. Of note, the Swiss case series mentioned above showed long-lasting high levels even after day

3 (Disanto et al. 2019), so did an earlier prospective study of Germany with 85 adult patients following CA (Rana et al. 2013), which measured levels up to day 7 post CA and showed that sNfL levels remained steadily high over time. Consequently, and as one initial measurement after 24 h following CA seems to have a better predictive value than NSE, using sNfL concentrations 24 h after CA might be sufficient as an outcome predictive assessment.

While different cutoffs for poor outcome have been calculated and suggested by three different study groups, a clear recommendation in this regard currently cannot be made. The individual sNfL levels should therefore be interpreted with great caution and in light of other predictive measures including neuroimaging, clinical course, and SSEPs, as no single parameter can reliably predict poor prognosis with certainty. Rather, it seems reasonable to incorporate the values into a clinical risk score. In one observational hypothesis-generating study (Hunziker et al. 2021), sNfL level at admission was on the one hand an excellent outcome predictor, and on the other hand could improve the significance of two clinical risk scores after CA (i.e., the Cardiac Arrest Hospital Prognosis [CAHP] score and the Out-of-Hospital Cardiac Arrest [OHCA] score). However, these risk assessments did not include the more complex neuroradiological and neurophysiological examinations such as with EEG, SSEPs, or cerebral MRI.

The development of status epilepticus (SE) after CA also indicates a poor outcome. Within the TTM trial collective, another study was performed in which patients with similar initial brain injuries were matched to patients who developed SE. Seventy-two hours after admission, significant elevated sNfL levels were observed in the SE group, indicating ongoing neuronal damage due to persistent seizures (Lybeck et al. 2021).

The concentrations of sNfL also provides a tool to assess the effects of other factors on neuronal damage within the management of the primary disease. Thus, in a post hoc analysis of the COMCARE trial (Wihersaari et al. 2021), in addition to the prognostic accuracy of sNfL for poor outcome, the positive influence of the higher mean arterial blood pressure (MAP) limit of 80-100mmHg, as reflected by lower sNfL levels compared to patients with lower MAP limits, was shown.

Epilepsy

Data for sNfL concentration in patients suffering from SE is scarce. The aforementioned study evaluating sNfL levels in patients with hypoxic-ischemic encephalopathies with SE (Lybeck et al. 2021) indicated ongoing neuronal damage reflected by increased sNfL levels and a smaller study on patients with autoimmune encephalitides revealed an association between higher CSF NfL levels and the presence of SE at admission (Constantinescu et al. 2016). It remains however unclear whether the elevated sNfL concentrations are with certainty caused by the neuronal consequences of SE or whether they are also contributed, at least partially, by the initial damage from CA. So far there have been explorative and pilot studies to quantify the levels of sNfL after single seizures that seem to show only

subtle postictal peaks of NfL concentrations (Nass et al. 2021b). However, sNfL could discriminate between patients with a single seizure and patients with a later diagnosis of epilepsy or post stroke epilepsy as shown in a small prospective observational case series of 62 adults from Sweden (Eriksson et al. 2021). In a Swedish study of adult patient with anterior ischemic strokes, sNfL concentrations after thrombectomy being above the median of the entire cohort was found to be associated with the development of post stroke epilepsy (Eriksson et al. 2020). These studies underscore the use of sNfL for the identification of marked neuronal damage in patients with repetitive seizures and the diagnosis of Epilepsy. The data to further strengthen the assumption that seizures are causally linked to increased NfL levels and additional brain injury is still pending. Interestingly, according to a case-control study of patients with preeclampsia, sNfL levels were elevated even without clinical or radiological indication of neurological damage (Andersson et al. 2021).

Neuroinflammatory Conditions of the CNS

sNfL is a well-known biomarker in multiple sclerosis, an inflammatory, demyelinating disease of the CNS. However, as patients are rarely transferred to the ICU due to multiple sclerosis, a further discussion of these studies is beyond the scope of this chapter.

In contrast to multiple sclerosis, little is known regarding sNfL levels in more fulminant variants, such as the acute disseminated encephalomyelitis (ADEM).

Concerning autoimmune encephalitis and autoimmune syndromes, one study showed an increase of CSF-NfL concentrations in both paraneoplastic and non-paraneoplastic autoimmune neurologic syndromes (Constantinescu et al. 2017). In autoimmune encephalitides, the type of antibody might play a significant role regarding the increase of sNfl levels (Day et al. 2021). A primarily receptor binding antibody that leads to the integration of the receptor and therefore to a primary functional mechanism leading to neurologic deficits might not lead to an axonal damage at disease onset per se, but later in the course of the disease. Therefore, different patterns regarding the evolution of sNfL levels might evolve over time. In concordance to this theory, Mariotto et al. found high sNfL levels in patients with autoimmune encephalopathy independent of the CNS inflammation profile or cerebral MRI studies. While in a retrospective case series of 13 patients with autoimmune-mediated epilepsy, the reason for elevated sNfL levels was found to be older age (Nass et al. 2021a), other studies point to the possible reflection of disease activity by CSF-NfL and/or sNfL levels, as they revealed decreasing concentrations with treatment (Constantinescu et al. 2017; Mariotto et al. 2019). Of note, NfL itself can function as a target for auto-antibodies, as shown in animal models with mice (Puentes et al. 2017), but respective data in humans are lacking.

Guillain-Barré Syndrome (GBS)

Guillain-Barré Syndrome (GBS) is a life-threatening neurological emergency. With its acute and often postinfectious onset with ascending paralyses reflecting a rapid immune-mediated demyelination of the peripheral nervous system it demands a fast recognition and adequate neurocritical care. While GBS primarily has a monophasic course, residual sequelae may impair daily activities with 20% of patients not being able to walk unaided after 6 months (Van Den Berg et al. 2014). The variety of symptoms, the range of disabilities, and the manifestation of the disease at every age, have an impact not only on an individual level, but also on society at large. The concentrations of CSF-NfL at the acute stage and sNfL within 5 days after symptom onset show predictive value for long-term disability and could potentially be used for decision making regarding optimal personalized treatment, including an early transferral to an intensive care unit (Altmann et al. 2020; Axelsson et al. 2018; Körtvelyessy et al. 2020). The CSF/serum ratio can help to distinguish between sNfL originating from peripheral disease and sNfL with central origin, as uncovered in a prospective case series of 21 adult patients with GBS and 19 healthy controls where low ratios indicated a disease of the peripheral nervous system, in contrast to higher levels that point toward diseases of the central nervous system (Körtvelyessy et al. 2020). In an earlier pilot study of 18 patients with GBS 9–17 years ago, a reassessment of outcome revealed that high NfL concentrations in the CSF at the acute stage of GBS predicted unfavorable long-term outcome with more prominent disability and worse quality of life (Axelsson et al. 2018). These findings were confirmed in another small study of 27 GBS patients in comparison with a control group of 22 patients with diagnoses not suggestive of any axonal damage.

Delirium, Anesthesia, and Surgery

Delirium and its complications are excessively adding to complications encountered in the ICUs. The pathomechanisms seem to be multifactorial and are not yet fully understood. A limited body of evidence indicates that neuronal damage occurs during episodes of delirium that could explain permanent cognitive decline (Rudolph and Marcantonio 2011). While biomarkers for neurodegeneration have been linked to postoperative cognitive dysfunction (Evered et al. 2016), no reliably biochemical markers have been established for delirium yet. NfL may be a potential marker for the incidence of delirium and concurrent damage and has been shown to reflect results from neurocognitive assessments in a study of 913 participants including patients with different neurodegenerative diseases and healthy controls (Olsson et al. 2019). While in principle this is of interest for all patients with delirium during intensive care, most investigations have focused on postoperative delirium. However, dementia as an important risk factor for delirium has to be looked at as a potential cofounder in this context and further studies regarding the predictive value of sNfL for the emergence of delirium in specific ICU populations and for certain outcomes are urgently needed.

The association between anesthesia, surgery, and elevated sNfL values independent of the type of surgery or anesthetic technique used has been previously demonstrated (Evered et al. 2018), but whether it is really independent stands on shaky ground.

In a prospective study including surgically treated adult patients with hip fractures, postoperative significant increases of sNfL were found as compared to preoperative measurements, regardless of whether they were demented or developed delirium (Halaas et al. 2018). However, patients with dementia had comparatively increased pre- and postoperative values. The magnitude of this increase was the same in the groups without dementia with and without delirium, but more significant in the group with both dementia and delirium. However, the delirium group included patients with preexisting delirium preoperatively and developing delirium postoperatively. When these groups were viewed individually, the group with preexisting delirium showed elevated values following surgery and the group with delirium following the operation showed a significantly larger increase of sNfL after surgery as compared to the group without delirium (Halaas et al. 2018). Thus, this study suggests that (peri-)operative stress and injury alone, and also the development of delirium, lead to neuronal damage that can be measured with sNfL, especially in pre-damaged vulnerable individuals with neurocognitive impairment. To what extent sNfL may contribute to diagnostics and prognostics in such complex scenarios remains uncertain and depends on future studies with careful designs to deal with such interactions and potential confounders.

However, the particular susceptibility of neurologically compromised patients to develop delirium was demonstrated in another study, which showed that sNfL levels increased with postoperative delirium on day 2 and 1 month after surgery and were significantly higher than in patients without delirium. Interestingly, patients with high preoperative baseline levels of sNfL were found to be at increased risk of developing delirium (Fong et al. 2020). The same study demonstrated elevated sNfL values even persisting after 1 month. Of note is also the timepoint of sNfL increase in relation to delirium onset. In a small case collection of three preoperatively neurologically unremarkable patients with cardiac surgery, sNfL levels were higher in postoperative measurements in patients who developed delirium with a latency of a few days (Saller et al. 2019). These results indicate that changes of sNfL concentrations could be used as a marker for the prediction and detection of delirium, especially when clinical examination is not fully possible in the post-surgical care with ongoing sedation and intubation. A study examining 114 adult surgical patients also demonstrated that sNfL increases on the first day after surgery in all patients, but more steeply in patients who were delirious on the first postoperative day or became delirious during the postoperative course (Casey et al. 2020). These results suggest that sNfL levels may be a promising marker for early and rapid detection of delirium and thereby may guide and optimize treatment. They further indicate that especially neurologically vulnerable patients are particularly at risk for additional neurological damage due to surgery itself and its possible consequences with the development of delirium.

Infectious Disease and Sepsis

Infectious disease affecting the CNS can be divided in directly invading infection and nervous system dysfunction without evidence of pathological infectious agents in the CNS, as seen with sepsis-associated encephalopathy. With direct infection, elevated sNfL concentrations can be easily explained by the direct alteration of axons by the infectious pathogens. In sepsis-associated encephalopathy (SAE), however, direct neuronal injury by the infectious pathogens itself is not observed by definition. The pathophysiology of SAE is not yet clearly defined, but is most likely multifactorial as a combination of alterations in BBB-function, cerebral microcirculation and metabolism, neurotransmission, and inflammatory cytokines, to name just a few suggested mechanisms. Two distinct patterns of neuroaxonal damage have been suggested from translational evidence: post-mortem samples of rat and human brains showed either signs of distinguished scattered ischemic lesions or diffuse axonal damage (Ehler et al. 2017). In another study, multifocal necrotizing encephalopathy was detected in the post-mortem brains of patients who died from septic shock (Sharshar et al. 2002). So far there have been two prospective longitudinal studies that both must be regarded as pilot studies which explore the increased levels of sNfL in patients with SAE. In a prospective, pilot observational study including 20 adult patients with septic shock and 5 patients without sepsis serving as controls, sNfL levels correlated with specific clinical appearances of SAE, structural neuronal damage on cerebral MRIs (as "white matter lesions"), and with survival (Ehler et al. 2019). As an indicator of ongoing neuronal damage, sNfL increased over time (from day 1 to 7) in patients with SAE. Another study collected sNfL concentrations shortly after SAE onset and could measure a correlation to mortality as well (Orhun et al. 2021). Nearly all patients in these last two studies subsequently developed septic shock. To which part neuronal damage has been the consequence of SAE or of the septic shock cannot be estimated at present. Formal studies concerning different types of shock and sNfL levels were not identified by the authors and are needed to clarify if NfL measurements can be used as reliable diagnostic and/or prognostic markers in such circumstances.

Coronavirus Infectious Disease (COVID)-19

In 2020, a global pandemic with a new variant of a severe acute respiratory syndrome (SARS) inducing coronavirus (SARS-CoV-2), discovered in 2019, occurred, flooding ICUs with critically ill patients, mainly suffering from acute respiratory distress syndromes (ARDS). Many neurological complications, such as prolonged delirium and encephalopathy, encephalitis and myelitis, epileptic seizures, GBS, and a frequent occurrence of critical illness associated polyneuropathies were readily observed. Since clinically typical deficits of the sense of smell were observed (Yachou et al. 2020) and neurotropic mechanisms were observed in other SARS viruses before, such a mechanism was suspected in COVID-19 as well. Due to the frequently observed mismatch of the respiratory clinic with severe

oxygenation disturbances, but no feeling of respiratory distress, an affection of the respiratory centers was postulated (Yachou et al. 2020).

Rapidly, studies were conducted and revealed increased sNfL values in adult critically ill COVID-19 patients and especially in nonsurvivors and higher levels as compared to other non-COVID-19 ARDS patients (Aamodt et al. 2021; Kanberg et al. 2020; Sutter et al. 2021). Other theories attribute a crucial role to the pronounced cytokine storm (Pilotto et al. 2021), comparable to the physical reaction in sepsis and the disease-specific altered coagulation. It is not to be neglected that these patients are severely ill with ventilation times of days to sometimes months and often develop acute renal insufficiencies with consequent need for renal replacement and potential cardiac complications. Consecutively, these patients required adequate analgosedation and circulatory support and were not yet exposed to established drug treatments due to the new occurrence of the disease. Arguably, one study group found elevated sNfL levels in all hospitalized COVID-19 patients regardless of neurological manifestation (Paterson et al. 2021). They did also not find the normal and pre-described correlation of NfL of the serum and the CSF, which was mainly driven by the cases with altered peripheral nerve systems. In fact another study demonstrated elevated sNfL in critically ill COVID-patients developing critical illness polyneuropathy compared to patients not developing critical illness associated polyneuropathy (Frithiof et al. 2021). COVID-19 patients generally presented with a more severe condition (e.g., longer ICU stays, prolonged and more frequent mechanical ventilation and more thrombotic events) as compared to a general ICU population. To a significant part, increased sNfL levels may reflect peripheral neuronal damage related to serve illness as opposed to direct neurotropic mechanism that damage the central nervous system.

In summary, Covid-19 represents the prime example of the difficulties encountered in dealing with sNfL levels in ICU populations. Increased sNfL levels are often, reliably and repetitively detected, but assignment to specific causes without looking at the patient's entire clinical picture is often impossible.

Applications to Prognosis

As seen with the critical illnesses discussed above, sNfL concentrations can indicate neuroaxonal damage but are not allocated to a specific causal mechanism and can be influenced by numerous factors affecting the central and/or peripheral nervous system that need to be considered (Fig. 1). Studies with well-characterized and carefully predefined populations need to be performed to investigate the respective share of individual factors. Longitudinal measurements could at least help characterize the damage into acute, recurrent, progressive, or persistent chronic damage. However, for applicability in prognostic assessments, disease non-specificity does not seem to be a major hurdle. In addition to the etiology of the primary damage, all other factors, such as the preexisting neurological conditions, concomitant diseases, cardiovascular factors, and complications occurring during the course of treatment

and the disease, play an important role in assessing the prognosis and seem to be reflected in cumulative increases of sNfL concentrations.

Consecutively, in a recent small prospective study on 35 adult ICU patients aimed to investigate whether sNfL levels are a potential biomarkers for prediction of outcome during intensive care, a correlation between sNfL levels on ICU admission and outcome at a median follow-up period of 26 days was uncovered (Fisse et al. 2021). According to these authors, this is especially applicable for patients with an altered CNS.

However, one factor must also be well considered in such a prognostic evaluation. NfL levels seem to be proportional to neuroaxonal damage, but the amount of damage is in specific clinical situations not the main indicator for functionality in daily activities and therefore the quality of life. A small strategically located ischemic stroke can be more devastating than a relatively large lesion in a more silent, e.g., the frontal brain area. Caution for prognostication is therefore especially given in disease with focal lesions. Not only the amount of damage but also the location is of utmost importance.

Conclusions

The conditions discussed above are good examples of where sNfL could be used in future everyday clinical practice. It has great potential to improve prognostic tools, such as, for example, in patients with persistent altered consciousness or coma after CA, can identify abundant neuronal damage potentially without the evidence of morphologic signs on CT or when advanced imaging techniques are not available, can possibly be used to prove ongoing neuronal damage after an initial acute event, as seen, for example, with SE, and could finally be used as a reliable marker for therapy responses.

With its long half-life and neuron specificity, reflecting combined neurological damage triggered by multiple mechanisms of injury, sNfL is a promising biomarker that may be a future piece of the daily clinical puzzles regarding prognostic assessments.

However, while promising progress has been made in recent years regarding the potential applications of sNfL, we are still in the early stages and more research is urgently needed to incorporate NfL into routine clinical practice in critically ill patients treated in the ICUs.

Mini-Dictionary of Terms

- ***Outcome prediction***. The attempt to predict whether a patient will survive or not and what disabilities will prevail at a given time after an illness or a life-threatening event.
- ***Confounder***. A confounder is a variable that influences both the dependent variable and independent variable, causing a spurious association. In other

words, a confounder is a third (often unmeasured) variable that influences both the supposed cause and the supposed effect. This is why confounders must be considered and excluded to ensure results regarding claimed associations are valid.

- ***Intensive care unit***. A specialized unit in a hospital for patients with life-threatening illnesses dependent of life supporting measures or in need of close surveillance.
- ***Neurological dysfunction***. A collective term encompassing cognitive or physical impairment as a consequence of a damaged neurological system.
- ***Serum neurofilament light chain***. A biomarker specific for neuroaxonal damage.

Key Facts of Serum Neurofilament Light Chain Levels

Neurofilament light chain is biomarker first reported to be purified in 1989. Studies related to its use as a diagnostic and/or prognostic marker in critically ill patients have multiplied in recent years.

Neurofilament light chains are cytoskeletal components found in the cytoplasm of neurons, especially their axons.

As the main function, neurofilament light chain promotes structural stability, promoting axonal growth, contributing to synaptic function of neurons.

Neurofilament light chain is found in central and in peripheral neurons and no other human cells constituting a major advantage compared to other biochemical markers for neuronal injury, such as, for example, the neuron-specific enolase.

Current in vivo studies point to a half-life of up to 3 weeks. The serum or cerebrospinal fluid concentration in humans largely depends on physiological turnover and neuronal damage, which may be acute, recurrent, or chronic in nature.

Summary Points

- Neurofilament light chain in the blood or cerebrospinal fluid is protein of the neuronal cytoskeleton and specific for neuroaxonal damage, but is not disease specific.
- When interpreting serum neurofilament light chain levels in clinical practice, the natural history of the disease and the temporal profile of neurofilament light chain must be considered.
- As confounding or neurofilament light chain influencing factors many conditions may be considered: age, other neurological comorbidities, renal function, and other non-neurological comorbidities, like cardiovascular risk factors, treatment-related factors, and many more.
- Serum levels of neurofilament light chain seem not to be influenced by hemolysis and therefor have an advantage over neuron-specific enolase in critically ill patients.

- The concentrations of neurofilament light chain in the blood or the cerebrospinal fluid have been evaluated in many life-threatening diseases treated in intensive care units. Among these are ischemic or hemorrhagic strokes, subarachnoid hemorrhage, traumatic brain injuries, epilepsies, Guillain-Barré syndrome, hypoxic-ischemic encephalopathy, delirium and postoperative states, sepsis, and COVID-19.
- The concentration of neurofilament light chain in the blood or the cerebrospinal fluid has great potential as a prognostic marker and could add value to clinical risk scores once added as an integral component.

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S100B As a Biomarker in Traumatic Brain Injury

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Contents

Abstract

Traumatic brain injury (TBI) is a leading cause of death and disability worldwide and constitutes a major public health concern. Over the recent years, there is a change in the epidemiology pattern of TBI, with falls being the most frequent. Biomarkers that have been used in TBI have been collected from a diverse range of biofluids and tissue samples such as blood, cerebrospinal fluid, urine, saliva, and cerebral microdialysis. Several biomarkers have been investigated. Herewith, we review the role of S100B in predicting positive CT findings in mild TBI, the possible prognostic value, and its role in predicting secondary injury development and treatment efficacy in TBI.

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Keywords

S100B · Traumatic brain injury · CT · Biomarker · Prognosis · Outcome

Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability worldwide and constitutes a major public health concern. Based on Glasgow Coma Scale (GCS) score, TBI is categorized as mild, moderate, and severe. Mild TBI is the most frequent form accounting for approximately 70% of cases. Over the last years, there is a change in the epidemiology pattern of TBI, with falls, mainly in the elderly, being the most common, followed by road accidents (Alexiou et al. 2020). In 2018 in the United States, there were approximately 223.050 TBI-related hospitalizations, and in 2019 there were 60.611 TBI-related deaths. Hospitalizations and deaths were more common in people aged 75 years and older and almost two times more common in males. Regarding children, there were 16.480 TBI-related hospitalizations in 2018 and 2.476 TBI-related deaths in 2019 (Centers for Disease Control and Prevention 2021a). Furthermore, TBI has several long-term consequences such as cognitive, sensory, behavioral, emotional, and physical impairments that ultimately affect quality of life and increase long-term mortality (Bramlett and Dietrich 2015). Even in mild TBI, 13.5% of patients had poor 1-year cognitive outcome (Christman Schneider et al. 2022).

Upon arrival in the emergency department (ED), patient's initial assessment by the trauma team is of key importance. Noncontrast CT represents the examination of choice, in the majority of cases, and is indicated in moderate and severe TBI. Regarding mild TBI, there is an intense debate over the indications that require a CT scan, considering the ionizing radiation the patient receives, the excessive cost, resource constraints, and the waiting hours at the ER (Gallaher et al. 2022; Alexiou et al. 2021). Therefore, the next important step is the identification of biomarkers that would indicate the presence of an underlying pathology and predict the need for a CT scan use in mild TBI. Other important fields that biomarkers would have a meaning are the assessment of patient's prognosis, monitoring response to treatment, and predicting coagulopathy occurrence (Alexiou et al. 2020; Gradisek et al. 2021).

Biomarkers

Biomarkers that have been used in TBI have been collected from a diverse range of biofluids and tissue samples such as blood, cerebrospinal fluid (CSF), urine, saliva, and cerebral microdialysis. Several biomarkers have been proposed such as calcium channel-binding protein S100 subunit beta (S100B), glial fibrillary acidic protein (GFAP), ubiquitin c-terminal hydrolase L1 (UCH-L1), and neuron-specific enolase (NSE) (Frankel et al. 2019). In order to enter clinical use, a biomarker should be easily evaluated, widely available, of low cost and of a high accuracy (Table 1)

Table 1 TBI biomarkers

Biomarker	Injury involved
S100B	Glial cells
Neuron-specific enolase (NSE)	Neurons
Glial fibrillary acidic protein (GFAP)	Astrocytes
Ubiquitin c-terminal hydrolase L1 (UCH-L1)	Neurons
Cleaved tau	Neurons
Neurofilament-light (NFL)	Axons
Glucose	Stress, inflammation
Neutrophil-to-lymphocyte ratio (NLR)	Inflammation

(Frankel et al. 2019). Cerebrospinal fluid sampling has several risks and constitutes an impractical method; saliva-based biomarkers although promising are still under evaluation (Yeung et al. 2021; Hicks et al. 2020), whereas blood-based biomarkers have been the most widely studied.

S100B

Biomarkers indicating neuronal, axonal, or astroglial damage have been evaluated in TBI patients. S100B is a Ca2 + −binding protein and belongs to S100 family of proteins. In the nervous system, S100B is primarily expressed in astrocytes and therefore is released following astroglial injury. Schwann cells also express S100B, and in lesser degree S100B can be found in other cells such as chondrocytes, melanocytes, bone marrow, and lymphocytes (Thelin et al. 2017). Renal excretion represents the major pathway for the elimination of S100B. A recent study investigated in 80 TBI patients, admitted to intensive care units (ICU), the concentration of 107 biomarkers in blood. These biomarkers were proteins related to inflammation, innate immunity, TBI, and central nervous system. Only 6 biomarkers showed to have improved prognostic accuracy at ICU admission, among them the S100B (Alexiou et al. 2021). Several commercially available immunoassays exist for the evaluation of serum S100B levels.

Predicting Positive CT Findings in Mild TBI

In 2014 in the United States, there were 2.9 million emergency room visits, hospitalizations, and deaths related to TBI (Centers for Disease Control and Prevention 2021b). Mild TBI is the most frequent (about 70%) form of TBI. Of these patients, about 5% will have positive CT findings and only 0.5% will require a neurosurgical intervention (Haydel et al. 2000). Thus, identification of patients of need of CT would be of paramount importance, to safely discharge the majority of mild TBI patients and reduce both radiation exposure and costs. Especially in children, CT is associated with increased lifetime risk of malignancy. A recent survey showed that

Table 2 Studies investigating the role of S100B for the detection of abnormal CT in mild TBI. The sensitivity, specificity, and cutoff value are presented

Author/year	No of patients	Population	Sensitivity	Specificity	Cutoff value
Jones et al. (2020)	679	Adults	84.6%	33.6%	0.10 μg/L
Çevik et al. (2019)	48	Mixed	95.8%	62.5%	0.47 μg/L
Biberthaler et al. (2006)	1309	Adults	99%	30%	0.10 μg/L
Egea-Guerrero et al. (2018)	260	Adults	95.5%	30.7%	0.10 μg/L
Asadollahi et al. (2016)	158	Adults	94.9% 98.7%	35.4% 39.2%	0.115 μg/L (3 h) 0.210 μg/L (6 h)
Manzano et al. (2016)	73	Peds	95% 100% (>2 yrs)	34% 37%	0.14 μg/L
Papa et al. (2014)	397	Adults	100%	5%	0.020 ng/mL
Calcagnile et al. (2013)	351	Adults	100%	30%	0.10 μg/L
Bazarian et al. (2013)	787	*Mixed*	100% 88.9%	12.3% 31.7%	0.060 μg/L 0.097 μg/L
Wolf et al. (2013)	107	Adults	33% 72%	91% 37%	0.48 mg/L 0.105 μg/L
Müller et al. (2011)	233	Adults	86.4%	12.2%	0.105 μg/L
Castellani et al. (2009)	109	Adults	100%	42%	0.16 μg/L
Müller et al. (2007)	226	Adults	95%	31%	0.10 μg/L
Bazarian et al. (2006)	96	*Mixed*	90%	n/a	0.15 μg/dL
Lagerstedt et al. (2018a)	132	Adults	100%	11%	0.06 ug/L
Ingebrigtsen et al. (2000)	182	Adults	90%	65%	0.2 μg/L
Lagerstedt et al. (2018a)	207	Adults	100%	18.4%	0.072 pg/mL
Lagerstedt et al. (2017)	261	Mixed	100%	9%	0.052 μg/L

CT is the main modality used in pediatric head trauma and shielding, or a specific pediatric imaging protocol is used only in about half of cases; thus, there is room for improvement (Argyropoulou et al. 2020). Although MRI was readily available in 68.6% of cases, it was used for head trauma imaging in about one-third of cases (Argyropoulou et al. 2020).

The identification of biomarkers that would predict which patient with mild TBI is of need of CT is of paramount importance. Several studies have investigated the role of S100B toward this scope (Table 2) (Jones et al. 2020; Biberthaler et al. 2006;

Egea-Guerrero et al. 2018; Asadollahi et al. 2016; Manzano et al. 2016; Papa et al. 2014; Calcagnile et al. 2013; Bazarian et al. 2013; Wolf et al. 2013; Müller et al. 2011; Castellani et al. 2009; Müller et al. 2007; Bazarian et al. 2006; Lagerstedt et al. 2017, 2018a, b; Ingebrigtsen et al. 2000). The most common cutoff value used is 0.10 μg/L. In Scandinavian guidelines for the initial management of minimal, mild, and moderate head injuries in adults, the S100B assessment has been incorporated. In detail, in TBI patients presented with a GCS score of 14 and no risk factors such as anticoagulant therapy, or patients with GCS 15 with loss of consciousness or repeated vomiting and without risk factors, if S100B is less than 0.10 μg/l, then the patient can be discharged without a CT (Undén et al. 2013). When S100B was compared to two clinical decision rules, namely the Canadian CT Head Rule and the New Orleans Criteria, for predicting traumatic intracranial injuries, S100B outperformed both clinical decision rules. Using S100B, more than one-third of patients could avoid a CT. The negative predictive value was 97.3%. When S100B was incorporated in both decision rules, there was a significant increase in the diagnostic performance. S100B should be evaluated within 6 hours after trauma (Jones et al. 2020).

Apart from S100B, other biomarkers have been investigated toward this scope such as GFAP, neutrophil to lymphocyte ratio, and admission glucose levels (Alexiou et al. 2020; Alexiou et al. 2019). GFAP has been approved by Food and Drug Administration to evaluate the need for head CT within 12 h after mild TBI. A recent study performed a head to head comparison, between S100B and GFAP in 1359 patients with TBI of all severities, to predict intracranial abnormalities on CT within 1d postinjury. Both biomarkers exhibited higher serum level in patients with positive CT findings. Nevertheless, using receiver-operating characteristic curves, GFAP outperformed S100B (GFAP AUC – 0.85, S100B AUC – 0.67) (Okonkwo et al. 2020). Apart from blood, S100B levels have been evaluated in the urine after mild TBI. Although a noninvasive procedure, the results showed that urine S100B levels were not useful in the emergency department during the acute phase after a mild TBI (Le Sage et al. 2019).

Predicting Outcome

Predicting clinical outcome of patients with moderate or severe TBI is of great importance. S100B, when assessed within 4 h of injury, could predict patients' outcome at 6 months, as assessed by Glasgow Outcome Scale-Extended (GOS-E). S100B and GFAP could predict outcome better than age, sex, GCS, and CT findings (Frankel et al. 2019). In a study of 265 TBI patients of all severities, there was a strong correlation between S100B levels in serum and outcome. In univariate analysis, S100B showed stronger relation to outcome than age, pupil response, GCS, and CT findings. The best predictive value had samples taken between 12 and 36 h postinjury (Thelin et al. 2013).

A recent study investigated if there is a correlation between certain genetic polymorphisms related to possible blood biomarkers and global neurological

outcome in TBI patients. For the S100B protein, after controlling for age, sex, and GCS at presentation, two SNPs (rs1051169 or rs9984765) were significantly associated with GOS score. Especially, the variant C allele of rs1051169 was found to be significant correlated with increased likelihood of having a better outcome at 3, 6, 12, and 24 months postinjury. On the contrary, the presence of variant C allele for rs9984765 was associated with poor GOS (1–2) outcome at 6 months post-trauma (Osier et al. 2018). Regarding pediatric head trauma, serum concentrations of S100B, NSE, and IL-6 were measured within 6 hours postinjury and after 1 week and were correlated with patients' outcome at 6 months. The median serum S100B level at presentation was 178.12, and after 1 week there was a significant decrease to 40.86 pg/mL. The serum S100B and NSE levels both at admission and 1 week post-trauma were significantly higher in the poor GCS group than in the favorable GCS group. No correlation was found for IL-6 levels (Park and Hwang 2018). Using a resting state functional MRI in TBI patients, there was a decrease in intrinsic brain connectivity that correlated significantly with the highest S100B protein levels in the acute phase of trauma (Thompson et al. 2016).

Predict Secondary Injury Development and Treatment Efficacy

S100B serum levels, assessed more than 48 h after TBI, were found to secondary increase in TBI patients with secondary radiological abnormalities present on CT or MRI. A cutoff value of $\geq$0.05 μg/L had 80% and 89% specificity, and a cutoff value of $\geq$0.5 μg/L had 16% sensitivity and 98% specificity to detect secondary radiological findings. Moreover, the secondary increase of S100B influenced treatment and diagnosis in 21% of the cases (Raabe et al. 2004). There are findings that suggest a correlation between ICP and serum S100B levels. Serial measurements of S100B may predict the increase of intracranial pressure and mortality after acute brain injury (Rezaei et al. 2017). Development of coagulopathy after TBI is a known event and is more frequent in severe TBI. Coagulopathy occurrence is a risk factor for secondary brain damage and has been associated with unfavorable outcome (Wada et al. 2021). A meta-analysis that included 22 studies showed that the pooled proportion of TBI patients with coagulopathy was 35.2% (Epstein et al. 2014). Assessment of S100B value for the prediction of coagulopathy occurrence would be interesting.

Other Biomarkers

The neutrophil-to-lymphocyte ratio (NLR) has been proven a useful and cost-effective biomarker to evaluate prognosis and the need for cranial computed tomography in patients with mild TBI. In a retrospective study of 130 adult patients

who presented with mild TBI and underwent a brain CT, 74 patients had positive CT-findings. Patients with abnormal CT-findings had significant higher NLR-levels than patients with normal CT. Receiver-operating characteristic curve analysis found a 2.5 value as best predicting patients with positive CT findings with 78.1% sensitivity and 63% specificity (Alexiou et al. 2020). NLR also proved useful for prediction of coagulopathy occurrence in TBI patients. In a retrospective study that included 173 patients, 37 patients had severe TBI, 19 moderate, and 117 mild TBI, and 40 patients (23.1%) developed coagulopathy. Their mean NLR was 7.5 ± 6.7. An NLR cutoff value of 4.2 could predict patients with coagulopathy with 87.5% sensitivity and 52.9% specificity (Alexiou et al. 2022). TBI has also been associated with increased blood glucose levels. Patients with mild TBI and positive CT findings show higher glucose levels than patients with normal CT. Serum glucose levels higher than 120 mg dl-1 at presentation in the emergency department have 74.4% sensitivity and 90.7% specificity for the detection of patients with abnormal CT after mild TBI (Alexiou et al. 2019). Furthermore, patients with a serum glucose of 151 mg dl(−1) or higher more often developed coagulopathy (sensitivity 91.5%, specificity 87.5%) (Alexiou et al. 2014).

Conclusion

Although S100B is most prominent in astrocytes, it can be found in several other cells such as osteocytes and adipocytes. Thus, S100B has been found elevated in trauma patient without TBI. This might be a limitation. Nevertheless, S100B is a useful biomarker in TBI patients for detecting patients of need for CT in mild TBI, predicting outcome in moderate to severe TBI and validating treatment effect.

Applications to Prognosis, Other Diseases, or Conditions

In this study, we reviewed S100B as a potential biomarker in TBI. The evidence so far showed that S100B levels at presentation in the emergency department correlate with patients' outcome. Increased S100B levels are associated with worse outcome. Furthermore, S100B predicts the outcome better than age, sex, GCS, and CT findings. Apart from that, in mild TBI which is the most frequent form of TBI, about 5% of patients will have positive CT findings and only 0.5% will require a neurosurgical intervention. S100B can detect patients that require a CT with high sensitivity and specificity. S100B should be evaluated within 6 hours of the time of trauma. S100B serum levels, assessed more than 48 h after TBI, were found to secondary increase in TBI patients with secondary radiological abnormalities present on CT or MRI. Moreover, the secondary increase of S100B influenced treatment and diagnosis in one-fifth of patients.

Key Facts

- Traumatic brain injury (TBI) is a leading cause of death and disability worldwide.
- Biomarkers that have been used in TBI have been collected from a diverse range of biofluids and tissue samples such as blood, cerebrospinal fluid, urine, saliva, and cerebral microdialysis.
- S100B is a Ca2 + −binding protein and belongs to S100 family of proteins. In the nervous system, S100B is primarily expressed in astrocytes and therefore is released following astroglial injury.
- Using a cutoff value of 0.10 μg/L, S100B can predict patients with mild TBI that require a CT scan.
- S100B, when assessed within 4 h of injury, could predict patients' outcome at 6 months, as assessed by Glasgow Outcome Scale-Extended.

Mini Dictionary of Terms

- Traumatic brain injury. A leading cause of death and disability worldwide.
- Biomarkers. Can be evaluated in blood, cerebrospinal fluid, urine, saliva, and cerebral microdialysis.
- S100B is a Ca2 + −binding protein and belongs to S100 family of proteins.
- Canadian CT Head Rule and the New Orleans Criteria. Clinical decision rules for the prediction of traumatic intracranial injuries.
- Glasgow Outcome Scale-Extended. An outcome instrument to categorize global outcomes of TBI survivors.

Summary Points

- Blood biomarkers have been utilized for the detection of mild traumatic brain injury (TBI) patients that require a CT.
- A S100B cutoff value of 0.10 μg/L has been used for the discrimination of mild TBI patients with abnormal CT findings.
- S100B and GFAP can predict TBI outcome better than age, sex, GCS, and CT findings.
- S100B serum levels may secondary increase in TBI patients with secondary radiological abnormalities present on CT or MRI.
- S100B have been found elevated in trauma patients without TBI.

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Biomarkers of Sepsis and a Focus on PCSK9

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Francesca Innocenti, Valentina Di Maria, Alice Poggi, and Riccardo Pini

Contents

Abstract

Sepsis is a time-dependent disease and a prompt diagnosis and treatment may improve the outcome. In the actuality, we do not have a reliable biomarker to support the early identification. Tens of mediators are released during the abnormal immune response that characterizes sepsis and several of them have been tested for this role. In the following paragraphs, we will report an overview of the most studied biomarkers, grouped by their role in the inflammatory activation during sepsis.

Thereafter, we will focus on the involvement of the lipid metabolism in the immune response. Lipids are one of the main components of the pathogens' cell wall, which is recognized by innate immune response. Besides, endogenous lipoproteins are key actors in the removal of these bacterial products from the

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bloodstream. Proprotein convertase subtilisin kexin type 9 regulates lipid metabolism in healthy subjects and during sepsis, and it could become a biomarker as well as a therapeutic target.

Keywords

Biomarkers · Sepsis · Immune response · Cytokines · Infections · Organ damage · Diagnosis · Prognosis · Lipid metabolism · PCSK9

Abbreviations

ACE2	Angiotensin-converting enzyme 2
AM	Adrenomedullin
Ang-2	Angiopoietin-2
APC	Activated protein C
ARDS	Acute respiratory distress syndrome
AT	Antithrombin
AUC	Area under curve
CK	Chemokines
CPR	C-Reactive Protein
CRAC	Cholesterol Recognition Amino-Acid Consensus
CSFs	Colony-Stimulating Factors
DAMPs	Damage-Associated Molecular Patterns
DC	Dendritic Cells
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic Acid
EGF-A first	Epidermal Growth Factor-like
ELAM-1/CD62E	Endothelial-Leukocyte Adhesion Molecule 1
Et-1	Endothelin
FDA	Food and drug administration
FGF21	Fibroblast growth factor 21
GalNac	N-acetylgalactosamine
Gas6	Growth arrest-specific protein 6
G-CSF	Granulocyte Colony-Stimulating Factors
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factors
GOF	Gain-of-function
GWAS	Genome Wide Association Studies
HBP	Heparin-binding protein
HDL	High-density lipoprotein
HFN1α	Hepatocyte Nuclear Factor 1α
HFNC	High flow nasal cannula
HMGB1	High-Mobility Group Box 1
IaIp	Inter-alpha Inhibitor protein
ICAM-1/CD54	Intercellular Adhesion Molecule-1
ICU	Intensive Care Unit
IFNs	Interferons

IL	Interleukin
INR	International Normalized Ratio
ISTH	International Society of Thrombosis and Hemostasis
LBP	Lipopolysaccharide-binding protein
LDL	Low density lipoprotein
LOF	Loss-of-function
LPS	Lipopolysaccharide
mABs	Monoclonal antibodies
MCF	Monocyte Chemotactic Factor
M-CSF	Macrophage Colony-Stimulating Factors
MIP	Macrophage Inflammatory Protein
miRNAs	microRNAs
MOF	Multiple Organ Failure
mRNA	Ribonucleic acid messenger
NASH	Nonalcoholic steatohepatitis
NC	Nasal cannula
NIV	Noninvasive ventilation
NK	Natural Killer
NLR	Nod-Like Receptors
NLRP3	Pyrin Domain-Containing Protein 3
PAI-1	Plasminogen Activator Inhibitor-1
PAMPs	Pathogen-Associated Molecular Patterns
PCSK9	Proprotein convertase subtilisin kexin type 9
PCT	Procalcitonin
PRR	Pattern-Recognition Receptors
PTX3	Pentraxin 3
RCT	Reverse cholesterol transport
RISC	RNA-induced silencing complex
SAA	Serum Amyloid A
SARS	Severe acute respiratory syndrome
Sars-Cov 2	Severe acute respiratory syndrome coronavirus 2
siRNA	Small Interfering RNA
SIRS	Systemic Inflammatory Response Syndrome
SOFA	Sequential Organ Failure Assessment
SREBR-2	Sterol Regulatory Element-Binding Protein 2
sTREM	Soluble triggering receptor expressed on myeloid cells
suPAR 17	Soluble urokinase plasminogen activator receptor
TAM	Tyro3, Axl, and Mer (tyrosine kinase family)
TF	Tissue Factor
Th cells	Helper T cell
TIR	Toll-Interleukin-1 Receptor
TLR	Toll-Like Receptors
TM	Thrombomodulin
TNF	Tumor Necrosis Factor
tPA	Tissue plasminogen activator

VCAM-1/CD106	Vascular cell adhesion molecule 1
VEGF	Vascular endothelial growth factor
VLDL	Very low density lipoprotein
VLPs	Virus-like particles
VWF	Von Willebrand factor
WHO	World Health Organization

Introduction

Sepsis represents a leading cause of in-hospital mortality and, despite declining age-standardized incidence and mortality, it remains a major cause of health loss worldwide (Rudd et al. 2020). The early recognition of the syndrome and a prompt treatment may improve prognosis, but several factors make the timely diagnosis difficult. In fact, we lack something like the chest pain for acute coronary syndrome, because sepsis may arise from different organs and at presentation chief complaints vary from dyspnea to abdominal pain, stranguria, or even nothing more than fever or reduced level of consciousness. The time lapse between the appearance of symptoms and the beginning of medical treatment may vary significantly, especially for patients who develop the disease outside the hospital. They often begin the treatment at home, before understanding the real severity of the illness. Besides the absence of specific symptoms, we lack the sepsis "troponin," a feasible and reliable biomarker to confirm the diagnosis. During sepsis, in the presence of an abnormal response to the infection, multiple pathways are activated in the course of the illness, and the released mediators remain in the bloodstream for different periods, with variable kinetic. The same biomarker, measured at different time intervals from the beginning of the disease, will present distinct values, and establishing a cutoff to confirm the diagnosis of sepsis becomes really difficult. In 2010, in a review about sepsis biomarkers, Pierrakos and coll. Reported that up to 178 substances had been tested for this role, but they concluded that none of them had an acceptable specificity or sensitivity to be routinely employed in the clinical practice (Pierrakos and Vincent 2010).

In the following paragraphs, we will present an overview of biomarkers evaluated for their diagnostic and prognostic performance in septic patients. We will group them based on their role in the complex network of the inflammatory activation during sepsis. We will then focus on mediators linked to lipid metabolism. In the final part, we will report possible future approaches to this issue, in order to overcome the limitations encountered in recent researches.

Biomarkers in Sepsis: An Overview

The Biomarkers Definitions Working Group defined a biomarker as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic

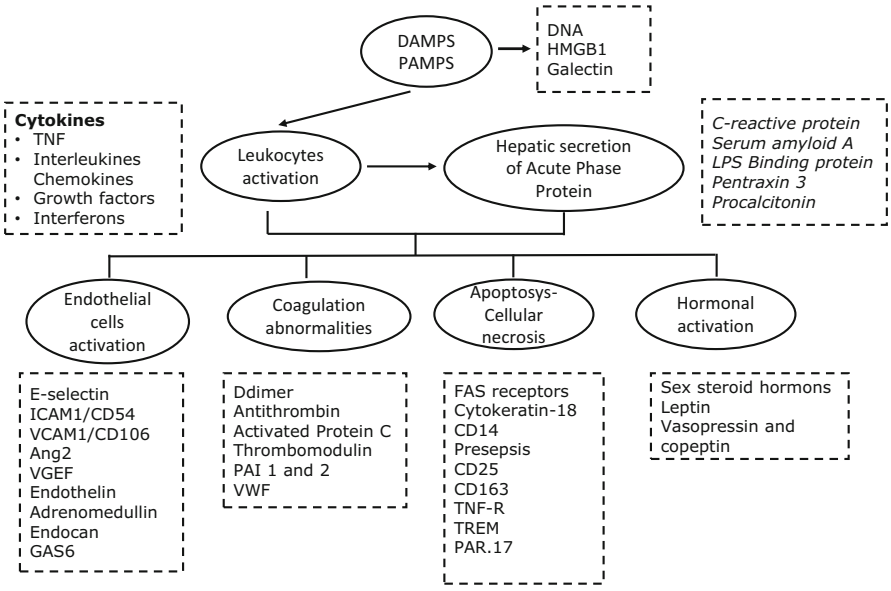

Fig. 1 Candidate biomarkers in sepsis, grouped by their role in the immune response

intervention" (Douglas and Roussel 2016). Every step in the development of sepsis is characterized by the release of several substances in the bloodstream, which can be used as biomarkers (Fig. 1).

During the interaction between the pathogen and the host, immune cells are activated by two categories of mediators: the pathogen-associated molecular patterns (PAMPs), released by pathogens themselves, and damage-associated molecular patterns (DAMPs). These are alarmins released by cells after their necrosis or by injured tissues, which behave as endogenous danger signals.

Two main substances are part of this group:

- Circulating deoxyribonucleic acid (DNA).
- High-mobility Group Box 1 (HMGB1): nuclear factor bound to DNA, secreted by activated monocytes and macrophages. It is passively released by necrotic or damaged cells and it further triggers inflammation (Angus et al. 2007).

In septic patients, plasma levels of DNA increase more than in critically ill patients for other reasons (Dwivedi et al. 2012). On the other hand, HMGB1 remains high up to 1 week. For both mediators, increased levels were associated with an adverse outcome.

Galectin-3 is a 30 kDa intracellular lectin, widely expressed in human tissues, including all types of immune cells. It is involved in several functions, including cell development and apoptosis, pre-mRNA splicing, as well as inflammation, fibrosis, and host defense and can be considered an alarmin. It can be passively released from

damaged cells, even in the absence of necrosis. Circulating galectin-3 concentrations are increased in patients with sepsis, higher in patients with septic shock and in nonsurvivors than in those with good prognosis (ten Oever et al. 2013).

Circulating PAMPs and DAMPS activate Pattern-Recognition Receptors (PRR), which trigger a cascade of activation/phosphorylation, inducing a stereotyped inflammatory response. Several types of PRR are known in humans and Toll-like receptors (TLRs) are the most studied. They have a leucine-rich repeat extracellular domain and an intracellular Toll-interleukin-1 receptor (TIR) domain and their activation determines the secretion of several pro-inflammatory cytokines. A different type of PRR with an emerging role in recent years is the Nod-like receptor (NLR), specifically the pyrin domain-containing 3 (NLRP3) member. NLRP3, combined with other structures, generates a complex called inflammasome that can convert pro-inflammatory procaspases into their mature form, with consequent release of important proinflammatory cytokines (Danielski et al. 2020).

Cytokines belong to a huge family of small proteins or glycoproteins, produced for the most part by helper T cells (Th cells) and macrophages. Virtually absent in the healthy population, cytokines perform the function of coordinating an effective immune response, a mechanism by which lymphocytes, inflammatory cells, and hematopoietic cells can communicate with each other, influencing both the innate and adaptive immune response (Chousterman et al. 2017).

After binding to specific receptors on various types of cells, cytokines induce activation, proliferation, or migration of target cells.

Cytokines can be divided into several categories: interleukins, chemokines, interferons, tumor necrosis factor, and growth factors. *Tumor necrosis factor* (TNF) is one of the main orchestrators of inflammation. It was the first cytokine to be identified in the blood of septic patients (Carswell et al. 1975). This discovery determined a shift in the traditional concept of the pathophysiology of the most severe consequences of infections. In fact, different types of infections cause the release of cytotoxic substances, which are the real cause of tissue damage and multiorgan failure. TNF proved to be able to induce shock and tissue injury (Tracey et al. 1986) and its neutralization by specific antibodies reduced the lethality of the infection in the experimental setting (Tracey et al. 1987). These data were not confirmed in humans, but several authors reported a correlation between high levels of TNF and a poor outcome.

Interleukins (IL) are the most important group of cytokines released during infectious processes. They encompass a wide group of proteins mainly secreted by leukocytes and endothelial cells, which contribute to cell signaling and promote the activation, proliferation, and death of immune cells. They include two main subgroups: pro- and anti-inflammatory interleukins. Pro-inflammatory interleukins are responsible for cell activation, tissue damage, and necrosis while anti-inflammatory interleukins aim to dampen and finally reverse the inflammatory process (Dinarello 2007). Many of them have been evaluated as potential biomarkers during sepsis with

Table 1 Pro- and anti-inflammatory cytokines

	Characteristics	Factors inducing secretion	Main actions
Pro-inflammatory cytokines			
IL-1β	Members of the IL-1 family (11 genes)	Inflammasome	• Activation of the JNK and p38-MAPK pathways. • Stimulation of NFκB. • Induction of the synthesis of various inflammatory genes such as IL-6, IL-8, MCP-1, COX-2, IκBα, IL-1α, IL-1β, and MKP-1.
IL-6	Not a single protein but includes a family of molecules Like IL-11, oncostatin M, ciliary neurotrophic factor, or cardiotrophin-like cytokine	Tissue macrophages	• Activation of the complement pathway. • Capillary leakage. • Induce the secretion of anti-inflammatory IL-10.
IL12	Heterodimeric structure including two subunits	Dendritic cells, macrophages, and lymphoblastoid Cells	• Induction of the differentiation of naïve T cells into type 1 helper T cells (TH1). Activation of NK cells, with consequent production of a high amount of INFγ.
IL-18	Member of the IL-1 superfamily	Formed from a precursor form Which is processed by caspase-1 following the inflammasome Activation	• Stimulation of the production of IFN-γ.
Anti-inflammatory cytokines			
IL-1RA	IL-1 receptor antagonist	Immune cells and epithelial cells	• Block of the action of IL-1α or IL-1β inflammatory signals.
IL-10	Main member of the IL-10 superfamily, which also includes IL-19, IL-20, IL-22, IL-24, IL-26, as well as type III IFN-γ subfamily	Wide variety of cell types including macrophages and T cell subsets	• Co-stimulation with IL-2 of cytolytic activity of T-cells. • In isolation, induction of anergic state of T cells.

variable results. In Table 1 we report a brief description of those mainly studied as biomarkers during sepsis.

Interleukin-6 (IL-6) has been regularly reported as a sensitive marker, whose levels are proportional to the intensity of the insult, being higher in patients with septic shock than in those with sepsis. IL-6 participates in the early innate immune response with pathogen detection and tissue damage and, although the activation occurs in the presence of other kinds of insults, infection prompts a more robust IL-6

response than other stimuli. Among 258 substances considered by Pierrakos and coll. in their review, it was one of the most studied biomarkers (Pierrakos et al. 2020). It was one of the three potential diagnostic biomarkers tested in a study population including >300 patients, with a good diagnostic performance (Area Under the Curve >80%) (Henning et al. 2020). Besides IL-6, another well-known cytokine is IL-10, a potent anti-inflammatory substance. Several authors reported that IL-10 levels correlate with the levels of other inflammatory cytokines, confirming that both pro- and anti- inflammatory responses occur simultaneously during sepsis. It has also been demonstrated that IL-10 level correlated with the severity of the septic process and with the outcome (Mannino et al. 2015).

Chemokines are a family of chemoattractant cytokines, which play a vital role in cell migration from blood into tissue and vice versa. Single chemokines are usually cell-specific and attract a single kind of cell. They induce not only leukocyte recruitment to the site of infection but also the release of immune cells from the bone marrow or spleen. Recruited leukocytes will clear bacteria and cell debris, but their activation will also amplify and propagate the inflammation at a distance from the initial site of infection. Anyway, they play a crucial role in an efficient immune response, as the lack of chemokines or their receptors leads to a quasi-immunosuppressed state and increased infection-induced lethality. Several of these substances have been evaluated as biomarkers, including the IL-8, one of the main chemokines that recruits neutrophils toward inflamed tissues, monocyte-chemotactic factor (MCF)-1 and macrophage inflammatory protein (MIP)-1α/β. The levels of all these substances were increased in patients with sepsis than in those with less severe infections, but these results were not definitively confirmed (Chousterman et al. 2017).

Different types of *growth factors* are secreted during sepsis and the most involved in the generation of the cytokine storm are the hematopoietic targeted colony-stimulating factors (CSFs): Granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), and granulocyte colony-stimulating factor (G-CSF). Alongside the cell development, they induce myeloid cell differentiation and proliferation, with a larger number of activated cells available for the immune response, but also for cytokines' production. Moreover, CSFs enhance the response to early-phase molecules such as IL-1β or TNF-α. Therefore, there is a tight interconnection and reciprocal reinforce among different pathway that contribute to an adequate defense against pathogens. On the other hand, it is utmost difficult to find a single biomarker, which is really the expression and measure of this complex network (Hamilton 2008).

Finally, *interferons* (IFNs) are classified into three major types according to their receptor specificity. IFN-γ is mainly produced by CD4 and CD8 T cells and, to a lesser extent, by Natural Killer (NK) cells. INFγ promotes the inflammatory response during sepsis, but its production is dampened during sepsis, probably due to the contemporary presence of pro- and anti-inflammatory stimuli (Schoenborn and Wilson 2007).

Acute Phase Proteins are produced by the liver in response to numerous inflammatory cytokines, especially IL-6, which was initially named as "hepatocyte-stimulating factor." In several ways they contribute to an efficient immune defense: they participate in the elimination of microbial products and cellular debris and they act to neutralize some inflammatory mediators, such as free radicals or proteases.

Procalcitonin (PCT) is the pro-peptide of calcitonin, produced by thyroid gland C-cell. In healthy subjects, plasma levels of this molecule are very low and start to increase during systemic infection or inflammatory conditions like inhalation injury, pulmonary aspiration, severe burns, pancreatitis, heat stroke, abdominal infarction, severe trauma, or after invasive surgery (Becker et al. 2008).

PCT slightly increases when the infection is limited to a tissue or organ without systemic involvement, but in the presence of a severe systemic reaction or poor organ perfusion PCT levels markedly increase (Reinhart and Carlet 2000). Such increment occurs within 2–4 h after the initial stimulus, reaches the highest level in 8–24 h and persists as long as the inflammatory process continues. This sensitivity and specificity in the diagnosis of infection differed greatly in different studies (specificity from 54 to 100% and sensitivity from 42 to 100%) (Becker et al. 2008), but it was a useful predictor of mortality at ICU admission (Sager et al. 2017). The 2016 Surviving Sepsis Campaign guidelines suggested using PCT to guide and limit the antibiotic treatment. However, subsequent meta-analyses found a modest reduction in the duration of the antibiotic therapy (from 1 to 1.5 days) based on PCT levels (Hamade and Huang 2020).

The comparison between PCT and C-reactive protein (CPR) showed that PCT correlated better than CRP with the overall course of the septic process and had a better correlation with the outcome than CPR (Becker et al. 2008). On the other hand, the second one had a higher accuracy in detecting localized infection, like acute appendicitis or endocarditis (Parlato and Cavaillon 2015).

C-reactive protein (CRP) was discovered by Tillett and Francis in 1930 as a serum entity present in rabbits with pneumonia, able to bind a polysaccharide fraction C prepared from pneumococci and absent in normal sera. Then, it was reportedly found independent to the type of infection. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria), in order to activate the complement system. Its increase usually begins 12–24 h after the initiation of the infection and reaches the peak after 2–3 days. In several studies, elevated levels of CRP were associated with a poor outcome (Tan et al. 2019). It proved to be a reliable tool to monitor the efficacy of initial antimicrobial therapy, as the level decreased more rapidly and to a greater extent in patients with a favorable response to antibiotics treatment than in those with poor outcome. However, the usefulness of CRP was not confirmed in all types of patients and, depending on the studies, the sensitivity of CRP varies from 30% to 97.2% and its specificity from 67% to 100% in adult and pediatric sepsis. This heterogeneity could be attributed to the different populations included in the studies (Aydemir et al. 2018) and the different timing of the evaluations.

Serum Amyloid A (SAA) is another major acute phase reactant. Present at very low concentration at homeostasis, it can reach levels higher than 1 mg/mL during inflammation. SAA induces extracellular matrix-degrading enzymes, acts as a chemoattractant for monocytes and neutrophils, inhibits the oxidative burst response, and prevents platelet aggregation. During inflammation, it has a role in cholesterol metabolism, either facilitating its delivery to cells or its removal from sites of tissue damage. In neutropenic patients, SAA performed better than CRP to differentiate between infectious and noninfectious febrile episodes and it was reported to be an early and accurate marker of neonatal early-onset sepsis (Casl et al. 1994; Enguix et al. 2001; Ucar et al. 2008). In adult sepsis, its variability was really high (Lannergard et al. 2009).

Lipopolysaccharide-binding protein (LBP) is an acute-phase protein that has been suggested as a marker of infection. It has a role in the innate immune response, as it binds to lipopolysaccharide and brings it to the CD14 receptors on the monocyte-macrophage cell lineage. CD14 receptors then interact with TLR-4, initiating the production of cytokines. As a potential biomarker, an interesting aspect of this protein is its long half-life. Interestingly, elevated levels of LBP are also seen in Gram-positive infections (Gaini et al. 2006) and a strong correlation between LBP and CRP has been described, suggesting a common activation or a common pathway for these acute phase proteins. It is present in the serum in healthy subjects (5–10 μg/mL) and increases during inflammation up to 200 μg/mL. Serum LBP levels have been regularly reported to be higher in sepsis than in SIRS patients. In patients admitted to the ED for suspected infection (Gaini et al. 2006), LBP performed similarly to IL-6 and CRP to distinguish between the systemic inflammatory response syndrome (SIRS) and sepsis (AUC = 0.86 versus 0.87 and 0.84, respectively) and was superior to PCT as a diagnostic marker for infection (AUC = 0.74).

Pentraxin 3 (PTX3) is a promising marker of infection and inflammation, and it correlates with disease severity. Pentraxins are key components of innate immunity, which is the first line of defense against microbes. C-reactive protein (CRP) belongs to the group of short pentraxins produced principally in the liver, while PTX3 is the prototype of long pentraxins produced by endothelial cells and phagocytic cells (Pierrakos and Vincent 2010). PTX3 is released in response to early pro-inflammatory cytokines (TNF and IL-1), but also after direct contact with microbial products. Therefore, its increase occurs in the earliest stages of the disease and it could represent a timely marker of infection, with both a diagnostic role and a prognostic value (Vanska et al. 2011). In fact, high PTX3 levels have been found to correlate with a poor outcome in severe infections (Uusitalo-Seppala et al. 2013).

The disproportionate leukocytes activation and recruitment into host tissues plays a pivotal role in causing **breakdown of the vascular endothelium**, which represents one of the causes of tissue injury and organ dysfunction (Zonneveld et al. 2014). The leukocyte recruitment is initiated by soluble mediators, including cytokines or bacterial-derived lipopolysaccharide, which upregulate the expression of adhesion molecules on both leukocytes and the endothelium. This upregulation results in a multistep adhesion cascade whereby circulating immune cells sequentially roll on, firmly adhere to, and transmigrate across the endothelium. By trans-endothelial

migration or diapedesis, leukocytes cross the endothelial barrier, either through the inter-endothelial junctions, or via the formation of transcellular pores, and accumulate in tissues. During the progression of the inflammatory response, soluble isoforms of the leukocyte recruitment adhesion molecules are shed from cell surfaces and accumulate within the bloodstream. These soluble isoforms have been evaluated as possible prognostic biomarkers of the severity of inflammation. However, we have to consider that shedding is mostly an active process, with complex regulatory mechanisms as it could serve to dampen inflammation and specifically to reduce leukocyte–endothelial interactions and protect the host from excessive collateral damage. Furthermore, age-related differences in both levels of soluble adhesion molecules and the enzymes that mediate shedding have been observed in both healthy and septic patients.

The list of molecules related to endothelial dysfunction is rich and the most studied substances are the following:

- *Soluble forms of adhesion molecules*: Increased levels of these biomarkers have been shown to be associated with increased sepsis severity, organ dysfunction, and mortality, with a possible value in the prognostic assessment of septic patients.
  - *E-selectin* (also known as endothelial-leukocyte adhesion molecule 1 (ELAM-1/CD62E): allows the rolling of the cells on the endothelium.
  - *Intercellular adhesion molecule-1 (ICAM-1/CD54)* and.
  - *Vascular cell adhesion molecule 1 (VCAM-1/CD106)* allows a firm adhesion before migration.
- An*giopoietin-2 (Ang-2)* is produced by endothelial cells and inhibits angiogenesis, through the inhibition of Angiopoietin 1.
- *Vascular endothelial growth factor (VEGF)* promotes proliferation, migration, and survival of endothelial cells, but it also favors the endothelial permeability, induces the expression of cell adhesion molecules, and upregulates the procoagulant activity.
- *Endothelin (Et-1)* is a peptide produced by endothelial cells, with potent vasoconstricting properties.
- *Adrenomedullin (AM)* is a hypotensive peptide acting locally as a vasorelaxant and as a systemic vasodilator, produced by vascular smooth muscle cells and endothelial cells.
- *Endocan* is a dermatan sulfate proteoglycan, expressed by lung and kidney endothelial cells.
- *Heparin-binding protein (HBP)*, contained within neutrophils, induces cytoskeletal rearrangement of endothelial cells leading to vascular leakage.
- *Growth arrest-specific protein 6* (Gas6) is a vitamin K-dependent protein, has a prosurvival and antiapoptotic effect, and regulates the inflammatory response by the downregulation of TNFa, IL-6, and interferon secretion in dendritic cells. Finally, Gas6 is involved in the activation of the endothelium in response to inflammation, increasing the leukocyte extravasation and the sequestration of

circulating platelets and leukocytes on activated endothelium (Ekman et al. 2010).

Levels of the aforementioned biomarkers were higher in patients with sepsis than in those with SIRS and their level paralleled those of other biomarkers, like IL-6 and TNF, as well as prognostic scores. However, data are not fully consistent, with sporadic reports of an inverse relationship, for example, for VEGF (Karlsson et al. 2008). In several studies, septic patients showed higher levels than in those with SIRS, but there was always a wide overlap of values between the two subgroups, dampening the possible utilization of these biomarkers for the early diagnostic assessment.

Coagulative Alterations are virtually recognized in all patients with sepsis; they range from a subtle asymptomatic activation of coagulation to a serious systemic involvement of small vessels (50–70% of patients), leading to the clinical picture of a Disseminated Intravascular Coagulation (DIC) (35% of patients). This spectrum of clinical manifestations is sustained by a derangement of coagulation and fibrinolysis mediated by several cytokines, such TNF, IL-6, and IL-1. These cytokines are responsible for the expression of tissue factor (TF) on monocytes and macrophages, as well as on the damaged endothelial cells, and it has been recognized as the main initiator of coagulation in sepsis, together with other clotting factors, such as factor VIIa, factor Xa, thrombin, and fibrin. On the other hand, especially in cases of overt DIC occurring in septic patients, the prothrombotic effects have shown to be significantly enhanced by the excessive suppression of fibrinolysis caused by over-production of plasminogen activator inhibitor (PAI)-1 (Fig. 2) (Levi and Poll 2015).

The main substances linked to the coagulation cascade candidates to the role of biomarkers are the following:

D-dimer is the name given to one of the families of fibrin fragments, which form and circulate in the bloodstream for several days after a thrombotic event or in the presence of an abnormal activation of the coagulation process. According to the International Society of Thrombosis and Hemostasis (ISTH), D-dimer evaluation has shown its utility in stratifying septic patients in the light of the severity of the coagulopathy, as part of the DIC score, altogether with the values of fibrinogen, international normalized ratio (INR), and platelets count (Innocenti et al. 2019).

Antithrombin (AT) is a glycoprotein that inactivates several enzymes of the coagulation cascade. Reduced levels of AT were reportedly associated with the occurrence of sepsis in Intensive Care Unit (ICU) patients and trauma patients. Among patients with sepsis, the levels of AT were lower in patients with organ dysfunction and in nonsurvivors as compared to survivors.

Activated protein C (APC) is generated following the cleavage of its precursor by thrombomodulin. APC is an inhibitor of the coagulation cascade and displays anti-inflammatory properties. The majority of septic patients have reduced levels of protein C and this deficiency is associated with increased morbidity and mortality. However, the treatment with recombinant human APC has been withdrawn for lack

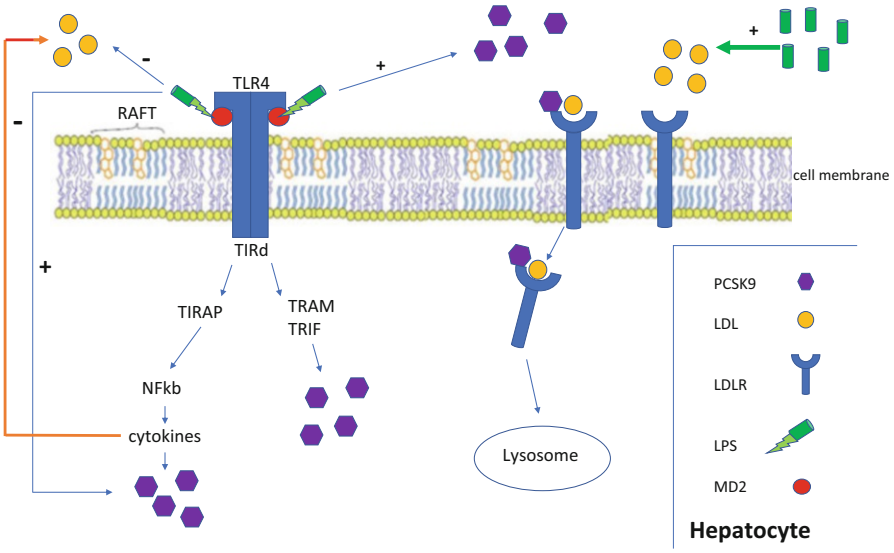

Fig. 2 LDL particles and PCSK9 in sepsis. The LDL receptor (LDLR) binds to LDL and mediates their endocytosis. PCSK9 binds to LDLR and targets it into lysosomes. Inflammatory cytokines reduce circulating LDL and stimulate receptor-mediated internalization of LDL. The LPS inhibits LDL production and promotes PCSK9 synthesis. Pathogen lipids are partially carried by circulating LDL. MD2 acts as a bridge in the crosstalk between LPS and TLR4

of efficacy, to confirm that the blockade of a single system can hardly affect septic patients' prognosis.

Thrombomodulin (TM) is a glycoprotein expressed on the surface of endothelial cell, mesothelial cell, monocyte, and a subset of dendritic cell that acts as a receptor of thrombin and neutralizes its clotting activity. The soluble form is considered as a marker of endothelial cell injury. The levels were higher in sepsis patients who developed organ failure and in nonsurvivors compared to survivors. However, preliminary data showed that the administration of recombinant TM in septic patients improved prognosis, supporting the concept that this mediator could be a marker of abnormal coagulation activity and not a deleterious mediator (Kato et al. 2013).

Plasminogen activator inhibitor (PAI) types 1 and 2 are mainly produced by endothelial cells and are markers of altered fibrinolysis. They counteract the action of tissue plasminogen activator (tPA) and urokinase that act upstream of fibrinolysis by converting plasminogen into plasmin. Patients with septic shock have significantly enhanced levels of PAI-1, which have a strong predictive value for multiple organ failure (MOF), DIC, and mortality.

Von Willebrand factor (VWF) is produced by the bone marrow and endothelial cells. It mediates the adherence of platelets to one another and to sites of vascular damage, promoting the formation of blood clots. It also acts as a carrier for factor VIII in the circulation. Plasma levels of VWF were found higher in septic shock

patients than in patients after traumatic shock and, among septic patients, they were higher in nonsurvivors.

Overall, biomarkers linked to the coagulation cascade may have a prognostic role and, in some cases, they could become a therapeutic target (Patel et al. 2019). Their utilization as diagnostic biomarkers is precluded by the difficulty to find a reliable cutoff, due to the wide dispersion of values.

During sepsis, **apoptosis** particularly affects lymphocytes, NK cells, and dendritic cells, as well as endothelial and epithelial cells. In innate and adaptive immune systems, cell death benefits the host by downregulating the inflammatory response, but the extensive loss of immune cells may compromise the ability of the host to eliminate invading pathogens and predispose to secondary infections (Cao et al. 2019). During the apoptotic process, receptors and membrane proteins are released in the bloodstream and they have been evaluated as possible biomarkers. *Fas receptors*, which are activated by Fas ligands and induce apoptosis in the activated form, can be shed from the cell surface and found as soluble receptors. During apoptosis of epithelial cells, activated caspases cleave *cytokeratin 18* into proteolytic fragments, which diffuse into the serum. Both full length and cleaved fragments can be found in the circulation of septic patients. The levels of both substances were higher in septic patients than in those with critical illnesses of other etiology, but their prognostic value was not confirmed by several studies.

Besides the soluble receptors released during the apoptotic process, several other receptors, usually embedded in the cell membrane, are shed from their location and released into the bloodstream. In Table 2, we reported the most studied soluble receptors.

A recent meta-analysis by Velissaris and coll (Velissaris et al. 2021). explored the utility of suPAR as a diagnostic biomarker in the Emergency Department. In fact, SuPAR can be easily and rapidly measured and, in this clinical setting, it could have a role in the exclusion of an infection and the management of sepsis, alone or in combination with other biomarkers. However, few studies investigated this potential utilization as well as the optimal cutoff value, and the timing of measurements has yet to be determined. The same observations can be applied to all the soluble receptors, which seem to be good candidates for the role of sepsis biomarker, but have been evaluated in heterogeneous studies, including small populations with different sepsis sources. These conditions make results hardly generalizable and we need further investigation to determine whether it is meaningful to introduce these substances in the clinical practice.

Profound **alteration of the levels of circulating hormones** is typical for sepsis. *Sex steroid hormones*, namely, estrone and estradiol, increase markedly in female patients, while in males testosterone is significantly decreased. *Leptin* is a hormone secreted by adipocytes that acts on the hypothalamus to regulate food intake. Critically ill patients showed threefold times plasma leptin levels compared to controls, with loss of the circadian rhythm normally observed. Leptin levels were found to be more increased in patients with sepsis than in those with shock of other aetiology. Among septic patients, nonsurvivors showed higher levels than survivors, but several studies failed to confirm these results. *Vasopressin* is a neurohypophyseal

Table 2 Soluble receptors candidate as biomarkers during sepsis

	Origin	Produced by	Diagnostic and prognostic role
CD14 sCD14-ST or presepsin	Part of the LPS receptor that shuttles the endotoxin to PRR	Hepatocytes Activated monocytes by shedding and secretion	● Higher levels in patients with MOF than in those without. ● Higher levels in nonsurvivors from gram septic shock. ● Significantly higher levels in patients with sepsis than in those with SIRS. ● Good correlation with PCT levels and sequential organ failure assessment (SOFA) score. ● Early increase and strong correlation with the clinical course of sepsis.
sCD25	One of the three chains (α-chain) of the IL-2 receptor Involved in the binding of IL-2	Activated activated lymphocytes	Higher levels in patients with sepsis than in those with uncomplicated infections, not definitively confirmed
sCD163	● Receptor for the haptoglobin-hemoglobin complexes, ● Acts as a scavenger receptor for hemoglobin.	Monocytes/ macrophages	● Higher levels in patients with sepsis than in controls. ● Higher levels in nonsurvivors than in survivors (Su). ● Independent association with increased mortality among septic patients altogether with SOFA score.
Soluble TNF receptors	● Two types of TNF receptors, both of which can be shed from the cell surface. ● Behave as inhibitors of TNF.		● Enhanced levels of sTNF R-I andR-II in patients with sepsis as compared to healthy controls. ● Higher levels were found in nonsurvivors than in survivors. ● Strong correlation between both soluble receptors and clinical scores.

(continued)

Table 2 (continued)

	Origin	Produced by	Diagnostic and prognostic role
sTREM	• One of the immunoglobulin superfamily receptor, activated by bacterial or fungal infections. • Induction of the release of proinflammatory cytokines.	Polymorphonuclear granulocytes and mature monocytes	• Good diagnostic performance, with AUC = 0.9 (Su). • Dynamic changes in serum sTREM-1 helpful for prognostic assessment (Vedi su, 33–34).
suPAR.17	• Three-domain glycosylated protein (D1–D3), which binds to glycosylphosphatidylinositol (GPI) anchor on cell surface to release its soluble form, i.e., suPAR.17. • Has chemotactic properties.	Neutrophils, lymphocytes, monocytes, and macrophages	• Better diagnostic performance than PCT to distinguish patients with sepsis from those with SIRS. • Weak prognostic performance.

peptide hormone produced by the hypothalamus. Its secretion is regulated by plasma osmolarity and venous and arterial baroreceptors. Minor changes (lower than 2%) in plasma osmolality already affect the vasopressin release, while large changes in circulating blood volume sensed by baroreceptors are needed to increase vasopressin release to the same extent (Wagener and Bakker 2015). In the early phase of a vasoplegic shock, the vasopressin level is markedly increased, up to ten times the normal levels, to maintain blood pressure via V1a vasopressin receptor stimulation. However, the prolonged stimulation may induce the depletion of the stores, with restoration of "normal" levels, inappropriate for the situation. This evolution compromises the possibility to use this molecule as a biomarker. *Copeptin* is co-released with AVP, from the same precursor, in response to osmotic and hemodynamic stimuli. Its half-life is twofold longer compared to vasopressin, making this substance suitable as a biomarker. Its prognostic value in septic patients has been studied by several groups and increased levels proved to be associated with increased mortality (Gomes et al. 2021).

PCSK9: What About This Molecule? Could PCSK9 Be a Useful New Sepsis Biomarker?

During inflammation and infection, significant changes in cholesterol metabolism and levels have been reported; in addition, cholesterol and pathogen's lipids play an essential role in generating intracellular signals and in regulating the systemic inflammatory response (Paciullo et al. 2017; Innocenti et al. 2021). Microbial cell walls contain lipid moieties such as lipopolysaccharide (LPS) in Gram-negative bacteria, lipoteichoic acid, a structurally similar glycolipid found in Gram-positive bacteria, and phospholipomannan in fungal pathogens. These pathogen-associated

lipids are major ligands for toll-like receptors family, in particular by TLR4, and thus figure prominently in the septic inflammatory response (Walley et al. 2014).

The TLR4 belongs to the family of transmembrane receptors, with an extracellular leucine-rich repeat domain that interacts with PAMPs and DAMPs or alarmines and an intracellular Toll/IL-1 receptor (TIR) signaling domain. TLR4 is the main receptor for the LPS that triggers the activation of nuclear factor-Kb and the production of pro-inflammatory cytokines (Medzhitov and Janeway Jr. 1997). Circulating LPS induces a strong upregulation of TLR4, normally expressed on monocytes and neutrophils (Parlato and Cavaillon 2015). Activated TLRs crosstalk with lipid rafts, which favor their recruitment and clustering. Lipid rafts consist of dynamic assemblies of transmembrane proteins, free cholesterol, which is the main component, and lipids that float freely within the bilayer of cellular membranes. Cholesterol serves as a spacer between the hydrocarbon chains of sphingolipids and acts as a dynamic glue, keeping the raft assembly together. It also favors the crosstalk between lipid rafts and TLRs, due to the presence of the Cholesterol Recognition Amino-Acid Consensus (CRAC) sequences in the intracellular juxtamembrane domain of several TLRs (Ruysschaert and Lonez 2015). The cytokine storm induced by this activation is one of the mechanisms that determines organ dysfunction.

On the other side, circulating lipoproteins are involved in the clearance of the LPS, with a modulating function on the abnormal immune response (Lee et al. 2015). High-density lipoproteins (HDL) modulate the inflammatory response due to their ability to sequestrate the LPS. This action leads to the attenuation of adhesion molecule expression, upregulation of endothelial nitric oxide synthase, and reduces oxidative stress (Murch et al. 2007). Structural microbial lipid molecules, which travel free or bound to carrier proteins, (e.g., LPS-binding protein and bactericidal/permeability binding protein), are assembled in HDLs, transferred to LDLs (low density lipoproteins), and VLDLs (very low-density lipoproteins), to be subsequently eliminated through the biliary system, by a mechanism involving LDL receptor. This mechanism is also called as reverse cholesterol transport (RCT), a mechanism by which the body removes excess cholesterol from peripheral tissues and delivers them to the liver, where it will be redistributed to other tissues or removed from the body by the gallbladder (Paciullo et al. 2017; Momtazi et al. 2017; Boyd et al. 2016). In this scenario proprotein convertase subtilisin-kexin type 9 (PCSK9) is another important factor.

PCSK9, initially called apoptosis-regulated convertase 1, was first identified in 2001 in studies of cerebellar neuron apoptosis, but the gene was characterized only in 2003 (Hess et al. 2018).

Human PCSK9 gene, located in chromosome 1p33–34.3, encodes a serine protease enzyme belonging to the proteinase K subfamily of subtilases. This soluble molecule is mainly secreted by the liver but also in the brain, kidney, intestine, steroidogenic tissues, pancreas, and others (Seidah et al. 2003). In the liver, PCSK9 synthesis is regulated by the hepatocyte nuclear factor 1α (HFN1 α), which is a liver transcription factor, and by the sterol regulatory element-binding protein 2 (SREBR-2) (Dong et al. 2010; Lagace 2014; Xiao et al. 2012), but also through the cytokines storm induced by the crosstalk between TLR4 and LPS.

PCSK9 is a key regulator of serum cholesterol level because it acts in the regulation of LDL, by targeting liver LDL receptors. The LDL receptor degradation promoted by PCSK9 occurs both in the intracellular and in the extracellular compartment. In the extracellular pathway, circulating PCSK9 binds to the LDL receptor on the surface of hepatocytes by the first epidermal growth factor-like (EGF-A) repeat domain and carries the complex into the lysosomes. PCSK9 binding inhibits the recycling of LDLR to the cell surface and enhances its lysosomal degradation. In the intracellular pathway, PCSK9 binds endocytosed LDL receptors and directs it to lysosomes for their degradation (Lagace 2014; Zhang et al. 2008). A similar action is performed on VLDL receptors expressed in the adipose tissue.

The regulatory role of PCSK9 on LDL receptors is exerted even during sepsis, when they mediate LPS removal from the circulation (Momtazi et al. 2017). Therefore, PCSK9 participates in the innate immunity response to infection.

The interest in PCSK9 has grown over the last few years. It was observed that gain-of-function (GOF) mutations of the gene encoding for this proprotein convertase were associated with increased LDL cholesterol levels and cardiovascular risk by the accumulation of plasma cholesterol in the vascular endothelial cell wall and consequent atherosclerosis. During sepsis, these mutations decrease LPS clearance, with consequent increased cytokines storm. On the other hand, loss-of-function (LOF) mutations of this gene are linked to low levels of serum LDLs, with reduced cardiovascular risk as well as enhanced LPS clearance during sepsis.

During sepsis, PCSK9 can play a dual role with even possible contradictory effects in the host.

The increasing PCSK9 activity during sepsis might have a beneficial role in the inhibition of reverse cholesterol transport and in the stimulation of immune cell functions, by the accumulation of intracellular cholesterol and possibly improved lipid raft composition. On the other hand, PCSK9 inhibition promotes pathogen lipid clearance by LDLR, reduces deleterious inflammatory signals, and may improve prognosis. Boyd and coll (Boyd et al. 2016) demonstrated that upon presentation, septic patients who developed cardiovascular and respiratory failure had low cholesterol levels and higher PCSK9 levels compared to those who did not present organ failure. The authors attributed these results to the enhanced PCSK9 production due to the low cholesterol level, with consequent reduced LPS clearance and abnormal immune response. Genga and coll (Genga et al. 2018). confirmed that septic patients with loss-of-function mutations of PCSK9 gene showed lower 1-year mortality rate and reduced risk of hospital readmission for infection compared to wild-type patients.

In the prospective study performed by our group, we observed that serum levels of PCSK9 were not closely associated with cholesterolemia and patients with PCSK9 level in the lower quartile showed increased sepsis severity and mortality rate, without significant differences between other subgroups. The mortality curve tended to be U-shaped and showed the lowest mortality in the intermediate subgroups and an increased mortality among patients with normal and very high serum PCSK9 level (Innocenti et al. 2021).

This phenomenon could be explained through the altered lipid metabolism caused by sepsis, when a reduction in cholesterol levels can occur, mediated by an accelerated clearance and a reduction in the synthesis of lipoproteins mediated by the cytokines storm (Lekkou et al. 2014). Inflammation and cytokines stimulate PCSK9 expression (Feingold et al. 2008), whose activity regulates both the infectivity of pathogens and the host immune response. It also influences cholesterol metabolism and lipid raft composition, by the regulation of the expression of LDL receptors. So, during sepsis, the persistence of a normal serum level of PCSK9 could represent an inadequate response to infection, with possible prognostic consequences. These conflicting results indicate the need for further studies (Innocenti et al. 2021; Walley 2016; Topchiy et al. 2016).

PCSK9 Inhibitors

In order to inhibit the action of PCSK9 we can evaluate different strategies, which are summarized below:

- Pharmaceutical agents: Monoclonal antibodies (mABs) and small interfering RNA (siRNA).
- Natural compound (Berberine).
- Endogenous inhibitor (FGF21).
- Other inhibitors.

MABs are involved in the disruption of PCSK9 interaction with LDL receptors. None of the PCSK9 LOF mutations have shown serious adverse effects and PCSK9 inhibition could be a feasible therapy. The mechanism of action of these molecules is to block the binding between PCSK9 and the EGF-A domain of the LDL receptors. Currently there are two FDA-approved PCSK9 mABs that are indicated only for hypercolesterolemic patients (mixed dyslipidemia, primary hyperlipidemia, homozygous familial hypercholesterolemia), who are refractory to usual treatments.

Small interfering RNA or siRNA act through the transcriptional suppression of PCSK9 mRNA by blocking the proprotein at the protein receptor level, in order to "silence" the mRNA and to prevent translation. The advantage of this class of molecules over mABs is that siRNA blocks the PCSK9 in the intracellular and extracellular pathway and is administered only semiannually.

Berberine belongs to the class of natural compounds. This is an isoquinoline plant alkaloid found in different plant species like *Berberis vulgaris* (barberry), *Hydrastis canadensis* (gold-enseal), and *Berberis aristata* (tree turmeric). The mechanism of action of this drug is to decrease PCSK9 intracellular expression by reducing the promoter activity of the PCSK9 gene. Berberine increases LDL receptors production and simultaneously decreases PCSK9 mRNA production.

Fibroblast growth factor 21 (FGF21) is a natural compound, which determines the inhibition of PCSK9 expression. The mechanism of action is still unknown.

There are also several other types of PCSK9 inhibitors under research. One innovative approach of inhibiting PCSK9 is through a *vaccine formulation*; in actuality, we have two types, peptide based and virus-like particles. Both of them seem to be useful in familial hypercholesterolemia and LDL management (Chackerian and Remaley 2016; Crossey et al. 2015; Galabova et al. 2014).

By the way, all of these molecules are now available only for the treatment of patients, who are refractory to usual treatments against hypercholesterolemia and at high risk of recurrent major cardiovascular events.

The only molecule that has been studied against sepsis is *LGT-209*, which has been developed to prevent and treat host inflammation in sepsis (2017). Phase I studies (2016) yielded favorable results in 2016 and actually it is in phase II.

PCSK9 Inhibitors and COVID-19

The role of PCSK9 inhibitors has also been studied in patients with infection by severe acute respiratory syndrome coronavirus 2 (Sars-Cov2). Once again, the key association between statins, PCSK9, and the pathophysiological pathways involved in the COVID-19 was confirmed.

SARS-Cov 2 is a beta-coronavirus that belongs to the same family of severe acute respiratory syndrome (SARS). This virus crosses the cell membrane and enters the cell by the crosslink to angiotensin-converting enzyme 2 receptor (ACE2) (Zhou et al. 2020). The majority of SARS-CoV 2 infections are mild to moderate, 14% of patients develop severe disease (dyspnea, hypoxia, or $>$ 50% lung involvement on imaging within 24–48 h) and 5% critical disease (respiratory failure, shock, multi-organ dysfunction). Mortality rates range from 0.9% to 12% depending on the study populations (Wu and McGoogan 2020). The downregulation of ACE2 receptors and the cytokines storm caused by this infection are due to the release of different classes of interleukins and TNFα.

By the way, at this moment we have no specific therapies against this virus; supportive oxygen delivery (nasal cannula-NC, high flow nasal cannula-HFNC, noninvasive ventilation up to invasive ventilation) seem to be the most useful resource, and dexamethasone has been shown to significantly reduce 28-day mortality in patients with critical COVID-19. Remdesivir has been proposed in hospitalized patients with severe COVID-19 requiring low-flow supplemental oxygen, but WHO recommends against the use of this drug because of the limited efficacy (Group RC et al. 2020; Lamontagne et al. 2020).

PCSK9 inhibitors seem to interfere with several pathophysiological pathways in COVID-19. They seem to suppress SARS-CoV-2 infection, by blocking the virus entry into the host cells and inhibiting its replication through the disruption of lipid rafts. PCSK9 inhibitors are also associated with improved endothelial function as well as reduced oxidative stress and platelet adhesion. Human and experimental studies suggest that PCSK9 inhibitors exert antithrombotic properties and these results could be encouraging for the use of PCSK9 inhibitors in COVID-19 (Barkas et al. 2021).

Future Perspectives

After this long travel across tens of pathways involved in the septic process, which generate a myriad of substances potential candidate as biomarkers, it seems impossible not to find a reliable parameter for the early diagnosis of sepsis. However, most of them were tested in small populations, including patients with different previous medical conditions and chronic treatments, heterogeneous for age, sex, and source of the infection. Sepsis has become a huge container, where probably patients with different diseases are included. In fact, sepsis shock due to pneumonia in an old patient is a different illness compared to abdominal sepsis in a neutropenic oncologic patient; it is hard to find a diagnostic, and even prognostic, biomarker, useful in both situations (Cavaillon et al. 2020).

Pierrakos et al. proposed that measuring several biomarkers as well as the combination of a mediator with clinical parameters could be useful to overcome the limitations of any single substance (Pierrakos et al. 2020).

Different combinations of biomarkers have been recently reported, most of which included PCT or CRP associated with another substance. Some examples (Parlato and Cavaillon 2015) are the combination of CPR and body temperature, PCT and proadrenomedullin, PCT plus sTREM1 plus CD 64, PCT and C3a, or IL1Ra plus CPR plus gelatinase-associated lipocalin.

Probably, in future years, a change of perspective will occur if we want to obtain significant results for clinical practice. The first necessary step is to select homogeneous populations, at least in terms of infection site and demographic characteristics of patients. Thereafter, new approaches will probably open opportunities to characterize patients' response to the infection, in order to tailor the most appropriate treatment.

The consistent body of research about sepsis has revealed that the evolution and the prognosis of septic patients are largely affected by the interactions of two factors: the host genetics and environmental factors. Advances in technology have allowed us to explore the cellular and subcellular pathologic changes that occur in a septic host in a new way.

The metabolomic approach is based on the study of the metabolome, which is a collection of small molecules produced by cells that are responsible for metabolic processes in the organism. There are targeted and untargeted approaches to studying the metabolome. The targeted metabolomics studies focus on measuring a specified number of metabolites in the pathway of interest. The untargeted metabolomics, or "global metabolomics," takes a biologic sample, which can be tissue lysates, cells, or blood and other biofluids and analyze it by mass spectrometry for data acquisition. A global metabolic profile of the biological sample is then generated, including thousands of metabolites in a single experiment. As any critical illness disrupts the normal metabolic profile, the comparison of the results between healthy and septic patients could allow to identify specific profiles for that pathological condition, with the opportunity to obtain new early diagnostic and prognostic tools (Lee and Banerjee 2020).

Finally, genomic, transcriptomic and proteomic studies are emerging, based on the awareness that genetic variations of the human host conditionate the development of infection and the immune response and represent critical factors from a prognostic point of view. Functional genomics apply the knowledge derived from the analysis of the structure of genomes to clinical medicine. It can focus on a single gene, and how this gene's sequence alters gene expression in response to a septic challenge can focus on a small number of the so-called candidate genes or on larger groups or even measure the whole genome (Douglas and Roussel 2016). Transcriptomics explores the expression of messenger RNA of activated leukocytes. Different authors have identified several sets of genes, both down- or upregulated, which characterize the response of the host to the infection. The utility of this kind of study is to discover disease-associated cytologic signatures and to provide insight into the cellular basis of immune dysregulation in bacterial sepsis (Reyes et al. 2020). Results are not consistent between different studies, as most of the analyses have been performed on circulating cells. Activated cells, which are responsible for the secretion of inflammatory mediators, migrate into tissues, while those present in the bloodstream are not an important source of circulating substances. Another promising marker are microRNAs (miRNAs), which are noncoding, single-stranded short RNAs, which control gene expression in the posttranscriptional phase by inhibiting the translation of mRNA or by degradation of the mRNA itself. They regulate important processes such as cell proliferation, adhesion, apoptosis, and angiogenesis. Their role has been evaluated in the context of endotoxin tolerance, proposing circulating microRNAs as diagnostic as well as prognostic markers in sepsis. Reithmair and coll. Recently performed a comprehensive profiling of cellular and extracellular miRNAs in sepsis patients. They found a different compartment-specific regulation of miRNAs between sepsis patients and healthy volunteers. This assessment allowed the identification of septic patients as opposed to healthy volunteers, alongside a prognostic assessment (Reithmair et al. 2017).

Proteomics is the study of proteins and formed as an extension of the Human Genome Project. The study of the proteome is more complex than studies of genes or mRNA expression due to changes in proteins such as post-translational modifications, alternative splicing, formation of complexes, protein degradation, and the fact that much of transcribed mRNA is not translated to protein. The aim of this approach is to find new early diagnostic biomarkers and to identify gene or protein patterns associated with a specific modality of reaction to the septic insult, which could have a heavy prognostic weight.

We probably have to abandon the hope to find a single biomarker for the early diagnosis of sepsis. The combination of inflammatory mediators dosage with the opportunities derived from the aforementioned new approaches will allow to better characterize septic patients, to individuate homogeneous groups, and to tailor specific sets of indices to confirm the diagnosis and to assess prognosis.

Applications to Prognosis, Other Disease or Conditions

In this chapter we reported an overview of the most studied mediators, candidates to the role of diagnostic or prognostic biomarker during sepsis. The results we reported are referred to the adult population and are not immediately applicable to children. The trend of IL-6 and IL-8 was similar in adults and children, but discrepancies have been frequently reported. Differences are even more evident in the study of the genome and its products; the early transcriptomic response of children with septic shock showed huge discrepancies of gene expression between neonates, toddlers, infants, and school-age children (Wynn 2019).

Mini-Dictionary of Term (5–15)

- **Pathogen:** Organism like bacteria, virus, or fungus that is able to infect a host.
- **Receptor:** Biological structure able to combine with complementary molecule to send an information.
- **Mediator:** Molecule that allows information exchange between two or more biochemical structures.
- **Apoptosis:** Biochemical process that lead to programmed cell death.
- **Inflammasome:** Intracellular multiprotein complex that belongs to innate immune system.

Key Fact of Sepsis

- Sepsis is a time-dependent syndrome, caused by a dysregulated host response to infection.
- Despite the improvement in the management of critically ill patients, the mortality is still as high as 30–60%.
- The early resuscitation and antibiotic treatment may improve prognosis.
- We actually lack a reliable biomarker, which could support the early diagnostic confirmation.
- Besides mediators involved in the abnormal immune response, genomics and trascriptomics could give new information for the early diagnostic and prognostic assessment of septic patients.

Summary Points

- Sepsis is a time-dependent disease and a prompt diagnosis and treatment may improve the outcome.
- In the actuality, we do not have a reliable biomarker to support the early identification.

- Tens of mediators are released during the abnormal immune response that characterizes sepsis and several of them have been tested for this role.
- None of them demonstrated an adequate sensitivity and specificity to support the diagnosis of sepsis.
- Lipids are one of the main components of the pathogens' cell wall, which is recognized by innate immunity mechanisms.
- Endogenous lipoproteins are key actors in the removal of these bacterial products from the bloodstream.
- Proprotein convertase subtilisin kexin type 9 (PCSK9) regulates lipid metabolism in healthy subjects and during sepsis, and it could become a biomarker as well as a therapeutic target.

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Urinary Potassium Excretion as a Biomarker in Critically Ill for the Identification of AKI: A Review

37

Sunilkumar Nadikuda and Munta Kartik

Contents

Abstract

Acute kidney injury is one among the common complications in critically ill patients. Early detection and prompt management can fetch out excellent results improving morbidity and mortality. Many biomarkers have been studied in this context. Blood-based biomarkers were not so promising and being shunned by physicians for their laboriousness to interpret, cost, and poor turnaround time. Urinary biomarkers are being studied as an alternative, out of which urinary potassium is being studied extensively in recent times. A thorough knowledge of body and renal handling of potassium is a must before understanding its effectiveness in diagnosing AKI in critically ill patients. This review familiarizes with physiological and pathological handling of potassium by the body and the role of urinary potassium as a biomarker for AKI in critically ill patients.

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Keywords

Acute kidney injury (AKI) · Urinary potassium (UrK) · Biomarker · Intensive care unit (ICU) · Fractional excretion of potassium (FeK) · Urinary sodium (UrNa) · Fractional excretion of sodium (FeNa) · Acute tubular necrosis (ATN) · Serum creatinine (SrCrt) · Urine output (UO)

Abbreviations

AKI	Acute kidney injury
ATN	Acute tubular necrosis
BK channels	Maxi K channels
CKD	Chronic kidney disease
ESRD	End-stage renal disease
FeK	Fractional excretion of potassium
FeNa	Fractional excretion of sodium
FeUr	Fractional excretion of urea
GFR	Glomerular filtration rate
H_2O	Water
ICU	Intensive care unit
K	Potassium
Na	Sodium
ROMK	Renal outer medullary K channels
SrCrt	Serum creatinine
UrK	Urinary potassium
UrNa	Urinary sodium
UO	Urine output

Introduction

Acute kidney injury is one among the leading causes of admissions toward hospitals and ICUs. Incidence of AKI among critically ill is approximately 22–67%, increasing the risk of morbidity and mortality. It is associated with multiple adverse outcomes like progression to ESRD, CKD, dialysis dependency (5–20%), and death (50–60%) (Bouchard et al. 2015; Burns and Ho 2018; Okusa and Chertow 2009). In hospital setup, maximum contribution to AKI cases is from pre-renal AKI or ATN. Outcomes due to AKI have not changed measurably over the past decade. So, efforts have been intensified in early diagnosis and management of AKI (Perazella and Coca 2012). Various studies have looked into blood and urinary indices to establish their roles in identifying early AKI, but still early diagnosis and monitoring of course of AKI is challenging, as the utility of biomarkers as bedside tools is questionable (Perazella and Coca 2012; Lima and Macedo 2018; Vaidya et al. 2008; Hoste et al. 2010). Urinary biochemistry and microscopy are no inferior to other biomarkers in diagnosing AKI. One among them is urinary K

excretion, which is coming up with some consistent and convincing results in diagnosing AKI early (Kumar et al. 2021).

Physiology of Potassium Homeostasis

Human body is a potassium (K) manufacturing unit; it plays a key role in maintaining cell function; most (98%) of the exchangeable K is in intracellular compartment, whereas only 2% is in extracellular compartment. Almost all cells possess sodium (Na)-potassium (K) pumps which pump out Na and K into the cells, maintaining the potential difference across the cell membrane. The kidney is prime responsible for maintaining K homeostasis, which averages about 3000–4000 mEq of K in a 70 kg person. It is reabsorbed from the glomerulus and is actively secreted in distal convoluted tubules of the kidney maintaining a normal serum K range of 3.5–5.3 mEq/L. Adjustment of renal K excretion occurs over several hours, so initial changes in K concentration are buffered by K reserves in skeletal muscles (Kumar et al. 2021; Palmer 2015; Palmer and Clegg 2019). The reabsorptive capacity of kidney during K handling is mostly independent of K intake (Palmer and Clegg 2019). Normal individuals who have a high UrK excretion are associated with less risk of hypertension, cardiovascular diseases, and death (Burns and Ho 2018; Koeze et al. 2017; Palevsky et al. 2013).

Renal Handling of Potassium

In kidneys, K is freely filtered from the glomerulus, of which most of it is reabsorbed by the proximal convoluted tubule and loop of Henle. This absorption is primarily passive and proportional to Na and water (H_2O) gradient, only 10% of the filtered K reaches distal convoluted tubule and collecting duct. K secretion starts from the distal convoluted tubule and gradually increases along the distal part of the nephron into collecting duct.

Under most homeostatic conditions, K delivery to the distal nephron is constant and small in amount, but according to the physiological needs, distal nephron regulates the K secretion rates (Palmer 2015).

The cellular determinants of K secretion at the principal cell level includes intracellular K concentration, luminal K concentration, voltage difference, and permeability across luminal membrane for K.

Conditions that decrease luminal K concentration, increase the cellular K concentration, or render lumen electronegative will increase the rate of K secretion. Two major determinants of K secretion are rate of distal delivery of Na-H_2O and mineralocorticoid activity.

Increased delivery of Na to the distal nephron stimulates distal Na absorption, leading to more negative luminal potential of distal nephron, increasing K secretion. An increased flow rate also dilutes the luminal secreted K concentration and increases the K secretion further. Major K channels which contribute to the secretion of K are ROMK (renal outer medullary K channel) which have low conductance and high probability of being open under physiological conditions and BK (maxi K

channels) which have large conductance and active under conditions with increased flow (Welling 2013).

Aldosterone is the primary mineralocorticoid present in humans which has several effects on K secretion; it has direct effect on luminal membrane permeability to increase K secretion (Stokes 1985).

Potassium Handling in Pediatric Age Group

Growing children have a positive K balance, which is due to ongoing growth and increase in cell number, associated with paucity of ROMK and maxi K channels in the distal nephron. As the child grows, K secretion capacity increases as the ROMK channel expression increases followed by maxi K channels. These features of K handling by the developing kidney explains the high incidence of nonoliguric hyperkalemia in infants (Thayyil et al. 2008).

Potassium Handling during Pregnancy

Pregnancy is another physiological state with positive K balance; approximately around 300 mEq of K is retained. Effect of high levels of circulating progesterone on distal nephron is the likely cause of such changes (Elabida et al. 2011).

High Dietary Intake

Adaptive responses in the form of biochemical changes in renal tubules of handling Na and K transport in distal nephron balance the body K levels after ingestion of a K-rich diet. After a protein-rich diet, the kidney increases K secretion by increasing GFR and increases K secretory channels, buffering the development of hyperkalemia and maintaining homeostasis (Satlin et al. 2006).

Enteric Sensors of K

Enteric sensors in the gut sense the sudden surge in Na, K, and phosphate levels that signal the kidney to rapidly alter exchange or reabsorption. Dietary intake of K through enteric sensors signals increase in K excretion, which is independent of plasma K change or aldosterone levels. The kaliuretic response to K load is maximum for oral intake rather than intravenous correction (Michell et al. 2008).

Potassium Physiology During Exercise

During the exercise, intracellular K moves into the interstitial space of the skeletal muscles, and interstitial K concentration can raise up to as high as 10–12 mM with severe exercise. This raise in interstitial K leads to vasodilatation and increases the blood flow to exercising muscle. Additionally this rise in interstitial K limits the excitability and contractile force of skeletal muscles leading to fatigue. In conditions of hypokalemia, K accumulation in the interstitial space is decreased leading to impaired skeletal muscle blood flow leading to rhabdomyolysis (Palmer 2015; Palmer and Clegg 2019).

Circadian Rhythm of K Secretion

Despite variation in K intake, spacing between the meals and daily activity, there exists a circadian rhythm in K secretion. K excretion is lower during early morning and night time and maximum during afternoon. This is attributed to intraluminal K concentration, aldosterone, and glucocorticoid levels. The change in circadian rhythm in secretion of K may not be clinically much significant, except for sustained levels of blood pressure even during night times (Gumz et al. 2009).

Potassium Handling During Pathological States

Effect of Plasma Tonicity and Acid-Base Disorders

Intracellular K serves as a reservoir to limit the fall in extracellular K concentration occurring under pathological conditions where there is loss of K from the body (Palmer 2015). Hyperglycemia shuffles intracellular water to the extracellular compartment; this favors efflux of K from the cells as a process of solvent drag, leading to cell shrinkage and further increase in K efflux from the cells. Mineral acidosis (hyperchloremic normal anion gap metabolic acidosis) greater than organic acidosis (high anion gap metabolic acidosis) causes a cell shift in K (Palmer and Clegg 2019; Aronson and Giebisch 2011).

Effect of Volume Status

Hypovolemia and hypervolemia have regulation of K secretion by balanced reciprocal relationship between Na-H_2O absorption and aldosterone levels. Overall the inverse relationship between aldosterone levels and delivery of Na-H_2O to distal nephron maintains the renal K excretion constantly independent of volume status (Palmer 2015; Palmer and Clegg 2019) (Fig. 1).

Hypertension

It has been seen that K intake is inversely related to prevalence of hypertension. Low K diet may increase blood pressure in hypertensive patients. Increase in dietary K intake increases the natriuretic response and decreases the blood pressure. Sympathetic stimulation increases catecholamine levels, leading to β_2 stimulation and increased uptake of K into skeletal muscles (Palmer and Clegg 2019; Appel et al. 2006).

Chronic Kidney Disease (CKD)

Intracellular and extracellular distribution of K is regulated by insulin and catecholamine's in normal conditions. In CKD or metabolic syndromes, insulin-mediated glucose uptake is impaired, whereas cellular K uptake remains normal demonstrating differential regulation of insulin-mediated glucose and K uptake (Palmer 2015).

In CKD, the loss in nephron mass is counteracted by an adaptive response of increased K secretion by nephrons, maintaining the homeostasis until the GFR falls below 15–20 ml/min. It has been studied that CKD patients, those who maintain a

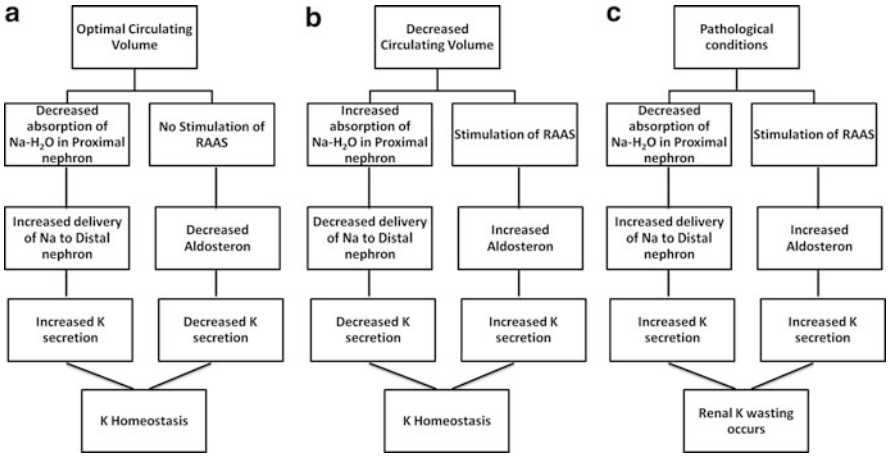

Fig. 1 Potassium handling by the kidney. (**a**): Potassium handling by the kidney in euvolemic state. (**b**): Potassium handling by the kidney in hypovolemic state. (**c**): Potassium handling by the kidney during pathological conditions

reasonably good amount of daily UrK excretion, have a better long-term prognosis than those who have a lesser excretion (Burns and Ho 2018; Kumar et al. 2021).

Major Determinants of K Excretion

(a) Distal nephron Na delivery and flow rate in renal tubules.
(b) Plasma K concentration.
(c) Circulating aldosterone.
(d) Arginine vasopressin levels.
(e) Acid-base status.

Acute Kidney Injury

AKI is a common complication in critically ill patients due to dehydration, sepsis, toxins, heart failure, liver failure, and shock because of various reasons. AKI is characterized by rapid reduction in renal function, which is manifested as raise in SrCrt levels and with or without drop in UO. AKI is defined as a rise in SrCrt by 0.3 mg/dl within 48 hrs; or a rise in SrCrt by 1.5 times from baseline, which is presumed to have happened within 7 days; or a decrease in urine output up to 0.5 ml/kg/hr. for consecutive 6 hours (Khwaja 2012). Early detection is prudent for risk stratification, to avoid nephrotoxic drugs, and to treat underlying pathologies (Burns and Ho 2018).

Urine Output

Urine output for predicting AKI in critically ill patients is a poor marker, it takes a median of 13 hrs for diagnosing AKI in critically ill patients with urine output as a

criterion, and 33% of patients with AKI are non-oliguric in ICUs. Non-oliguric AKI are seen in most of the AKI types; in ICUs, factors which contribute to the non-oliguric state include aggressive fluid resuscitation, volume expansion, renal vasodilators, and diuretic usage (Lima and Macedo 2018; Macedo et al. 2011).

Serum Creatinine

SrCrt is another modality to define an AKI; it takes a median time of 24 hrs to diagnose an AKI with SrCrt as a criterion. Apart from GFR, SrCrt is also influenced by gender, age, muscle mass, hydration, muscle metabolism, and drugs. In patients who are critically ill, there is a rise in volume of distribution, leading to reduction in SrCrt levels, which leads to delay in diagnosis of AKI, over that in septic patients there is decreased production and release of creatinine from muscles. These all lead to underestimation of decrease in renal function in initial 48 hrs of ICU admission (Macedo et al. 2010; Doi et al. 2009).

As per definitions, AKI is being diagnosed depending on UO and SrCrt levels. But, they have their own limitations in diagnosing AKI in early stages in ICU (Koeze et al. 2017; Palevsky et al. 2013). Literature in the past has proven that changes in SrCrt and UO do not pick up early changes in kidney injury, which is an idle time for pharmacological interventions in such patients. Similarly, other renal biomarkers have their own limitations, over that they are costly and non-specific, requires a battery of biomarkers to be tested and slow turnaround time, and has limited bedside risk stratification, leading to diagnosis of AKI more challenging in critically ill patients (Maciel et al. 2016).

There is need for easily accessible, bedside blood or/and urine tests that are useful in diagnosing and monitoring AKI in resource-limited settings. Urinary biochemistry is another emerging domain in diagnosis of AKI, they are being studied extensively in recent times, and they are being considered as a cost-effective, noninvasive, simple bedside tool for diagnosing of AKI (Lima and Macedo 2018; Maciel et al. 2016; Saha et al. 1987).

Urinary Biochemistry as Biomarkers in the Assessment of AKI

Urinary diagnostics in this era are more focused on developing a novel biomarker for AKI, which can be utilized as point of care test for AKI. Many bedside, cost-effective tests have been studied in the past, few among them are urinary microscopy, fractional excretion of urea, fractional excretion of Na, spot UrNa, UrNa/UrK ratio, and fractional excretion of K.

Urinary Microscopy

Urinary microscopy is an inexpensive and easily available bedside test for differential diagnosis and clinical outcome in AKI. Graber and co-workers in 1991 demonstrated the utility of urinary microscopy in ATN patients. It can be used to differentiate types of AKI and also predict the severity of AKI and death (Graber et al. 1991). But doing so requires lots of training and experience and it's a time consuming process (Burns and Ho 2018).

Fractional Excretion of Urea (FeUr)

Fe Urea % = [Urine/Plasma] Urea ÷ [Urine/Plasma] Creatinine X 100

Fractional excretion of urea is based on the hypothesis that intact tubules will reabsorb urea normally, whereas injured tubules as in ATN will not do so. Proximal tubule is the major site of urea reabsorption.

A FeUr <35% reflects pre-renal AKI, whereas FeUr >50% reflects loss of tubular function due to ATN. In 1992 Kaplan and Khon noted discordance between FeNa and FeUr in pre-renal AKI (Kaplan and Kohn 1992).

Fractional Excretion of Sodium (FeNa)

FeNa % = [Urine/Plasma] Na ÷ [Urine/Plasma] Creatinine X 100

In 1976 Espinel tested the hypothesis of fractional excretion of Na in selected oliguric AKI patients (Espinel 1976). It is based on the similar hypothesis that intact tubules will reabsorb Na normally and injured tubules do not. Later this was used to differentiate the type of AKI, prerenal (FeNa <1%) versus ATN (FeNa >3%). The major drawback of this test is its reduced utility in diseased states, like AKI in liver failure, AKI in sepsis, non-oliguric ATN, and early and late stages of AIN (Perazella and Coca 2012).

Spot Urine Na and Urine Na/ Urine K Ratio

In 1970 Bricker et al. proposed a phenomenon of testing urinary spot Na to differentiate types of AKI (PRA vs ATN). Urinary Na concentration of <20 mEq/L was observed in PRA patients and urinary Na concentration of >40 mEq/L in ATN patients. Later over few decades, multiple studies came up with differentiating methods of PRA and ATN. Shankel T et al. in 2019 proposed UrNa/UrK ratio for differentiation of types of AKI. The authors propose this method to be 100% specific and sensitive and would replace many other tests in the future (Shankel and Shankel 2019).

Fractional Excretion of Potassium (FeK)

FeK % = [Urine/Plasma] K ÷ [Urine/Plasma] Creatinine X 100

Potassium handling is different from Na and Ur handling in the kidney; K is secreted distally in the renal tubules that may lead to such more obvious variation in FeK. Urinary FeK excretion is inversely related to creatinine clearance in patients who are not exposed to recent diuretic therapy. In recent times, FeK is being studied as an another domain in the diagnosis of AKI, with evidence emerging from studies done by Maciel AT et al., Burns AR et al., and Nadikuda et al. (Burns and Ho 2018; Kumar et al. 2021; Maciel et al. 2014). Median FeK increases in two days prior to AKI diagnosis. Loop diuretics have their effect on loop of Henle leading to altered K reabsorption and altered K secretion in distal part of nephron, increasing K excretion independent of GFR. Theoretically increased K intake and diuretic therapy interferes with the FeK and its use in diagnosing AKI. This poses major limitations in the utilization of FeK in diagnosing AKI, but evidence is emerging that FeK is not altered by use of diuretics and oral intake (Maciel et al. 2014). Low UrNa, FeUr, and

higher FeK values may signal toward a drop in GFR at ICU admission. But FeNa and FeUr are less specific and controversial in diagnosing AKI in critically ill, these have not shown any significant difference in differentiating non-AKI and AKI patients, these are mostly utilized in special group of patients, and thier utility is mostly useful in differentiating type of AKI. FeK is also dependent on the duration and severity of the AKI; it increases as the AKI progresses, possibly due to drop in GFR and activation of aldosterone; FeK values are maximum from days 1 to 3 of onset of AKI, so this seems to be a more reliable biomarker over FeNa and FeUr in diagnosing and monitoring of AKI (Maciel et al. 2014).

In 2013 Maciel AT et al. has studied on FeK in the course of AKI in critically ill patients and concluded that FeK is related to the duration and severity of AKI. Its increase is due to drop in GFR and increase in aldosterone. FeK seems to be more useful tool than FeNa and FeUr, which can ring the alarm for AKI much before the raise in creatinine levels (Maciel et al. 2014).

Studies by Burn et al. and Nadikuda et al. had shown that single spot urinary biochemical assessment once daily at same time in a day would be equivalent to 24 hrs urine sample (Kumar et al. 2021; Maciel et al. 2016). A sequential evaluation of these parameters in spot urine test at same time in a day seems to be useful (Maciel et al. 2013).

A study done by Burn et al on UrK excretion in 2018, concluded that a cut-off point of $\leq$3.8 mmol of UrK can predict AKI within 7 days of testing with a specificity of 85% and sensitivity of 77%. Major limitations of that study were, it was a single-center study with a specific category of patients (trauma patients) and over that effect of diuretic therapy on FeK has not been studied (Burns and Ho 2018).

In 2021 a similar kind of study was done by Nadikuda et al. on hundred critically ill patients had concluded that mean UrK excretion of 4.39 mmol/L was linearly correlated with CrCl and was a better predictor of AKI in such patients. A UrK excretion value of 3.49 had a sensitivity of 87% and specificity of 74% in predicting AKI in critically ill patients. The author has concluded that two hour UrK excretion can be a simplest bedside accessible tool for AKI prediction (Kumar et al. 2021).

Conclusion

AKI continues to be a common complication in ICU patients, and there is no major improvement in the outcomes of AKI in critically ill patients in terms of morbidity and mortality in near past. Though there have been many reasons for this, one among them is delayed pickup of such high-risk patients in ICU. In the area of early diagnosis of AKI, blood biomarkers are extensively studied but they are costly and laborious as a battery of investigations needs to be studied at a time and poor turnaround time. Urinary biochemical analysis is emerging as a new domain of interest in diagnosis of AKI among critically ill patients. Out of various urine biochemical analyses, UrK excretion is emerging up with some promising results, though bigger studies are awaited. The chase for a new simple, bedside,

cost-effective, point of care tool for diagnosis of AKI in critically ill patients continues. It's a new alluringly ripe area for research urging for larger studies.

Summary Points

- Acute kidney injury is one among the common complications in critically ill patients.
- Early detection and prompt management can fetch out excellent results improving morbidity and mortality.
- Many biomarkers have been studied in this context. Blood-based biomarkers were not so promising and being shunned by physicians for their laboriousness to interpret, costly, and poor turnaround time.
- Urinary biomarkers are being studied as an alternative, out of which urinary potassium is being studied extensively in recent times. A thorough knowledge of body and renal handling of potassium is a must before understanding its effectiveness in diagnosing AKI in critically ill patients.
- This review familiarizes with physiological and pathological handling of potassium by the body and the role of urinary potassium as a biomarker for AKI in critically ill patients.

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Blood Cortisol as a Biomarker in Intensive Care Unit

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Mehmet Yasir Pektezel and Leyla Das Pektezel

Contents

Abstract

Despite some issues are limiting the use of blood cortisol as an excellent biomarker in intensive care units, it is seen in literature that blood cortisol is being used as a predictor mostly with its association to delirium and with respect to mortality especially in patients with trauma and sepsis-septic shock. Although some researches with contradicting results could be found, blood cortisol might still be a promising predictor in intensive care units.

Keywords

Blood cortisol · Delirium · Trauma · Sepsis · Septic shock

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Abbreviations

ACTH	Adrenocorticotropic hormone
CIRCI	Critical illness-related corticosteroid insufficiency
GCR	Glucocorticoid receptor
ICU	Intensive care unit
LOS	Length of stay
TBI	Traumatic brain injury

Introduction: Blood Cortisol as a Biomarker in Intensive Care Unit

Being one of the most important output mediator of hypothalamo-pituitary-adrenal axis, having a major role on stress response (Kyrou and Tsigos 2009), can make blood cortisol a good indicator in intensive care unit (ICU). However, efforts of evaluating blood cortisol in a critically ill patient followed in an ICU is not without hardship.

Despite advanced technology, it is still a major problem to homogenize the variability of blood cortisol assays which can cause over- or underestimation of actual cortisol levels (Arafah 2006). Furthermore, susceptible nature of blood cortisol with regard to many circumstances in critically ill patients such as medications, type-severity-duration of underlying illness, hemodilution, and genetic variations among individuals has to be stated as another restriction (Arafah 2006). At last, intricate conditions in terms of a synthetic adrenocorticotropic hormone (ACTH) injection for the dynamic evaluation of blood cortisol, despite a consensus reported (Arafah 2006), make the use of blood cortisol in critically ill patients further troublesome.

Notwithstanding, blood cortisol measurements not only become a predictor in some important ICU circumstances such as delirium, trauma, and sepsis-septic shock, it also modulates the treatment strategy in critically ill patients struggling with critical illness-related corticosteroid insufficiency (CIRCI) (Huang et al. 2021).

Blood Cortisol and Delirium

Delirium is a frequently encountered pathology in intensive care unit (ICU), and it is of importance regarding its relationship with ICU/hospital length of stay (LOS) and mortality (Devlin et al. 2018). Biomarkers accurately predicting delirium before its development would be helpful. Blood cortisol has already been investigated in this perspective in surgical patients mostly at peri-operative phases (Mcintosh et al. 1985; Shi et al. 2010; Mu et al. 2010; Hauer et al. 2012; Kazmierski et al. 2013; Guo et al. 2016; Eshmawey et al. 2019; Sun et al. 2016; Plaschke et al. 2010) with vague results (Table 1). Probably, owing to the fact that both surgery's own stress and anesthesia given had affected the renowned "circadian clock" might cause different individual blood cortisol responses (Coppola et al. 2020).

Table 1 Studies investigating cortisol and delirium association in patients undergone surgery

Article	Patient/way cortisol obtained	Result
Production of inflammatory cytokines, cortisol, and Abeta1-40 in elderly oral cancer patients with postoperative delirium (Sun et al. 2016)	257 patients with tumor surgery (oral cancer) Blood cortisol ‡	No differences in preoperative cortisol levels Higher cortisol levels in patients with delirium at postoperative phases
Preoperative Depression and Plasma Cortisol Levels as Predictors of Delirium after Cardiac Surgery (Eshmawey et al. 2019)	183 patients undergoing elective cardiac surgery Blood cortisol †	No association between preoperative cortisol levels and delirium formation
Impact of multicomponent, nonpharmacologic interventions on perioperative cortisol and melatonin levels and postoperative delirium in elderly oral cancer patients (Guo et al. 2016)	160 patients with tumor surgery (oral cancer) Urine cortisol ‡	Higher postoperative, but not preoperative, urine cortisol levels in patients who developed delirium
Cortisol levels and neuropsychiatric diagnosis as markers of postoperative delirium: a prospective cohort study (Kazmierski et al. 2013)	113 patients undergoing cardiac surgery Blood cortisol ‡	Both pre- and postoperative higher cortisol levels associated with delirium formation
High serum cortisol level is associated with increased risk of delirium after coronary artery bypass graft surgery: a prospective cohort study (Mu et al. 2010)	243 patients undergoing CABG* Blood cortisol §	Higher cortisol levels associated with delirium
Early postoperative delirium after open-heart cardiac surgery is associated with decreased bispectral EEG and increased cortisol and interleukin-6 (Plaschke et al. 2010)	114 patients with cardiac surgery Blood cortisol §	Higher cortisol levels in delirium patients
Incidence and risk factors of delirium in critically ill patients after non-cardiac surgery (Shi et al. 2010)	164 patients undergoing non-cardiac surgery Blood cortisol §	Higher cortisol levels in delirium patients

†Cortisol level obtained prior to surgery
§Cortisol level obtained after surgery
‡Cortisol level obtained prior to and after surgery
*Coronary artery bypass graft surgery

Importance of serum cortisol level for further delirium development has been studied not only in patients solely undergoing surgery but also in patients with sepsis and septic shock in which cortisol level was found to be higher at first three consecutive days in patients with brain dysfunction compared to those without (Nguyen et al. 2014). In that study, it is important to emphasize that cortisol levels had been obtained at admission to ICU and during the next four consecutive days (Nguyen et al. 2014). Restricting factor in the mentioned article was that its results

were not able to be generalizable regarding cortisol effects on delirium. Because brain dysfunction had been defined as a combination of a Glasgow Coma Scale (GCS) of <13 and delirium positivity according to Confusion Assessment Method for the ICU (CAM-ICU) criteria (Nguyen et al. 2014).

Blood Cortisol and Trauma

Although CIRCI is a well-known phenomenon, diagnosing CIRCI in ICUs is still challenging (Huang et al. 2021). It is known that steroid insufficiency might be detected up to 70% of patients with trauma (Huang et al. 2021). In a recent prospective observational study, patients were separated to have a severe low blood cortisol level (<15 mcg/dl), relative low blood cortisol level (15.01–25 mcg/dl), and normal blood cortisol level (> 25 mcg/dl), in which 12% of patients had a severe low blood cortisol level (Kwok et al. 2020). According to the study, higher blood product requirement, higher vasopressor use, and increased mortality were detected in those having a severe low blood cortisol level (Kwok et al. 2020). In another important multicenter prospective cohort involving patients with multiple trauma while CIRCI has been detected in 54.3% of patients, it is found that only delta cortisol level of less than 9 mcg/dl after a synthetic ACTH injection (250 mcg) to be an independent risk factor for mortality (Yang et al. 2014). However, some inconsistent reports might also be found in terms of low cortisol-related higher mortality (Olivecrona et al. 2013).

There are still debates while stratifying blood cortisol levels in patients. In a study, patients with traumatic brain injury (TBI) were separated according to different blood cortisol levels of <83 nmol/L (3 mcg/dl), <276 nmol/L (10 mcg/dl), and <414 nmol/L (15 mcg/dl). It is shown that the highest mortality was detected in patients having a blood cortisol level of <276 mol/L (10 mcg/dl) and the authors suggested a hydrocortisone replacement be required for those (Bensalah et al. 2018). Another interesting point of the study was that adrenal insufficiency at the end of the first week, determined by an insulin tolerance test, has been found in nearly a third of patients with normal baseline cortisol levels obtained at the admission (Bensalah et al. 2018). Another interesting study have stated that TBI patients with adrenal insufficiency who did respond to a hydrocortisone replacement therapy seemed to have a more favorable neurologic outcome assessed with Glasgow Coma Scale than those did not (Bernard et al. 2006).

It has to be kept in mind that pretraumatic stressful periods might also have an effect on cortisol levels. In a study involving patients with TBI and subarachnoid hemorrhage, it indicated that patients with chronic diseases and/or stressful event have a significant lower hair cortisol level than those without (Sorbo et al. 2020). Thus, the time of blood cortisol sample obtained might be another crucial point in trauma patients (Pandya et al. 2014). In a small retrospective study, blood total cortisol was randomly drawn from patients (not at admission but in the first seven days) and it was shown that patients with a random total cortisol level of greater than

Table 2 Studies investigating cortisol level and trauma association in patients followed in ICU

Article	Patient/way cortisol obtained	Result
Prospective evaluation of admission cortisol in trauma (Kwok et al. 2020)	189 patients with trauma Severe low blood cortisol level (<15 mcg/dl) Relative low blood cortisol level (15.01–25 mcg/dl) Normal blood cortisol level (> 25 mcg/dl)	Increased mortality was detected in those having a severe low blood cortisol level
Critical illness-related corticosteroid insufficiency after multiple traumas: a multicenter, prospective cohort study (Yang et al. 2014)	70 patients with multiple injuries Baseline blood cortisol $_{T30\text{-}60min}$ blood cortisol after 1 mcg synthetic ACTH	Only delta cortisol level of less than 9 mcg/dl after a synthetic ACTH injection (250 mcg) to be an independent risk factor for mortality
Pre-traumatic conditions can influence cortisol levels before and after a brain injury (Sorbo et al. 2020)	55 patients with TBI and subarachnoid hemorrhage Hair cortisol level at admission and at the third month	Lower hair cortisol level after trauma remained at the third month
Increased total serum random cortisol levels predict mortality in critically ill trauma patients (Pandya et al. 2014)	242 patients with trauma Blood cortisol level randomly obtained in a day at the first week of the admission	A random total cortisol level of greater than >30 mcg/dl to be related with a greater mortality rate while compared to those with a that of less than 30 mcg/dl
Cortisol evaluation during the acute phase of traumatic brain injury-A prospective study (Bensalah et al. 2018)	277 patients with TBI Blood cortisol level obtained in a day at the first week of the admission	Hydrocortisone replacement is advised for TBI patients with a blood cortisol level of less than 276 nmol/L (10 mcg/dl)

ACTH adrenocorticotropic hormone, *ICU* intensive care unit, *TBI* traumatic brain injury

>30 mcg/dl to be related with a greater mortality rate while compared to those with a that of less than 30 mcg/dl (Pandya et al. 2014).

Although it is not in scope with this chapter, in another study patients with higher cerebrospinal fluid cortisol levels were found to show poorer outcome at 6 months while compared to others (Kumar et al. 2016). Some details with respect to cited articles have been shared in Table 2.

Blood Cortisol and Sepsis-Septic Shock

While patients with septic shock are being discussed, prior to blood cortisol, glucocorticoid receptor (GCR), an important molecule especially with regard to its highly variable expression in septic shock patients, has to be emphasized. Although this variability, glucocorticoid sensitivity was shown in septic patients not to differ from

controls (Cohen et al. 2016). In a recent original investigation, a more complicated course of septic shock was found in patients with lower GCR and higher blood cortisol (> 25 mcg/dl) levels (Alder et al. 2018). It was stated in the study that while 75% of patients with a lower GCR and higher blood cortisol (> 25 mcg/dl) levels showed a poor prognosis, only 13% of patients with lower cortisol (< 15 mcg/dl) and higher GCR levels did the same (Alder et al. 2018).

Another original investigation showed that non-survived patients with septic shock have higher median blood cortisol level (24 mcg/dl) than survived group (19 mcg/dl). The interesting part of the study was that no baseline blood cortisol level difference has been detected between responders and non-responders determined after a 250 mcg synthetic ACTH injection (Dalegrave et al. 2012). Some studies (Maqbool et al. 2009) are conducted not only based on blood cortisol level but also a maximum blood cortisol increase response after a synthetic ACTH injection with respect to its biomarker feature. In a small study, a baseline blood cortisol level of >20 mcg/dl and a maximal blood cortisol increase response of <9 mcg/dl after a synthetic ACTH injection were found as independent predictors of 28-day mortality (Bollaert et al. 2003).

Blood cortisol level might be also a good indicator while distinguishing patients with sepsis from those without at the ICU admission. In a prospective study, a baseline cortisol level of >450 nmol/L (16.3 mcg/dl) was found to differentiate septic conditions from non-septic ones. Furthermore, in multiple regression models, it was stated that a combination of mentioned blood cortisol level and an ACTH level of <233 nmol/l might be a more reliable parameter than solely procalcitonin level or a sepsis score of > 7 while estimating septic patients at the ICU admission (Lesur et al. 2010). In another detailed original study, the median blood cortisol levels of 39.2 mcg/dl and 28.6 mcg/dl were found in patients with sepsis and septic shock, respectively. In that study, higher blood cortisol level was found to be an independent factor for both progression of sepsis to septic shock and a higher mortality by a cut-off point of 34.2 mcg/dl (Zhang et al. 2014).

In a small cohort, blood free cortisol level was investigated in septic shock patients. While a mean of 170 nmol/L (6 mcg/dl) free blood cortisol level was detected, it is reported that increasing free blood cortisol level is associated with increased noradrenaline infusion dose and APACHE-III score (Sturgess and Venkatesh 2012). Notwithstanding, it has to be emphasized that free cortisol levels must be assessed with caution in critically ill patients especially in terms of measurement variabilities (Cohen et al. 2013).

According to the literature, it seems that predictive feature of blood cortisol level in patients with sepsis and septic shock differs from that in patients with trauma and delirium. Relative higher blood cortisol levels are mentioned in patients with septic shock (Dorin et al. 2015; Vardas et al. 2014; Vassiliadi et al. 2014; Cohen et al. 2012), and though not evidence based, this might be an explanation for negative results detected in big prospective randomized controlled studies (Venkatesh et al. 2018; El-Nawawy et al. 2017; Keh et al. 2016) investigating effects of hydrocortisone replacement on prognosis of septic shock patients, even irrespective of measured blood cortisol levels (Nichols et al. 2017), if any. And still inter-assay

variations in blood samples of patients being evaluated for cortisol levels are needed to be remembered while these results are being assessed (Briegel et al. 2009). Some details with respect to cited articles have been shared in Table 3.

Table 3 Studies investigating cortisol and sepsis-septic shock association in patients followed in ICU

Article	Patient/way cortisol obtained	Result
The glucocorticoid receptor and cortisol levels in pediatric septic shock (Alder et al. 2018)	164 patients with sepsis and septic shock Baseline blood cortisol Complicated course: Two or more organ failure at day 7 or death by day 28	Patients with high cortisol level (> 25 mcg/dl) had a more complicated course than patients with low cortisol level (<25 mcg/dl)
Relative adrenal insufficiency as a predictor of disease severity and mortality in severe septic shock (Dalegrave et al. 2012)	69 patients with septic shock Baseline blood cortisol	Median blood cortisol level in survived group was higher than median blood cortisol level in non-survived group
Baseline cortisol levels, cortisol response to corticotropin, and prognosis in late septic shock (Bollaert et al. 2003)	82 patients with septic shock Baseline blood cortisol $_{T30min}$ blood cortisol after 1 mcg synthetic ACTH	A blood cortisol level of >20 mcg/dl and a maximal blood cortisol increase response of <9 mcg/dl were found to be independent predictive of a 28-day mortality
Prognostic significance of hypothalamic-pituitary-adrenal axis hormones in early sepsis: a study performed in the emergency department (Zhang et al. 2014)	461 patients with sepsis Blood cortisol level obtained in 24 hours after admission	Higher blood cortisol level was found to be an independent factor for both progression of sepsis to septic shock and higher mortality by a cut-off point of 34.2 mcg/dl
Plasma free cortisol and B-type natriuretic peptide in septic shock (Sturgess and Venkatesh 2012)	21 patients with septic shock Free blood cortisol obtained in 72 hours after shock developed	Increasing free blood cortisol level to be associated with increased noradrenaline infusion dose and APACHE-III score
Prevalence of occult adrenal insufficiency and the prognostic value of a short corticotropin stimulation test in patients with septic shock (Maqbool et al. 2009)	30 patients with septic shock Baseline blood cortisol T_{60min} blood cortisol after 1 mcg synthetic ACTH	Baseline blood cortisol >34 mcg/dl and a delta maximum blood cortisol of <9 mcg/dl has a 28-day mortality of 82%
Basal serum cortisol levels are not predictive of response to corticotropin but have prognostic significance in patients with septic shock (Kwon et al. 2007)	68 patients with septic shock Baseline blood cortisol	A baseline blood cortisol level of >30 mcg/dl mortality of 58.8%

ACTH adrenocorticotropic hormone, *ICU* intensive care unit

Applications to Prognosis, Other Diseases, or Conditions

In this chapter, blood cortisol was reviewed as a circulation biomarker. Blood cortisol level might be helpful in different circumstances by different perspectives. While it might have prognostic value in patients with delirium followed in ICU, on the other hand, it can be used as an outcome predictor in patients with trauma and sepsis. In particular, some studies conducted in ICU showed that higher blood cortisol is related with higher postoperative delirium frequency; however, caution must be exercised with regard to differences in blood cortisol sample obtaining methodologies among studies. Despite measuring issues to be solved, it seems that blood cortisol will keep being a promising biomarker in the future.

Mini-Dictionary of Terms

Cortisol: An endocrine hormone.
Glucocorticoid receptor: A receptor in which blood cortisol binds on.
Sepsis: A dysfunctional response of human body to an infectious agent.

Key Facts of Blood Cortisol as a Biomarker in Intensive Care Unit

Blood cortisol is a frequently measured parameter in ICU owing to its predictive and prognostic importance. It might be used in different patient populations being followed in ICU. Despite some sample obtaining and measuring issues waiting to be solved, it is still a promising biomarker for ICU patients.

Summary Points

- Blood cortisol measurement in critically ill patients may become troublesome; thus, caution must be exercised.
- Blood cortisol level as a biomarker in ICU has been investigated mostly for patients with delirium, trauma, and sepsis-septic shock.
- Although there is not a consensus respecting the use of blood cortisol as a biomarker in ICU, this area is still of importance.

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Citrullinated Histone H3 as a Biomarker in Sepsis and Critical Care

39

Fatih Cakmak and Seda Ozkan

Contents

Abstract

Sepsis occurs when the body's response to these chemicals is unbalanced and changes are triggered that can damage multiple organ systems. Histones are structures located in the cell nucleus and can be released into the bloodstream and tissues when the cell is damaged or during programmed cell death, with the formation of neutrophil extracellular traps. Citrullinated histone H3 level is associated with disease severity in the sepsis. Citrullinated histone H3 levels are correlated with the severity of septic shock. Neutrophil extracellular trap

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components and citrullinated histone H3 were detected in patients with resuscitation, intoxication, cerebrovascular accident, and heat stroke. Citrullinated histone H3 is considered the most specific NET marker due to the critical role of histone citrullination in NETosis.

Keywords

Histone · Cit-H3 · NETosis · Sepsis · Septic shock · Critical care · Neutrophil extracellular traps · Sepsis biomarkers

Abbreviations

ALI	Acute lung injury
ARDS	Acute respiratory distress syndrome
Cit-H3 mAb	Anti-histone H3 monoclonal antibody
Cit-H3	Citrullinated histone H3
DIC	Disseminated intravascular coagulation
IL-1β	Interleukin-1β
IL-6	Interleukin 6
LPS	Lipopolysaccharide
MODS	Multi-organ dysfunction syndrome
NETs	Neutrophil extracellular traps
PAD	Peptidylarginine deiminase
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor α
Wbc	White blood cell

Introduction

Activated neutrophils secrete chromatin fibers known as neutrophil extracellular traps (NETs). NETs are fibrous structures released from activated neutrophils in the event of bacterial infection and as a systemic response in any inflammatory process (Savchenko et al. 2011; Vitkov et al. 2009; Garcia-Romo et al. 2011; Kessenbrock et al. 2009). This distinctive feature of neutrophils was first revealed by Brinkmann et al. in 2004 (Brinkmann et al. 2004). As a result of some reactions that occur in neutrophils that encounter pathogens or foreign molecules, granule and nuclear contents are released out of the cell. During this event, called NETosis, NET structures are formed. It has been determined that the function of these structures in the organism is to physically surround the pathogen in the infection area and prevent its spread, as well as to reduce its virulence and antibacterial effect on the pathogen with the histones, granular enzymes (myeloperoxidase and elastase), and some cytoplasmic proteins (lactoferrin and cathepsins) in its structure. When NETosis is formed in the organism at the wrong time, in an undesirable area, or when a disorder develops in the removal of extracellular traps formed in the organism, it leads to some undesirable situations. The type of cell death involved in the formation of

NETs is referred to as NETosis, different from apoptosis and necrosis. The formation of NETs is thought to be different from apoptosis, since caspase is not needed and DNA fragmentation is not observed (Fuchs et al. 2007). Neutrophils secrete highly condensed nuclear chromatin structures during the event called NETosis, and hyper-citrullination of histone H3 via the peptidylarginine deiminase 4 (PAD4) enzyme plays a key role in chromatin condensation. Inhibition of the PAD4 enzyme prevents its citrullination during H3 and NET formation (Remijsen et al. 2011; Neeli et al. 2008; Wang et al. 2009). Measuring citrullinated histone H3 (Cit-H3) in association with the formation of NETs can help quantify the response of NETs to the systemic inflammatory response. In studies, the presence of NETs was determined immuno-cytochemically in blood and sputum samples obtained from patients treated in the intensive care unit, whereas NET structures were not found in samples taken from healthy individuals (Hirose et al. 2012; Hamaguchi et al. 2013). The respiratory system is one of the areas of the human body where it is easiest for bacteria to multiply, and NETs can begin to form as a precursor to pathogens before respiratory infections are fully established.

Sepsis and Cit-H3

Sepsis is a name given to a condition that can be life-threatening in some cases, caused by the human body's response to an infection. Definitions of sepsis and septic shock are given in Table 1. The immune system normally releases a variety of chemicals into the bloodstream to fight infections that occur in the body. Sepsis occurs when the body's response to these chemicals is unbalanced and changes are triggered that can damage multiple organ systems. Post-translational modifications of histone proteins, such as citrullination and acetylation, can lead to changes in structure and function. The degradation or citrullination of histone proteins via the enzyme PAD4 can be stimulated by powerful stimulants of the immune system called lipopolysaccharide (LPS). PAD4 is an enzyme that catalyzes the citrullination of histones resulting from the decondensation of DNA chromatin in the NET

Table 1 Sepsis and septic shock definitions

Sepsis	Life-threatening organ dysfunction caused by a dysregulated host response to infection
Sepsis clinical criteria	Organ dysfunction: Defined as 2 or more elevations in the SOFA score Hypotension: SBP 100 mmHg or less Altered mental status: GCS less than 15 Tachypnea: RR 22 or higher
Septic shock	A condition in which the underlying circulatory and/or cellular/metabolic disorders are severe enough to seriously increase mortality
Septic shock clinical criteria	Both of the following with sepsis despite adequate volume resuscitation: Persistent hypotension requiring vasopressors to maintain MAP at 65 mmHg Lactate 2 mmol/L or more

structure and converts the amino acid arginine to citrulline (Vossenaar et al. 2003). The PAD4 enzyme was first detected in human HL-60 leukemia cells and is highly expressed in peripheral blood (Nakashima et al. 2002). It is known that LPS-induced citrullination of histone proteins is an early response of neutrophils to inflammatory stimuli (Neeli et al. 2008). Cit-H3 has been identified as part of the NETs produced, and Cit-H3 is released into the extracellular space as a result of the neutrophil response in the event of infection (Neeli et al. 2009). Our data show links between citrulline proteins and the mechanisms by which diseases such as rheumatoid arthritis, multiple sclerosis, and psoriasis occur. As a result of a study investigating the structure and amounts of citrulline proteins in the arthritic joints of animals, it was found that the severity of inflammation and the amount of citrulline protein were related (Lundberg et al. 2005). It is unknown whether citrullinated histone proteins can be involved as a biomarker in predicting the severity of sepsis during the response of neutrophils to infection. A study has revealed that extracellular histones, particularly H3 and H4, can serve as biomarkers of disease prognosis and therapeutic targets in sepsis (Xu et al. 2009).

Histones are structures located in the cell nucleus and can be released into the bloodstream and tissues when the cell is damaged or during programmed cell death, with the formation of NETs (Brinkmann et al. 2004). It has been shown that many NET components such as histones increase in the systemic circulation in many inflammatory conditions such as sepsis, acute lung injury, autoimmune diseases, and malignancies and damage the vascular endothelial structure by stimulating them (Czaikoski et al. 2016; Caudrillier et al. 2012; Bosmann et al. 2013; Villanueva et al. 2011; Berger-Achituv et al. 2013). In many inflammatory injuries, endothelial barrier dysfunction causes fluid leakage and leukocyte infiltration, resulting in tissue damage and multi-organ failure (Opal and Poll 2015; Kumar et al. 2009). In order to make targeted therapies more effective in inflammatory diseases, it is necessary to elucidate the molecular mechanisms of endothelial barrier regulation (Pfeifer et al. 2013). Endothelial dysfunction, which is the main cause of multi-organ failure in sepsis, causes tissue edema and hemostasis and causes death (Ince et al. 2016). Studies have shown that Cit-H3 increases the permeability of human umbilical vein endothelial cells in vitro. Infusion of Cit-H3 protein into experimental animals has been shown to induce extravasation of fluorescently labeled albumin in mouse mesenteric microvessels without cell death (Meegan et al. 2018). These observations suggest that Cit-H3 may be one of the causes of endothelial damage, vascular leakage, and tissue edema in sepsis (Deng et al. 2020).

A study revealed that serum Cit-H3 level is associated with disease severity in the sepsis model induced by LPS. According to this result, early detection of circulating Cit-H3 level can help predict prognosis in patients with septic shock (Li et al. 2011). Many studies have shown that citrullination of histone proteins is a very important molecular mechanism for NET formation (Papaynnopoulos and Zychlinsky 2009). There are studies showing that histone deimination in neutrophils represents a rapid response to responses elicited by bacterial infections or inflammatory processes (Neeli et al. 2008). In the light of these findings, the idea that histone citrullination plays a role in the emergence of Cit-H3 and H3 in the bloodstream is supported. The

first 3 h is a very important time in the treatment of sepsis. Studies suggest that the detection of Cit-H3 in the sera of ex-patients in less than 3 h is a link between Cit-H3 and the severity of septic shock. In a study comparing the efficacy of Cit-H3 and TNF-α as a mortality biomarker, it was determined that all subjects with Cit-H3 in their serum died. According to this result, it has been claimed that serum Cit-H3 level is a better biomarker than TNF-α in order to distinguish between mild and severe sepsis. In the same study, it was suggested that Cit-H3 not only reflects the changes in TNF-α but is also more useful than TNF-α in monitoring the severity of the shock model induced by LPS (Li et al. 2011). Considering that NETs are the main source for Cit-H3 after infection formation, serum Cit-H3 level was thought to be specific for septic shock, and histone H3 has been suggested as a biomarker in sepsis. Cit-H3 has been isolated in the sera of animals exposed to *E. coli*, and increased levels of Cit-H3 have been correlated with renal impairment (Xu et al. 2009).

Our biomarkers for sepsis are quite inadequate due to their non-specificity for the disease, short half-lives, and insensitivity to monitoring treatment response. It is emphasized that Cit-H3 released by NETs in severe infections may be a good biomarker for sepsis. It has been shown that circulating serum Cit-H3 is not elevated in hemorrhagic shock but is elevated in LPS-induced septic shock. In addition, it has been reported that Cit-H3 in the blood can be detected within 30 min after LPS administration and remains elevated in the blood for 24 h. Cit-H3 is thought to be a reliable biomarker due to its detectability in serum in the early stages of sepsis, its specificity, duration, and response to treatment. Sepsis is the most important cause of death in patients hospitalized in the intensive care unit (Mayr et al. 2014). Excessive NETosis is the most important distinguishing feature of sepsis (Clark et al. 2007). In neutrophils that are activated within 2–3 h after direct stimulation by the pathogen, the nuclear and granular membranes disappear, the plasma membrane is ruptured, and the nuclear content containing granular proteins spreads to the extracellular space (Guimaraes-Costa et al. 2012). There are studies showing that Cit-H3 is released into the circulation within 3 h after peritoneal injection of LPS in the LPS-induced shock model and that circulating serum Cit-H3 levels are correlated with the severity of septic shock (Li et al. 2011).

Comparison of Cit-H3 and Other Sepsis Biomarkers

In a study comparing serum Cit-H3 level with procalcitonin, IL-1β, and IL-6, serum Cit-H3 level was found to be more sensitive for endotoxemia detection. The results show that Cit-H3 is a reliable biomarker in endotoxic shock due to its early detection, high specificity for sepsis, long half-life, and ability to monitor response to therapeutic intervention. Characteristics of an ideal biomarker are given in Table 2. It is thought that serum Cit-H3 levels can diagnose sepsis and septic shock (Pan et al. 2017). It has been thought that extracellular histones in the bloodstream may be associated with tissue damage in the event of infection or during the systemic inflammatory response to trauma (Hampson et al. 2017; Abrams et al. 2013). The release of cytokines, IL-1β and TNF-α, can cause activation of inflammatory cells

Table 2 Characteristics of the ideal biomarker

It should not be affected by disturbances outside the system it belongs to
Analysis should not require expensive hardware
The results of the biomarker should be easily interpreted
The amount should not show widespread variation in the general population
It must be precise, sensitive, and specific for the condition of the disease
Its measurement should be practical and easy, and its levels should be compatible with the stages of the disease
It should be obtained from body tissues and fluids in appropriate and reliable amounts

and initiate migration to the site of infection. An excessive or unregulated release of cytokines is quite common in sepsis, resulting in systemic and local detrimental effects. A relationship was found between TNF-α level and mortality in patients diagnosed with sepsis (Martin et al. 1997). Sustained elevations of cytokine concentrations in patient plasma, including TNF-α and IL-1β, are associated with poor prognosis in septic patients with acute respiratory distress syndrome (ARDS) (Meduri et al. 1995). The reducing effect of anti-histone H3 monoclonal antibody (Cit-H3 mAb) on circulating IL-1β and TNF-α levels can reduce the negative effects caused by excessive cytokine secretion (Deng et al. 2020). Biomarkers used in sepsis diagnosis are listed in Table 3.

Acute lung injury (ALI) occurs in the majority of septic patients and is one of the most common causes of death (Hudson et al. 1995). NETs can immobilize and kill a wide variety of pathogens such as bacteria, fungi, viruses, and protozoa. They try to control infections by preventing the spread of pathogens to the organism, inactivating and killing virulence factors (Brinkmann et al. 2004; Guimaraes-Costa et al. 2009; Baker et al. 2008; Beiter et al. 2006; Buchanan et al. 2006; Saitoh et al. 2012; Urban et al. 2006). However, they also have roles in tissue and endothelial damage, increase intravascular coagulation and thrombosis, and cause the immune system to lose its function (Papayannopoulos 2018).

NETs have very important roles in the pathogenesis of sepsis. NETs can capture pathogens and trap them in a confined space with antimicrobial agents where NET contents are present in high amounts (Nauseef 2007). It also has antimicrobial activity in DNA itself (Halverson et al. 2015). Histones and histone-like proteins take an active role in the fight against pathogens in many different ways (Hoeksema et al. 2016). If the formation of excessive amounts of NET and NET components cannot be controlled, they can cause serious and irreversible harm to sepsis patients. Histones and citrulline histones are specifically identified as Cit-H3 NET components and have been found to be increased in sepsis. Histones bind to endothelial cells and damage endothelial cells, causing an increase in endothelial wall permeability and influx of calcium into the cell, leading to cell death (Saffarzadeh et al. 2012). Histones also cause intravascular thrombosis by disrupting thrombomodulin-dependent protein C activation (Ammollo et al. 2011; Semeraro et al. 2011). In addition to all these, extracellular histones have also been found to cause liver damage through toll-like receptor 2 (TLR2) and TLR4. Cit-H3 secretion has been

Table 3 Sepsis biomarkers

Procalcitonin
C-reactive protein
Interleukin-1β
Interleukin 6
Tumor necrosis factor α
cfDNA

detected in the blood and peritoneal fluid of sepsis patients (Liang et al. 2018; Pan et al. 2017; Biron et al. 2017).

The specificity of blood parameters such as white blood cells, C-reactive protein, and procalcitonin used in suspected inflammatory conditions for sepsis and urosepsis is limited (Mierzchała-Pasierb and Lipinska-Gediga 2019; Pierrakos and Vincent 2010; Giannakopoulos et al. 2017). In patients with suspected urosepsis, biomarkers with high specificity and facilitating diagnosis are needed before clinical worsening. Cit-H3 is a subtype of histone protein that has long been studied as an inflammatory cytokine. Previous studies have shown that histone H3 promotes sepsis by damaging endothelial cells, causing pulmonary dysfunction and inducing disseminated intravascular coagulation (DIC) (Xu et al. 2009; Semeraro et al. 2011; Tang et al. 2012; Abrams et al. 2013; Gould et al. 2016; Xu et al. 2022). At the same time, several clinical studies have confirmed that serum histone H3 concentrations are associated with an increased risk of DIC and death in septic patients (Ito et al. 2019; Wildhagen et al. 2015).

Cit-H3 in Urosepsis

Urosepsis is a clinical condition that is difficult to diagnose and critical to treat in the field of urology. It is very important to provide early diagnosis in urosepsis patients; for this purpose, biomarkers that predict disease development in the early period are needed to improve prognosis and increase survival. There are studies supporting that Cit-H3 values are good predictors in the onset of the disease in urosepsis patients. Vital signs of patients such as blood pressure, heart rate, fever, and respiratory rate are the first indicators in the diagnosis of urosepsis and in the evaluation of the postoperative period. Excess Cit-H3 is released into the bloodstream from NETs and necrotized cells when patients encounter severe trauma conditions, severe infections, and different pathological conditions. This causes damage to the vascular endothelium, leading to platelet aggregation and intravascular coagulation (Meegan et al. 2018; Tang et al. 2012; Denning et al. 2019; Rhodes et al. 2017; Allam et al. 2014; Ekaney et al. 2014; Carestia et al. 2013). Cit-H3 can greatly impair the phagocytosis capacity of macrophages, and extracellular Cit-H3 accumulation may be responsible for the initiation of inflammatory processes leading to sepsis (Friggeri et al. 2012). In a study on urology patients who underwent surgery, histone H3 concentrations were found to be temporarily high in the control group shortly after the operation. The results of many different studies have also shown that high Cit-H3 concentrations are

found in sepsis patients. In a study examining the third and sixth hour Cit-H3 concentrations of postoperative patients, serum Cit-H3 value was found to be more effective than other blood parameters in predicting urosepsis. It is thought that measurement of postoperative serum Cit-H3 concentration will help to improve patient prognosis and to detect urosepsis early. Studies show that serum Cit-H3 concentrations and early changes in the postoperative period in patients with postoperative urosepsis differ significantly from those in patients without postoperative urosepsis (Ito et al. 2019; Wildhagen et al. 2015).

Cit-H3 in Critical Care Patients

In a study aiming to detect NETs and Cit-H3 present in the blood circulation of critically ill patients to be followed up in the intensive care unit, NET components and Cit-H3 were detected in patients with resuscitation, intoxication, cerebrovascular accident, and heat stroke. No NETs or Cit-H3 were found in the blood circulation of patients treated in the intensive care unit for cardiac complaints or exposed to trauma (Hirose et al. 2014). NETs occur in humans in response to pathogens such as bacteria, viruses, and fungi. One study reported that NETs trap circulating bacteria and aid intravascular immunity to stop bacterial spread in case of septic infection. When these results are examined, the presence of NETs or Cit-H3 in the bloodstream of infected and sepsis candidate patients should be suspected. When the sera of patients with cardiopulmonary arrest were analyzed, NETs or Cit-H3 were detected in the bloodstream in a high proportion (62.5%) of these patients (Remijsen et al. 2011; McDonald et al. 2012).

Although there have been many developments in intensive care practices, sepsis continues to threaten life with a mortality rate of 20–30%. Early diagnosis of sepsis is very difficult and very important because early interventions and treatments increase the survival rate of patients (Stoller et al. 2016; Kumar et al. 2011). Although NETs are protective in the initial stages of infection, harmful effects on human life such as promoting sepsis-induced coagulopathy and causing tissue and organ damage have been reported. Cit-H3 is considered the most specific NET marker due to the critical role of histone citrullination in NETosis (Paues Göranson et al. 2018). After severe neutrophil activation in the first step of NETosis, the enzyme PAD4 enters the cell nucleus and induces citrullination of histone H3, leading to chromatin condensation. Therefore, a Cit-H3-specific antibody has been used to detect NETs and to assess neutrophil formation of NETs in vitro, but quantification of Cit-H3 in the living circulation has been difficult (Biron et al. 2017). In addition, Cit-H3 has been detected in rat plasma by ELISA and Western blot methods, as well as in the blood of critically ill and septic patients by Western blot and immunofluorescence (Hirose et al. 2014). In recent studies with the ELISA method, Cit-H3 was detected in a small number of plasma samples in the human LPS-induced endotoxemia model. Cit-H3 was also detected in the plasma of cancer patients by the same ELISA method.

Human data on the increase in circulating Cit-H3 in the sepsis model induced by LPS injection are consistent with studies showing elevations of plasma Cit-H3 in rat models where inflammation is triggered by LPS (Goldmann and Medina 2013; Konig and Andrade 2016). Studies have shown elevations of Cit-H3 in the plasma of mice with only LPS-induced septic shock compared to mice with hemorrhagic shock. As a sepsis marker, Cit-H3 is more sensitive than procalcitonin, which is routinely used as a septic biomarker in endotoxemia and the inflammatory cytokines IL-1β and IL-6. When these data are evaluated, it indicates that the circulating Cit-H3 level will provide great convenience in detecting patients with sepsis. In the human endotoxemia model induced by LPS injection, Cit-H3 could be detected in plasma within 2 h after LPS injection. In addition to being a possible diagnosis and prognostic marker in sepsis, Cit-H3 may also be useful in monitoring the treatment response. Therapeutic agents targeting Cit-H3 can be tried to identify new treatment strategies to combat sepsis and related complications (Czaikoski et al. 2016; Thålin et al. 2017). There are studies showing the essential role of NETs in sepsis by showing the increases in serum Cit-H3 in the human endotoxemia model (Paues Göranson et al. 2018). Plasma Cit-H3 level can be a promising blood biomarker in the prediction, early diagnosis, and prognosis of sepsis (Thålin et al. 2018).

Cit-H3 in ARDS

Histones are highly positively charged core proteins that regulate gene expression through post-translational modifications such as acetylation, methylation, and phosphorylation (Berger 2002). Five subtypes of histones have been described. Of these subtypes, H2A, H2B, H3, and H4 are called core histones. H1 is defined as linker histone. Histone subtypes are shown in Table 4. Histones remain dormant in the nucleus, but when they migrate into the extracellular space, they can produce severe cytotoxic effects (Felsenfeld and Groudine 2003; Chen et al. 2014). ARDS and severe sepsis are the most common causes of mortality in critically ill patients. ARDS criteria are shown in Table 5. Recent studies have associated histone levels in the circulation with disease severity and associated histones with the pathogenesis of these diseases. Treatment strategies that target histone inactivation and degradation have proven effective in correcting sepsis and inflammatory lung injury in animal models (Xu et al. 2009). Histone proteins are released from damaged or activated cells into the extracellular space in cases of sepsis, trauma, ARDS, and other acute organ injuries where they act as potent proinflammatory mediators (Berger 2002). The major source of extracellular histones is the release of intracellular contents out of the cell due to rupture of the plasma membrane after necrotic cell death. Studies have shown that histones facilitate the formation of ARDS and sepsis by disrupting the endothelial function. There are studies showing that extracellular histones, especially H3 and H4, cause endothelial cell death (Xu et al. 2009). Studies have shown that histones in the NET structure and histone blocking antibodies or treatment with histones are responsible for NET-induced cell death in endothelial and

Table 4 Histone protein subtypes

Super family	Family	Subfamily	Members
Linker	H1	H1F	H1F0, H1FNT, H1FOO, H1FX
		H1H1	HIST1H1A, HIST1H1B, HIST1H1C, HIST1H1D, HIST1H1E, HIST1H1T
Core	H2	H2AF	H2AFB1, H2AFB2, H2AFB3, H2AFJ, H2AFV, H2AFX, H2AFY, H2AFY2, H2AFZ
		H2A1	HIST1H2AA, HIST1H2AB, HIST1H2AC, HIST1H2AG, HIST1H2AI, HIST1H2AK, HIST1H2AL, HIST1H2AM
		H2A2	HIST2H2AA3, HIST2H2AC
		H2BF	H2BFM, H2BFS, H2BFWT
		H2B1	HIST1H2BA, HIST1H2BB, HIST1H2BC, HIST1H2BD, HIST1H2BG, HIST1H2BH, HIST1H2BJ, HIST1H2BK, HIST1H2BN, HIST1H2BO
		H2B2	HIST2H2BE
	H3	H3A1	HIST1H3A, HIST1H3B, HIST1H3C, HIST1H3D, HIST1H3E, HIST1H3G, HIST1H3H
		H3A2	HIST2H3C
		H3A3	HIST3H3
	H4	H41	HIST1H4B, HIST1H4D, HIST1H4H, HIST1H4I
		H44	HIST4H4

Table 5 ARDS Berlin criteria

Timing	Acute onset (starting within a week or worsening respiratory symptoms)
Radiological	Bilateral diffuse infiltrates (effusion, nodule, not compatible with mass or atelectasis)
Clinical presentation	Clinical condition unexplained by the presence of heart failure or fluid overload
Hypoxemia	PaO2/FiO2 $\leq$ 300 with other criteria

epithelial cells. As a result of a study on histones in plasma, it was shown that histone release, NET formation, and endothelial damage are responsible for the pathogenesis of ARDS. In rats, inflammation and respiratory dysfunction caused by vascular barrier disruption, infiltration of inflammatory cells into the alveolar space, and potent release of pro-inflammatory cytokines and chemokines have been demonstrated by direct intratracheal injection of histone complex (Abrams et al. 2013). In a study performed by administering histone complexes to rats, it was determined that histones formed endothelial damage by binding to pulmonary and hepatic endothelial cells following the application, and this endothelial damage was the earliest pathological result, resulting in multiple organ failure. There are studies suggesting that serum Cit-H3 levels and platelet counts may play a role as early biomarkers of coagulopathy in sepsis patients at high risk of death (Ito et al. 2019). Endothelial damage, neutrophil accumulation, and pulmonary edema triggered by histones are the hallmarks of ARDS and sepsis (Matthay and Zemans 2011). In a study

demonstrating the role of histones in sepsis, endothelial cells were found to be the primary target of histone-induced cytotoxicity. Different studies have shown that as an alternative mechanism of histone-induced endothelial damage, Cit-H3 disrupts the integrity of the endothelial layer by breaking down the adherent connections between cells and remodeling the cytoskeleton with increased actin stress. As a result of the study, it was also found that the increase in endothelial permeability caused by Cit-H3 was not associated with Rho GTPase activation but was inhibited by forskolin, a cAMP-raising agent (Meegan et al. 2018).

Cit-H3 in Pediatric Sepsis

The kinetic change of the Cit-H3 level in the circulation of the patients within 48 h was associated with the progression of the disease (worsening, change, and improvement). In a study on sepsis conducted in the clinical sepsis group, it was found that the zero-hour Cit-H3 level was higher in patients who died than in those who survived, but there was no significant difference in 48th hour Cit-H3 values (Li et al. 2014). Cit-H3 level in pediatric sepsis patients can be considered both as an early biomarker for sepsis and as a prognostic determinant. The ELISA method has been suggested as the most reliable method for measuring Cit-H3 level as a NET marker and has been confirmed by studies on humans (Thålin et al. 2017; Hoppenbrouwers et al. 2018). In a study in adult patients, Cit-H3 was found to be significantly higher in septic shock patients. Studies conducted in both adult and pediatric patients showed that the sepsis group showed significantly higher Cit-H3 levels than healthy controls. There are studies showing that the Cit-H3 levels of the sepsis group are statistically higher than the suspected sepsis group (Li et al. 2014). The most appropriate time for Cit-H3 detection in pediatric meningococcal sepsis and adult sepsis has been suggested as the acute critical phase after the formation of NETs (Hirose et al. 2014; Hoppenbrouwers et al. 2018). Studies on sepsis in mouse models have shown that Cit-H3 can be used as a biomarker in endotoxemia. In studies in which a sepsis model was created with LPS application, an increase in plasma Cit-H3 level was detected within 0.5 h after the application, and it was found to remain high up to 24 h. Compared to other sepsis biomarkers used (procalcitonin, IL-1β, and IL-6), Cit-H3 was found to be more specific because it is increased only in cases of infection, is rapidly detected in the blood, and has a long half-life (Pan et al. 2017). A study in adult burn patients showed that plasma Cit-H3 level is an early and specific biomarker for sepsis and severe sepsis (Hampson et al. 2017). The use of Cit-H3 as a new biomarker in pediatric sepsis patients shows very promising results. Plasma Cit-H3 level is significantly correlated with disease severity and survival rate in pediatric sepsis patients.

Results of an observational study showed that sepsis patients had higher Cit-H3 levels than trauma patients. The same study confirmed the utility of histones in predicting disease progression in sepsis patients and suggested that Cit-H3 could be used as a reliable indicator to monitor response to antibiotic therapy in sepsis patients (Jackson Chornenki et al. 2019). Increasing evidence suggests that there is a

significant association between histone levels and cardiac dysfunction, MODS, and death in patients with sepsis.

There are studies indicating that the role of circulating Cit-H3 levels in predicting mortality is higher than traditional inflammatory biomarkers such as wbc, C-reactive protein, and cfDNA (Yokoyama et al. 2019). In a study investigating plasma NET levels of 199 patients with DIC admitted to intensive care units, plasma Cit-H3 values were analyzed to assess the severity of coagulation and predict clinical outcome. It was found that increased Cit-H3 levels were correlated with the DIC score and D-dimer increase used in the detection of coagulopathy (Kim et al. 2015).

In a study that aimed to measure histone H3 and H2B levels in septic patients, it was shown that plasma Cit-H3 level can significantly distinguish septic shock cases from healthy controls (García-Giménez et al. 2017). The biggest challenge in the use of such biomarkers is that histones can be found to be high in diseases such as stroke, trauma, diabetic retinopathy, cancer, and rheumatoid arthritis (Allam et al. 2014; Jung et al. 2019; Song et al. 2019).

Cit-H3 and Liver Dysfunction

Liver dysfunction, a component of the multiple organ dysfunction syndrome, is associated with poor prognosis in patients with sepsis. Therefore, predicting liver dysfunction prevents the occurrence of severe organ failure in sepsis patients (Nomura et al. 2019). Being able to assess and predict the extent of organ failure will greatly facilitate clinicians' work and will affect the determination of treatment strategies for patients with sepsis. Citrullinated histone, particularly Cit-H3, has been identified in the formation of NETs (Leshner et al. 2012). Cit-H3 may therefore be considered a biomarker for NETs in sepsis patients and may be a new therapeutic target (Li et al. 2014). NETs play very important roles in the inflammatory environment during infection, but excessive and prolonged exposure to NETs has been reported to cause endothelial damage (Saffarzadeh et al. 2012). Interaction between neutrophils and platelets accelerates NET formation and thrombosis resulting from aggregation of intravascular platelets. Excess microvascular thrombosis can lead to microcirculation disturbances, resulting in organ dysfunction. It is known that endothelial damage and extravasated platelet aggregation that occur after NET formation are the main causes of organ dysfunction such as liver damage. Serum Cit-H3 concentrations allow to differentiate between septic and non-septic shock patients and correlate with disease severity (Nomura et al. 2019).

Cit-H3 and Cardiopulmonary Resuscitation

In a study conducted in patients undergoing cardiopulmonary resuscitation, it was shown that NETs 30 days after successful resuscitation may be associated with poor neurological function in patients (Mauracher et al. 2019). In contrast to the predicted coagulation agents associated with poor prognosis after cardiac arrest, NET

components may also serve as therapeutic targets (Adrie et al. 2005; Buchtele et al. 2018). Administration of agents that target NETs immediately after or during resuscitation may contribute to the prevention of secondary brain injury and improvement in neurological function. In order to determine treatment strategies, it is necessary to determine which step of NET formation should be targeted and which component of NETs is most suitable as a target structure. When the results of the studies are examined, it is thought that the early inhibition of histone H3 citrullination by selective PAD 4 enzyme inhibitors may be a promising approach.

Apoptotic and necrotic cell death are cell destruction mechanisms frequently seen in cancer patients. The cfDNA and nucleosome release, which increase in cell death, do not conclusively indicate NET formation. However, plasma Cit-H3 level during cell death is widely accepted as a specific biomarker for NET formation. A univariate relationship was found between Cit-H3 and cfDNA, two biomarkers of NET formation, and the risk of death in cancer patients. However, in multivariate analyses, Cit-H3 alone is the best biomarker independently for mortality risk in patients with cancer. Studies provide further evidence that NETs are associated with poor survival and mortality in cancer patients (Grilz et al. 2019).

Cit-H3 and Patient Follow-Up

Early diagnosis and rapid initiation of appropriate antibiotic therapy in microbial infections are the most important steps in the treatment of sepsis patients (Rhodes et al. 2017). In order to develop new and rapid diagnosis and treatment methods, it is necessary to understand the molecular mechanisms underlying the pathogenesis of sepsis. In one study, serum Cit-H3 concentrations were positively correlated with PAD2 and PAD4 enzyme concentrations, consistent with preclinical studies. It was found that the serum Cit-H3 level measured at the first admission to the emergency department of patients with septic shock was significantly higher not only compared to healthy volunteers but also compared to patients with other forms of shock. The rate of elevation in serum Cit-H3 is directly proportional to the severity of the disease, and serum Cit-H3 level that continues to rise 48 h after admission to the emergency department is considered an indicator of mortality. Therefore, serum Cit-H3 may represent a crucial component in understanding the pathophysiology of septic shock and may be a useful biomarker in systemic infections (Tian et al. 2021).

There are studies showing that serum Cit-H3 is a possible diagnostic and prognostic blood biomarker for severe inflammatory response in advanced cancer patients (Thålin et al. 2018). It has been found in studies conducted during the pandemic in recent years that serum Cit-H3 levels increased in COVID-19 patients. According to the results of the study, high serum Cit-H3 values measured at the first admission of the patients to the hospital suggest the diagnosis of septic shock and indicate the presence of a microbial infection in the patient. These study findings are consistent with rodent models of endotoxic and septic shock, which reveal that Cit-H3 is released during activation of the host defense mechanism against bacteria.

High serum Cit-H3 concentrations may be useful in identifying patients who may have an undiagnosed infection. Therefore, elevated serum Cit-H3 values in patients without suspected sepsis at the time of first admission to the hospital may lead to the use of empirical antibiotics and to expand investigations in terms of infection. In a study, SOFA scores of patients with high SOFA scores (Table 6) showed a positive correlation with serum Cit-H3 concentration. Patients with elevated serum Cit-H3 levels 24 and 48 h after the start of treatment have a high risk of early death. In addition, patients with the highest serum Cit-H3 levels were observed to be patients with fungemia, which may represent NETosis occurring in response to common fungal infections. These findings may indicate uncontrolled infection, inadequate antimicrobial therapy, or a maladaptive inflammatory response in patients with persistently elevated serum Cit-H3 concentrations. In these patients, the addition of broader antibiotics, antifungal agents, and a larger diagnostic study are required (Tian et al. 2021; Zuo et al. 2020).

Applications to Prognosis

In this chapter, a new biomarker have been reviewed which may represent a crucial component in understanding the pathophysiology of septic shock and may be a useful biomarker in systemic infections (Thålin et al. 2018). In particular, serum Cit-H3 levels are correlated with the severity of septic shock (Li et al. 2011). Preliminary studies suggest that Cit-H3 level is significantly correlated with disease

Table 6 Sofa score criteria

SOFA score					
	0	1	2	3	4
Respiratory PaO2/FiO2	>400	<400	<300	<200 MV	>100 MV
Cardiovascular Hypotension	MAP >70	MAP <70	Dopamine <5	Dopamine 5.1–15 or Epinephrine<0.1 or norepinephrine <0.1	Dopamine >15 or Epinephrine>0.1 or norepinephrine >0.1
Liver Bilirubin	<1.2	1.2–1.9	2.0–5.9	6.0–11.9	> 12
Coagulation Platelet 10^3/mm3	>150	<150	<100	<50	<20
Kidney Creatinine mg/dL, urine output	<1.2	1.2–1.9	2.0–3.4	3.5–4.9 Output <500 ml/d	<1.2 Output<200 mL/d
Neurological Glasgow coma score	15	13–14	10–12	6–9	<6

severity and survival rate in pediatric sepsis patients. It is possible that these biomarkers may be used clinically in investigating prognosis in patients. For example, histone proteins are released from damaged or activated cells into the extracellular space in cases of sepsis, trauma, ARDS, and other acute organ injuries where they act as potent proinflammatory mediators (Berger 2002).

Applications to Other Diseases or Conditions

In this study, we review a new biomarker in sepsis and critical care. Application of this methodology suggests that histone proteins plays a role in the mechanisms of diseases such as rheumatoid arthritis, multiple sclerosis, and psoriasis. At the same time, several clinical studies have confirmed that serum histone H3 concentrations are associated with an increased risk of DIC and death in septic patients. Serum Cit-H3 concentration will help to improve patient prognosis and to detect urosepsis early. The biggest challenge in the use of such biomarkers is that histones can be found to be high in diseases such as stroke, trauma, diabetic retinopathy, cancer, and rheumatoid arthritis (Allam et al. 2014; Jung et al. 2019; Song et al. 2019).

Mini-Dictionary of Terms

Cit-H3: A histone protein released from neutrophils and helps to diagnose sepsis.
NETosis: An important innate immune mechanism to combat pathogenic bacteria, fungi, and parasites.
Lipopolysaccharide-induced sepsis: They can activate cells of the innate immune system, such as macrophages and neutrophils, and cause inflammation.
Histones: They are highly positively charged core proteins that regulate gene expression through post-translational modifications.
Sepsis: Life-threatening organ dysfunction that can lead to death can occur from an infection in human body.

Key Facts of Cit-H3 in Sepsis and Critical Care

Scores used in the diagnosis of sepsis correlate with Cit-H3 levels.
Cit-H3 concentrations remain high in patient plasma for up to 48 h and are effective in diagnosis and follow-up.
Post-cardiac arrest patients have higher plasma Cit-H3 levels, which may predict mortality.
Compared with CRP, procalcitonin, and IL-6, Cit-H3 was found to be a better biomarker in the diagnosis of sepsis.
Drugs targeting Cit-H3 may be useful in the treatment of sepsis.

Summary Points

- Early detection of circulating Cit-H3 level can help predict prognosis in patients with septic shock.
- Increased levels of Cit-H3 have been correlated with renal impairment.
- Excessive NETosis is the most important distinguishing feature of sepsis.
- Histone H3 promotes sepsis by damaging endothelial cells, causing pulmonary dysfunction and inducing disseminated intravascular coagulation.
- Serum Cit-H3 value was found to be more effective than other blood parameters in predicting urosepsis.
- Histone release, NET formation, and endothelial damage are responsible for the pathogenesis of ARDS.
- Sepsis patients had higher Cit-H3 levels than trauma patients.
- Cit-H3 levels were correlated with the DIC score and D-dimer increase used in the detection of coagulopathy.

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Tumor Necrosis Factor-Alpha (TNF-Alpha) as a Biomarker in Trauma and Critical Care

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Serap Biberoglu and Seda Ozkan

Contents

Abstract

Tumor necrosis factor-alpha (TNF-α) is mainly synthesized from monocytes and macrophages. It is a kind of glycoprotein that also contributes to the production of

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T-lymphocyte, neutrophil, mast cell, fibroblast, and endothelial cells. The most important stimuli that start TNF-α synthesis and release are lipopolysaccharides.

TNF was first described by Carswell in 1975 and was named TNF because of its cytotoxic activity to tumor cells through immune cells.

Tumor necrosis factor-alpha (TNF-α) plays a role in some late consequences of trauma such as sepsis, multi-organ failure, and ischemia-reperfusion injury.

Severe trauma is still the most common cause of death in people under the age of 40. Severe brain injuries, significant blood loss (hemorrhagic shock) after blunt, and penetrating injuries determine the causes of sudden and early death in traumas. After multitrauma, hemodynamic disorders may occur resulting in direct organ damage or organ failure.

Endothelial cell damage, leukocyte accumulation, disseminated intravascular coagulation (DIC), and microcirculatory dysfunction eventually lead to programmed cell death (apoptosis) and necrosis of parenchymal cells (microenvironment theory) caused by multiple organ dysfunction syndrome (MODS) or multiple organ failure.

Researching TNF-α and TNFRs levels in multitrauma patients were important in terms of TNFα being a biomarker. Studies have shown that increases in TNFR1 and TNFR2 expression levels in monocytes and lymphocytes were significantly associated with the severity of traumatic injury.

In this chapter, the relationship between tumor necrosis factor-alpha (TNF-alpha) as a biomarker in trauma and critical care was discussed in light of scientific studies.

Keywords

TNF-α · Tumor necrosis factor-alpha · Trauma · Critical care · Biomarker · Soluble tumor necrosis factor receptor 1 · Traumatic brain injury · Tumor necrosis factor receptor 1 · Multiple organ dysfunction syndrome · Cytokines · Sepsis

Abbreviations

ADAM17	A disintegrin and metalloproteinase 17 domain
Ag	Antigen
ALI	Acute lung injury
APC	Antigen-presenting cells
ARDS	Acute respiratory distress syndrome
CARS	Compensatory anti-inflammatory response syndrome
CPP	Cerebral perfusion pressure
CSF	Cerebrospinal fluid
DAI	Diffuse axonal injury
DIC	Dissemine intravascular coagulation
GCS	Glasgow Coma Scale
h	Hour
ICP	Increased intracranial pressure
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6

IL-8	Interleukin-8
ISS	Injury severity score
MODS	Multiple organ dysfunction syndrome
MOF	Multiple organ failure
mRNA	Messenger ribonucleic acid
PMNL	Polymorphonuclear leukocytes
PTSD	Postconcussive syndrome and posttraumatic stress disorder
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SIRS	Systemic inflammatory response syndrome
TACE	TNF-α converting enzyme
TBI	Traumatic brain injury
TH	T-helper cells (lymphocytes)
TNF	Tumor necrosis factor
TNFR1	Tumor necrosis factor receptor 1
TNFR1s	Soluble tumor necrosis factor receptor 1
TNFR2	Tumor necrosis factor receptor 2
TNFRS	Soluble tumor necrosis factor receptor
TNF-α	Tumor necrosis factor-alpha

Introduction

TNF-α

Tumor necrosis factor-alpha (TNF-α) (cachectin) is mainly synthesized from monocytes and macrophages. It is a kind of glycoprotein that also contributes to the production of T-lymphocyte, neutrophil, mast cell, fibroblast, and endothelial cells (Tables 1 and 2). It plays an important role in the initiation and continuation of inflammation in many autoimmune diseases, as it is the first cytokine that can be detected in the blood with tissue damage and physical stress (Vilcek 2008).

Table 1 The effect of TNF-α on cells

Macrophage	***Fibroblast***
Support in maturation	Supporting development and ensuring reproduction
Increase in chemokine release	Suppression of type 1 collagen gene and decrease in collagen synthesis
Increased IL-1 production	Prevention of proteoglycan synthesis and increase in cartilage resorption
Monocyte	***Hepatocyte***
Support in maturation	Increase in the acute phase response
Increase in IL-1 production	
T cell	***Glia***
Support in maturation	Modification of synaptic communication
Proliferation	
B cells	***Synoviocyte***
Support in maturation	Increase in metalloproteinase synthesis and development of cartilage damage
Antibody production	

Table 2 The effect of TNF-α on endotel cells

Increase in chemokine release
Increased vascular endothelial growth factor secretion
Increase in IL-1 production
Adhesion molecules, especially selectin, increase in expression
Increased cell infiltration and angiogenesis
Inhibition in thrombomodulin synthesis stimulation of the extrinsic coagulation pathway
Decreased in plasminogen activators, as a result increased procoagulant effect

The most important stimuli that start TNF-α synthesis and release are lipopolysaccharides. Although the release of TNF-α can be induced even by itself, its release is increased by gram-positive bacteria, parasites, viruses, tumor cells, immune complex, complement system activation, interleukin (IL)-1, IL-2, and interferon-γ (Tseng et al. 2018). Expression begins within 30 min after stimulation, peaks at 90–120 min, and decreases to undetectable levels within 240 min. Thanks to the 50-fold increase in the transcription rate in the promoter region of the TNF gene in macrophages and hundreds of times increase in the translational capacity of messenger RNA during the production phase, a thousand-fold increase in its production can be achieved (Parameswaran and Patial 2010).

History of TNF

TNF was first described by Carswell in 1975 and was named TNF because of its cytotoxic activity to tumor cells through immune cells (Carswell et al. 1975; Kany et al. 2019).

Actually, it started when physician P. Bruns, who lived in France in the 1860s, noted in his notes that patients with bacterial infections developed regression in tumor sizes. In the following period, a surgeon named W. Coley preferred to treat sarcomas with bacterial extracts. Tumor size regression was observed after the use of these products; however, toxic effects were also occurring. Because of these observed effects, this product was later named "Coley toxin" (McCarthy 2006).

In the 1940s, it was understood that the potent site in the tumor toxin was the lipopolysaccharides found in the cell wall of gram-negative bacteria. Loyd Old, who worked in the field of cancer and immunology in the 1960s and 1970s and was the discoverer of T-helper and killer cells, observed that antitumor activity developed in mice vaccinated with *Mycobacterium bovis*, but this effect did not develop when BCG vaccine was administered directly on tumor cells. Thereupon, he stated that the regression in tumor size could not be achieved by lipopolysaccharides alone, and he theorized that the mice might actually have secreted a substance indirectly (Carswell-Richards and Williamson 2012).

In 1975, Carswell, in his experiments on mice, stated that there is a cytotoxic factor that provides the development of necrosis in sarcoma. Thus, the name of "tumor necrosis factor" started to be mentioned for the first time in the literature (Carswell 1975).

Anthony Cerami conducted studies on the molecule, and he named it as "Cachectin"on patients with cancer and tuberculosis (Sherry and Cerami 1988).

Finally, together with Old Ceramini and Bruce A. Beutler, they succeeded in purifying this mysterious substance in 1980 (Bashyam 2007). By 1985, it was realized that cachectin and TNF-α are actually the same molecule. Over time, Goeddel succeeded in elucidating the receptor structure and signaling pathways leading to apoptosis (Chen and Goeddel 2002).

The Structure of TNF-α

A three-monomer, bell-shaped transmembrane glycoprotein formed from the use of 212 amino acids. It contains cysteine residues associated with disulfide bonds, which is determinative in biological function.

TNF-α, a kind of metalloproteinase called ADAM17, is converted to monomers by the converting enzyme (TACE) and released into the circulation to exhibit autocrine and paracrine effects in the form of a soluble TNF-α molecule (Cantarini et al. 2012).

The Role of TNF-α in Trauma

Tumor necrosis factor-alpha (TNF-α) plays a role in some late consequences of trauma such as sepsis, multi-organ failure, and ischemia-reperfusion injury (Rabinovici et al. 1993).

After trauma, hemodynamic disorders that will result in direct organ damage or organ failure in the body may be accompanied.

Severe trauma is still the most common cause of death in people under the age of 40. Severe brain injuries, significant blood loss (hemorrhagic shock) after blunt, and penetrating injuries determine the causes of sudden and early death in traumas. Late deaths are determined by secondary brain injuries and host defense failure. These first hits represent a greater challenge, as local tissue damage such as contusions or lacerations, hypoxia, and hypotension induce greater local and systemic host responses to preserve immune integrity and stimulate reparative mechanisms. SIRS (Table 3) is characterized by the local and systemic production and release of different mediators, such as proinflammatory cytokines, complement factors,

Table 3 Systemic inflammatory response syndrome is defined by the satisfaction of any two of the criteria below

Body temperature over 38 or under 36 °C
Heart rate greater than 90 beats/minute
Respiratory rate greater than 20 breaths/minute or partial pressure of CO_2 less than 32 mmHg
Leucocyte count greater than 12,000 or less than 4000/microliters or over 10% immature forms or bands

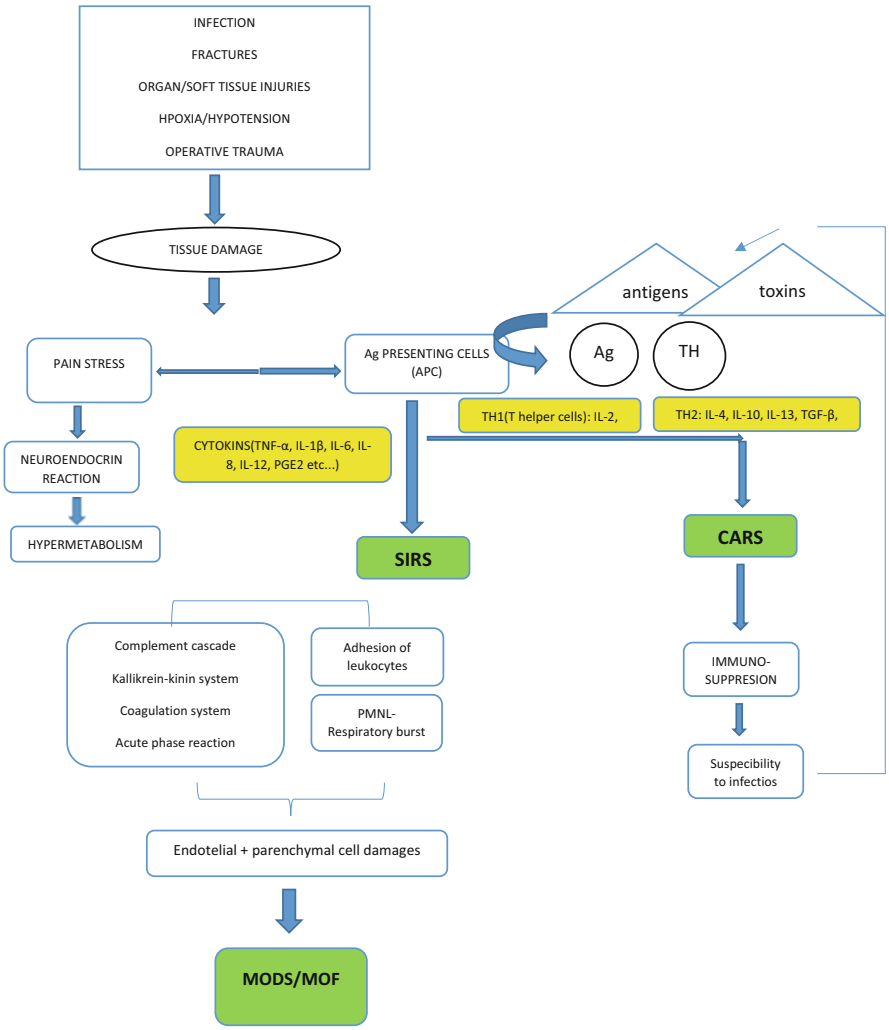

Fig. 1 Host defense response after trauma. *APC* antigen-presenting cells, *TH* T-helper cells (lymphocytes), *SIRS* systemic inflammatory response syndrome, *CARS* compensatory anti-inflammatory response syndrome, *PMNL* polymorphonuclear leukocytes, *MODS* multiple organ dysfunction syndrome, *MOF* multiple organ failure

proteins of the contact phase and coagulation systems, acute phase proteins, neuro-endocrine mediators, and accumulation of immunocompetent cells in the local tissue injury (Fig. 1) (Keel and Trentz 2005; Lenz et al. 2007; Clark et al. 2010). And also, this systemic inflammation increases with second hits such as ischemia/reperfusion injuries, surgery, or infections (two-hit theory) (O.D. Rotstein 2003; Rose and Marzi 1996).

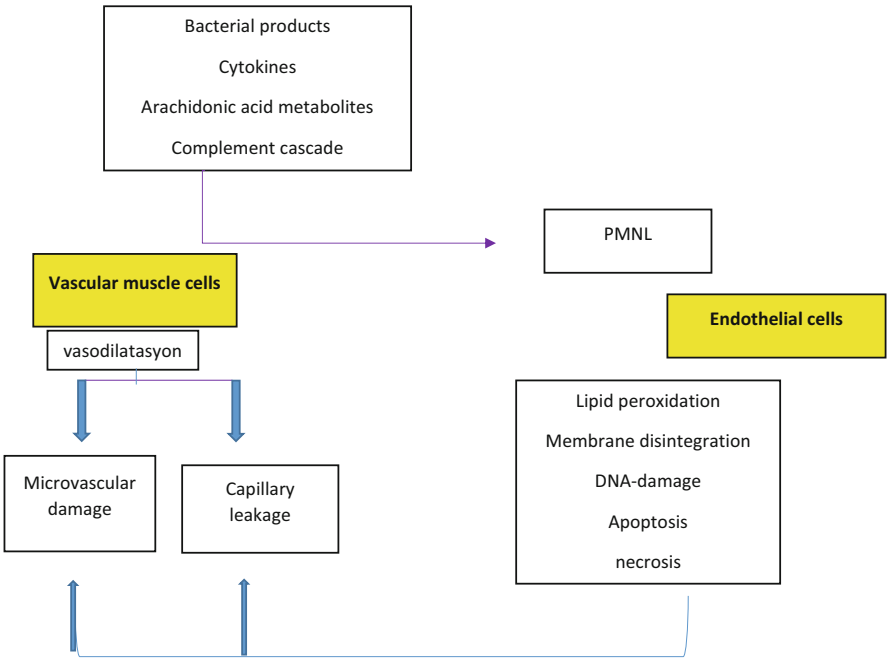

Fig. 2 Endothelial cell damages and the development of a capillary leakage. *PMNL* polymorphonuclear leukocytes

In addition, different clinical trials have shown that, in parallel with the proinflammatory reaction, anti-inflammatory mediators are produced (compensatory anti-inflammatory response syndrome (CARS)) to avoid the autodestructive effects of immunocompetent cells (Fig. 1) (Keel and Trentz 2005). An instability between these two immune responses, with excessive release of pro- or anti-inflammatory mediators, appears to be responsible for organ dysfunction and increased sensitivity to infections and sepsis (Hensler et al. 2002; Martin 1997).

Endothelial cell damage (Fig. 2), leukocyte accumulation, disseminated intravascular coagulation (DIC), and microcirculatory dysfunction eventually lead to programmed cell death (apoptosis) and necrosis of parenchymal cells (microenvironment theory) caused by multiple organ dysfunction syndrome (MODS) or multiple organ failure.

Tissue injury induces in proportion to the severity of trauma, genetic factors, general condition of the host, and type of antigens, both local and systemic release of proinflammatory cytokines and phospholipids. Tissue macrophages (e.g., alveolar macrophages), polymorphonuclear leukocytes (PMNL), monocytes lymphocytes, natural killer cells, and parenchymal cells are involved in a complex network of this host defense response. Hyperinflammation (proinflammatory response) leads to the

clinical appearance of SIRS and finally to host defense failure (MODS, MOF) (Keel and Trentz 2005; Giannoudis et al. 2004).

In a study conducted with 47 multitrauma patients in 2001, it differed depending on time. TNFα and soluble TNF receptor levels were elevated relative to those of healthy subjects. TNFα and TNF-R2 levels measured 4 h after trauma were found to be above the normal range during the entire observation period. Mean values of sTNFR1 plasma levels were higher than normal, but no significant difference was found. Severe trauma resulted in increased levels of sTNFR1 at the scene and during hospitalization. SIRS development with elevated TNFR1s started on the spot. It was present at presentation with sTNF-R2 increasing from day 1 to day 4. The outcomes of the patients were not associated with either TNF-α or TNF receptor levels (Spielmann et al. 2001).

In a study conducted with 84 patients with multiple trauma, TNF-α levels were examined to investigate correlations between injury severity score (ISS) and new biochemical parameters. TNF-α levels were found to be significantly higher. It was found to be significantly higher than the control group and it was found to be correlated with trauma severity, sand also can be used to evaluate trauma severity (Alper et al. 2016).

A study investigating TNF-α and TNFRs (soluble tumor necrosis factor receptor level) in multitrauma patients had important data in 2013. Blood samples were analyzed within 2 h after hospital admission, 6–8 h and 1–5 days after admission. TNF-α and TNFRs were found to be higher than the control group in the first 2 h and remained significantly higher until 3–5 days. In trauma patients, increased levels of TNF-α and TNFR were correlated with the severity of traumatic injury early after injury, supporting the hypothesis that trauma-induced organ dysfunction may result from an auto-destructive inflammatory response (Liu 2013).

TNFR1 and TNFR2 were analyzed in leukocytes under control conditions with high expression levels in neutrophils, moderate in monocytes, and lower expression levels in lymphocytes. Traumatic injury resulted in an increase in the highest expression levels of TNFR1 and TNFR2 in the H-ISS patient group. In particular, prominence of monocytes and lymphocytes in the early stages following trauma resulted in injury-induced increase in expression levels of TNFRs. In addition, increases in TNFR1 and TNFR2 expression levels in monocytes and lymphocytes were significantly correlated with the severity of traumatic injury (Liu 2013).

In response to severe trauma, plasma levels of TNF and related receptors increase. In contrast, results for elevated TNF-α levels following trauma are generally negative. It has been reported that low TNF-α levels promote the remodeling or replacement of injured tissue by stimulating fibroblast growth (Spielmann et al. 2001; Cinat et al. 1994, 1995; Rabinovici et al. 1993).

Depending on the severity of injury and the post-traumatic course, anti-inflammatory mediators are also produced. Natural inhibitors of receptors, like soluble TNF-receptors (TNF-R1 and TNF-R2), or IL-1 receptor antagonist (IL-1ra) are detectable in the serum of injured patients, correlating also with the ISS and the incidence of post-traumatic complications (Ertel et al. 1995; Hensler et al. 2002).

TNF-α is mainly produced by inflammatory cells (such as monocytes and macrophages). Nonimmune cells (such as fibroblasts, neurons, keratinocytes, and smooth muscle cells) also produce TNF. TNF-α is an acute phase protein involved in the local inflammatory response. As a result of increased vascular permeability with high TNF levels, it leads to the recruitment of macrophages and neutrophils to the injured/infectious area. The action of TNF-α is mediated by TNFRs on the cell surface. TNFRs has two different members. TNFR1, known as p55, is constitutively expressed in most cell types, while TNFR2, called p75, is restricted to hematopoietic cells (Lui 2014; Woodcock and Morganti-Kossmann 2013; Loetscher et al. 1990).

Cytokines composed of polypeptides exhibit para- or autocrine behavior. In addition to hyperacute proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) or interleukin-1β (IL-1β), there are also subacute (secondary) cytokines that act after 1–2 h. Increased serum levels of TNF-α, IL-1β, or IL-8 are observed in the bronchoalveolar lavage fluids of patients with systemic inflammation, as well as those with chest trauma or acute respiratory distress syndrome (Keel and Trentz 2005; Martin 1997; Donnelly et al. 1994).

After the typical cell death proteins TNF-a or Fas ligand (CD95 ligand) bind with TNF-R1 and Fas antigen (CD95 antigen) receptors, and after activation of complex intracellular cascades such as intracellular proteases (e.g., calpains, caspases) and effector enzymes, the cell they induce death. Cellular expressions of TNF-RI and Fas antigen or their soluble molecules are elevated in serum from injured patients after surgery or during sepsis (Freitas et al. 2004; Keel and Trentz 2005).

Local (Kupfer cells) and systemic release of proinflammatory cytokines such as TNF-α, IL-1β, IL-6 induces the acute phase reaction in the liver. So, it improves tissue protective and antimicrobial mechanisms (Whicher and Evans 1990; Keel and Trentz 2005).

The Role of TNF-α in Sepsis

Sepsis is one of the most serious complications seen in patients after major trauma. Although TNF-α was found to be high in some of the previous studies performed in the intensive care unit (Hashmi and Zeng 2006; Cua and Tato 2010; Ahmed Ali 2018) in multitrauma patients, no significant elevation was observed.

In recent studies analyzing the diagnostic value of TNF-α in patients with multiple abdominal trauma in the intensive care unit, significant elevations were found in the sepsis groups (Zhai et al. 2021).

TNF-α releases other cytokines in the early stage of infection. Therefore, it plays a central role in the systemic inflammatory response due to its direct effect on septic shock, and TNF-α plasma levels are associated with sepsis-induced death (Georgescu et al. 2020).

In contrast to TNF-α and sTNF-R2, increased levels of sTNF-R1 in the very early stage were thought to be associated with the severity of trauma in studies, and both soluble receptors are clinically associated with SIRS and MODS in the early post-traumatic stage. In the case of early SIRS, sTNF-R1 rises soon after the injury, while

sTNF-R2 levels rise 24 h later. These results confirm other research showing that both receptor subtypes are involved in the inflammatory response (Spinas et al. 1992; Van Zee et al. 1992; Spielmann et al. 2001).

Conversely, some data show elevated levels of sTNF-R2 to be significantly associated with MODS at hospital admission (Spielmann et al. 2001). Others also found a correlation for MODS and a membrane-bound TNF-α/sTNF-R2 ratio (Pellegrini et al. 1996). However, trauma-induced ischemia/reperfusion injury has been shown to produce extremely high levels of sTNF-R2, but not correlated with the development of a MODS at any time (Seekamp et al. 1998).

The Role of TNF-α in Traumatic Brain Injury (TBI)

In the brain, microglia and astrocytes produce TNF. TNF, a potent multifunctional proinflammatory cytokine, has shown in early studies that TNF expression is detrimental, mostly in rat models of TBI (Table 4).

In a study conducted on veterans and soldiers after 9/11, it was found that there were changes in biomarker activity, especially in those with recurrent TBI, and this was associated with chronic neurological and behavioral symptoms. Cytokines, including IL-10 and TNF-α, are key components of inflammatory responses in the CNS in a study that showed an association between TNF-α concentrations and symptoms of postconcussive syndrome and posttraumatic stress disorder (PTSD) (Guedes et al. 2020). Again, in a study supporting this study, TNF-α levels were found to be associated with the severity of Neurobehavioral Symptom Inventory and PTSD symptom scores, and this confirms the previous findings of the group describing increased blood TNF-α levels and chronic TBI symptoms in military personnel (Devoto et al. 2017).

Eight cytokines like TNF-α were studied as a biomarker in 60 patients with intracranial infection due to traumatic brain injury and were found to increase significantly, closely related to the severity of the infection (Zhang et al. 2021).

The Role of TNF-α in Lung Injury/ARDS

Recent studies evaluating biomarkers in patients with ALI/ARDS (acute lung injury/acute respiratory distress syndrome) have identified a number of biomarkers that predict clinical outcomes; one of them was TNF-α. In addition, studies have provided information about the pathogenetic mechanisms underlying ALI/ARDS (Calfee et al. 2007; Ware et al. 2004; Uchida et al. 2006; Fremont et al. 2010). Patients with ALI had higher severity of illness scores, more days of mechanical ventilation, longer hospital stays, and higher mortality versus controls. Biomarker TNF-α had a high diagnostic accuracy as reflected in differentiating ALI from controls (Fremont et al. 2010).

Although they found significant differences in this study comparing a panel of eight biomarkers (including TNF-α) in patients with traumatic ALI/ARDS with

Table 4 Studies relevant to the development of TNF as biomarkers of TBI

Species	Tissue/fluid	Findings	Reference
Rat	Brain homogenates, brain slices	Increased mRNA and protein expression detectable at 1 h, and peak expression between 4 and 8 h post-TBI	Taupin et al. 1993; Shohami et al. 1994; Fan et al. 1996; Knoblach et al. 1999; Dalgard et al. 2012
Rat	Brain homogenates	TNF expression increases after severe TBI, but not mild TBI	Knoblach et al. 1999
Rat	Brain homogenates	DAI and post-traumatic hypoxia lead to increased expression of TNF versus DAI alone	Yan et al. 2011
Rat	CSF	Peak levels of TNF in CSF are not reached until 24 h after TBI	Stover et al. 2000
Human	CSF, serum, plasma	TNF is increased in CSF, serum, and plasma following TBI	Goodman et al. 1990; Ross et al. 1994; Morganti-Kossmann et al. 1997; Csuka et al. 1999
Human	Postmortem tissue	TNF mRNA and protein can be detected in the brain within minutes of injury	Frugier et al. 2010
Human	CSF	TNF protein concentrations peak in the CSF within 24 h	Hayakata et al. 2004
Human	CSF, serum	Six hours after TBI, TNF expression is higher in CSF than in serum. TNF expression does not correlate with outcome Increased serum TNF levels correlate with increased ICP and decreased CPP, but not outcome. TNF concentrations in CSF not linked to ICP, CPP, or outcome	Shiozaki et al. 2005

CSF Cerebrospinal fluid, *CPP* Cerebral perfusion pressure, *ICP* Increased intracranial pressure, *TBI* Traumatic brain injury, *TNF* Tumor necrosis factor

patients with nontraumatic ALI/ARDS, however, this study did not examine the role that biomarkers may play in the diagnosis of ALI/ARDS (Calfee et al. 2007).

In a 2017 study with seven biomarkers, it was shown to have high diagnostic accuracy in distinguishing trauma patients with ARDS from those without.

Applications to Prognosis, Other Diseases or Conditions

Applications to Prognosis

It has been determined that TNF, as a proinflammatory cytokine, triggers diseases such as rheumatoid arthritis, insulitis, inflammatory bowel disease, in which abnormal TNF production is shown after discovery. It is characterized in inflammation of

various diseases such as sepsis, chronic immune and autoimmune pathologies, cancer and neurodegeneration, peripheral and central nervous system diseases (Probert 2015).

Applications to Other Diseases or Conditions

In the study that measured TNF levels in CSF (Cerebrospinal fluid) in patients with mild cognitive impairment, the detection of Alzheimer's was much higher in those with higher TNF levels, making it explanatory in terms of approach to the question of whether intrathecal inflammation is a response to the onset of Alzheimer's or its presence (Tarkowski et al. 2003). Another study took advantage of the increased sensitivity of the assay for soluble TNF receptors rather than TNF itself. They found good evidence on the levels of these receptors in serum and CSF that predict conversion to clinical Alzheimer's disease over a 4–6-year period (Buchhave et al. 2009).

Major Depression: A meta-analysis of 24 studies reported significantly higher concentrations of TNF and IL-6 in depressed subjects than in control subjects (Dowlati et al. 2010).

The Role of TNF-α in Covid-19: Inflammatory mediators such as TNF-α have been reported to be higher in critically ill patients (Huang et al. 2020). TNFα released from SARS-CoV-2-infected macrophages and monocytes lead inflammation-derived injurious cascades causing MODS/MOF (Iwasaki et al. 2021).

Neuroinflammation is important in the pathological process of neurological disease. Microglial activation is a criterion feature of neuroinflammation (Lv et al. 2011). Previous data from in vitro or chronic neurodegenerative disease have shown that microglia activation contributes to neuroinflammation and exacerbation of neuronal damage. In contrast, several recent studies have demonstrated that activated microglia provide a neuroprotective role and prevent neuronal loss after brain injury (Napoli and Neumann 2010; Wang et al. 2013).

Mini-Dictionary of Terms

- **SIRS:** Systemic inflammatory response syndrome is an exaggerated defense response of the body to a noxious stressor (infection, trauma, surgery, acute inflammation, ischemia or reperfusion, or malignancy, to name a few) to localize and then eliminate the endogenous or exogenous source of the insult.
- **DIC:** Abnormalities of the hemostatic system in patients result hypercoagulation and hyperfibrinolysis.
- **ISS:** The injury severity score (ISS) is an established scoring system to assess trauma severity. It correlates with mortality, morbidity, and hospitalization time after trauma. It is used to define the term major trauma. A major

trauma (or polytrauma) is defined as the injury severity score being greater than 15.

- **Cytokine:** A type of protein that is made by certain immune and nonimmune cells and has an effect on the immune system. Some cytokines stimulate the immune system and others slow it down.
- **ARDS:** Acute respiratory distress syndrome is a life-threatening condition where the lungs cannot provide the body's vital organs with enough oxygen. It is a type of respiratory failure characterized by rapid onset of widespread inflammation in the lungs.

Key Facts of TNF-Alpha

- TNF-α is mainly synthesized from monocytes and macrophages.
- A three-monomer, bell-shaped transmembrane glycoprotein formed from the use of 212 amino acids.
- TNF-α plays a role in trauma such as sepsis, multi-organ failure, and ischemia-reperfusion injury.
- The most important stimuli that start TNF-α synthesis and release are lipopolysaccharides.
- TNF-α levels are changed in multitrauma.
- TNF-α levels are correlated with injury severity score (ISS).

Summary Points

- Tumor necrosis factor-alpha (TNF-α) plays roles in some late consequences of trauma such as sepsis, multi-organ failure, and ischemia-reperfusion injury.
- Tissue injury induces in proportion to the severity of trauma, genetic factors, general condition of the host, and type of antigens, both local and systemic release of proinflammatory cytokines and phospholipids.
- Studies have shown that increases in TNFR1 and TNFR2 expression levels in monocytes and lymphocytes were significantly associated with the severity of traumatic injury.
- In trauma patients, increased levels of TNF-α and TNFR were correlated with the severity of traumatic injury early after injury, supporting the hypothesis that trauma-induced organ dysfunction may result from an auto-destructive inflammatory response.
- In contrast to TNF-α and sTNF-R2, increased TNFR1s levels in the very early period were thought to be associated with the severity of trauma in studies, and both soluble receptors can be said to be clinically associated with SIRS and MODS in the early posttraumatic period.
- Although more evidence is needed, TNF-α levels can be used as an auxiliary biomarker in determining the prognosis of multitrauma patients.

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ADAM10 as a Biological Marker in Traumatic Brain Injury

41

Sathiya Sekar and Changiz Taghibiglou

Contents

Abstract

Mild Traumatic brain injury (mTBI)/concussion is a growing epidemic health issue throughout the world. Concussion/mTBI is relatively common among children and adolescents mostly due to sport-originated incidences. In fact, according to the Centers for Disease Control and Prevention (CDC), concussions are a major public health concern, which disproportionately affect youth, with more than half of mTBI occurs in children and adolescents. Concussions in the adolescent patients are of particular concern as their brains are still developing and are more susceptible to injury. Misdiagnosis and/or mismanagement of mTBI victims is of greater concern in healthcare sectors due to the low detectability through imaging and a complex spectrum of symptoms. Accurate and on-time diagnosis is of greater importance as it would assist in decision making

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on return to play and to provide rapid deployment of treatment and rehabilitation services for the patients. In the last decade, there has been an intensive search for blood-based biomarkers of mTBI, and recently FDA has approved the first blood-based mTBI diagnostic kit. In the present chapter, we discuss about the ADAM10 and its plasma levels as a potential diagnostic biomarker for TBI.

Keywords

Traumatic brain injury · Mild injury · Concussion · ADAM10 · Blood biomarker

Abbreviations

ADAM10	A disintegrin and metalloproteinase 10
CD-US	Color Doppler ultrasound
CT	Computed tomography
E9.5	Embryonic day 9.5
GCS	Glasgow Coma Scale
HIV	Human immunodeficiency virus
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
mTBI	Mild traumatic brain injury
NG2	Nerve-glia antigen 2
PrPC	Cellular prion protein
SPECT	Single-photon emission computed tomography
TBI	Traumatic brain injury

Introduction

Traumatic brain injury (TBI) is one of the leading causes of death worldwide. It is caused by the external mechanical forces to the head leading to temporary or permanent impairment in physical, cognitive, and/or psychosocial functions (Reis et al. 2015). The damages to the brain may be either direct or transmitted through falls, motor vehicle collisions, sports-related, blasts, or any abuse/assaults. Further, injuries to the brain also occur by surgeries, infections, brain tumors, ischemia, or stroke. Depending on the severity of the brain insult, TBI was categorized into mild, moderate, and severe (Reviewed in Najem et al. 2018).

Global incidences of 69 million individuals have been reported for TBI every year (Dewan et al. 2019; Biessels et al. 2006; GBD 2016 Traumatic Brain Injury and Spinal Cord Injury Collaborators 2019). The incidence of mild, moderate, and severe TBI was found to be 131, 15, and 14 cases per 100,000 people, respectively (Dawodu 2021). This data clearly showed that about 82% of the patients affected with brain injury are mild injured ones (Fig. 1). In Canada, it was reported that one in every three Canadians was affected by brain injury. Further, brain injury accounts for

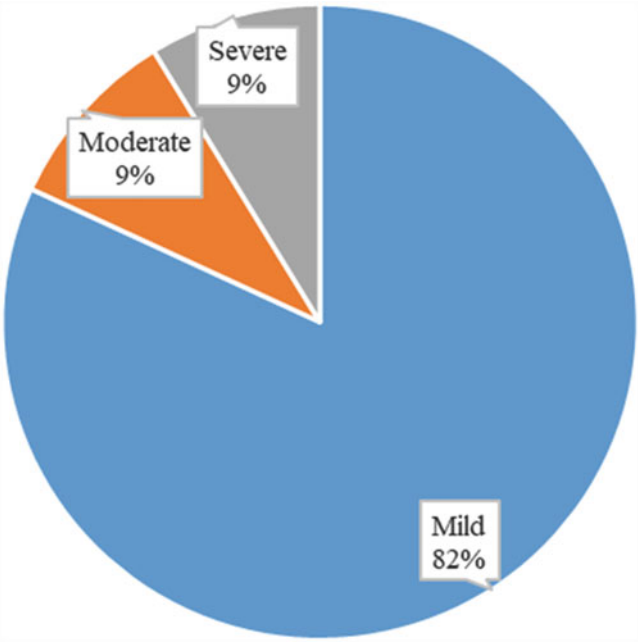

Fig. 1 Percentage of traumatic brain injury cases based on the severity of the brain insults

about 85% of all cases of brain insults including HIV, neurodegenerative diseases, cancer, and spinal cord injury (Brain Injury Canada; Acquired Brain Injury 2015). Though there are various numbers reported, the occurrence of TBI are not well documented as the patients with mTBI are not hospitalized due to the mild injury.

Mild TBI (mTBI) or concussion is most common among children and adolescents due to sports-related injuries. In addition, the Centers for Disease Control and Prevention (CDC) reported mTBI as a major public health problem, as more than half of the patients were children and adolescents (CDC 2022). Furthermore, the TBI recovery time is longer in younger patients than in adults (Harmon et al. 2019; Topolovec-Vranic et al. 2011; McCrory et al. 2009). Misdiagnosis and/or mismanagement of mTBI victims is of great concern in healthcare sectors due to the low detectability through imaging and a complex spectrum of symptoms. The clinical diagnosis of mTBI still remains challenging and suitable blood-based biomarkers not only ease this challenge, support diagnosis, but also provide more insight into pathophysiology of TBI.

Recently, we reported that A disintegrin and metalloproteinase 10 (ADAM10), a proteolytic enzyme, was increased in the plasma of patients and animal models of brain injury (Persad et al. 2021; Linsenmeier et al. 2018; Endres et al. 2017) and therefore has a potential to be used as a biomarker in the diagnosis of TBI. The present chapter focuses on ADAM10 as the biomarker for TBI diagnosis.

Pathophysiology of TBI

The insults on the head induce various biochemical and pathological alterations in TBI, and these modifications depends on the severity or nature of the insult (Young et al. 2015). The severity of TBI can be measured using Glasgow Coma Scale (GCS) and few imaging modalities (Menon and Maas 2015; Teasdale et al. 2014). The brain injury is classified into primary and secondary injuries. Primary injuries occur as a direct result of impact at the time of injury that leads to epidural or subdural hematomas, cortical contusions, vascular injuries, and axonal modifications. Secondary injuries are the injuries that initiate over hours or days after injury, resulting in the alterations of various biochemical cascade at the cellular and neuronal network disruption (Puntis and Smith 2017; Kubal 2012; Maas et al. 2008; Besenski 2002; Giza 2001). This disruption results in the increased calcium influx, vascular protein degradation, release of excitatory neurotransmitters, decreased energy store, induction of apoptosis, and finally neuronal death. In addition to this, inflammatory responses by activation of glial cells and release of free radicals contribute to neuronal damage (Werner and Engelhard 2007) (Fig. 2). Since the time window for the occurrence of secondary injuries is delayed, it provides the opportunities to develop various neuroprotective strategies to halt the neuronal death cascade which have become much important in TBI research. However, the promising pre-clinical

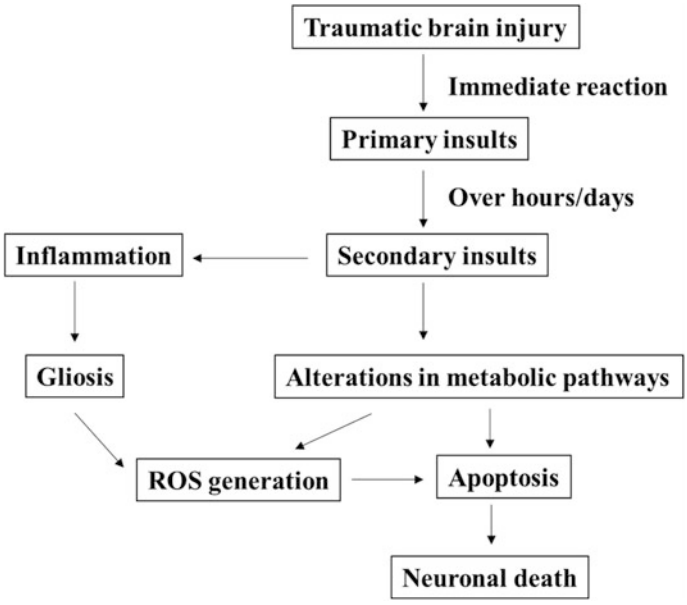

Fig. 2 Pathophysiology of traumatic brain injury. Primary insults will be the immediate reactions after injury. Secondary reaction may occur after few hours or days after injury. The secondary reaction may lead to various other metabolic alterations including gliosis, reactive oxygen generation (ROS), and finally apoptotic cell death

results obtained in the in vivo experiments failed in clinical trials (Puntis and Smith 2017; Kochanek et al. 2016).

Recent Advances in Imaging Diagnostics

Numerous imaging techniques and modalities have been recently used to study the severity of the insults in TBI patients. Computed tomography (CT) is one of the most commonly used technique to readily assess the lesions and intracranial problems that need immediate attention for initial evaluation and interventions (Marehbian et al. 2017). It helps to identify the lesions including cerebral edema, hemorrhages, infarctions, and bone fractures in specific brain regions through multiple cross-sectional imaging. In the past few decades, CT severity scores like Marshall CT and Rotterdam CT scorings have been developed to determine the severity of the brain insults (Schweitzer et al. 2019; Useche and Bermudez 2018; Bodanapally et al. 2015; Maas et al. 2005).

Magnetic resonance imaging (MRI) is used as the advanced imaging technique that produces high-resolution two- or three-dimensional brain images. It uses magnetic field and radio waves to produce structurally sensitive images, better tissue contrast, and fewer artifacts over CT images (Smith-Bindman et al. 2012; Gallagher et al. 2007; Hollingworth et al. 2000). In addition, MRI producing high sensitivity images particularly detects axonal injury (Marehbian et al. 2017). However, it has various limitations that include inability to be used by patient quickly after injury, its availability, cost, and length of the scanning time.

The next occasionally used imaging method is color Doppler ultrasound (CD-US) that screens the post-traumatic cerebral vasospasm (LaRovere et al. 2016; Kramer et al. 2013). CD-US is noninvasive and less expensive than MRI, but not suitable as an immediate replacement for MRI. Although there are imaging models used to detect the changes following brain insults, their limitations fail to detect the abnormalities in mTBI patients (Shin et al. 2017). Recent advances in other neuroimaging models like magnetic resonance spectroscopy (MRS) and single-photon emission computed tomography (SPECT) have been also developed. Since most of mTBI patients become asymptomatic after few days, the persistence of post-injury symptoms like cognitive and psychological symptoms may not be notified by these imaging techniques and left untreated (McInnes et al. 2017; Young and Tsao 2017).

Need for TBI Biomarkers

In the last few decades, significant advances have been developed in understanding complexity of the TBI pathophysiology. Understanding the pathological events at cellular and subcellular levels is of greater importance in order to develop a novel therapeutic intervention in the treatment of TBI. Due to the complexity in brain injury, clinicians need to have access to reliable sensitive blood-based biomarker(s) which would be usable at the point of care as a rapid and simple TBI diagnostic tool

(Zetterberg and Blennow 2016). We have studied blood-based biomarkers of mTBI/concussion in rodent models and concussed individuals which resulted in identification of cellular prion protein (PrPc) and ADAM10 as potential biomarkers of mTBI (Persad et al. 2021; Sekar et al. 2021; Sekar et al. 2019; Pham et al. 2015a, b).

A Disintegrin and Metalloproteinase 10 (ADAM10)

ADAM10, an active member of the ADAM family of proteinases, has been found to be synthesized in the endoplasmic reticulum and localized in the Golgi apparatus as mature form (Hsia et al. 2019). The cytoplasmic domain of ADAM10 had various binding sites that regulate the physiological functions. The binding site for other proteins includes calmodulin (Horiuchi et al. 2007), endophilin-A2, Lck, or ZDHHC6 and two SH3 binding domains (Ebsen et al. 2014). The presence of SH3 binding site in ADAM10 guides it to the postsynaptic membrane for their regulatory activities in neurons (Marcello et al. 2007). Phage library analysis revealed that ADAM10 consists of 305 SH3 binding sites and 38 candidate binding proteins at the C-terminus (Ebsen et al. 2014). Although the functions of the several ADAM10 binding partners need to be investigated, their interactions at the C-terminus may play a vital role in the regulation of various cellular events.

The role of ADAM10 in brain development has been investigated in animal models. Conventional ADAM10 knockout animals die on E9.5 (Hartmann et al. 2002) suggesting the greater importance of ADAM10 in the developmental processes (Saftig and Lichtenthaler 2015). Further, conditional ADAM10 knockouts showed various abnormalities including epileptic seizures, synaptic dysfunction, perinatal lethality, and behavioral deficits (Prox et al. 2013). On the other hand, Zhuang et al. (2015) established an adult ADAM10 knockout animal model and reported similar results. This clearly indicates that ADAM10 is a key factor in the developmental processes. Loss of its function leads to several abnormalities in the brain (Saftig and Lichtenthaler 2015).

ADAM10 binds to other proteins, influencing them in activating/inactivating various signaling processes at the cellular and subcellular levels. Marcello et al. (2007) showed that ADAM10 co-localized with postsynaptic protein SAP-97, but not with the presynaptic synaptophysin. In contrast, Lundgren et al. (2015) reported that ADAM10 was associated with synaptophysin in mouse primary hippocampal neurons. This clearly suggested that ADAM10 presents both in pre- and postsynaptic terminals, and is essential for normal synaptic specifications and functions. It is well known that neurons and glial cells are highly interdependent and regulate synaptic functions (Allen and Lyons 2018). Identification of one of the ADAM10 partner nerve-glia antigen 2 (NG2), glial marker, clearly showed that ADAM10 also influences the network activities through glial cells (Sakry et al. 2014). Based on the above observations, it is notable that the study of ADAM10 and its major anchoring proteins may indeed a powerful tool in elucidating various pathological processes and also in developing novel therapeutic interventions.

ADAM10 and Traumatic Brain Injury

Several research teams have reported an increased level of ADAM10 in injury site and in various regions of the brain following TBI (Del Turco et al. 2007; Warren et al. 2012; Zohar et al. 2011). In addition, increased ADAM10 also activates astroglial cells, but not microglia following injury (Warren et al. 2012; Del Turco et al. 2007). Due to its different proposed modes of action, the exact role of ADAM10 in TBI has been poorly understood. Deller et al. (2000) reported that ADAM10 may be involved in the reorganization of the extracellular matrix in the affected brain regions. In addition, ADAM10 may also involve in processing the synaptic proteins like N-cadherin, neuroligins, or ephrins in the postsynaptic membranes (Suzuki et al. 2012; Malinverno et al. 2010; Warren et al. 2012; Janes et al. 2005) to halt/restore the damages of degenerating terminals. Further, studies showed that ADAM10 cleaves and releases APPs-alpha and thus protects neurons in in vitro and in vivo models following injury (Del Turco et al. 2007; Kögel et al. 2012; Plummer et al. 2016). These observations suggest that the ADAM10 is involved in the neuronal protection against brain injury and its levels return to normal within a few days. On the other hand, long-term elevation of ADAM10 levels not only failed to reorganize the synapses, but also resulted in functional abnormalities in neurons (Warren et al. 2012). In the above conditions, pharmacological inhibition of ADAM10 may prevent these deteriorating effects and restore neuronal functions (Appel et al. 2021). This collectively showed a two-faced role of ADAM10 in TBI: both beneficial in early stage and detrimental at the later stages of brain injury. Thus, ADAM10 plays a plasticity enhancing and neuroprotective role in the early phase of brain injury.

Diagnosis of mTBI and/or concussion at the point of care is still a challenging issue for the health care providers. Short-comings of current imaging technologies in detecting mTBI can often result in misdiagnosis, leading to further complications in the future. Misdiagnosed concussion patients may develop delayed symptoms and are highly vulnerable to repeated mTBI. Therefore, the use of blood-based protein biomarkers for the screening and diagnosis of mTBI is urgently needed to avoid catastrophic long-term complications. In case of children and adolescents, early accurate diagnosis of concussion is very important as it would help in making decisions on return-to-play or to provide rapid treatment options and rehabilitation services for the patients (Register-Mihalik et al. 2013). The clinical evaluation of the patients is the current gold standard for mTBI diagnosis. As it was discussed earlier in this chapter, ADAM10 cleaves enormous number of protein substrates near the extracellular membrane and the active form of ADAM10 involved in the shedding of the membrane spanning proteins (Wetzel et al. 2017; Pruessmeyer et al. 2009). Further, Taghibiglou and team showed that plasma ADAM10 levels were raised in mTBI (Persad et al. 2021), the enzyme primarily responsible for PrPC cleavage from the plasma membrane (Linsenmeier et al. 2018; Enres et al. 2017). Warren et al. (2012) reported the rearrangement of ADAM10 in the synapse following TBI. Persad et al. (2021) recently reported that ADAM10 act as a potential biomarker for TBI and ADAM10 exhibited greater expression in patients' blood with worse

clinical grade. In addition, increased plasma ADAM10 levels were observed in patients with intracerebral hemorrhage and TBI models (Feng et al. 2022; Zhang et al. 2016). Furthermore, increased plasma ADAM10 levels have been reported in sports-related concussion especially ice hockey athletes in children and adolescence (Taghibiglou et al. unpublished data). Here we speculate that shedding of cell membrane ADAM10 into the circulation may also involve in the shedding of many other proteins that result in long-term serious complications like neurodevelopmental disorders and neurodegenerative diseases. This finding may benefit the physicians in accurate diagnosis of the TBI victims.

On the other hand, we also demonstrated that cellular prion protein (PrPC) is one of the novel biomarkers for mTBI (Pham et al. 2015a; Pham et al. 2015b) and their increased plasma concentration was observed in repeated mild TBI mice brains (Sekar et al. 2019; Sekar et 2021). Based on these findings, we speculate one of the possible mechanisms of action of ADAM10 alterations in neuronal damage (Fig. 3). Following brain insults, ADAM10 sheds off from the cell membrane to the peripheral circulation along with PrPC. Decreased membrane ADAM10 and PrPC further results in the alterations of secondary metabolic cascades including inflammation, ROS production, and finally apoptotic neuronal cell death.

In conclusion, increased plasma ADAM10 level has a value as a potential diagnostic biomarker for screening TBI and concussion. Investigating the clear molecular events on the dislodgement of ADAM10 in mTBI may provide better

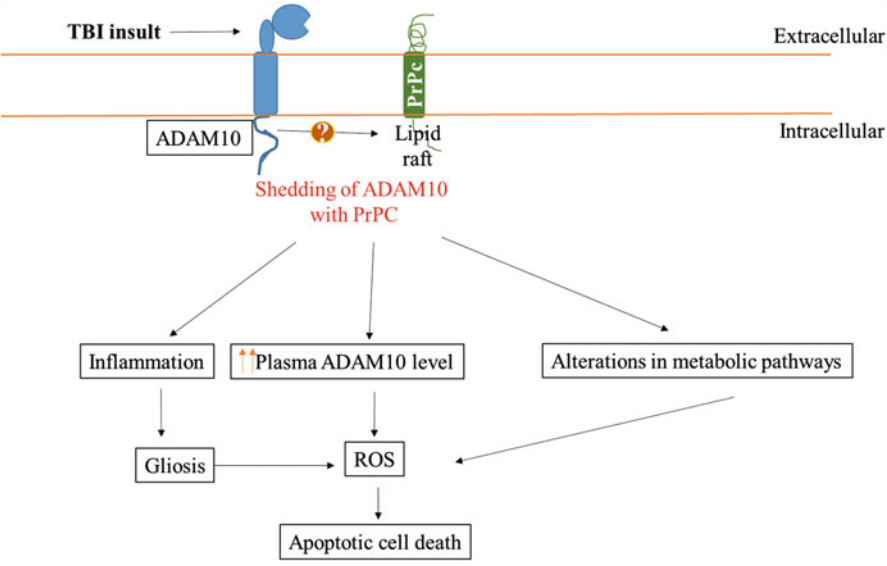

Fig. 3 One of the possible mechanisms of action of ADAM10 in the pathogenesis of traumatic brain injury. Once insults occur on the brain, ADAM10 sheds off from the cell membrane to the peripheral circulation along with PrPC by unknown mechanism. Decreased membrane ADAM10 and PrPC further result in the alterations of secondary metabolic cascades including inflammation, ROS production, and finally apoptotic neuronal cell death

insight in understanding pathobiology of concussion. ADAM10 may also serve as a promising diagnostic tool alone or in combination with other blood-based biomarkers in the diagnosis and early treatment of concussion-related neuronal injuries.

Mini-Dictionary of Terms

TBI: Traumatic brain injury is an injury to the brain that occurs through some types of trauma or force such as skull fractures, contusions, and intracranial hematoma.

Types of TBI: There are three main types of TBI, mild, moderate, and severe TBI. Moderate and severe TBI are easy to detect, while mild is difficult as the victims shows no symptoms.

mTBI: Mild traumatic brain injury is a type of brain injury in which the patients showed asymptomatic but may induce long-term cognitive and neurological deficits.

ADAM10: A disintegrin and metalloproteinase 10 (ADAM10) an active member of the ADAM family of proteinases that has been found in cell membrane and is involved in various physiological functions.

Interacting partner: A protein (in this case ADAM10) which interacts with another protein to perform its physiological role is called interacting partner.

Summary Points

- Traumatic brain injury is the growing epidemic throughout the world.
- Mild traumatic brain injury is asymptomatic and hence it is quite challenging to diagnose and interventions.
- Development of biomarker is of greater importance to avoid misdiagnosis and mis-interventions.
- ADAM10, a cell membrane metalloproteinase, sheds from the cell membrane following injury.
- Increased plasma ADAM10 levels in brain injury victims reveal that ADAM10 could be the potential biomarker for the diagnosis of TBI and for the development of novel therapeutic interventions.

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Part VI

Biomarkers in COVID-19

Laboratory Markers of COVID-19 in the Emergency Room

42

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Contents

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Abstract

After the December 2019 outbreak in China, infection by the novel Coronavirus SARS-Cov2 (COVID-19) has quickly overflowed worldwide. Different studies preliminarily observed that some laboratory tests resulted altered in COVID-19. The Royal College of Pathologist defined Laboratory Medicine as "*the hidden science to save lives.*" Following this sentence, several studies preliminarily observed that some laboratory tests are characteristically altered in COVID-19. These biomarkers have been proposed as rapid and sensitive alternatives in identifying likely COVID-19 cases in Emergency Room. The chapter dissertation shows how the baseline level of certain biomarkers can predict disease progression and outcome in COVID-19 patients, already in Emergency Room. Finally the evaluation of biomarkers concentrations during the disease course can define the disease progression, predicting the multi-organs failure.

Keywords

COVID-19 · Laboratory Medicine · Biomarkers · C-reactive protein · Emergency Room · Cytokines storm · RT-PCR · Hematological markers · Neutrophil to Lymphocyte ratio · Intensive care unit · Disease trend

Abbreviations

ACE-2	Angiotensin-converting enzyme 2
ALT	alanine aminotransferase
ARDS	acute respiratory distress syndrome
AST	Aspartate aminotransferase
ATP	adenosine triphosphate
BB	CK brain isoform
BGA	Blood gas-analyses
BNP	natriuretic peptides type B
CBC	complete blood count
CK	Creatine Kinase
CNS	central nervous system
COV-HI	COVID-19-associated hyperinflammation
COVID-19	Novel Coronavirus SARS-Cov2
COX	cycloxygenase
CRP	C-reactive protein
CT	Computer Tomography
CVD	cardiovascular disease
DIC	disseminated intravascular coagulation
DVT	deep vein thrombosis
E	Envelope
EDTA	ethylene diamine tetraacetic acid
Hs-Tn	High-sensitivity Troponin
ICU	Intensive Care Unit

IL-6	interleukin-6
LDH	Lactate dehydrogenase
M	Matrix
MB	CK heart tissue isoform
MM	CK striated muscle isoform
MODS	Multiple Organ Dysfunction Score
N	Nucleocapsid
NLR	neutrophil-to-lymphocyte ratio
NSPs	non-structural proteins
ORFs	Open Reading Frames
PCT	Procalcitonin
PE	pulmonary embolism
P_{O2}	partial pressure of O2
POCT	Point of Care Testing
RBC	red blood cell
RdRp	RNA-dependent RNA polymerase
RT-PCR	polymerase chain reaction
S	Spike
S_{aO2}	oxygen saturation
SAPS	Simplified Acute Physiology Score
TLM	Time-LYM% model
WBC	white blood cell
WHO	World Health Organization

Introduction

After the December 2019 outbreak in China, infection by the novel Coronavirus SARS-Cov2 (COVID-19) has quickly overflowed worldwide. It has been declared a pandemic by the World Health Organization (WHO) on March 11th and is now a World public health crisis (Rodríguez-Morales 2020). During the pandemic events Scientists and Governances had obtained decisive results regarding the capability of detecting and monitoring the virus spread. COVID-19 is phylogenetically considered as a part of beta-coronaviruses family. Similar to other coronaviruses, the genome of COVID-19 is a positive-sense single-stranded RNA [(+) ssRNA] with a 50-cap, 3'-UTR poly(A) tail (Ksiazek et al. 2003). This positive-sense 30 kb genome length hosts 14 Open Reading Frames (ORFs), encoding non-structural proteins (NSPs) for virus replication and assembly processes, structural proteins including spike (S), envelope (E), membrane/matrix (M), and nucleocapsid (N) (Ksiazek et al. 2003). The development of diagnostic methods based of genome structure was the first step of COVID-19 molecular diagnosis. Reverse transcriptase polymerase chain reaction (RT-PCR) is now considered the elective method for COVID-19 diagnosis. However, this technique has been affected by several drawbacks. The control of pre-analytical and analytical conditions is necessary for better performance of all diagnostic assays. According to the natural

history of the virus and their viral kinetics in several districts, sampling procedures largely considered a source of pre-analytical troubles. Several studies had reported sputum as the most accurate sample for laboratory diagnosis of COVID-19, followed by nasopharyngeal swabs, while throat swabs were not recommended for the diagnosis (Tahamtan and Ardebili 2020). Emergency Room conditions obviously elicit the use of nasopharyngeal swab specimens; however the appropriate procedure to obtain a representative specimen requires expertise and training. About this, using chest computer tomography (CT) images as "*gold standard*" for the diagnosis of interstitial pneumonia, Gili and colleagues explored diagnostic performances of RT-PCR during first pandemic period in 539 individuals admitted to the Emergency Room. This work evidence that RT-PCR had poor accuracy (moderate sensitivity with poor specificity), maybe relate to inappropriate and poor representative swab collection (Gili et al. 2021). The control of analytical conditions represents the second step of diagnostic accuracy. The first application of the RT-PCR in COVID-19 diagnosis, targeting the spike gene region S, has shown considerable specificity but however, limited sensitivity (Gili et al. 2021). The combined use of other, more specific probes targeting viral genes, such as RNA-dependent RNA polymerase (RdRp) in the ORF1ab region, N (Nucleocapsid) and E (Envelope), improved the sensitivity of the method. The comparison of the results obtained from Literature and the European validation in approximately 30 Centers exhibited that the RdRp gene is the appropriate target because of its highest sensitivity (Tahamtan and Ardebili 2020). In addition, the WHO recommends the E or N gene assay as a first-line screening, followed by the RdRp gene assay as a confirmatory test. Although it was attempted to design the RT-PCR assay based on the COVID-19 conserved regions, the mismatching between the primers, probes, and target sequences can affect the assay performance, generating potential false-negative results. Multiple target gene amplification could be used to avoid invalid results. Despite all, primers mutation and changing in probe target regions or the presence of amplification inhibitors in the sample affect final results.

In conclusion, pre-analytical troubles, suboptimal sensitivity, long production time results, and other intrinsic methodological features affect RT-PCR. This method cannot be yet considered a rapid and specific tool that can be used to help in the Emergency Room. Several studies preliminarily observed that several laboratory tests have been shown as characteristically altered in COVID-19 and they have been proposed as rapid and sensitive alternatives in identifying likely COVID-19 cases. In a very recent work appeared on *PloS ONE,* Gili and co-worker demonstrated that the use of Charlson comorbidity index identified patients with a worse outcome (Gili et al. 2021). However, the use of any type of comorbidity index was not totally appropriate to quickly evaluate patient outcomes in the Emergency Room. Several studies showed that some baseline laboratory parameters have been clearly linked to clinical features (Lippi and Plebani 2020b). For example, in a very recent work, Assandri and coworker observed that several laboratory markers are characteristically altered in COVID-19 patients (Assandri 2020). These parameters have been proposed as rapid and sensitive alternatives in identifying likely COVID-19 cases, during Emergency Room activity (Assandri

2020). Concerning hematological tests at admission showed low white blood cell count in over 80% of cases, and lymphocyte count below 1×10^9/L in over 55%. Also C-reactive protein (CRP) serum levels were higher in patients with worst clinical presentation. Finally Assandri and colleagues purposed the use of few but selective laboratory parameters to classify patients and easily identify false negative results on RT-PCR assays (Assandri et al. 2020a, b; Assandri and Montanelli 2020; Lippi and Plebani 2020a, b).

Disease Progression and Laboratory Markers in Emergency Room

COVID-19 could be considered a multisystem viral disease, classifying into different clinical phases, caused by a complex interplay of the immunological, inflammatory, and coagulative pathways. Laboratory medicine has always supported the clinical decision from screening to diagnosis and prognosis. All individuals admitted to the Emergency Room activate the diagnostic classification pathway. Laboratory data are therefore a whole of the patient's general assessment. It is therefore not possible to ignore the meaning of the different laboratory markers. A biomarker is defined as a "*characteristic that can be objectively measured and evaluated as an indicator of normal biological and pathological processes, or pharmacological responses to a therapeutic intervention*" (Biomarkers and surrogate endpoints: preferred definitions and conceptual framework 2001). Their feedback and their value in numerical terms will help in the correct classification of disease staging and consequently, the right therapeutic approach.

Disease Progression

Viral Entry and Replication

Similar to other Coronaviruses, the genome of COVID-19 is positive-sense single-stranded RNA [(+) ssRNA] transmitted through exposure to micro-droplets from infected patient (Paraskevis et al. 2020; World Health Organization. Naming the Coronavirus Disease 2020). Molecular interplay between the viral S glycoprotein and the Angiotensin-converting enzyme 2 (ACE-2) in distal airways and alveoli represent the first step of viral infection (Shang 2020a; Mohamadian et al. 2021). ACE-2 is a type I transmembrane carboxypeptidase with homology to ACE, a key player in the rennin-angiotensin system. ACE-2 is expressed in several anatomic district, respectively, on the nasal, oral, nasopharyngeal, and oropharyngeal epithelium, gut epithelia, renal proximal tubular cells, and in the central nervous system (CNS) (Mao et al. 2020) The presence of different targets can explain the different clinical manifestations during COVID- 19 disease**.** The main substrate of the ACE-2 enzyme is angiotensin-II, which plays a key role in counteracting the activity of the angiotensin converting enzyme (ACE), reducing the amount of angiotensin II.

Internalization of the ACE-2 after viral interaction modify the angiotensin II concentration (Devaux et al. 2020). This deregulation activates the downstream inflammatory pathways, leading to the "cytokine storm" that adversely affects multiple organs (Valizadeh et al. 2020). Viral replication leads to the production of virions that interplay between their main targets, epithelial cells, lymphocytes, and vascular endothelial cells. The potentiated virion building causes an extraordinary pulmonary and systemic inflammation responsible to the clinical presentation of severe COVID-19 disease (Mohamadian et al. 2021).

Immune Response and Inflammation

COVID-19 disease was the result of dysregulation of innate and adaptive immune response (Catanzaro et al. 2020). The uncontrolled immune response leads to the massive production of cytokines and chemokines event named the "cytokine storm." The antiviral activity of CD4+ and CD8+ T cell is expressed by destruction of infected cells and by activation of the T-dependent B cells, with consequence production of specific antibodies. The balance between two cell types (naïve and memory T cells) is crucial for the host defensive response performance (Catanzaro et al. 2020). An imbalance favoring naïve T cell activity, concur to hyperinflammation events. This event evolves through the NF- kB, JAK/STAT pathway, and the macrophage activation too, leading to the release of interleukin-6 (IL-6) and TNF-alpha (Catanzaro et al. 2020). IL-6, as a key player, activates different cell types, upregulating the expression levels of its final product, CRP. The activation of these molecular pathways stimulate inflammatory cytokines and chemokines production, that recruit several target cell and trigger coagulative cascade (Catanzaro et al. 2020).

The Coagulative Cascade and Growing Inflammation

A normal vascular endothelium is considered a balance system in which both antithrombotic and anti-inflammatory events allow the tissue to function properly. This homeostasis is disturbed by COVID-19 infection that cause thrombosis and inflammation, mainly triggered and driven by thrombin (Mitchell 2020). Platelets activation, the first step of hemostasis, leads to the recruitment of more platelets, linking themselves via fibrinogen. Platelets promote the production of pro-inflammatory cytokines and pro-angiogenic factors that recruit leukocyte and cause extravasation. The enhanced inflammatory state, neutrophils, and macrophages recruitment to growing thrombi also recruit plasmin, through which fibrin is degraded to D-dimers (Mitchell 2020). After COVID-19 lung invasion, the high fibrinolytic capacity cause vigorous fibrinolytic events and consequently production of D-dimers, which pour out into the blood circle (Mitchell 2020).

Laboratory Markers in Emergency Room

Blood Gas-Analyses (BGA): Partial Pressure of Oxygen and Oxygen Saturation

The term BGA is a set of laboratory tests referred to the acid-base balance and oxygenation status of a patient. Oxygenation status is evaluated using partial pressure of oxygen (P_{O2}), measured by amperometry and hemoglobin oxygen saturation (S_{aO2}), measured by co-oximetry. Emergency and intensive care unit use BGA as a part of clinical evaluation (Casagranda 2010). For this reason the correct choice of samples became essential to right patients' evaluation. Arterial blood is a sample prototype for evaluation gas exchange, oxygenation status, and metabolic acid-base disorders. For its intrinsic nature, arterial blood is considered a uniform sample type, not affected by interference changes depending of systemic/local circulation. S_{aO2} is a very essential tool of patient care and is considered a measure of how much hemoglobin is currently bound to oxygen, compared to the amount of hemoglobin unbound. Due to the critical nature of tissue oxygen consumption, clinicians are able to monitor current oxygen saturation. After these considerations and in order to speed up the Emergency Room triage process, different authors investigated the role of BGA during COVID-19 pandemic. Elezagic and co-workers showed that all patients tested positive in a 50 patients cohort exhibited statistically significant reduction of P_{O2} and S_{aO2} levels, compared with COVID-9 negative patients (Elezagic et al. 2021). Also Turcato and colleagues explain that the blood gas-parameters have inverse correlation between the extension of pulmonary inflammatory process, evidenced by CT scan. Arterial blood gas test value is correlated with the median percentage of the pulmonary inflammatory process extension. Over 14.3% pulmonary inflammation processes corresponded to a low S_{aO2} (92.8%) (Turcato et al. 2020). In another study, hypoxemia was strictly associated with increasing mortality risk. Compared to normoxemic patients, hypoxemic subjects with S_{aO2} under 92% showed a 1.8-to 4.0-fold increased mortality risk, depending directly on initial S_{aO2} measured in Emergency Room (Chatterjee et al. 2021). In this direction Shang et al. demonstrated a correlation between the chest CT scan abnormality and the P_{O2} status, namely, ground-glass opacities and pulmonary fibrosis (Shang et al. 2020b). Finally Canetta and Co-workers described 35 patients experiencing one or multiple syncope, in which they recorded low P_{O2} and ratio of arterial oxygen partial pressure (Canetta et al. 2020). The use of BGA in Emergency Room is certainly useful for preliminary approach to patient but several attentions are needed (Fig. 1). Stressful work conditions often exasperate the probability to incorrect withdrawal, resulting in adverse patient evaluation and outcome. In fact BGA is exposed to risks of errors caused by improper sampling, transport, and storage conditions.

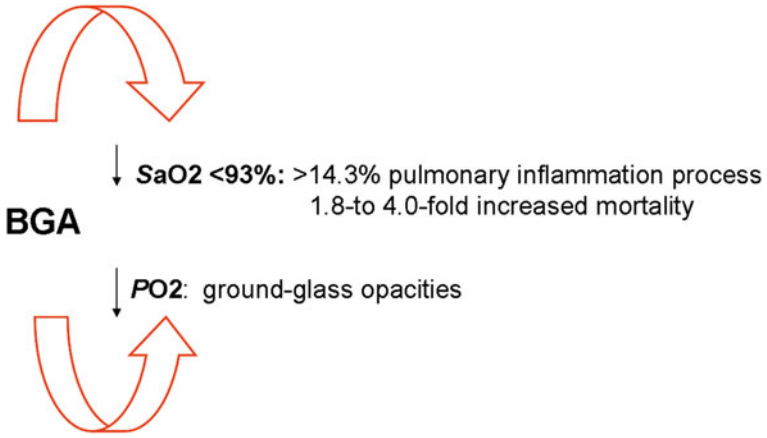

Fig. 1 Evaluation of BGA biomarkers in Emergency room and their predictive value

Hematological Biomarkers

The complete blood count (CBC) is the most common laboratory procedure and the "starting point" of medical investigation. This assay provides information about cells in peripheral blood. For correct evaluation of blood cells, the use of ethylene diamine tetraacetic acid (EDTA) tube is necessary. CBC parameters that are analyzed from an automated instrument are red blood cell (RBC)-related numbers, white blood cell (WBC)-related numbers, and platelet-related numbers.

Hemoglobin Quantification

Low hemoglobin concentration obviously complicate clinical picture in patients with suboptimal S_{aO2}. About this, a retrospective study revealed that anemia and altered iron homeostasis, very common in hospitalized COVID-19 patients, were associated with increased mortality (Bellmann-Weiler et al. 2020).

WBC-Related Numbers: Absolute Lymphocyte Count (Cells/μL) and Neutrophil to Lymphocyte Ratio

Lymphopenia represents the most frequent biomarkers alteration in COVID-19 and it's now considered the hallmark of this infection (Terpos et al. 2020). Several studies indicate that a lymphocyte counts under 1500 cell/μL could be considered a hallmark of first stage of COVID-19 infection (Gili et al. 2021). However distinctions between cell types are needed. A higher total T cell count, including both CD4+ and CD8+, has been shown to be a predictor of less severe disease and a more favorable clinical outcome. After discharge, lymphocyte counts resulted normal in almost all cases (Terpos et al. 2020). In contrast, the decrease in B cell counts among

severe COVID-19 patients is not as consistently observed as the decrease in T cell counts (Liu et al. 2020c). Several mechanisms have been proposed to explain the reduced lymphocytes count. It has been speculated that COVID-19 directly infect T lymphocytes, inducing the depletion of CD4+ and CD8+ cells, through ACE2 cell-membrane receptor. Also, IL-6 and TNF-alpha as pro-inflammatory cytokines are probably responsible for lymphocyte deficiency and consequently low lymphocyte count. Meta-analysis of 28 reported studies evidenced that condition of lymphopenia is linked with higher risk of poor outcome (Zheng et al. 2020). Lymphopenia, more prominent in nonsurvivors, however persisted throughout in all patients and progressively fell to reach nadir at 8–9 days (Samprathi and Jayashree 2021). For this purpose Tan et al. established a model using lymphocyte percentage, named Time-LYM% model (TLM), for disease classification and outcome. A lymphocyte percentage under 20% at time 10–12 days represent the operative cutoff as first step to distinguish patients. A timeline reduction define curable (5–20%) or ICU patients (<5%) (Tan et al. 2020). Leukocytosis, especially neutrophilia, is another alteration detectable at the CBC of COVID-19 patients (Amgalan and Othman 2020). Some authors proposed neutrophil-to-lymphocyte ratio (NLR) as an independent risk factor for severe disease (Samprathi and Jayashree 2021). Elevated NLR, resulting from the increased neutrophil count and decreased lymphocyte count, has been reported to be significantly associated with an increased risk of death (Samprathi and Jayashree 2021). Other studies define the use of NRL as a good prediction factor to pneumonia severity. Imran et al. showed a positive correlation between NLR and CRP, in severe COVID-19 patients with an accurate ratio cutoff at 4.795 (Imran et al. 2021). According to previous study, Borghetti and co-workers showed a positive correlation between a higher NLR at admission and more severe outcome. With an operative cutoff at upper 4 (NLR > 4) NLR became an independent predictor factor of ICU at admission (Ciccullo et al. 2020). In conclusion, lymphocyte count in number (<1500 cell/μL), percentage (<20%), their progressive fall, and high NLR (>4) are independent risk factor of worse outcome. This evidence should be applied in Emergency Room to rapidly and better evaluate patient at admission (Fig. 2).

Platelet Count (Cells/μL)

A ready to use and very simple laboratory test, platelet count is considered an accurate biomarker associated with disease severity and worse outcome. Thrombocytopenia is defined as count less than 150,000 platelets per microliter, in adults. Literature reported that thrombocytopenia is associated with disease severity and mortality scores according to Acute Physiology and Chronic Health Evaluation II, Multiple Organ Dysfunction Score (MODS) and Simplified Acute Physiology Score (SAPS) II (Vanderschueren et al. 2000). According to these uses, thrombocytopenia has been described in COVID-19 patients and associated with the progression and prognosis of the disease. Lippi and co-workers conducted a meta-analysis of 9 studies and 1779 patients. This study revealed that thrombocytopenia was strictly associated with disease severity in COVID-19 patients.

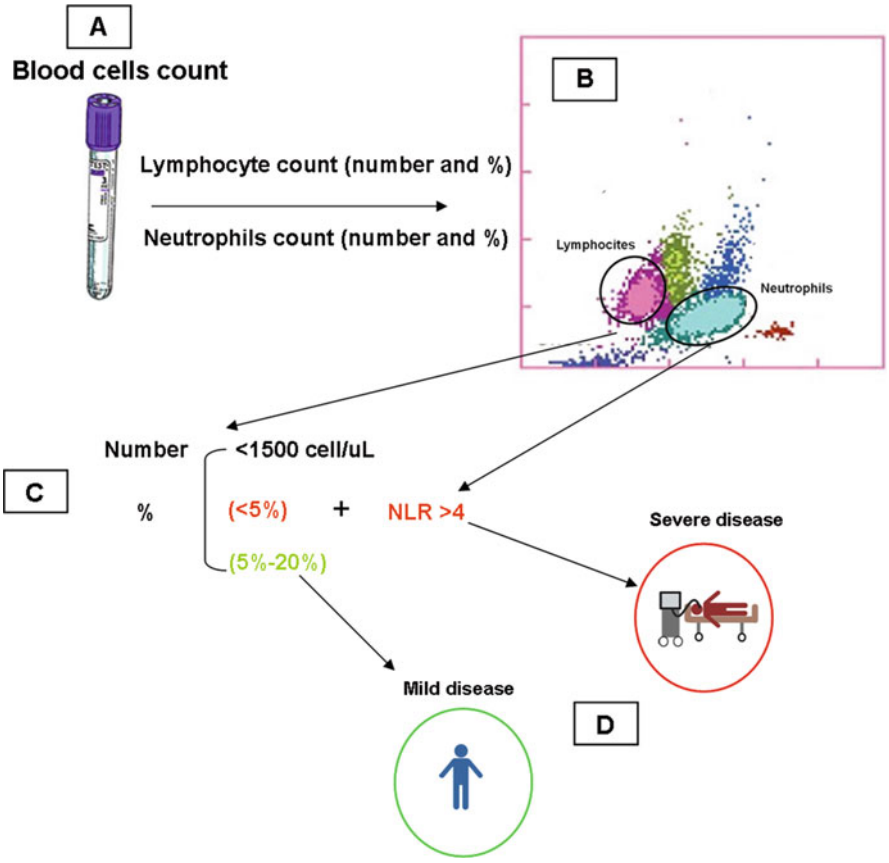

Fig. 2 (**a**) Blood cells count and its predictive value. (**b**) Scattering of blood cell count and their population were evaluated at admission in emergency Room (**c**) Lymphocyte (number and percentage) and NLR were used as predicting biomarkers in Emergency Room (**d**)

However this parameter, following the thrombocytopenia definition, is fulfilled only in four patients' cohort (Lippi et al. 2020). The direct infection of hematopoietic cells and bone marrow can induce platelet reduction. Also the lung injury could contribute to platelet depletion, leading to decreased platelet production and increased consumption.

Inflammatory Biomarkers

The systemic inflammatory response to the severe acute respiratory syndrome during COVID-19 is a hallmark of the infection.

C-Reactive Protein (CRP)

C-reactive protein (CRP) is now considered the acute-phase reactant protein "*par excellence,*" measured by immunoassays techniques, based on an antigen-antibody analytical approach. Discovered by Tillett and Francis, CRP is a pentameric inflammatory protein of the pentraxin superfamily (short pentraxin) produced by the liver in response to inflammation, induced by the IL-6 activity on the target gene. Located into 1q23.2 on the long arm of chromosome 1, CRP binds to phosphocholine in pathogens and membranes of host cells to enhance phagocytosis and facilitate clearance. CRP also efficiently activates the classical pathway of the complement system, an important component of innate host defense. During bacterial infection-mediated inflammation, this molecule activates in fact the classic complement pathway and phagocytic activity through Fc receptors to remove cellular debris and damaged cells, also helping the clearance of foreign pathogens (as pattern recognition molecule) (Nehring et al. 2021). Compared to the erythrocyte sedimentation rate, CRP levels are considered a direct marker of inflammation, which rapidly rise and fall with the inflammatory stimuli (Nehring et al. 2021). Several causes explain the increasing CRP, including acute and chronic conditions, with or without infective etiology. Scientific Literature remarks that up to 90% of all marked elevations in CRP concentration were attributed to an infectious disease, most often caused by bacterial pathogens (Anderschueren 2006). Sepsis-3 criteria 2020 considered a CRP level $>$ 100 mg/L an independent predictor of ICU recovery and 30-day mortality (Koozi et al. 2020). However elevated CRP concentrations have also been reported in severe viral infections, which correlate with disease progression and severity (Ko et al. 2016; Vasileva and Badawi 2019). Similarly, CRP levels have been reported to be elevated in hospitalized patients with COVID-19, and their levels correlate with severity and mortality. Liang and colleagues showed that, in a cohort of 298 subjects with COVID-19, patients who died had an initial CRP level tenfold higher than that of survivors (Liang et al. 2020). Two different reports identified an association between CRP levels and respiratory failure. Levels of CRP $>$5 mg/L are correlated with a fivefold risk of acute respiratory distress syndrome (ARDS) (Wu et al. 2020). In a large cohort of COVID-19 patients hospitalized in New York, the authors reported a systemic median CRP concentration 40-fold higher than the normal CRP value at the time of hospitalization. These patients presented a worse outcome and more cardiovascular /thrombotic events than other patients with lower initial CRP value (Smilowitz et al. 2021). Sharifpour observed that the CRP level trajectory grew in a linear trend during 7 days of hospitalization, with a peak on day 5. According to other studies, survivor patients showed a lower CRP peak than worse outcome patients (Sharifpour et al. 2020). These evidences are supported by explicit high cytokine levels measured in different studies. The analysis of 1400 patients hospitalized with COVID-19 revealed IL-6 levels correlate with CRP concentration and patients survival (Del Valle et al. 2020). In a definitive and very exhaustive paper, Mueller et al. demonstrated that CRP trending can predict respiratory deterioration in hospitalized COVID-19 patients. This trend showed that a rapid rise in CRP levels predict respiratory failure and ICU

admission, while CRP concentration plateau in stable patients. Progressive worse outcome compared to mild too is represented by a more rapid rise in CRP levels curve at 24–48 h after admission in Emergency Room. Entry levels and trend of CRP concentration during the first 48 h of hospitalization is a higher sensitivity predictor for respiratory failure (Mueller et al. 2020). In this regard, a very interesting work that appeared on Lancet investigates a new operational definition of COVID-19-associated hyperinflammation (COV-HI). Manson and co-workers support the concept that COV-HI phenotype is linked with a worse clinical outcome. To note, the CRP trend differs between severe disease and a milder course. This study has defined CRP threshold as a concentration greater than 150 mg/L or doubling within 24 h from greater than 50 mg/L (Manson et al. 2020). Finally, Literature reported that CRP concentration correlated with myocardial injury (Guo et al. 2020; Lala et al. 2020; Shi 2020; Basso et al. 2020).

In summary CRP measurement and concentration reflect disease severity and the magnitude of the acute inflammatory response. Defining the trends and prognostic thresholds of CRP concentration in COVID-19 patients help clinician in risk stratification and management (Fig. 3). Evaluation at admission in Emergency Room (CRP $>$ 150 mg/L) and trend 24–48 h after admission (doubling within 24 h from greater than 50 mg/L) are the hallmarks of COVID-19 hyperinflammation and severity. The use of CRP concentration at admission and its trend allows distinguishing patients into three categories: (1) mild; (2) progressive; or (3) severe (see Fig. 3). How to explain these evidences? Is there a plausible molecular mechanism? The major accredited pathways regard ACE2 receptor and its cellular entrance. The internalization of COVID-19-ACE2 receptor determines the hyperactivity of Ang II, via NF-kB pathway. This event induces the synthesis of CRP that produces dangerous effects mediated by complement, binding to Fc receptors, and induction of apoptosis. Molecules that block Ang II receptors and the use of ACE inhibitors ameliorate COVID-19 clinical evolution by decreasing of pro-inflammatory cytokines, especially IL-6 with reduction of CRP concentration (Manson et al. 2020).

Procalcitonin (PCT)

As a routine biomarker, PCT is considered a good specificity molecule to distinguish bacterial from non-bacterial infections and its serum concentration is now used as an

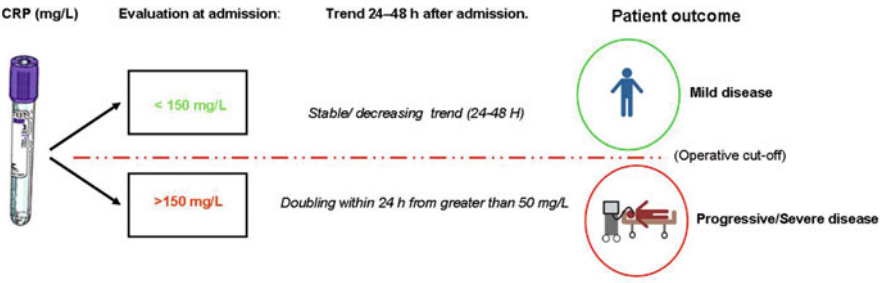

Fig. 3 CRP serum concentration at admission and its trend (24–48 h) define clinical outcome

essential part of decision protocol and antibiotic approach (Dorizzi et al. 2006). PCT is a glycoprotein calcitonin pro-hormone produced by the thyroid parafollicular cells. During a pathological event as a microbial infection, PCT levels significantly increases and it is released by all parenchymal tissue cells under the control of pro-inflammatory stimuli and related cytokines. Since it was discovered in 1993, a significant correlation between serum PCT level and infectious diseases has been reported (Assicot et al. 1993). It is now noted that in physiological condition, PCT serum levels do not exceed 0.05 ng/mL (Vijayan et al. 2017). After bacterial infection, PCT increases its levels, which are detectable 2–6 h after the pathological stimulus (Schuetz et al. 2017). To further validate the use of PCT in COVID-19 patients, 51 different studies were conducted. However, the literature provides conflicting data regarding PCT levels in COVID-19 patients. Li and coworkers showed the absence of significant association between PCT and disease outcome in COVID-19 patients (Li et al. 2020). Conversely, Lippi and coworkers reported that the PCT serum levels quintuplicate in severe cases. This considerable increase from baseline PCT levels could reflect critical onset of COVID-19 infection (Lippi et al. 2020). Several studies reported the independent use of cutoff >0.05 ng/mL, which partially uniform with the Literature results (Ahmed et al. 2021). Following this operative cutoff, Liu and co-workers showed that PCT levels greater than 0.07 ng/mL, which can generate a particular assay's accuracy (sensitivity and specificity of 73.15 and 84.85%, respectively), can be used for the prediction of morbidity combined with other routine biochemical markers (Liu et al. 2020a). Once the association between high PCT values in COVID-19 and increased mortality rates has been established, it is necessary to evaluate the serum PCT concentration as a discriminant between bacterial and viral infection. Bacterial coinfection in COVID-19 patients occurred between 14% and 28% of cases (Langford et al. 2020). International Guidelines alert the clinicians to not prescribe antibiotics therapy in patients with PCT value lower than 0.1 ng/mL and strongly recommended it in patients with marker values above 0.5 ng/mL (Albrich and Harbarth 2015). However, Vanhomwegen and colleagues referred to an over-treatment in 87% of COVID-19 patients with PCT level above 0.5 ng/mL (Vanhomwegen et al. 2021). At the moment, initially elevated PCT levels can predict worse outcomes but is not strictly correlated with bacterial coinfection in COVID-19 patients.

Creatine Kinase (CK)

Creatine kinase (CK) catalyzes the phosphorylation of creatine through adenosine triphosphate (ATP). During muscle contraction CK catalyzes the re-phosphorylation of ATP combined with creatine phosphate. The three CK isoenzymes are a dimer composed of subunits derived from striated muscle (MM), heart tissue (MB), and brain (BB). As a marker of muscle damage, CK is routinely used as a simple, immediate biomarker in Emergency Room and now is proposed to be potentially associated with severe COVID-19 condition. In a very recent meta-analysis, the association between the elevated CK and COVID-19 severity and mortality were analyzed. The systematic literature search revealed that in 2471 patients from 14 studies elevated CK serum levels were associated with poor outcome and this

effect did not vary with age, gender, or comorbidities. Beside a poor sensitivity, CK has a good specificity and confers to a 49% probability for poor outcome (Akbar et al. 2021).

Comprehensive Metabolic Panel Biomarkers

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)

Gastrointestinal manifestations during COVID-19 ranged 10–15% of patients (Buscarini et al. 2020). The evaluation of liver cytolysis through classical enzyme measurement in COVID-19 patients includes aminotransferases, aspartate aminotransferase (AST), and alanine aminotransferase ALT (AASLD expert panel consensus statement 2020). In patients with COVID-19, elevated liver enzymes were associated with direct liver injury, in response to cytokines storm, and drug-induced liver, and muscle injuries. However the prognostic significance of abnormal liver enzymes remains unclear (Schaefer et al. 2020). Some authors underline the difficulties to evaluate elevated liver enzymes in COVID-19 patients. Bloom et al. showed that, after the enzymes follow-up, liver synthetic status was generally normal. The probable diagnosis was of COVID-19-related and drug-induced liver injury. The consensus statements of scientific societies invite the clinician to find alternative etiologies of elevated liver enzymes during COVID-19 (AASLD expert panel consensus statement 2020). Current recommendations were absent for or against liver biopsy procedure in patients who developed acute liver injury. However this diagnostic procedure should rarely be used in patients with abnormal liver enzymes elevation (AASLD expert panel consensus statement 2020).

Creatinine

Serum creatinine is one of the most frequent assay products in clinical chemistry laboratories worldwide. Serum creatinine can be frequently measured by enzymatic methods. Renal injuries were evaluated in Emergency Room throughout clinical observation and creatinine measurement. Different studies have revealed that the number of COVID-19 patients with renal dysfunction are more in severe patients than in mild patients and this condition is positively correlated with the disease severity on admission (Wang et al. 2020). Also Literature evidences suggest that male gender, older age, diabetes, and hypertension may aggravate the risk of renal dysfunction in COVID- 19 patients as in other clinical condition (infection and sepsis), demonstrating that these elements are independent risk factors of renal dysfunction (Xiang et al. 2021). Creatinine represents fast and secure biomarkers to evaluated acute kidney injuries during COVID-19 infection. Proteinuria and/or hematuria is however indicative of kidney injury, without serum creatinine elevation (Legrand et al. 2021). In conclusion, prognosis of renal dysfunction remains

unknown in COVID-19 patients, their evolution unclear, and therapeutic strategies are lacking.

High-Sensitivity Troponin (hs-Tn) and Natriuretic Peptides Type B (BNP)

Troponins and natriuretic peptides focus on different aspects of cardiovascular involvement, but are complementary in evaluation of cardiac events. The risk of acute myocardial damage, ischemia, and infraction following infection is well known and is related to inflammatory state (Musher et al. 2019). Troponins are the classical myocardial injury biomarkers, reflecting myocardial ischemia/infarction as a direct consequence of oxygen acute coronary syndromes or pulmonary embolisms. The Fourth Universal Definition of Myocardial Infarction defines myocardial injury as "*Tn concentrations >99th percentile upper reference limit.*" Dynamic changes define the two different condition, acute or chronic injury. Laboratory assays are precise and the use of high-sensitivity (hs) Tn tests are strongly recommended. A hs assay had an imprecision of <10% at the 99th percentile, with possibility to detect values above the limit of detection in 50% or more of a healthy population (Apple and Collinson 2012). hsTn is a robust prognostic biomarker for a short- and long-term cardiovascular risk, including acute respiratory failure setting. Not surprising that hs-Tn is a robust prognostic marker in different circumstances. In fact, according to International Guideline (American College of Cardiology), for any patients with hsTn increases>99th percentile, this elevation should be classified as chronic myocardial injury, acute non-ischemic injury, and acute myocardial infarction (Sandoval et al. 2020). Patients can already have prior cardiovascular diseases (CVD) or can develop CVD during COVID-19. The high rate of chronic cardiovascular diseases is the most frequent comorbidity condition in COVID-19 patients. In these cases, chronic and stable hs-Tn increase is considered as chronic injury and associated with adverse prognosis (Sandoval et al. 2020). In the absence of prior CVD the increasing inflammatory responses plus pro-thrombotic status can contribute to development of acute non-ischemic injury and infraction. In these conditions, increasing or dynamic changes in hs-Tn are the hallmark of the pathological situation (Sandoval et al. 2020). Finally, in a critical illness, such as sepsis or respiratory failure, the increase of hs-Tn levels categorizes the events as acute myocardial injury (ischemic, if clear ischemia is present). The recent COVID-19 studies reinforced several important details and recommendations for hs-Tn in Emergency Room. First of all, a direct correlation exists between myocardial injury and adverse outcome (Sandoval et al. 2020). Shi et al. in their study reinforced the concept of a continuous relationship between hs-Tn concentrations and patients' outcomes, demonstrating that mortality rates correlate with higher hs-Tn concentrations (Shi et al. 2020). Up to date, patients with chronic CVD have higher risk for developing acute conditions, but also have higher mortality risk (Guo et al. 2020). Several other studies underscore the use of serial measurements of hs-Tn to monitor changing patterns (Zhou et al. 2020) to rapid probable survivors and nonsurvivors,

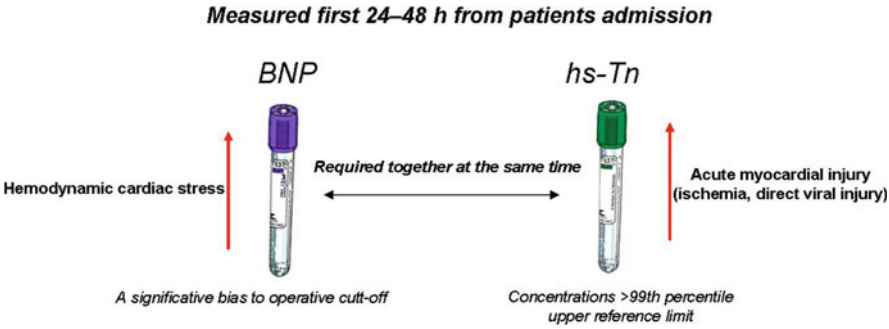

Fig. 4 hs-Tn and BNP for evaluation of cardiac involvement in COVID-19 patients in Emergency Room

and facilitate the identification of patients in whom further evaluation are needed (Fig. 4).

Different to hs-Tn, BNP are considered a sensitive biomarker of hemodynamic cardiac stress, due to ischemic/inflammatory left ventricular dysfunction and heart overload leading to pulmonary involvement (Oremus et al. 2014). Literature presents 44 studies, including 18,856 patients. Emerging data reflect the importance of BNP concentrations, significantly higher in high severity or nonsurvivor patients, when compared to others low severity or survivor patients (Zinellu et al. 2021). In meta-regression analysis, the BNP concentration was significantly and positively associated with D-dimer, LDH, and PCT concentrations (Zinellu et al. 2021). BNP plasma concentrations measured within the first 24–48 h from patients' admission were significantly higher in COVID-19 with severe disease and in patients with worse outcome (Zinellu et al. 2021). The association between plasma BNP level, disease severity, and mortality is likely to reflect the heart failure status. In this context, these data confirmed that BNP plasma concentrations are significantly associated with severe disease and mortality in COVID-19 patients. Stefanini and co-workers suggested hs-TnI 19.6 ng/L and BNP 100 pg/mL (Stefanini et al. 2020). Qin et al. showed no uniformity of cutoff value (different cutoffs according to different hospital sites) (Qin et al. 2020). It is therefore necessary to rely on the values proposed by the different laboratories and evaluate the biomarkers trends with respect to the critical differences.

In conclusion, hs-Tn and BNP can be a complementary use for cardiac involvement evaluation in COVID-19 patients in Emergency Room (Fig. 4).

Other Biomarkers

D-Dimer

The formation of fibrin clots by the coagulation system after perturbation of hemostasis in response to vascular injury is counterbalanced throughout the clot

breakdown by the fibrinolytic system. D-dimers are fragments produced when plasmin (fibrinolytic pathway) cleaves fibrin to break down clots. D-dimer is composed of two covalently bound fibrin D domains, cross-linking by factor XIII during clot formation and progression. The D-dimer concentration is measured using various commercial kits, based on monoclonal antibodies, with different accuracy and performances (Linkins and Takach 2017). Declared sensitivity and specificity of D-dimer kits is strictly linked to the intrinsic structure of the method, such as type of monoclonal antibody, the capture method, and the instrumentation used (Schrecengost et al. 2003). Assay methods to D-dimer concentration measurement conceptually divided into two different steps, such as fragment capture by monoclonal antibodies, and the D-dimer-monoclonal antibodies complexes detection and quantification (Linkins and Takach 2017). Regardless of methods used in clinical laboratories, the evaluation of D-dimer concentration in clinical practice is used to exclude a deep vein thrombosis (DVT) and pulmonary embolism (PE) diagnosis and also to confirm the disseminated intravascular coagulation (DIC). Several studies demonstrated that COVID-19 is predisposing to thrombotic events and their complication in up to 25% of patients (Cui 2020; Klok et al. 2020). Endothelial dysfunction represents the main trigger of thrombotic event, occurring through multiple mechanisms and factors. Endothelial cells virus infection lead to cellular damage that changes the asset of intercellular junctions and consequently exposes prothrombotic subendothelial collagen. The ACE2 receptor internalization causes an imbalance in the accumulation of AngII, which promotes the endothelial expression pro-coagulative factors (P-selectin, tissue factor and von Willebrand factor). Intracellular viral replication lead to the expression of prothrombotic proteins that immediately activate the extrinsic coagulation cascade, with platelets recruitment, activation, and consequently hypercoagulability. Also local hypoxia exacerbates the pro-thrombotic phenotype, and consequently induction of the cyclooxygenase (COX) pathway-mediated vasoconstriction (Fig. 5) (Loo et al. 2021) Several clinical evidences support these biological data, showing an increase of D-dimer concentrations, three- to fourfold rise, in the early stages of COVID-19 disease and this level is strictly linked to poor outcome (Bikdeli et al. 2020). A recent study on 191 COVID-19 patients reported that D-dimer levels greater than 1 mg/ml on admission correlate to an 18-fold increase in mortality risk (Zhou et al. 2020). A very recent meta-analysis reported that D-dimer concentrations have only moderate accuracy to distinguish severe COVID-19 patients (Rostami and Mansouritorghabeh 2020). Also this study suggests that D-dimer can predict fatal outcome with acceptable specificity, with low risk of misdiagnosis, and a good diagnostic accuracy in patients (Rostami and Mansouritorghabeh 2020). Literature showed that a low D-dimer concentration can exclude the diagnosis of DVT without other and repeating tests, only in particular situations, using high sensibility methods (>98%) (Linkins and Takach 2017). The use of different thresholds can improve the clinical utility of the biomarker. COVID-19 is considered a high clinical pre-test probability condition. In this situation literature and clinical practice suggested the use of recommended threshold as <500 μg/L (0.5 mg/L) (Linkins and Takach 2017). Following this suggested cutoff and despite the heterogeneity of units used in laboratory around

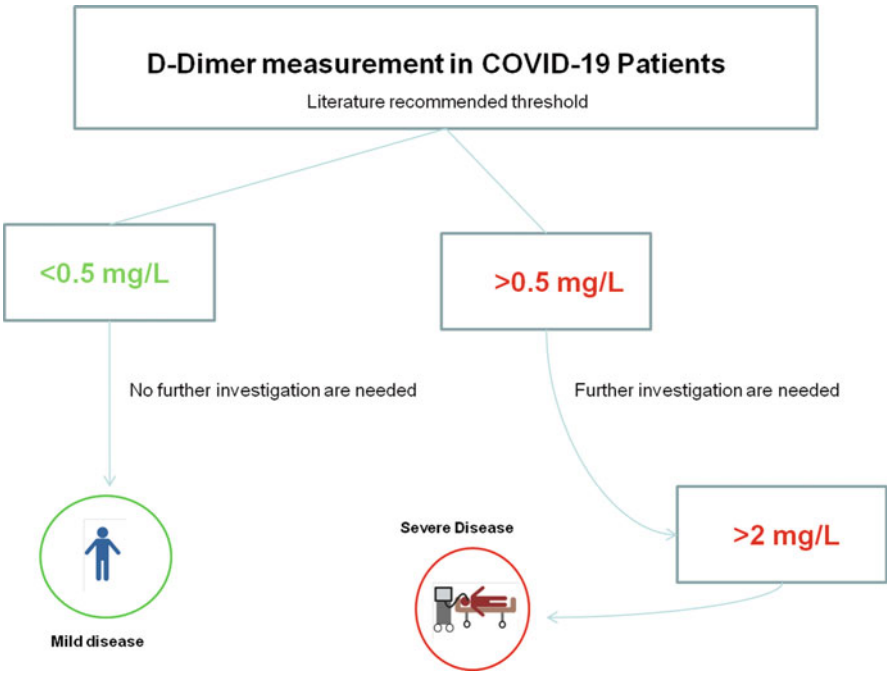

Fig. 5 D-dimer evaluation at admission in Emergency Room

the world, two different studies identified similar cutoff value (>2.14 mg/L and > 2.0 mg/L) as predictor of in-hospital mortality, while baseline value was not associated with DVT (Yao et al. 2020; Liu et al. 2020b). The same result is referred by a Chinese study predicting DVT and mortality using a cutoff value of 2.025 mg/L rather than the first test at admission with great accuracy (He et al. 2021).

In conclusion, D-dimer can predict worse outcome in COVID-19 patients with moderate accuracy but can intercept DVT with high sensitivity. D-dimer should be used in Emergency Room and values over the cutoff threshold (0.5 mg/L and 2 mg/L) allowed quickly classification and right addressed (Fig. 5).

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase (LDH) is a key enzyme of the anaerobic metabolic pathway, catalyzing the reversible oxy-reductive reaction of lactate to pyruvate throughout the reversible reduction of NAD+ to NADH . Using this knowledge, LDH activity was measured by assessing the rate of production of NADH that changes the optical density of the sample measured spectrophotometrically at 340 nm (Schumann et al. 2002). In clinical practice, the incidence of LDH was associated with the presence of diabetes and affects the prognosis of several pathological conditions, including cancers (Erez et al. 2014). High LDH serum levels are associated with poor prognosis in COVID-19 patients, with moderate sensitivity and specificity. Also several studies reported that elevation of LDH for at least >250 U/L was associated

with worse outcome in patients with COVID-19. LDH concentration could be use to predict poor outcome: high LDH serum level would indicate a 44% of probability and normal LDH level 11% for poor prognosis (Martha et al. 2021).

Biomarkers: From Bench to Patient

The Royal College of Pathologist defines Laboratory Medicine as "*the hidden science to save lives.*" In this book chapter, the dissertation of different biomarkers has showed that entry-level and differential change in biomarkers concentration can predict disease progression and outcome of COVID-19 patients. The temporal variations of biomarkers during the disease course are important to understand possible disease progression and to better patient management.

Based on the currently available evidences this book chapter would like to offer the following recommendations to the use of biomarkers in Emergency Room.

- For asymptomatic patients, without notably comorbidities, no investigations are needed.
- For all patients in the mild/moderate category, with notably comorbidities: 1) first step: CRP, CBC, with particular attention to lymphocytes (1500 cell/uL, 5–25%), neutrophils (numbers, NLR > 4) count, P_{O2}, S_{aO2} ($<93\%$) are needed at admission. 2) Second step: If any of these biomarkers are abnormal, creatinine, D-dimer (0.5 mg/L), PCT (>0.05 ng/mL), cardiac biomarkers (BNP and troponin I), and LDH are advisable.
- For all patients in the severe category, in addition to the markers mentioned above (1), creatinine, D-dimer, PCT, cardiac biomarkers (BNP and troponin I), and LDH are advisable.
- To strictly monitor patients CBC, CRP $P_{O2,}$ and S_{aO2} should be repeated 48–72 h after admission or earlier.

Diagnosis will be confirmed by using RT-PCR. Test interpretation problems however rose in relation to: i) negative results occurred in suspected COVID-19, with CT scan images compatible with COVID-19 pneumonia. In this case, the combined use of CT images and laboratory markers can identify RT-PCR false negative results in Emergency Room (Fig. 6) (Assandri et al. 2020a). ii) at low viral load ("weak positives, threshold cycle $\geq$ 35 "), patients can be in the initial (more rarely) or late (more frequently) stage of infection. The interpretation of the "weak positive" results particularly difficult in the absence of laboratory data attesting to previous results. Blood cells count, with particular attention to lymphocyte and platelet count, CRP, serum creatinine, and liver enzymes are needed at admission. If any of these biomarkers are abnormal with a clinical picture compatible with COVID-19 infection, monitoring of clinical and biochemical parameters are necessary (Fig. 7). Finally the combined use of CT images and laboratory markers confirm RT-PCR positive results in Emergency Room (Fig. 8).

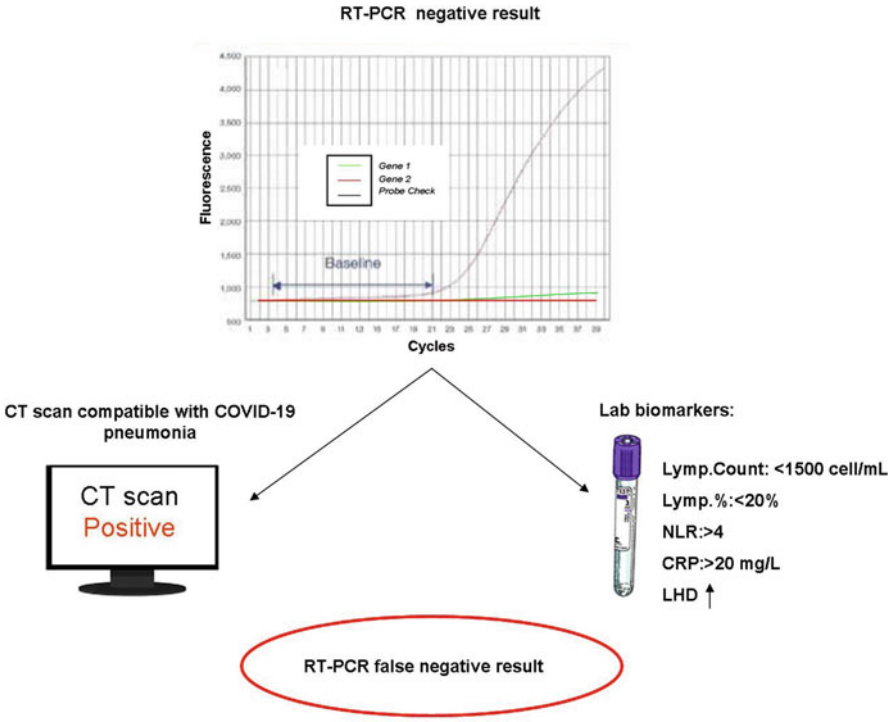

Fig. 6 The combined use of CT images and laboratory markers identifies RT-PCR false negative results in Emergency Room

Applications to Prognosis

This book chapter will explain biochemical, pathological, and molecular pathways that justify the use of several laboratory biomarkers to categorization patients in Emergency Room. Pre-analytical troubles, suboptimal sensitivity, long production time results, and other intrinsic methodological features affect RT-PCR (Gili et al. 2021; Lippi et al. 2020; Assandri et al. 2020b). This method cannot be yet considered a rapid and specific tool that can be used to help the Emergency Room. In this book chapter, the dissertation of different biomarkers has showed that entry level and differential change in biomarker concentration can predict disease progression and outcome of COVID-19 patients. The temporal variations of biomarkers during the disease course are important to understand possible disease progression and to better patient management.

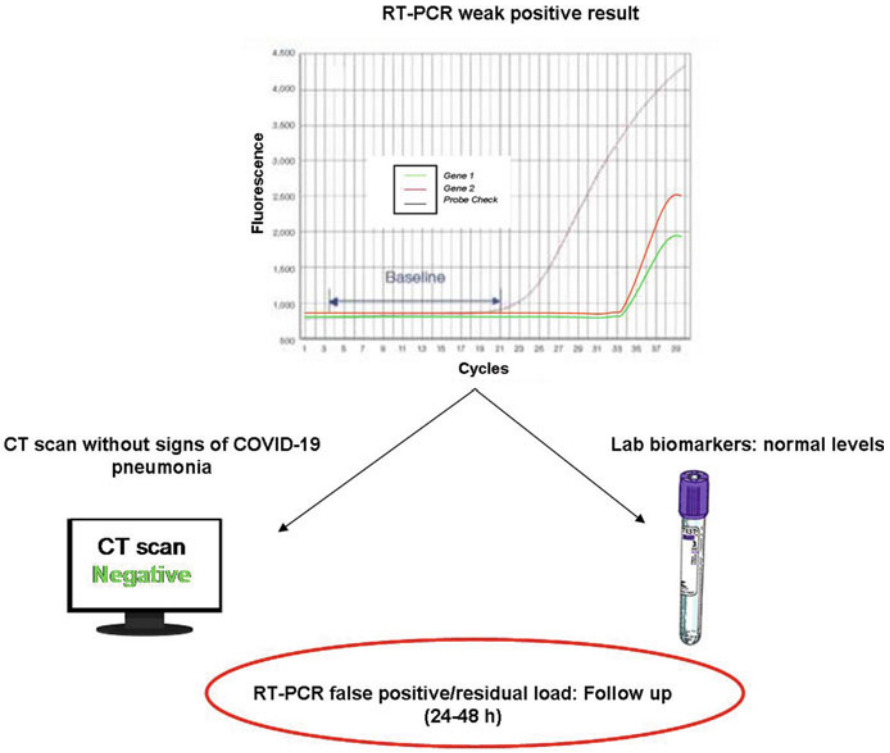

Fig. 7 RT-PCR threshold cycle ≥35 interpretation in patients with negative CT scan and normal level of laboratory markers

Mini-Dictionary of Terms

- SARS-Cov2: Positive-sense single-stranded RNA virus responsible of multi-system viral disease (COVID-19 disease), classifying into different clinical phases, caused by a complex interplay of the immunological, inflammatory, and coagulative pathway.
- CRP: C-reactive protein a pentameric, acute-phase reactant protein of the pentraxin superfamily (short pentraxin). This concentration and its temporal trend define COVID-19 phenotype and patient's outcome in Emergency Room.
- Lymphocyte: Lymphocytes are a type of white blood cells and represent 20–40% of total white blood cells. Low percentage and count (number) could be considered a hallmark of first stage of COVID-19 infection.
- Blood Gas Analysis (BGA): A set of laboratory tests referred to the acid-base balance and oxygenation status of a patient. Oxygenation status is evaluated using partial pressure of oxygen (P_{O2}), measured by amperometry and hemoglobin

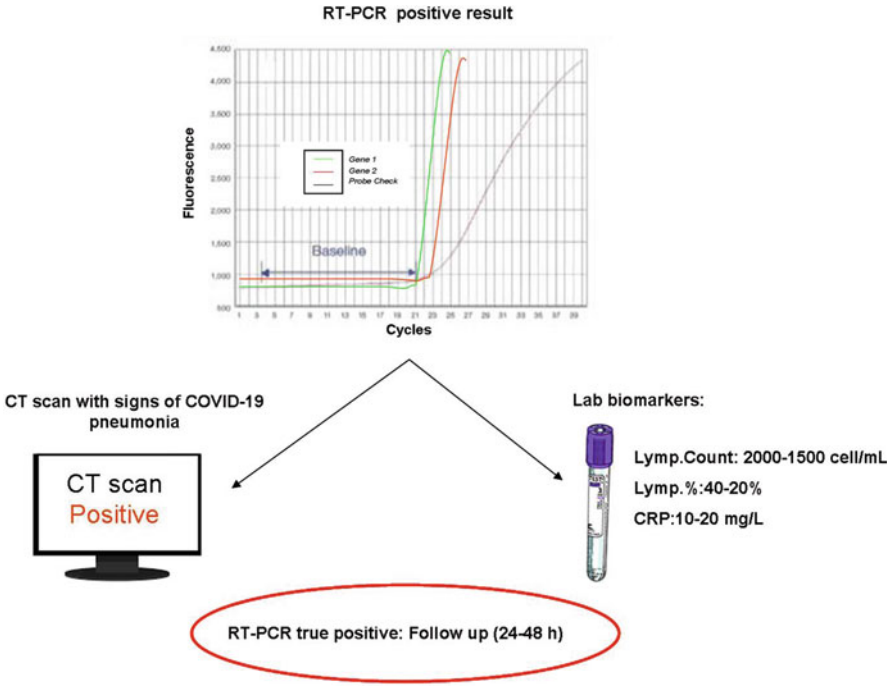

Fig. 8 The combined use of CT images and laboratory markers identifies RT-PCR true positive results in Emergency Room

oxygen saturation (S_{aO2}), measured by co-oximetry. This parameter is used for patient evaluation in Emergency Room.

- Emergency Room: Is a medical treatment unit specializing in emergency medicine, dedicated to the acute care of patients, usually found in a hospital or other primary care center.

Key Facts of COVID-19 Biomarkers

- SARS-CoV-2 is an ethological agent of COVID-19 disease.
- This condition is now considered a multisystem viral disease that involved different body districts, with different clinical manifestation and outcome (mild to severe and death).
- RT-PCR methods are not sufficient in evaluating patients in Emergency Room.
- Some laboratory biomarkers are related to disease severity and outcome.
- Measuring their concentration, and evaluation of temporal trend, can help clinicians to classify patients in Emergency Room.

Summary Points

- The ongoing pandemic of COVID-19 poses several challenges to clinicians. Time diagnosis and hospitalization, risk evaluation, and effective use of intensive care units and services are essential.
- Clinical assessment is extremely important to correct patients' evaluation, but laboratory markers can provide additional, essential information that significantly impact patients care (diagnosis, prognosis, and outcome).
- A biomarker is defined as a "*characteristic that can be objectively measured and evaluated as an indicator of normal biological and pathological processes, or pharmacological responses to a therapeutic intervention*."
- Entry level and differential changes in biomarker concentration can predict disease progression and outcome of COVID-19 patients. The temporal variations of biomarkers during the disease course are important to understand possible disease progression and to better patient management.
- For asymptomatic patients, without notably comorbidities, no investigations are needed.
- For all patients in the mild/moderate category, with notably comorbidities: 1) first step: CRP, CBC, with particular attention to lymphocytes (1500 cell/uL, 5–25%), neutrophils (numbers, NLR > 4) count, P_{O2}, S_{aO2} (<93%) are needed at admission. 2) Second step: If any of these biomarkers are abnormal, creatinine, D-dimer (0.5 mg/L), PCT (>0.05 ng/mL), cardiac biomarkers (BNP and troponin I), and LDH are advisable.
- For all patients in the severe category, in addition to the markers mentioned above (1), creatinine, D-dimer, PCT, cardiac biomarkers (BNP and troponin I), and LDH are advisable.
- To strictly monitor patients CBC, CRP P_{O2} and S_{aO2} should be repeated 48–72 h after admission or earlier.

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Serum Angiotensin II as a Biomarker in COVID-19

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Seda Ozkan and Afsin Ipekci

Contents

Abstract

Angiotensin II, synthesized by the cleavage of angiotensinogen to angiotensin I by renin and then conversion to angiotensin II by the angiotensin-converting enzyme, is the main effector peptide of the renin-angiotensin system.

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Transmembrane angiotensin-converting enzyme 2 (ACE2), the enzyme that metabolizes angiotensin II, is the host receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2, which binds to transmembrane angiotensin-converting enzyme 2, affects angiotensin II levels by causing an imbalance in the renin-angiotensin system.

Angiotensin II triggers inflammation, coagulopathy, vasculopathy, fibrosis, oxidative stress, and thrombosis through the angiotensin II type 1 receptor (AT1R). Angiotensin II is thought to contribute to pneumonia, sepsis, acute respiratory distress syndrome, diffuse thrombosis, and multi-organ damage in coronavirus disease 2019 (COVID-19).

Studies conducted in the preclinical and early period of the SARS-CoV-2 pandemic on severe acute respiratory syndrome coronavirus (SARS-CoV) shared findings that serum angiotensin II levels increased. However, recent studies have shown an increase in circulating soluble ACE2 levels and a decrease in serum angiotensin II levels in COVID-19.

Despite conflicting results, the majority of studies have shown that decreased serum angiotensin II levels are associated with the severity, prognosis, and mortality of COVID-19.

In this chapter, the relationship between COVID-19 and angiotensin II and the renin-angiotensin system was discussed in light of scientific studies conducted during the SARS-CoV-2 pandemic.

Keywords

SARS-CoV-2 · COVID-19 · Angiotensin II · Renin-angiotensin system · Angiotensin-converting enzyme · Angiotensin-converting enzyme 2 · Angiotensin II type 1 receptor · Soluble angiotensin-converting enzyme 2 · Angiotensin 1–7 · Biomarker · Prognosis

Abbreviations

ACE	Angiotensin-converting enzyme
ACE2	Angiotensin-converting enzyme 2
ADAM17	A disintegrin and metalloproteinase 17 domain
ADH	Antidiuretic hormone
ALI	Acute lung injury
Ang	Angiotensin
AP	Aminopeptidase
ARDS	Acute respiratory distress syndrome
AT1R	Angiotensin II type 1 receptor
AT2R	Angiotensin II type 2 receptor
AT4R	Angiotensin II type 4 receptor
COVID-19	Coronavirus disease 2019
CP	Carboxypeptidase
MasR	Mas receptor
MrgD	Mas-related G protein-coupled receptor member
NEP	Neprilysin

POP	Prolyloligopeptidase
RAS	Renin-angiotensin system
sACE2	Soluble angiotensin-converting enzyme 2
SARS-CoV	Severe acute respiratory syndrome coronavirus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
TF	Tissue factor
TMPRSS2	Human androgen-sensitive transmembrane serine protease type 2

Introduction

The SARS-CoV-2 virus, which appeared in late 2019 and caused the pandemic, uses angiotensin-converting enzyme 2 (ACE2) as a receptor to infect the host cell. ACE2 is a metalloproteinase type I transmembrane enzyme responsible for the metabolism of angiotensin II (Ang II). With the identification of ACE2 as a receptor, it has been revealed that the renin-angiotensin system (RAS) and angiotensin II, the main effector peptide of RAS, play an important role in the pathogenesis of coronavirus disease 2019 (COVID-19) (Wallentin et al. 2020; Gheblawi et al. 2020; Li et al. 2020).

After the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to transmembrane ACE2 through its S-protein, it transfers its RNA to human cells that are translated to produce new viral particles. As a result of the binding of SARS-CoV-2 with ACE2 on the cell, an imbalance occurs in the RAS. The use of ACE2 as a host receptor and viral replication was first demonstrated in severe acute respiratory syndrome coronavirus (SARS-CoV). Preclinical studies reported that after SARS-CoV, identified in 2003, binds to the ACE2 receptor, ACE2 is downregulated and reduced, resulting in excessive Ang II production (Kuba et al. 2006; Huang et al. 2014; Zou et al. 2014; Miesbach 2020). Current data suggest that SARS CoV-2 binds to the ACE2 receptor with 10–20 times the affinity of SARS-CoV (Wrapp et al. 2020). The decrease in transmembrane ACE2 activity due to viral blockade was thought to cause an increase in Ang II levels in COVID-19 (Miesbach 2020; Dean et al. 2021; Zhang et al. 2022). Thus, Ang II was held responsible for the pathogenesis of COVID-19 disease and lung injury due to its vasoconstriction, inflammation, fibrosis, oxidative stress, cell proliferation, hypertrophy, and apoptosis-enhancing effects (Kuba et al. 2006; Dean et al. 2021; Zhang et al. 2022).

The first two studies on SARS-CoV-2 reported that serum ACE2 decreased and Ang II levels increased (Liu et al. 2020; Wu et al. 2020). In the second group of studies, it was reported that there was no change in serum levels of Ang II and renin-angiotensin-aldosterone system peptides in COVID-19 (Kintscher et al. 2020; Henry et al. 2020; Rieder et al. 2021; Files et al. 2021). However, recent studies measuring broad angiotensin peptides, angiotensin II, and soluble ACE2 (sACE2) have reported an increase in sACE2 levels and a correspondingly significant decrease in serum Ang II levels. They reported that low Ang II levels are associated with poor prognosis and mortality in COVID-19 (Ozkan et al. 2021; Eleuteri et al. 2021; Bolay et al. 2021; Ipekci et al. 2022; Montiel et al. 2022; Wang et al. 2022; Krenn et al. 2022).

In this chapter, the relationship between COVID-19 and RAS and its effect on Ang II levels are discussed based on scientific studies conducted during the SARS-CoV-2 pandemic.

Renin-Angiotensin System (RAS)

The RAS, which represents the most important regulatory mechanism in both physiological and pathological conditions, includes a complex network of enzymes, peptides, and receptors (Ferrão et al. 2014; Hrenak and Simko 2020; Gheblawi et al. 2020). The RAS was first recognized as the hormonal system that maintains the balance of cardiovascular circulation. Later it was understood that it also includes a local tissue system that works synergistically or independently with the circulating one. Evidence suggests that tissue RAS locally produces mediators with homeostatic regulatory functions, thus contributing to some degree of organ dysfunction or disease (Mascolo et al. 2020; Mascolo et al. 2021).

The first component of RAS, renin, was discovered in 1898 with the understanding of the suppressive effects of extracts from rabbit kidneys. In 1934, it was determined that narrowing of the renal artery causes hypertension. Hypertensin and angiotonin were discovered in 1939 and named angiotensin (Ang). Ang I and Ang II were obtained by purification of Ang in 1954. Upon suspicion of the presence of a converting enzyme, angiotensin-converting enzyme (ACE) was isolated and described by Skeggs et al. in 1956. Angiotensin receptors were discovered in 1970, mas receptor (MasR) in 1986, and Ang 1–7 in 1988. In 2000, ACE2 was found as a close relative of the ACE (Gheblawi et al. 2020; Donoghue et al. 2000). ACE2 expression was initially detected in the heart, kidney, and testis. However, subsequent studies showed that it is found much more widely throughout the body, including the upper respiratory tract, lungs, intestine, and liver (Gheblawi et al. 2020; Bánhegyi et al. 2021). Sequence comparison of ACE and ACE2 showed that, like ACE, ACE2 is an integral transmembrane protein (and ectoenzyme) with a transmembrane anchor (type I membrane protein) close to its C-terminus. It was hypothesized that the two proteins would have similar substrate specificities and involvement in RAS. With the discovery of ACE2, the counterregulatory axis of the RAS was also defined (Gheblawi et al. 2020; Bánhegyi et al. 2021).

In 2003, it was discovered that ACE2 acts as a cell surface receptor for SARS-CoV. Recent studies have identified the ACE2 protease domain as the receptor for the SARS-CoV-2 virus, which causes pandemics in the world (Li et al. 2003; Gheblawi et al. 2020; Walls et al. 2020; Yan et al. 2020).

The RAS plays a crucial role in regulating renal, cardiac, and vascular physiology. Activation of the RAS is central to many common pathological conditions, including hypertension, heart failure, and kidney disease (Bánhegyi et al. 2021; Fisher 2022). Components of RAS function as signaling molecules that regulate inflammation, oxidative stress, cell proliferation, tissue remodeling, and apoptotic or necrotic cell death in various organs and systems (Hrenak and Simko 2020).

Angiotensin II

Angiotensin II (Ang II) is the main effector peptide of RAS. It is synthesized by the cleavage of angiotensinogen by renin to angiotensin I (Ang I) and then converted to Ang II by ACE (Unger 2002; Mascolo et al. 2021; Biberoğlu et al. 2021). However, Ang II can also be synthesized locally via pathways involving other enzymes such as chymase, chymostatin-sensitive Ang II producing enzyme, and cathepsin G (Mascolo et al. 2020; Mascolo et al. 2021).

The Role of Angiotensin II in the Classical Renin-Angiotensin System (First Axis)

The classical RAS pathway begins with the release of angiotensinogen from the liver and the release of renin from the kidneys, stimulated by low blood arterial pressure and low sodium chloride levels (Dean et al. 2021). Renin is synthesized by the juxtaglomerular cells of the renal afferent arteriole and stored in its inactive form known as prorenin. Activation of the juxtaglomerular cells causes the cleavage of prorenin to renin. Renin is secreted in response to activation of the sympathetic nervous system, hypotension detected by baroreceptors, or decreased sodium load in the distal nephron tubules (Alam et al. 2022; Fountain and Lappin 2022). When renin is released into the circulation, it uses angiotensinogen as a substrate to produce Ang I. Angiotensinogen is a large α2-globulin synthesized in the liver and is constantly present in the circulation. Ang I is a physiologically inactive decapeptide but acts as a precursor for Ang II (octapeptide) (Fountain and Lappin 2022). Ang I is converted to Ang II by ACE, which is primarily transcribed as a membrane-bound enzyme and then separated from the cell membrane by proteolytic cleavage (Dean et al. 2021; Xavier et al. 2021; Alam et al. 2022). Specifically, the C-terminal portion of ACE is responsible for the metabolism of Ang I to Ang II, while the N-terminal portion cleaves bradykinin to other peptides (Xavier et al. 2021; Morris and Sanghavi 2022).

Ang II demonstrates its effects such as aldosterone secretion, vasoconstriction, increased renal tubular sodium reabsorption, endothelial function, protein synthesis, oxidative stress, fibrosis, hypertrophy, and inflammation by binding to the transmembrane angiotensin II type 1 receptor (AT1R) (Alam et al. 2022; Dean et al. 2021; Fisher 2022; Silva et al. 2022). This part is the first axis of the RAS, which includes Ang II (Fig. 1).

The Role of Angiotensin II in the Non-classical Renin-Angiotensin System (Second Axis)

The main effector of non-classical RAS is ACE2. ACE2 is a metalloproteinase type I transmembrane enzyme characterized by a small cytoplasmic domain, a transmembrane domain, and an ectodomain (Xavier et al. 2021). ACE2 is expressed by many

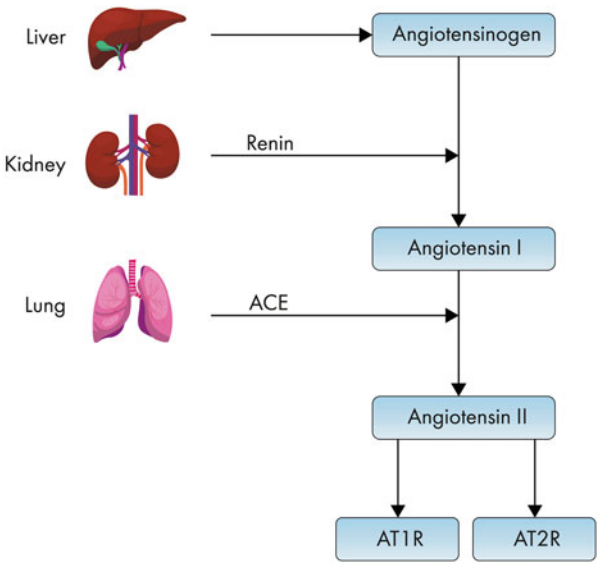

Fig. 1 The classical renin-angiotensin system. ACE, angiotensin-converting enzyme; AT1R, angiotensin II type 1 receptor; AT2R, angiotensin II type 2 receptor

organs including vascular endothelium, epithelial, and smooth muscle cells. So far, it has been shown to be found in the lungs, heart, kidneys, gastrointestinal tract, nervous system, bladder, testis, ovaries, prostate, pancreas, placenta, blood vessels, and liver (Sungnak et al. 2020; Catarata et al. 2020; Gheblawi et al. 2020; Miesbach 2020; Xavier et al. 2021; Beyerstedt et al. 2021; Mascolo et al. 2021; Shylesh et al. 2022; Fisher 2022). It is more expressed in endothelial and epithelial cells, especially in the lung, heart, and kidney (Gheblawi et al. 2020). The sACE2 protein containing the enzyme's catalytic domain enters the circulation after the membrane-bound molecule is cleaved by the disintegrin and metalloprotease 17 enzyme (ADAM17). ACE2 exists in both tissues and circulation in soluble form. Information on the regulation of ACE2 expression in different cells and the relationship between circulating ACE2 levels and cellular ACE2 levels is limited (Wallentin et al. 2020; Mascolo et al. 2020; Mascolo et al. 2021).

After the discovery of ACE2, a second axis of the RAS with an opposite function, also called the "non-classical RAS," was identified (Fig. 2). In non-classical RAS, the main peptide is Ang 1–7 heptapeptide, whose synthesis may involve two different enzymatic pathways. The first pathway begins with Ang II cleavage by ACE2 into Ang 1–7. The second pathway is the cleavage of Ang I by ACE2 to Ang 1–9 and the sequential conversion of Ang 1–9 to Ang 1–7 by ACE (Patel et al. 2016; Gheblawi et al. 2020; Mascolo et al. 2021; Fisher 2022). Ang 1–7 negatively regulates RAS activation by binding to both MasR and MrgD receptors and reduces the harmful effects mediated by Ang II/AT1R (Dean et al. 2021; Patel et al. 2016). The main action of the vasodepressor Ang 1–7 is the activation of the Mas receptor and the induction of kinases leading to the activation of endothelial nitric oxide synthase (Fisher 2022).

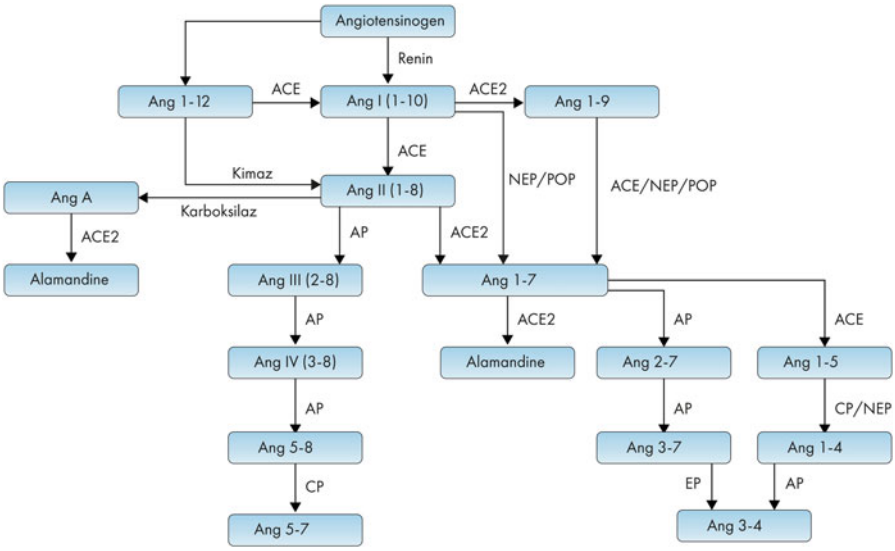

Fig. 2 The non-classical renin-angiotensin system. (*Ang* angiotensin, *ACE* angiotensin-converting enzyme, *ACE2* angiotensin-converting enzyme 2, *NEP* neprilysin, *POP* prolyloligopeptidase, *CP* carboxypeptidase, *AP* aminopeptidase)

Recent studies have shown that Ang II also binds to the transmembrane angiotensin type 2 receptor (AT2R), which has physiological effects different from the AT1 receptor. Ang II plays a protective role by inducing anti-inflammatory, antioxidative, anti-fibrotic, natriuresis, and vasodilation effects via AT2R (Mascolo et al. 2021; Unger 2002; Alam et al. 2022). AT2Rs are less frequently expressed after fetal life but maybe upregulated in response to injury (Fisher 2022).

The discovery of Ang II-derived peptides has provided a better understanding of the role of the RAS. Ang 1–7 produces two more peptides, Ang 1–5 by the action of ACE and Ang 2–7 by the action of aminopeptidases. Ang 2–7 produces Ang 3–7 by aminopeptidase. Ang 1–5 is converted to Ang 1–4 by neprilysin or carboxypeptidases. Finally, both Ang 3–7 and Ang 1–4 are converted to Ang 3–4 by aminopeptidases and endopeptidases (Fig. 2) (Ferrão et al. 2014; Gheblawi et al. 2020; Hrenak and Simko 2020; Xavier et al. 2021).

Ang II is cleaved and converted to Ang A by the carboxylase enzyme in a different pathway. In a third pathway, it is cleaved by aminopeptidase A to form Ang III (2–8). Angiotensin III is cleaved by aminopeptidase N to form Ang IV (3–8). Ang IV is cleaved by another aminopeptidase to form Ang 5–8. The pathway ends with Ang 5–8 cleaved by a carboxypeptidase to form Ang 5–7 (Fig. 2) (Ferrão et al. 2014; Hrenak and Simko 2020; Gheblawi et al. 2020; Xavier et al. 2021).

Ang A, which is produced by the cleavage of Ang II, is converted to Alamandin, an angiotensin peptide with alanine, by the ACE2. Ang 1–7 can also be converted to Alamandine by ACE2. Alamandine acts through the MrgD receptor and is part of the

Table 1 Receptors affected by Ang II and Ang II-derived peptides

Detrimental effects	Protective effects			
AT1R	AT2R	AT4R	MasR	MrgD
Ang II	Ang II	Ang IV	Ang (1–7)	Ang (1–7)
Ang III	Ang III	Ang III		Alamandine
Ang A	Ang 1–9	Ang 3–7		Aldosterone

Ang angiotensin, *AT1R* angiotensin II type 1 receptor, *AT2R* angiotensin II type 2 receptor, *AT4R* angiotensin II type 2 receptor, *MasR* Mas receptor, *MrgD* Mas-related G protein-coupled receptor member

protective RAS (Ferrão et al. 2014; Hrenak and Simko 2020; Xavier et al. 2021; Fisher 2022).

Peptides derived from Ang II have effects similar to those of Ang II through the AT1 and AT2 receptors, depending on the tissues and systems in which they occur. It is estimated that Ang IV exerts its effects on AT4R, Ang A on AT1R, Ang 1–9 on AT2R, and alamandine on MrgD receptors. Ang 1–7 acts on both Mas and MrgD receptors. Ang III binds to AT1, AT2, and AT4 receptors. AT2R, MaS, and MrgD receptors have opposite effects of AT1R and are on the protective side of RAS. AT4R shows the same effects as AT1R (Table 1) (Ferrão et al. 2014; Hrenak and Simko 2020; Gheblawi et al. 2020; Xavier et al. 2021).

The Role of Angiotensin II in the Tissue Renin-Angiotensin System

In addition to circulating RAS, it has been proven that there are tissue (local) RAS that works independently of each other and from circulating RAS. Many tissues and cells have all the necessary RAS components to form Ang II in situ. The main enzymes involved in Ang II formation in tissue-specific RAS are serine proteases (especially kallikrein-like enzymes, also called tonins), cathepsin G, and chymase (Ferrão et al. 2014; Xavier et al. 2021). In the kidney, 40% of Ang II is produced by non-ACE pathways, while chymase is the predominant producer of Ang II in the human heart, coronary arteries, and atherosclerotic aorta (Xavier et al. 2021). The formation of Ang II in tissue has more significant physiological effects than circulating Ang II in some cases. RAS in tissue is thought to undertake paracrine (cell to different cell), autocrine (cell to the same cell), and intracrine (intracellular) action mechanisms in addition to classical circulating RAS, which has endocrine effects (Zhuo et al. 2013; Ferrão et al. 2014).

Locally synthesized Ang II acts on the cell surface, nuclear, and cytoplasmic AT1 and AT2 receptors. Ang II activation in these local systems is thought to play a role in the development of various diseases with its detrimental effects including tissue remodeling, endothelial dysfunction, and fibrosis (Ferrão et al. 2014; Xavier et al. 2021; Fisher 2022).

The Effects of Angiotensin II

Ang II acts through specific Ang II receptors (AT1R and AT2R) (Fig. 3).

The effects of Ang II via the AT1R are the following:

- Stimulation of the AT1R causes vasoconstriction, the release of catecholamines, and an increase in systemic vascular resistance (Unger 2002; Mascolo et al. 2021; Alam et al. 2022; Mascolo et al. 2021).
- Ang II induces the secretion of aldosterone, a steroid hormone synthesized by the adrenal cortex. Aldosterone, the downstream effector of Ang II, increases sodium reabsorption and potassium excretion by increasing Na-H exchange in the proximal convoluted tubule of the kidney. Increased Na levels in the body increase the osmolarity of the blood, causing fluid to shift into the blood volume and extracellular space. Thus, the arterial pressure increases (Ferrão et al. 2014; Alam et al. 2022; Fountain and Lappin 2022; Fisher 2022).
- Ang II has three effects on the brain via the AT1R. First, it stimulates thirst and increases water intake by binding to the hypothalamus. Second, it stimulates the release of antidiuretic hormone (ADH) by the posterior pituitary. ADH increases water reabsorption in the kidney by adding aquaporin channels to the collecting duct. Third, angiotensin II reduces the sensitivity of the baroreceptor reflex. This reduces the baroreceptor response to an increase in blood pressure, which would be counter to the purpose of the RAS (Fountain and Lappin 2022).
- It stimulates fibrosis and reduces collagenase activity and expression of mitogen-activated protein kinase (Mascolo et al. 2021).
- It stimulates the production of pulmonary fibroblast procollagen via AT1R in lung injury (Marshall et al. 2004). It causes unfavorable tissue remodeling. It promotes pulmonary vasoconstriction, increased vascular permeability, production of inflammatory cytokines, and extracellular matrix synthesis, thus contributing to

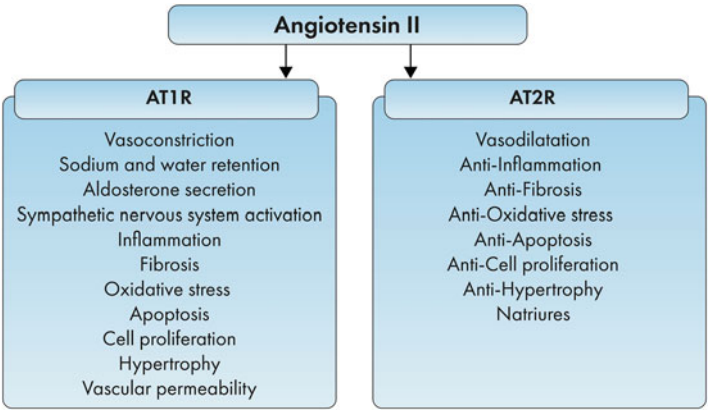

Fig. 3 Ang II acts through specific Ang II receptors (AT1R and AT2R). (*AT1R* angiotensin II type 1 receptor, *AT2R* angiotensin II type 2 receptor)

many of the core features of acute respiratory distress syndrome (ARDS) (Rieder et al. 2021).

- Angiotensin II acts as an inflammatory mediator through a variety of mechanisms, including intercellular and vascular adhesion molecules, reactive oxygen species, nuclear factor-kB, and superoxide. It produces a proinflammatory effect on leukocytes, endothelial cells, and vascular smooth muscle cells (Fisher 2022).
- It induces cellular hypertrophy, which plays a role in the pathology of hypertension and atherosclerosis by promoting the growth of vascular smooth muscle cells (Fisher 2022; Miesbach 2020).
- It induces the generation of reactive oxygen species in the endothelial cell through activation of AT1R and NADPH oxidase-2, thereby limiting nitric oxide bioavailability (Montiel et al. 2022).
- It provides NLRP3-inflammatory activation and subsequent release of pro-inflammatory cytokines that recruit inflammatory cells (Shah 2020).
- Ang II stimulates tissue factor (TF) expression both in vitro and in vivo. TF initiates blood coagulation. TF activation becomes dominant over the TF pathway-inhibitor and forms the prothrombotic endothelium (Celi et al. 2010).
- It stimulates platelet-derived growth factor production and increases platelet aggregation (Miesbach 2020).
- It stimulates the expression and release of plasminogen activator inhibitor 1, which is the main inhibitor of the fibrinolytic system (Skurk et al. 2001; Miesbach 2020).

The effects of Ang II via AT2R are the following:

- Ang II plays a protective role by inducing anti-inflammatory, anti-oxidative, anti-fibrotic, natriuresis, and vasodilation via AT2R (Mascolo et al. 2021; Unger 2002; Alam et al. 2022).

The half-life of Ang II in plasma is 1–2 min; at this point, peptidases reduce it to Ang III and IV. It has been reported that Ang III has 100% of the aldosterone-stimulating effect of Ang II, but 40% of the suppressive effects (Fountain and Lappin 2022). The physiological level of Ang II is determined by the balance between ACE and ACE2 activities (Bánhegyi et al. 2021).

The Role of Angiotensin II in COVID-19

ACE2 has gained importance in the pathogenesis of COVID-19 due to its host receptor for SARS-CoV-2 (Fig. 4) (Wallentin et al. 2020; Gheblawi et al. 2020).

ACE2 is widely expressed in many tissues in the human body, including epithelial cells in the nasal and oral mucosa, pneumocytes in the respiratory tract, vascular endothelial cells, and smooth muscle cells (Beyerstedt et al. 2021). This explains the multi-organ dysfunction observed in COVID-19 in addition to the respiratory system, which is the primary target of the virus (Miesbach 2020). ACE2 is expressed

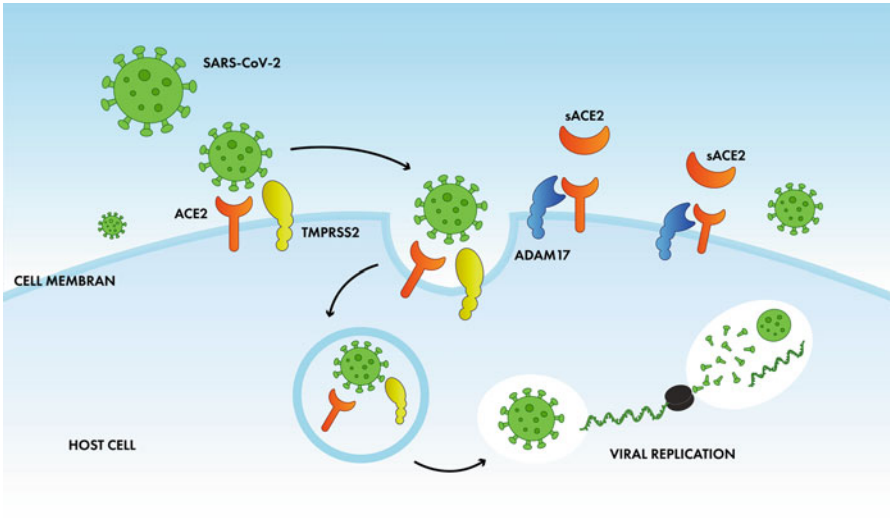

Fig. 4 Intracellular entry of SARS-CoV-2. (*ACE2* angiotensin-converting enzyme 2, *TMPRSS2* human androgen-sensitive transmembrane serine protease type 2, *ADAM17* a disintegrin and metalloproteinase 17 domain, *sACE2* soluble angiotensin-converting enzyme 2)

mostly in type I and II pneumocytes, type II alveolar epithelial cells, and tracheal luminal ciliary epithelial cells in the lungs (Jia et al. 2006; Shylesh et al. 2022).

SARS-CoV-2 invades the host cell by binding to ACE2 receptors on the epithelial surface of the respiratory system (Li et al. 2020). SARS-CoV-2 needs both ACE2 and human androgen-sensitive transmembrane serine protease type 2 (TMPRSS2) and a disintegrin and metalloproteinase 17 domain (ADAM17) enzymes to enter cells (Hoffmann et al. 2020). SARS-CoV-2 binds to the ACE2 receptor by the glycosylated spike protein. TMPRSS2 mediates the formation of this bond by cleaving the spike protein into spike-1 and spike-2 subunits (Mascolo et al. 2020; Hoffmann et al. 2020; South et al. 2020). The spike-1 subunit binds to ACE2 and facilitates viral attachment. The spike-2 subunit carries out membrane fusion and viral internalization (intracellular viral replication) in the pulmonary epithelium (Hoffmann et al. 2020; Zhang et al. 2020; Mascolo et al. 2021). SARS-CoV-2 downregulates ACE2 and also upregulates ADAM17, which may assist viral particle fusion to the cytoplasmic membrane (Shylesh et al. 2022). ADAM17 is the main sheddase responsible for the cleavage of transmembrane ACE2 into its soluble form, sACE2. Different sheddases can also contribute to inducible shedding (Xavier et al. 2021; Lundström et al. 2021). ADAM17 acts with TMPRSS2 to cleave ectodomains of various cytokines anchored in cell membranes, receptors, and enzymes. Both enzymes have the same specificity for transmembrane ACE2 (tACE2) (Lambert et al. 2005; Zipeto et al. 2020; Xavier et al. 2021). ADAM17 constitutively cleaves tACE2. TMPRSS2 supports the entry of the virus into the cell host (Xavier et al. 2021). ADAM17 cleaves ACE2 from the cell surface and releases the soluble, enzymatically active ectodomain form of the sACE2 into the circulation (Fig. 4)

(Lambert et al. 2005; Dean et al. 2021; South et al. 2020). ADAM17-mediated sACE2 is a biologically active form of sACE2, capable of degrading Ang II and countering the effects of Ang II (some say blocking circulating viral particles, others virus-carrying) (Heurich et al. 2014; Xavier et al. 2021). ADAM17-induced ACE2 shedding is activated by phorbol ester. Calmodulin, which is considered a sACE2 inhibitor, is found in plasma and blocks sACE2 activity. It was discovered that calmodulin's interaction with the cytoplasmic tail of ACE2 inhibits ACE2 shedding independently from phorbol ester-mediated shedding. Calmodulin and ADAM17 are thought to be involved in ACE2 proteolytic cleavage (Lambert et al. 2005; Wang et al. 2022).

It is hypothesized that sACE2 binds to SARS-COV-2, transporting the virus in circulation, facilitating the entry of virus into the cell via the AT1R, and spreading the virus to other organs (Wang et al. 2022). On the other hand, it is hypothesized that sACE2 blocks circulating viral particles (Heurich et al. 2014; Xavier et al. 2021).

Although the main source of sACE2 in COVID-19 is pulmonary endothelial and/or alveolar cells, data from a new study suggest that sACE2 continues to increase late in the disease process and that other cells or vascular beds also contribute to sACE2 formation (Lundström et al. 2021). Circulating sACE2 levels in healthy individuals are very low and difficult to measure. ACE2 functions predominantly in tissues, because the physiological activity of sACE2 is extremely low and can be masked by endogenous inhibitors such as calmodulin in human plasma. Accordingly, elevation in sACE2 naturally reflects increased shedding of tissue ACE2 and decreased protection against tissue RAS (Wang et al. 2022).

Preclinical studies have reported that after SARS-CoV, identified in 2003, binds to its receptor, ACE2 activates the RAS, leading to downregulation of ACE2 expression, resulting in overproduction of Ang II (Kuba et al. 2006; Huang et al. 2014; Zou et al. 2014). Current evidence indicates that SARS CoV-2 binds to the ACE2 receptor with 10–20 times the affinity of SARS-CoV and that ACE2 is required for viral replication (Wrapp et al. 2020). As a result of the entry of SARS-CoV-2 into the cell, the decrease in membrane-bound ACE2 disrupts the balance of the angiotensin system. Loss of ACE2 leads to downregulation of the ACE2/Ang 1–7/MasR pathway and increased Ang II levels (Dean et al. 2021; Miesbach 2020; Zhang et al. 2022).

Increased Ang II levels mediate inflammation by vasoconstriction, cell proliferation, angiogenesis, generation of reactive oxygen species, fibrosis, the release of proinflammatory cytokines, inflammatory cell chemotaxis, and epithelial cell apoptosis via AT1R. Ang II also mediates tissue microvascular injury (Kuba et al. 2006; Dean et al. 2021; Zhang et al. 2022).

The primary target of the virus is the respiratory system and major symptoms start from here. Respiratory and flu-like symptoms are the main symptoms of COVID-19. Lymphopenia and elevated proinflammatory cytokine levels lead to acute respiratory distress syndrome (ARDS), organ failure, and diffuse coagulopathy (Guo et al. 2020; Mascolo et al. 2021).

An imbalance between Ang II and Ang 1–7 levels can exacerbate lung injury caused by SARS-COV-2, which contributes to decreased lung function and increased fibrosis and inflammation (South et al. 2020). Both AT1 and AT2 receptors are present in normal and pathological human lungs (Mascolo et al. 2021). AT1 receptors are located on vascular smooth muscle cells, alveolar macrophages, and the stroma under the airway epithelium, while AT2 receptors are located on the bronchial epithelium and endothelial cells (Bullock et al. 2001). In studies conducted before COVID-19, it was reported that Ang II also plays a role in the development of lung diseases such as idiopathic pulmonary fibrosis, sarcoidosis, pulmonary hypertension, acute respiratory distress syndrome, and lung cancer. Ang II contributes to lung injury and different lung diseases by regulating cell proliferation, immune-inflammatory response, hypoxia, and angiogenesis (Mascolo et al. 2020; Catarata et al. 2020).

Ang II stimulates tissue factor expression, thereby causing endothelial dysfunction, the development of arteriosclerosis, and microvascular thrombosis. In addition, Ang II stimulates platelet-derived growth factor production and increases platelet aggregation. Ang II inhibits the fibrinolytic system by stimulating the expression and release of plasminogen activator inhibitor 1 (Miesbach 2020; Puurunen et al. 2018; Skurk et al. 2001). These effects of Ang II are thought to contribute to thrombosis in COVID-19 disease.

The increase in Ang II triggers proinflammatory cell activation, neutrophil infiltration into the lungs, and initiation of inflammatory processes. Ang II is not only a chemotaxis factor for mononuclear cells. Ang II also activates adhesion molecules such as chemoattractant cytokines, P-selectin, intercellular cell adhesion molecule type I, and vascular cell adhesion molecule type I in vascular endothelial cells and smooth muscle cells. It causes the adhesion of monocytes and neutrophils to endothelial cells. The increase in Ang II is considered one of the early responses during inflammatory processes (Ruiz-Ortega et al. 2001; Xavier et al. 2021). Combined with the enhanced neutrophil heterogeneity of COVID-19, Ang II triggers NETosis. Thromboembolism caused by Ang II-mediated neutrophil extracellular traps causes severe damage to many organs (Zhang et al. 2022).

Ang II acts as a stimulatory molecule that assists T lymphocyte activation. Thus, it plays a role in inflammatory responses by promoting cellular proliferation, differentiation, effector function, migration, and adhesion. T lymphocytes can produce both AT1R and Ang II by activating immune cells through autocrine mechanisms (Coppo et al. 2008; Hoch et al. 2009; Xavier et al. 2021).

In addition, Ang II has been shown to induce IL-6 transcription and increase IL-6 levels in vascular smooth muscle cells. IL-6 is a multifunctional cytokine that mediates the proliferation of B-lymphocytes during antibody synthesis. Ang II stimulates the release of IL-6, leading to cytokine storms and worse outcomes in COVID-19 (Miesbach 2020).

Ang II induces inflammation, coagulopathy, vasculopathy, and thrombosis, contributing to pneumonia, sepsis, ARDS, diffuse thrombosis, and multiple organ damage in COVID-19 (Miesbach 2020; Zhang et al. 2022).

Serum Angiotensin II Levels in COVID-19

The role of SARS-CoV-2 in the pathogenesis of COVID-19 disease, with its entry into the cell using the ACE2 receptor and its viral replication, is well defined. However, conflicting results have been reported regarding the levels of serum Ang II in studies conducted on COVID-19 (Montiel et al. 2022). While reports in the early period of the pandemic found an increase in serum Ang II levels in COVID-19 patients (Liu et al. 2020; Wu et al. 2020), recent studies using gold standard assays have shown the opposite (Gerard et al. 2021; van Lier et al. 2021).

Plasma Ang II levels were found to increase in the first study performed in a small patient group immediately after the definition of COVID-19. It was also reported that there was a strong correlation between both viral load and lung injury and Ang II levels (Liu et al. 2020). In another early study, higher plasma Ang II levels were reported in COVID-19 patients with severe symptoms compared to mild cases (Wu et al. 2020).

Similarly, in previous studies on H5N1 and H7N9 patients, serum Ang II levels were found to be high (Huang et al. 2014; Zou et al. 2014). In experimental lipopolysaccharide-induced lung fibrosis, Ang II levels were found to be slightly elevated in plasma, but much more increased in bronchoalveolar lavage fluid. These changes were also accompanied by higher AT1R expression in pulmonary tissue (Cao et al. 2019). Activation of the ACE/Ang II/AT1R cascade was associated with an increased risk of pneumonia and poor prognosis (Nie et al. 2014). In another study in mice infected with the H7N9 influenza virus, Ang II levels were found to increase with decreased ACE2 protein expression in lung tissue 3 days after infection (Yang et al. 2014). Plasminogen activator inhibitor 1 is a biomarker of ARDS (Bhargava and Wendt 2012) and was shown to increase with Ang II in a rat ventilator-induced lung injury model (Annoni et al. 2019). Based on these early studies of COVID-19 and the results of previous studies on SARS-CoV, it was predicted that ACE2 levels should be downregulated and Ang II levels should be increased in SARS-CoV-2 infection (Krenn et al. 2022).

In the COVID-19 studies conducted in the second period, no changes were detected in serum Ang II levels and serum levels of renin-angiotensin-aldosterone system peptides (Rieder et al. 2021; Kintscher et al. 2020; Henry et al. 2020; Files et al. 2021). Some studies also found no change in ACE and ACE2 levels (Kutz et al. 2021).

Recent studies measuring circulating angiotensin peptides, Ang II, and soluble ACE2 in COVID-19 patients have reported divergent results from the theory of decreased ACE2 and increased Ang II levels reported in early pandemic studies (Krenn et al. 2022). Possible reasons for conflicting angiotensin II results in studies are shown in Table 2.

In new studies on COVID-19, it was determined that Ang II levels in the systemic circulation decreased significantly in parallel with increased ACE2 levels. The decrease in Ang II levels was even more evident in patients with moderate-severe respiratory failure, ARDS, requiring intensive care, and death (Ozkan et al. 2021;

Table 2 Reasons for conflicting angiotensin II results in studies

Reasons
Different levels of angiotensin II in tissue and circulation
Variations in sample sizes
Variations in patient profiles
Differences in the distribution of mild, moderate, and severe patient groups
Variations in angiotensin II measurements
Differences in the ELISA kits used
Different treatments applied to patients
Unexplored features of angiotensin II and renin-angiotensin system
Differences in sample collection times
Various components of the renin-angiotensin system have a circadian pattern throughout the day
Age, gender, and smoking status of the patients
Comorbidities of patients

Eleuteri et al. 2021; Bolay et al. 2021; Ipekci et al. 2022; Montiel et al. 2022; Wang et al. 2022; Krenn et al. 2022).

Low Ang II serum levels (especially if lower than Ang I levels) are thought to result from the increased conversion of Ang II to Ang 1–7 by ACE2 or decreased ACE activity. It should also be noted that Ang II is converted to Ang 1–7 not only by ACE2 but also by prolyloligopeptidase and carboxypeptidase (Ferrão et al. 2014; Krenn et al. 2022; Triposkiadis et al. 2021).

In COVID-19, there is a decrease in both tissue and circulating ACE levels secondary to endothelial damage (Zhu et al. 2020; Files et al. 2021; Gerard et al. 2021; Krenn et al. 2022; Montiel et al. 2022; Wang et al. 2022). In severe COVID-19, Ang 1–7 levels also increase and Ang II/Ang 1–7 ratio is impaired (van Lier et al. 2021; Reindl-Schwaighofer et al. 2021; Valle Martins et al. 2021).

Pulmonary endothelial damage causes significant decreases in ACE activity in non-COVID acute lung injury (ALI) and ARDS. It has been found that the decrease in ACE causes a decrease in the capacity to convert Ang I to Ang II and a decrease in the Ang II/Ang I ratio. A low Ang II/Ang I ratio was associated with poor prognosis and increased mortality (Orfanos et al. 2000; Reddy et al. 2019; Lundström et al. 2021; Krenn et al. 2022). The reasons for the decreased Ang II/Ang I ratio in ARDS are thought to be increased metabolism of Ang II by ACE2, increased activity of other proteases that process Ang II, and inhibition of ACE by an as yet unidentified endogenous serum albumin (Reindl-Schwaighofer et al. 2021; Bánhegyi et al. 2021; Krenn et al. 2022).

The data suggest that circulating Ang II and pulmonary ACE activity are decreased in COVID-19 as well as in non-COVID-19-related ARDS correlated with disease severity (Zhu et al. 2020; Ozkan et al. 2021; Montiel et al. 2022; Wang et al. 2022). The primary site of circulating Ang II formation is pulmonary ACE. Downregulation of pulmonary ACE activity is possible in cases of extensive pulmonary vascular endothelial damage caused by COVID-19 (Wang et al. 2022). A recent study found that serum ACE levels decreased and ACE2 levels increased in

COVID-19 and non-COVID-19 ARDS. In addition, it showed that ACE decreased and ACE2 increased in lung tissue. It was defined that the increase in ACE2 level is primarily derived from endothelial cells. A selective loss of type 2 alveolar epithelial cells was observed in ARDS associated with COVID-19 (Gerard et al. 2021). Another study reported a 199-fold upregulation of ACE2 in cells in bronchoalveolar lavage fluid from patients with COVID-19 (Garvin et al. 2020). However, in a small autopsy series of patients who died from COVID-19 or influenza A (H1N1), high ACE2 protein expression was detected in alveolar and pulmonary endothelial cells for each disease (Ackermann et al. 2020). In bacterial pneumonia models, ACE2 levels in BAL and in the lungs were initially decreased to allow entry of immune cells but later increased to limit vascular permeability and relieve inflammation (Sodhi et al. 2018; Sodhi et al. 2019).

New findings in both COVID-19-related and non-COVID-19-related ARDS are in contrast to the findings of decreased ACE2 expression and increased Ang II shown in previous studies of lung injury in experimental animal models (Krenn et al. 2022). Differences in the regulation of ACE2 expression between species and the relatively short duration of experiments modeling ARDS in animals are held responsible for these results. In contrast to animal experiments, a dysregulated interferon response is thought to contribute to the induction of ACE2 expression in patients with prolonged COVID-19 and ARDS (Krenn et al. 2022; Ziegler et al. 2020; Gerard et al. 2021; Lundström et al. 2021).

Ang II levels are further reduced with the development of sepsis and ARDS in COVID-19. In COVID-19-associated ARDS, Ang I cannot be hydrolyzed to Ang II without functional ACE. Ang II deficiency contributes to hypotension and septic shock. Therefore, exogenous Ang II administration has also been considered for the treatment of vasodilatory shock associated with COVID-19 (Chow et al. 2020).

Increased sACE2 levels decrease circulating Ang II levels. It has been shown that sACE2 levels increase in various diseases such as inflammatory diseases, cardiovascular diseases, and type II diabetes. Increased sACE2 levels have been accepted as a marker of disease severity and clinical prognosis (Soro-Paavonen et al. 2012; Ramchand et al. 2018; Xavier et al. 2021; Zhang et al. 2022). ADAM17, which is activated by the binding of the virus in COVID-19 patients, increases the level of biologically active sACE2 in the circulation. It is thought that the increase in sACE2 is associated with the severity of the COVID-19 disease and poor prognosis, as in other diseases. Increased levels of ACE2 in the blood of patients with COVID-19 have been measured by ELISA as well as mass spectrometry-based assays (Vassiliou et al. 2021; Lundström et al. 2021; Patel et al. 2021; Nagy Jr et al. 2021; Kragstrup et al. 2021; Rahman et al. 2021; Reindl-Schwaighofer et al. 2021; Krenn et al. 2022; Wang et al. 2022; Zhang et al. 2022).

Pro-inflammatory cytokines induce the activation of ADAM17, further increasing sACE2 levels. On the other hand, the activation of ADAM17 can be increased by Ang II and bradykinin. ACE is the most important enzyme that inactivates bradykinin. Thus, pulmonary endothelial damage may play a role in increasing sACE2 levels as well as decreasing ACE. However, several other stimuli can increase ACE2

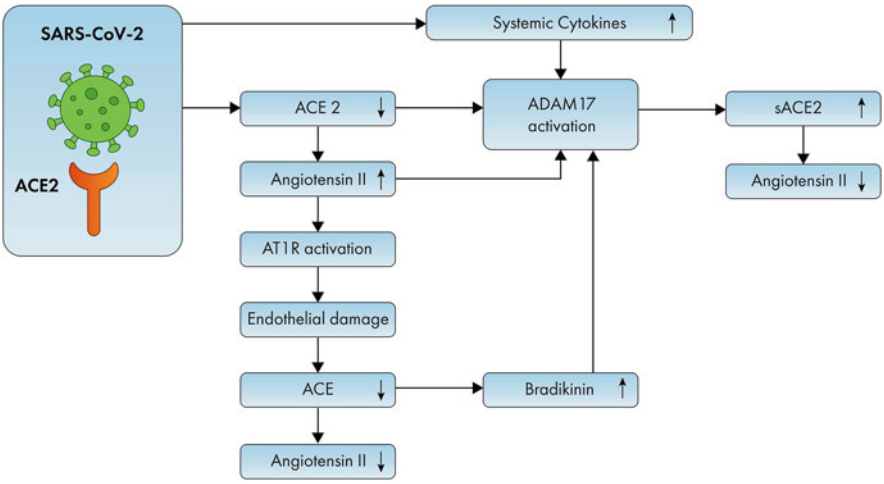

Fig. 5 Angiotensin II levels in COVID-19. (*AT1R* angiotensin II type 1 receptor, *ACE* angiotensin-converting enzyme, *ACE2* angiotensin-converting enzyme 2, *sACE2* soluble angiotensin-converting enzyme 2, *ADAM17* a disintegrin and metalloproteinase 17 domain)

shedding, including hypoxia and significantly elevated Ang II levels (Fig. 5) (Lundström et al. 2021; Krenn et al. 2022; Rahman et al. 2021; Xavier et al. 2021).

Two studies published in 2021 showed that sACE2 binds to SARS-COV-2, spreads throughout the body through the systemic circulation, and mediates its entry into different cells (Karthika et al. 2021; Yeung et al. 2021). The proteomic analysis detected evidence of the circulation of the virus-sACE2 complex throughout the body. It revealed that viremia was associated with sACE2 elevation in blood and endovascular damage (Li et al. 2021). After the endothelium is damaged, an activated inflammatory response causes a cytokine storm in COVID-19. Moreover, the impaired endothelial barrier cannot limit the permeation of the virus-sACE2 complex through vessels. The virus-sACE2 complex infects additional organs and tissues (Wang et al. 2022; Li et al. 2021).

Current clinical findings contradict the hypothesis of increased Ang II and decreased ACE2 in the systemic circulation in COVID-19. However, these changes may play a role locally in the lung and other tissues. While transmembrane ACE2 enters the circulation as active sACE2 via ADAM17, tissue ACE2 can be catalytically weakened by the influence of the virus. This may increase susceptibility to Ang II-mediated microvascular complications, inflammation, fibrosis, and oxidative stress secondary to loss of protective RAS in the tissue.

Ang II plays an important role in the initiation and progression of inflammatory responses therefore possible that an Ang II peak may be the first biological response to SARS-CoV-2 virus entry (Xavier et al. 2021). However, increased Ang II and cytokines activate ADAM17. Activation of ADAM17 decreases circulating Ang II

levels due to reasons such as increased circulating sACE2 levels, exacerbation of endothelial damage, and inhibition of ACE.

Contrary to the early studies, the relationship between RAS peptides was better demonstrated by studying more numerous RAS peptides and enzymes in subsequent studies. In the future, the study of Ang II and other RAS peptides in tissues will enable us to reach more enlightening data.

Despite the conflicting results of studies to date, the majority of studies have shown that reduced circulating Ang II levels are associated with severity, prognosis, and mortality of COVID-19 disease. Although more studies are needed for evidence, it can be predicted that serum Ang II levels can be used in the prognosis determination of COVID-19 disease.

Applications to Prognosis, Other Diseases, or Conditions

Applications to Prognosis

Despite the conflicting results of studies in COVID-19 patients, recent studies using gold standard methods have found that angiotensin II levels in the systemic circulation are significantly reduced. They reported that low angiotensin II levels were even more pronounced in patients with moderate-severe respiratory failure, requiring intensive care unit hospitalization, ARDS, and mortality (Ozkan et al. 2021; Krenn et al. 2022; Eleuteri et al. 2021; Bolay et al. 2021; Ipekci et al. 2022; Montiel et al. 2022; Wang et al. 2022). Although further studies are needed for evidence, serum angiotensin II levels can be used to determine the prognosis of COVID-19.

Applications to Other Diseases or Conditions

In recent studies, it has been shown that there is a decrease in serum angiotensin II levels with the decrease of ACE secondary to pulmonary endothelial damage in ALI and ARDS caused by different reasons (Orfanos et al. 2000; Reddy et al. 2019; Gerard et al. 2021; Lundström et al. 2021; Krenn et al. 2022). Angiotensin II levels can be used as an auxiliary biomarker in prognosis follow-up in non-COVID-19 ARDS cases.

It has long been known that angiotensin II plays a role in the pathogenesis of hypertension and other cardiovascular diseases (Unger 2002; Ferrão et al. 2014; Patel et al. 2016; Alam et al. 2022). Ang II levels can be used in the follow-up and treatment of these diseases.

Studies have shown that angiotensin II levels decrease in sepsis and septic shock and angiotensin II can be used in the treatment (Chow et al. 2020; Alam et al. 2022). Angiotensin II levels can be used to predict prognosis and mortality in sepsis.

Mini-Dictionary of Terms

- *SARS-CoV-2*: Enveloped virus with single-stranded RNA with protein protrusions on the surface.
- *COVID-19*: Infectious disease caused by SARS-CoV-2 virus.
- *Host receptor*: The region where the virus will bind for entry to the organism to be infected.
- *Sheddaz*: Enzyme that cleaves ectodomains of cytokines, receptors, and enzymes.
- *Transmembrane enzyme*: Membrane proteins that participate in intracellular and extracellular reactions embedded in the cell surface membrane.

Key Facts of Angiotensin II

- Angiotensin II is the main effector peptide of the renin-angiotensin system.
- RAS is responsible for the most important regulatory mechanisms in both physiological and pathological conditions.
- The renin-angiotensin system includes a complex network of enzymes, peptides, and receptors.
- Angiotensin II and the renin-angiotensin system play a role in the pathogenesis of COVID-19.
- Angiotensin II levels are changed in COVID-19.

Summary Points

- SARS-CoV-2 uses ACE2 as its host receptor and causes an imbalance in the RAS.
- Imbalance in the RAS affects serum angiotensin II levels.
- Angiotensin II has been associated with the pathogenesis of COVID-19 because it triggers inflammation, coagulopathy, vasculopathy, fibrosis, oxidative stress, and thrombosis.
- Despite conflicting data, the majority of studies have demonstrated a decrease in serum angiotensin II levels in COVID-19.
- Decreased serum angiotensin II levels are associated with ARDS and mortality in COVID-19.
- Although more evidence is needed, angiotensin II levels can be used as an auxiliary biomarker in determining the prognosis of COVID-19.

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Polyunsaturated Fatty Acid-Derived Lipid Mediators as Biomarkers in Critical Care

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Applications to COVID-19 and Putative Biomarkers

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Abstract

Inflammation is a necessary and beneficial process that protects against injury and infection. However, dysregulated and unrestrained inflammation underpins nearly every pathology related to critical illness. Inflammation is increasingly understood as a coordinated phenomenon between pro-inflammatory processes and restorative resolution of inflammation, both working in concert to restore homeostasis. There is a growing body of literature supporting the concept that resolution is controlled by derivatives of dietary polyunsaturated fatty acids termed specialized pro-resolving lipid mediators (SPMs) and that pathologic inflammatory processes are characterized by absence or dysregulation of SPMs leading to failure of inflammatory resolution. In this chapter, we will discuss the role of SPMs in several diseases frequently encountered in the ICU – including sepsis, ARDS, and viral illness, specifically influenza – and their potential uses as diagnostic and prognostic biomarkers. This discussion will provide a framework to discuss how emerging research implicates SPMs and polyunsaturated fatty acids in the clinical manifestations of COVID-19, itself a viral illness that causes sepsis and ARDS. Therapies modulating SPMs and SPMs themselves are emerging as candidate therapeutic molecules that may dampen pro-inflammatory cytokine storms, promote resolution of inflammation, and induce a return to homeostasis in COVID-19 and other severe illnesses caused by aberrant inflammation.

Keywords

Specialized pro-resolving mediator · Sepsis · Acute respiratory distress syndrome · COVID-19 · n-3 polyunsaturated fatty acids · n-6 polyunsaturated fatty acids · Arachidonic acid · Docosahexaenoic acid · Eicosapentaenoic acid · Resolvins · Maresins · Protectins · Lipoxins

Abbreviations

17S-diHDHA	17*S*-dihydroxy-docosahexaenoic acid
AA	Arachidonic acid
ALI	Acute lung injury
ALOX	Arachidonate 5-lipoxygenase
ALX/FPR2	Formyl peptide receptor 2

ARDS	Acute respiratory distress syndrome
COVID-19	Coronavirus disease 2019
COX2	Cyclooxygenase-2
cPLA2	Cytoplasmic phospholipase A2
CYP450	Cytochrome P450
DAMP	Damage-associated molecular pattern
DHA	Docosahexaenoic acid
DRV1	D-resolvin receptor 1
DRV2	D-resolvin receptor 2
EMT	Epithelial-mesenchymal transition
EPA	Eicosapentaenoic acid
GPCR	G-protein coupled receptor
HLH	Hemophagocytic lymphohistiocytosis
hsCRP	high sensitivity C-reactive protein
IL	Interleukin
LC-MS	Liquid chromatography/mass spectrometry
LXA	Lipoxin A series
LXB	Lipoxin B series
MarR	Maresin
NF-κB	Nuclear factor-kappa B
PAMP	Pathogen-associated molecular pattern
PCTR	Protectin conjugates of tissue repair
PD1, PDX	Protectin-D1, DX
PG	Prostaglandin
PMN	Polymorphonuclear neutrophil
PUFA	Polyunsaturated fatty acid
RvD	D-series resolvin
RvE	E-series resolvin
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SIRS	Systemic inflammatory response syndrome
SPM	Specialized pro-resolving mediator
TGFβ	Tumor growth factor-beta
TNF-α	Tissue necrosis factor-alpha
TX	Thromboxane

Introduction

Critical illness is a broad term describing severe disease and organ dysfunction usually in response to insults including infection, trauma, major surgery, vascular thrombotic events, and others. While the inciting event differs, critical illness is often related to an inordinate inflammatory response referred to as systemic inflammatory response syndrome (SIRS) (Bone et al. 1992). This syndrome is characterized by pathologic responses to inflammatory mediators leading to hallmark features of critical illness such as fever, hypotension, acute respiratory distress syndrome

(ARDS), vascular leak, and reduced oxygen delivery to tissues (Sheu et al. 2010; Joffre et al. 2019). In this chapter, we will discuss the role of polyunsaturated fatty acid (PUFA)-derived mediators of inflammation and resolution with a focus on specialized pro-resolving mediators (SPMs). We will review these lipid molecules in association with critical illness and lung disease, specifically sepsis, ARDS, and influenza A virus. We will then explore the role these SPMs may play in coronavirus disease 2019 (COVID-19), the disease caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus (Hu et al. 2021).

Discovery of Resolution as the Flip Side of Inflammation

Inflammation is a beneficial process that protects healthy tissue from injury and infection. Infections or injury expose cells to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular pattern (DAMPs), which activate key pathways including NF-κB that lead to release of pro-inflammatory chemokines and cytokines including TNF-α, IL1-β, IL-6, IL-8, and others (Tabas and Glass 2013; Basil and Levy 2016). These cytokines function as powerful extracellular signals to attract and activate the initial cellular-mediated immune response in the form of macrophages and T cells. Macrophages and neutrophils are primary effectors of the initial immune response (Glass and Saijo 2010). Structural cells including epithelial and endothelial cells also respond to injury or infection by producing chemokines that recruit circulating neutrophils and monocytes to the site of the insult, while altering their barrier function to allow circulating immune cells to invade the site of injury.

Eicosanoids have long been recognized as an important class of pro-inflammatory mediator.

Eicosanoids are derived from enzymatic or nonenzymatic oxidation of arachidonic acid (AA) and other PUFAs. The first eicosanoids to be recognized in inflammation include prostaglandins (PGs), leukotrienes (LTs), and thromboxanes (TXs) and generally have pro-inflammatory functions including mediating pain, fever, recruitment of lymphocytes and neutrophils, platelet activation, and altering endothelial barrier function leading to edema (Tapiero et al. 2002; Hanna and Hafez 2018). The first step in production of this class of pro-inflammatory eicosanoids is the release of AA from the cell membrane by phospholipase A2 (PLA2) or phospholipase C, followed by the conversion of AA to prostaglandin H_2 (PGH_2) by cyclooxygenase (COX) 1 or 2. PGH_2 is further converted to the final effector PGs by a family of specific synthetic enzymes. COX2 is upregulated by NF-κB and other pro-inflammatory stimuli, leading to increased output of pro-inflammatory PGs (Fig. 1).

Under the classical framework of inflammation, the eventual success of the immune system's ability to address an insult depended on the magnitude and timely onset of this acute phase of inflammation. Resolution was thought to occur passively as the concentration of biochemical mediators of the acute inflammatory response decreased locally (Buckley et al. 2013). In this model, resolution of inflammation

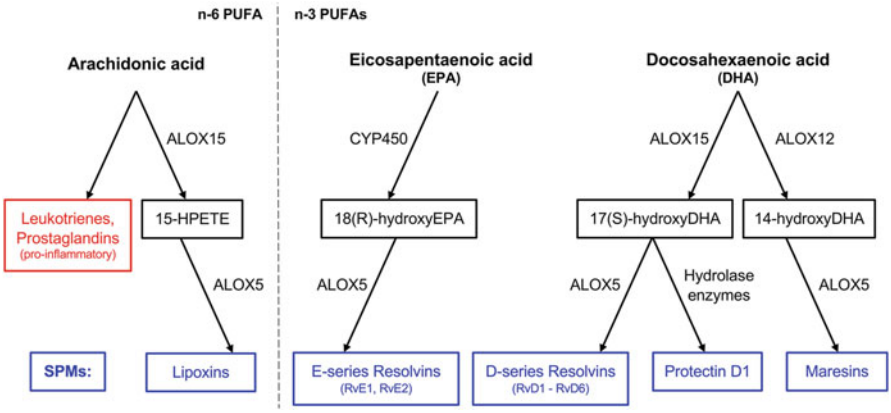

Fig. 1 The synthesis of specialized pro-resolving mediators from polyunsaturated fatty acids. Specialized pro-resolving mediators are a novel class of biomarkers that orchestrate resolution of inflammation. SPMs are mainly derived from dietary n-3 PUFAs including DHA and EPA, through a series of lipoxygenase and hydrolase reactions, although the lipoxin class of SPMs is derived from AA, an n-6 fatty acid, by the action of lipoxygenases rather than cyclooxygenases

was a consequence of how efficacious the acute inflammatory response was in addressing the initial insult. Chronic inflammation was explained by a combination of outsized initial response and recurrent insults resulting in increased serum concentrations of acute inflammatory mediators.

Serhan and colleagues were among the first investigators to isolate mediators of inflammatory resolution (Serhan et al. 1984a, b). These lipid mediators, like eicosanoids, were also downstream metabolites of AA but synthesized by lipoxygenase enzymes rather than cyclooxygenases (Fig. 1). These molecules, now known as lipoxin A4 and B4, are notable for their protective effects during inflammation (Ariel et al. 2006). These molecules ameliorate the collateral cellular damage that results from the indiscriminate utilization of reactive oxygen species to drive oxidative metabolism during acute inflammation (Wang et al. 2020).

The discovery of lipoxins prompted the discovery of other mediators of inflammatory resolution. These include molecules derived from n-3 PUFAs, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). DHA gives rise to the D-series resolvins, protectins, and maresins, while EPA gives rise to E-series resolvins (Fig. 1; Serhan et al. 2015). These mediators make up the novel class of biomolecules known as specialized pro-resolving mediators (SPMs) and are responsible for orchestrating resolution of inflammation by effecting unique responses in nearly every cell of the immune system (Basil and Levy 2016). SPMs induce neutrophils and macrophages to increase microbial clearance, natural killer cells to increase efferocytosis, lymphocytes to promote the transition from innate to adaptive immunity, epithelial cells to inhibit trans-migration of neutrophils, and dampen pro-inflammatory cytokine production by many cell types (Julliard et al. 2022). SPMs have been identified as key players in numerous conditions hallmarked by

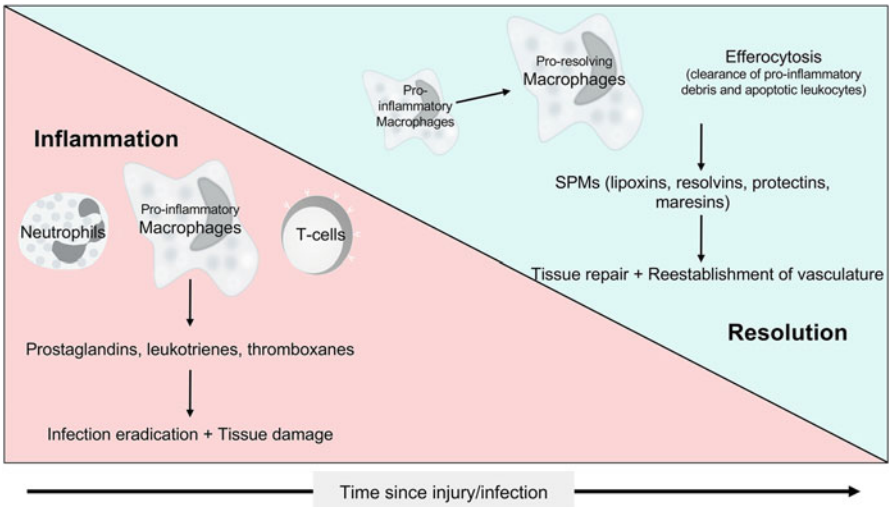

Fig. 2 Coordination of inflammation and resolution. The process of inflammatory resolution begins at the onset of inflammation. Arachidonic acid-derived mediators and omega-3 fatty acid-derived specialized pro-resolving mediators are synthesized concurrently. The initial inflammatory milieu is characterized by higher concentrations of mediators of acute inflammation, which gives way to the mediators of inflammatory resolution as the host returns to homeostasis

inflammatory dysregulation. These include viral infections, mycobacterial infections, asthma, chronic obstructive pulmonary disease (COPD), diffuse parenchymal lung disease, and, now most saliently, SARS-CoV-2 (Basil and Levy 2016; Krishnamoorthy et al. 2018; Kytikova et al. 2019). It is now understood that inflammation and resolution is a single, tightly regulated process that is usually beneficial, but sometimes goes awry (Fig. 2).

Inflammation Contains the Seeds of Resolution

Mediators of inflammation and resolution share the same biochemical inciting triggers (Serhan and Petasis 2011). Tissue injury sets off the biochemical pathways of inflammation by causing an intracellular calcium influx via exposure to either PAMPs or DAMPs via interaction with toll-like receptors on cell surfaces (Assimakopoulos et al. 2018). This influx of Ca^{2+} marks the onset of the inflammatory response to injury and activates enzymes like PLA2 that liberate PUFAs from phospholipids found in cellular membranes (Burke and Dennis 2009). These PUFAs include the n-6 fatty acid, AA (precursor of pro-inflammatory eicosanoids), and the n-3 fatty acids EPA, DHA, and alpha-linolenic acid, which serve as metabolic precursors to various SPMs (Miki et al. 2013). Synthesis of pro-inflammatory eicosanoids or SPMs from these precursors is temporally regulated; early expression of cyclooxygenases gives way to later expression of lipoxygenases, resulting in what

has been termed "lipid class switching," in which the same cells produce either pro-inflammatory or pro-resolving mediators depending on the time, cell type, and location (Levy et al. 2001). Thus, a timeline characterized by the coordinated rise and fall of the biomarkers of acute inflammation followed by the rise and fall of the biomarkers of inflammatory resolution is exhibited. The chronology of inflammatory resolution following acute inflammation serves to re-affirm homeostasis via a transition from the acute inflammatory phase to the resolution phase (Fig. 2; Serhan and Savill 2005; Langlois et al. 2018).

Analysis of cellular biomarker data reveals some clues for how inflammatory homeostasis is achieved after insult. Assays of the ratio of n-3 PUFAs to n-6 PUFAs, specifically AA, within the membrane and cytoplasm of cells exposed to injury can serve as a proxy to identify the dominant phase of the inflammatory process, acute inflammation vs resolution (Serhan and Petasis 2011). At the onset of an inflammatory insult, AA and its metabolites predominate. As time passes, there is a gradual increase in the concentrations of PUFAs responsible for resolution, resulting in an inversion of the PUFA:AA ratio. This inversion serves as one marker of the transition from the acute phase of inflammation to that of inflammatory resolution (Serhan and Petasis 2011).

PUFAs can also drive lipid class switching through positive feedback mechanisms. For example, some n-3 PUFAs have inhibitory effects on the pro-inflammatory transcription factor NF-κB (Singer et al. 2008). Furthermore, efferocytosis, the clearance of apoptotic cells from the site of inflammation via phagocytosis, leads to absorption of cell phospholipid bilayers by macrophages and increases the available substrate for new biosynthesis of SPMs (Arnardottir et al. 2014).

Not only have SPMs been useful as markers of inflammation resolution, they also actively drive the resolution process. SPMs inhibit differentiation of classically pro-inflammatory (M1) macrophages and promote differentiation of alternatively activated (M2) macrophages. M2 macrophages have increased phagocytic activity toward pathogens and apoptotic leukocytes, and they increase production of immunomodulatory (downregulating) cytokines. SPMs also inhibit antigen presenting function of macrophages and dendritic cells and promote differentiation of regulatory T cells and antibody production by B cells (Duffney et al. 2018; Julliard et al. 2022). This integral role of SPMs in the inflammatory response and the understanding of aberrant inflammation as a driver for critical illness have led to investigation of SPMs as biomarkers for severe manifestations of disease including COVID-19.

The Role of Dysregulated Inflammation in the Pathogenesis of COVID-19

Since COVID-19 was first recognized in 2019, the virus has resulted in over six million deaths and reached every country in the world (Carvalho et al. 2021; Worobey 2021). While the rapid rate of new cases has declined, new variants and possible endemic status make understanding the way in which SARS-CoV-2 causes

severe COVID-19 disease imperative (Torjesen 2021). One important aspect of understanding severe COVID-19 is the role of unchecked inflammation and aberrant resolution within the disease.

Although the process of infection is very much mediated by SARS-CoV-2, the severity of inflammation is often decoupled from viral load (Abdulrahman et al. 2021). There is only modest correlation between viral load gathered in respiratory or plasma samples and disease severity. This discordance implies that morbidity and mortality is due to a dysregulated inflammatory response rather than direct viral injury (Abdulrahman et al. 2021). This unchecked inflammatory response has been termed "cytokine storm" and overlaps with SIRS. Cytokine storm is not well defined but is characterized by high circulating cytokine levels, abrupt onset of systemic inflammatory symptoms, and secondary organ dysfunction due to inflammation beyond that which could be attributed to a normal response to a pathogen (Fajgenbaum and June 2020).

SARS-CoV-2 penetrates host defenses by utilizing a two-step mechanism to which alveolar epithelial cells are particularly vulnerable (Wiersinga et al. 2020; Lamers and Haagmans 2022). The viral structural spike (S) protein allows the virus to gain entry to lung epithelial cells by binding to the angiotensin-converting enzyme 2 and then uses cellular machinery to replicate hundreds of copies from a single host cell. The virus can invade immune cells, including lymphocytes, as well as structural cells including pulmonary endothelial cells that can produce immune-modifying cytokines and chemokines. Viral invasion of lymphocytes can result in profound lymphopenia while endothelial injury allows for an influx of monocytes and neutrophils (Helal et al. 2020). These recruited inflammatory cells release additional cytokines that activate a profound, systemic response resulting in the inflammatory pathologies of acute respiratory distress syndrome (ARDS) and multiple organ failure (Kox et al. 2020). Current evidence, discussed below, supports the hypothesis that this pathogenic, pro-inflammatory cascade represents a profound imbalance between pro-inflammatory and pro-resolving processes. We will discuss the possible utility of using SPMs as biomarkers for diagnosis, prognosis, and as potential therapies.

Pathologic Implications of Dysregulated Inflammation

Aberrant resolution of the inflammatory response has been identified as a contributor to the most severe manifestations of numerous diseases and syndromes cared for in the ICU. Three common and particularly salient conditions are ARDS, sepsis (defined as the SIRS response with documented infection), and severe influenza. As discussed above, the most severe cases of COVID-19 result in acute respiratory failure due to ARDS alongside sepsis, which causes hypotension due to increased vascular permeability and dysregulation in vascular tone (Wiersinga et al. 2020). Influenza can, more rarely, cause a similar end-result as COVI0D-19, manifesting as sepsis with shock and ARDS. These data will be reviewed here to provide

background for SPMs as potential as biomarkers in critical illness and to provide context for the studies examining SPM and lipid mediator roles in COVID-19.

Sepsis

Sepsis is defined as a dysregulated inflammatory host response to bacterial, fungal, or viral infection (Pinsky 2004; Singer et al. 2016). The response to infection is initially mediated by PAMPs that promote the release of a variety of cytokines – most importantly, IL-6 and TNF-α – and lipid mediators via interaction with toll like receptors (Savva and Roger 2013; Kieser and Kagan 2017). Lipid metabolism is altered in sepsis. Studies have demonstrated tissue predilection for inflammatory eicosanoids by examining the ratio of n-6- to n-3-PUFAs, similar to the previously mentioned PUFA:AA ratio. During episodes of acute inflammation, when compared to healthy controls, serum levels of both n-6 and n-3 PUFAs were decreased; however, the ratio of these lipid mediators skewed toward n-6-PUFAs, which are precursors to the pro-inflammatory metabolites of AA (Calder 2006; Duan et al. 2014). This finding led to studies investigating the association of n-6 and n-3 levels with sepsis and mortality. In one study of plasma fatty acids as a prognostic marker, a high n-6 to n-3 ratio was associated with elevated risk of death. Multiple studies corroborate this by reporting significantly lower n-3 PUFA concentrations in erythrocytes or plasma of sepsis patients compared to healthy controls (Mecatti et al. 2018).

Given the link between severe inflammation, PUFA metabolism, and sepsis, there has been significant interest in studying SPMs contribution to the pathophysiology of sepsis. One large study used LC/MS to examine 50 lipid mediators in patients with sepsis admitted to the ICU to establish characteristic profiles at different times (day 1, 3, and 7 of ICU stay) and compared survivors, nonsurvivors, and healthy controls. The study found minimal separation in lipid mediator profiles on day 1 between healthy patients and survivors with mild increases in classic pro-inflammatory eicosanoids (PGE_2 and PGD_2) and pro-resolving DHA-derivatives (RvD1, RvD5, and PD1). Among nonsurvivors, there was significant elevation in RvE2, LXB4, RvD2, and 15-epi-LXB4. There was greater separation between survivors and controls on day 3, with elevated levels of lipoxins, RvD5, RvE1, 17-epi-PD1, and 17-epi-RvD1. Nonsurvivors also had elevations in $PGF_2\alpha$ in addition to the aforementioned SPMs. Day 7 samples revealed similar profiles to day 3 but with further increases in RvD5 and RvE2 among survivors and increased $PGF_2\alpha$ among nonsurvivors (Dalli et al. 2017).

As previously discussed, RvD1 and RvD5 enhance phagocytosis of bacterial pathogens and immune cells that experienced apoptosis, while PD1 inhibits the pro-inflammatory cytokines TNF-α and IL-6 (Chiang et al. 2012). The elevated levels of these SPMs suggest efforts to reign in the inflammatory cascade (Bannenberg and Serhan 2010). The D- and E-series resolvins also exercise anti-inflammatory actions on PMNs by blocking TNF-α-induced IL-1β transcripts (potent regulators of PMN infiltration in various tissues) (Serhan et al. 2004).

$PGF_2\alpha$, which was elevated in nonsurvivors, is a potent pro-inflammatory mediator that is elevated in animal models of sepsis and is also a mediator of airway hyperresponsiveness and small airway constriction (Kawikova et al. 1996; Basu 2007). Finally, the investigators also reported that day 3 levels of protectin DX (PDX) was an excellent predictor of the development of ARDS. Elevated PDX levels are associated with survival in animal models of sepsis and treatment with PDX reduces experimental sepsis-induced ARDS in mice (Körner et al. 2018; Xia et al. 2020).

Another study evaluated the role of RvD1 and RvD2 on leukocyte activation and function on sepsis clinical severity by mapping expression of SPMs by LC/MS and expression of their receptors by flow cytometry (Jundi et al. 2021). SPMs exert their effect on immune cells by interacting with G-protein coupled receptors (GPCRs) located on the cell membrane and in turn increase the concentration of GPCRs on cell surfaces via positive feedback mechanisms (Chiang and Serhan 2020; Park et al. 2020). When compared to healthy individuals, patients with at least 72 hours of sepsis had a three-fold increase in DRV1, a five-fold increase in ALX, and a four-fold increase in DRV2 expression on circulating PMNs and monocytes. Further, administration of RvD1 or RvD2 to PMNs collected from patients with sepsis partially reversed their deficits in phagocytosis (Jundi et al. 2021).

In summary, these studies show that the lipidomic profile of PUFAs and SPMs can provide information on disease severity during sepsis. As sepsis increases in severity, there is increasing concentration of both pro-inflammatory and pro-resolving mediators of inflammation. Among survivors of sepsis, the n-6/n-3 ratio skews toward pro-resolving mediators, while nonsurvivors have an inverse phenomenon resulting in increased levels of n-6-derived AA metabolites. This finding implies SPMs not only have a protective role in sepsis but mediate the return of homeostasis. Moreover, the measurability of these lipid mediator profiles from peripheral blood samples increases the viability of SPMs as biomarkers of disease course. This data can potentially provide meaningful information to clinicians looking for clues regarding the efficacy of interventions and therapy.

Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome is characterized by severe hypoxemia and is caused by direct or indirect lung injury resulting in vascular leak and noncardiogenic pulmonary edema (Thompson et al. 2017). ARDS is a common and grave complication of critical illness, with mortality ranging from 30% to 45% even with proper supportive care (Group et al. 2016; Maca et al. 2016). It is diagnosed by bilateral infiltrations on chest x-ray coupled with severe hypoxemia and usually requires the use of invasive mechanical ventilation (Group et al. 2016). ARDS is increasingly recognized as a heterogeneous entity with several phenotypes distinguished by specific physiologic and molecular characteristics (Sinha and Calfee 2019;

Matthay et al. 2020). Lipidomic profiles of ARDS and acute lung injury (ALI) have provided evidence for the contribution of SPM dysregulation to pathologic inflammation within this disease process, and early studies have shown lower EPA concentrations in ARDS patients prompting further studies of lipid biology in ARDS (Cotogni et al. 2016).

There have been attempts to correlate lipidomic profiles in ARDS with mortality. One study used mass spectrometry to evaluate the concentrations and composition of 359 distinct lipids in the peripheral blood of 30 patients with ARDS (Maile et al. 2018). These membrane lipids were catalogued according to class (i.e., triacylglycerols, phosphatidylcholine, sphingomyelin, cholesteryl esters). Of these 359 lipids, 90 were found to differ significantly between survivors and nonsurvivors in composition or concentration. The cluster of lipids with the greatest correlation with survival included diacylglycerols, triacylglycerols, and phosphatidic acids. It is also notable that for the 90 lipids identified, only five were lower in survivors, indicating that in general higher levels of PUFAs may be associated with a survival benefit (Maile et al. 2018). It is feasible that a panel consisting of these glycerolipids may serve as an assay for disease severity or differentiate one phenotype of ARDS from another. Distinguishing phenotypes among a heterogeneous disease state like ARDS using glycerolipids or other lipid groups may be able to assist clinicians tailor more specific therapies in the future, although more research is needed.

Studies with human cells ex vivo and in animal models also support the concept that dysregulated resolution contributes to ARDS severity. Lipopolysaccharide (LPS) is a component of the bacterial wall of Gram-negative bacteria and is a powerful danger signal and pro-inflammatory stimulus. Introduction of LPS into the lungs or peritoneal cavity in animals is a well-understood injury model that causes ARDS-like injury (Vernooy et al. 2002). RvD1, RCTR1, and RvE1 have each been reported to limit lung inflammation in mouse and rat models of ARDS (Zhang et al. 2019; Yang et al. 2021; Luo et al. 2022). RvD1, RvD2, and MaR1 also suppressed IL-1β, TNF-α, and IL-8 production in LPS-stimulated human monocytes ex vivo (Gu et al. 2015; Koch et al. 2020). Moreover, these SPMs also induced IL-10 release via the GSK3β pathway, which is important because IL-10 has been demonstrated to enhance neutrophil apoptosis and facilitates resolution of inflammation (Cox 1996).

Another study demonstrated that DNA containing CpG motifs, another well-known PAMP, impaired phagocytosis of labeled *E. coli* and delayed apoptosis in human PMNs ex vivo. Normal bacterial phagocytosis and apoptosis were restored by treatment of the PMNs with 15-epi-LXA4 or 17-epi-RvD1 (Sekheri et al. 2020). Both 15-epi-LXA4 and 17-epi-RvD1 also reduced lung inflammation and enhanced bacterial clearance and neutrophil apoptosis in a mouse model of ARDS induced by intratracheal administration of live *E. coli* plus CpG DNA (Sekheri et al. 2020).

Although cystic fibrosis is a distinct entity from acute inflammatory syndromes like sepsis and ARDS, pro-inflammatory and pro-resolving pathways are dysregulated in similar ways. A study of airway fluid from CF patients demonstrated significantly lower concentrations of LXA4 and suppression of the LXA4/neutrophil ratio in patients with CF compared with controls and those with other inflammatory

lung disease, despite similar IL-8/neutrophil ratios. Administration of LXA4 analogs to a mouse model of chronic airway infection also resulted in increased neutrophil phagocytosis of bacteria as well as increased monocyte phagocytosis of apoptotic neutrophils (Karp et al. 2004). Similarly, RvD1 blocked transendothelial migration of human PMNs in an ex vivo flow chamber in a receptor-dependent manner, inhibited PMN transmigration, and reduced the levels of pro-inflammatory eicosanoids in PMN exudates in a mouse sepsis model (Norling et al. 2018). Taken together, these studies support the concept that SPMs can suppress neutrophilic lung inflammation induced by bacterial PAMPs and other damage signals and that reduced levels of pro-resolving mediators in patient blood and tissues signal poor prognosis.

As well as inhibiting inflammation, pro-resolving mediators can promote a return to homeostasis by restoring cellular architecture (Wang et al. 2013). Alveolar fluid accumulation as manifested by noncardiogenic pulmonary edema is a hallmark of ARDS (Thompson et al. 2017). LXA4 reduced lung edema in rat model of acid-induced acute lung injury by upregulating the sodium channel Na, K-ATPase. The effect of LXA4 was dependent on activation of the LXA4 receptor, as blockade of the receptor eliminated the effects of LXA4 (Vadász et al. 2007). Another consequence of ARDS is pulmonary fibrosis. While several mechanisms have been proposed, one of the best-supported is that mechanical ventilation leads to injury from repeated ventilator-induced stretching (Gattinoni et al. 1984; Zhang et al. 2002). A recent study demonstrated that RvD1 could attenuate stretch-induced EMT in human bronchial epithelial cells ex vivo and could also inhibit pro-fibrotic changes caused by ventilation in mice (Yang et al. 2019). The effect was dependent on ALX/FPR2, one of the RvD1 receptors, and was accompanied by decreased activation of key pro-fibrotic transcription factors.

Given the contribution of impaired omega-3 fatty acid metabolism to ARDS development, there have been clinical studies investigating supplementation of these fatty acids in patients with ARDS or who are at risk for ARDS; moreover, guidelines previously recommended omega-3 supplementation (Pontes-Arruda et al. 2008; Rice et al. 2011; Dushianthan et al. 2016). However, following results from the OMEGA trial which showed a potential of harm, the guidelines for routine omega-3 supplementation were modified, and now such a recommendation cannot be made (McClave et al. 2016; Rice et al. 2011). Despite these results, there is considerable evidence to date that SPMs play an important role in ARDS. Plasma studies of certain SPMs reveal correlation with survival, while ex vivo and murine models demonstrate powerful roles in inflammation resolution via decreased activation of neutrophils, inhibition of cytokine production, enhancement of pathogenic and efferocytotic phagocytosis, and promotion of a return to tissue homeostasis. It is worth noting that most reports to date involve RvD1, RvD2, and LXA4, which may represent biologic effect or bias toward SPMs that were discovered earlier. Future studies may uncover important roles for other SPMs in ARDS.

Influenza

COVID-19 and influenza are both viral infections that can result in severe disease characterized by sepsis and ARDS, making a consideration of SPMs in influenza especially relevant to COVID-19. While most studies evaluating biomarkers in influenza have focused on proteomics and metabolomics, there has been some important work investigating lipids' role in severe influenza infection (Imai 2015).

Studies in cultured human lung epithelial cells identified alterations in cholesterol and lipid metabolism with influenza infection (Lin et al. 2010). This finding led researchers to perform lipidomic profiling in mice infected with two strains of influenza: one with high pathogenicity and another with low pathogenicity. The researchers found that ALOX5 metabolites correlated with increased pathogenicity, while ALOX12/15 metabolites increased during disease resolution (Tam et al. 2013). Additionally, they found that the ratio of 13-hydroxylated linoleic acid (which is anti-inflammatory) to 9-hydroxylated linoleic acid (which is pro-inflammatory) predicted outcomes. The same group also reported that the ratio of 13- to 9-hydroxylated linoleic acid in nasopharyngeal lavage from children with flu obtained during the 2009–2011 H1N1 was associated with worsened severity and suggested this ratio might be a useful biomarker (Tam et al. 2013).

Another study revealed that endogenous production of PD1 is suppressed in a mouse model of influenza A virus infection, while addition of PD1 to human alveolar epithelial cells resulted in inhibition of influenza A viral replication in those cells (Morita et al. 2013). Treatment with PD1, but not RvD1, RvD2, nor LXA4, reduced inflammation in the mouse influenza model. While the authors proposed PD1 as a potential therapy for influenza, much about the role of SPMs during influenza infection remains unclear.

Pro-inflammatory Cascade and Inflammation Resolution in COVID-19

Pathogenesis of Severe COVID-19

Aside from taking over cellular machinery in service of viral replication, SARS-CoV-2 infection results in an inflammatory syndrome with highly variable clinical consequences. Clinical characteristics of COVID-19 range from mild infection characterized by fever, cough, or change in taste or smell, to critical infection, characterized by devastating respiratory failure due to ARDS, shock, and multiorgan dysfunction (Gandhi et al. 2020). There is ample evidence that the severe manifestations of COVID-19, like many other critical illnesses, are related to the inflammatory response. Numerous studies have correlated inflammatory markers such as IL-6, ferritin, CRP, and others with development of severe disease and death (Zeng et al. 2020). Furthermore, anti-inflammatory therapies such as corticosteroids, JAK-Kinase inhibition, and IL-6 targeted therapies have all shown benefit in clinical trials (van de Veerdonk et al. 2022). It is important to note again that the severity of

illness is not directly commensurate to the viral load. This also supports the conclusion that a dysregulated host response contributes to the severity of disease (Abdulrahman et al. 2021). Given the importance of lipids in inflammation, there have been several studies examining lipid mediators pertaining to COVID-19 which we discuss below.

PUFAs in COVID-19

A systematic review of the literature with a cutoff date of August 2021 identified 18 studies of PUFAs in COVID-19 (Mazidimoradi et al. 2022). The review concluded there was good evidence from multiple studies that increased serum levels of arachidonic acid or decreased levels of n-3 PUFAs were associated with increased risk of COVID-19 infection. Higher levels of n-3 PUFAs or a higher ratio of n-3:n-6 PUFAs were associated with decreased risk of severe disease, including the need for hospitalization, supplemental oxygen, intensive care, and mechanical ventilation. Regarding mortality, three studies reported an association between higher n-3 PUFA levels and decreased mortality, although several other studies were less clear. A more recent randomized prospective study of 4101 COVID-19 patients and 20,626 controls found that higher levels of n-3 PUFAs and DHA in blood were associated with lower susceptibility to testing positive for COVID-19 and decreased severity of disease (this study did not report mortality), while a smaller study reported that an increased n-6:n-3 ratio in erythrocyte membranes was associated with increased IL-6 (Sertoglu et al. 2022; Sun et al. 2022).

Beyond PUFAs: Lipidomics and SPMs in COVID-19

Lipidomic profiling has uncovered clear evidence of disruption of pro-inflammatory and pro-resolving mediators in COVID-19. A small study in Germany of serum and plasma from COVID-19 patients and healthy controls found increased SPMs in the COVID-19 cases, including RvD1, 17-HDHA, MaR1, and MaR2. However, they also found significant increase in pro-inflammatory mediators, PGD_2, PGE_2, $PGF_2\alpha$, TXB2, and LTB4, and the ratio of pro-inflammatory mediators:SPMs was 30-fold higher in the COVID-19 group (Regidor et al. 2021). A larger study from Canada reported similar findings of increased pro-inflammatory mediators (PGD_2, PGE_2, $PGF_2\alpha$, thromboxanes, and leukotrienes) and SPMs (17-HDHA, RvD1, and PDX) in BAL fluid from intubated COVID-19 patients, although they did not report the ratio of pro-inflammatory to pro-resolving mediators (Archambault et al. 2021). An interesting study from Brazil compared critically ill COVID-19 patients requiring intubation, with COVID-negative critically ill patients also requiring intubation, and they also reported increased AA, prostaglandins, and thromboxanes in plasma and tracheal aspirates of the COVID-19 cases (Pérez et al. 2022).

The role of PGE_2 has been further explored in a study using human lung epithelial cells and precision cut lung slices in culture. It was reported that infection of lung

epithelial cells by SARS-CoV-2 upregulated expression of COX2 which promoted the release of PGE_2. Because increased PGE_2 can inhibit B and T cell function, this suggests a mechanistic link between infection, lipid metabolism, and impaired adaptive immune responses to the virus (Ricke-Hoch et al. 2021). However, patient serum PGE_2 levels did not correlate with mortality.

Two groups have performed comprehensive lipid profiling in COVID-19 with similar results. A research group affiliated with the National Institute of Allergy and Immunology and Yale University performed lipidomic analysis of moderate and severe COVID-19 compared to healthy controls. (Moderate disease severity was defined by the need for hospital admission and supplemental oxygen therapy, while severe disease was defined by need for ICU admission.) While concentrations of all lipid mediators increased with disease severity, distinct characteristics between pro-inflammatory and pro-resolving mediators emerged. Moderate disease was associated with higher levels of COX2 products including PGE_2, PGD_2, and $PGF_2\alpha$, while severe disease was associated with increased products of ALOX5, ALOX12, and ALOX15 (see Fig. 1), including RvD1, RvD2, RvD3, RvD4, PD1, PDX, and LXA4. However, it was unclear whether this profile was a driver of severity, a treatment effect, or the result of preexisting conditions (Schwarz et al. 2020). A similar study was performed by a group in London, who found that COVID-19 patients exhibited elevations of SPMs in plasma and in specific immune cells including PMNs and monocytes, including ALOX5 products and the novel DHA-derived conjugates, maresin conjugates in tissue regeneration (MCTR), and protectin conjugates in tissue regeneration (PCTR) (Koenis et al. 2021). Also noteworthy, expression of SPM receptors was elevated on immune cells including PMNs and monocytes. Unlike the Yale study, these researchers found that production of ALOX15-derived SPMs including RvD2, PCTR2, RvT2, RvT3, and $RvD5_{n\text{-}3\ DPA}$ was decreased in severe cases compared to mild-to-moderate cases, although they confirmed the finding that severe cases had higher levels of AA-derived pro-inflammatory leukotrienes. Finally, among patients who had experienced complete resolution of symptoms, another distinct lipid mediator profile emerged. Compared to healthy individuals who had never experienced COVID-19, this group had comparably higher elevation uniformly across both SPMs such as RvD1, RvD2, and RvT2 and pro-inflammatory eicosanoids such as PGE_2, $PGF_2\alpha$, and LTE_4 (Koenis et al. 2021).

Finally, a group in the UK performed lipidomics profiling on plasma from 38 COVID-19 patients who were either severely ill (requiring supplemental oxygen) or critically ill (requiring mechanical ventilation). This analysis identified a subset of seven lipid mediators (TxB_4, LTD_4, RvE_4, 20-COOH-LTB_4, 20-OH-MaR1, RvD1, and RvD3) that provided an 87% accuracy in discriminating between severe and critical illness. A higher ratio of SPMs:pro-inflammatory mediators was associated with survival (Palmas et al. 2021).

These studies provide insight into profiles of lipid mediators in the peripheral blood and lung tissue of patients affected by COVID-19. While differing methodology between research teams may account for some differences, the findings trended toward the same direction. Among patients experiencing mild or moderate

disease severity, lipid mediator profiles were skewed toward relatively higher concentrations of DHA-derived pro-resolving mediators, like D-series resolvins, MCTR3, and PCTR3, and away from COX-2 metabolites like prostaglandin. As disease severity increased, the balance of n-3 PUFA-derived lipid mediators vs AA-derived mediators favored more pro-inflammatory metabolites. Furthermore, a subset of seven lipid mediators including D-series resolvins was able to discriminate between severe and critical illness with 87% accuracy. These consistent findings suggest a potential role for these metabolites in the mechanism of severe COVID-19 and the development and testing of lipid panels as biomarkers to determine who is most at risk of severe disease and detrimental outcomes. Additionally, some early studies have shown differences in the lipid profile of serum and lung tissue in COVID-19 and other inflammatory diseases, which may provide insight into pathogenesis and also, potentially assist in diagnosis of inflammatory conditions with similar clinical manifestations.

Therapeutic Targets and Future Directions for Specialized Pro-resolving Mediators

The RECOVERY trial determined the use of dexamethasone for hospitalized COVID-19 patients that require respiratory support with supplemental oxygen results in lower 28-day mortality (Group et al. 2020). One proposed reason for the efficacy of dexamethasone in the treatment of COVID-19 is its effect on SPMs. In a study of allergic airway inflammation in mice, dexamethasone induced the D-series pro-resolving lipid mediator pathway leading to the formation of 17S-HDHA, PD1, and PDX (Pyrillou et al. 2018). This finding was confirmed in COVID-19 by a group at the William Harvey Research Institute in which peripheral blood of patients treated with dexamethasone was found to have significantly reduced levels of pro-inflammatory eicosanoids when compared to COVID-19 patients not treated with dexamethasone. Dexamethasone significantly upregulated ALOX15-derived SPMs as well as SPMs receptors among all phagocyte subsets (Koenis et al. 2021). It should be noted that glucocorticoids such as dexamethasone block the release of important pro-inflammatory mediators including PGE_2 and leukotrienes. However, they also block the release of SPM precursors from membrane lipids, resulting in an SPM-deficient state, at least in animal models, so it is somewhat puzzling that dexamethasone was reported to increase SPM levels in COVID-19 patients (Das 2021). A possible explanation for the beneficial effects of dexamethasone on SPMs levels has been proposed by Serhan and co-workers (Andreakos et al. 2020).

Elevated leukotriene levels have been identified in tracheal aspirates of non-COVID ARDS patients, and LTE4, a biomarker of cysteinyl-LT production, has been identified in bronchoalveolar lavage of hospitalized patients with severe COVID-19 syndrome (Sala et al. 1991; Archambault et al. 2021). Furthermore, a recent work demonstrated that montelukast, an LT receptor antagonist, inhibits platelet activation induced by plasma from COVID-19 patients by preventing the surface expression of tissue factor and P-selectin (Camera et al. 2022). These

findings along with retrospective data on improved COVID-19 outcomes in those taking montelukast have spurred a clinical trial (ClinicalTrials.gov Identifier: NCT04714515) investigating its use as a therapy in COVID-19 (Khan et al. 2021).

Dietary supplementation with n-3 polyunsaturated fatty acids has been previously suggested as a therapy for ARDS and is also of interest in COVID-19. A randomized clinical trial of 101 patients hospitalized with severe disease and receiving enteral feeding reported significantly improved survival when the feeding formula was supplemented with a fish oil capsule containing DHA and EPA (Doaei et al. 2021). However, the patients were severely ill, it was single center and a small sample size, and the results are contrary to the earlier OMEGA study of PUFA supplementation in acute lung injury, which reported that n-3 fatty acid supplementation offered no benefit and may in fact be harmful (Rice et al. 2011). However, it is worth noting that the OMEGA trial accepted patients with all-cause ALI, while the recent trial was limited to patients with COVID-19, and the roles of lipid mediators may be different depending on the cause of the injury. In any event, there is reasonable evidence that higher n-3 fatty acid levels prior to infection, including dietary supplementation, can reduce the risk of testing positive and of acquiring severe disease.

Although no SPM-based drug is approved for human use, in vitro studies support the idea of using SPMs as therapies. Blood-derived monocytes from healthy controls and cystic fibrosis patients were exposed to the SARS-CoV-2 virion spike 1 glycoprotein (S1) with or without treatment with RvD1 and RvD2. The addition of S1 resulted in the immediate release of chemokines IL-8, IL-6, and TNF-α and resulted in the delayed and attenuated generation of endogenous SPMs. The addition of exogenous RvD1 and RvD2 dampened the pro-inflammatory response via the restoration of micro-RNAs that regulate resolution, decreasing NF-κB activation, and inducing polarization of macrophages to the M2 phenotype (Recchiuti et al. 2021). It was also reported that addition of MCTR3, PCTR3, or 17R-RvD3 to human PMNs from COVID-19 patients partially reversed a severe impairment in phagocytic function in vitro (Koenis et al. 2021). These studies show consistent benefits of SPM treatment on human leukocytes isolated from COVID-19 patients, supporting the concept that SPMs may represent an important new direction in COVID-19 therapy (Fig. 3).

Applications to Prognosis, Other Diseases, or Conditions

Each of the diseases mentioned previously in this chapter has signatures of SPM dysregulation that seem to be related to disease development and severity. For patients who eventually recover from critical illness fully without ongoing impairment, there is generally a gradual return of SPM concentrations to predisease states as the subjects regain homeostasis (Serhan et al. 2015). On the other hand, as we have highlighted, numerous studies demonstrate worsening dysregulation of the lipidome in subjects who have more severe disease or succumb to their illness (Drobnik et al. 2003; Madenspacher et al. 2019). These pathology-specific patterns

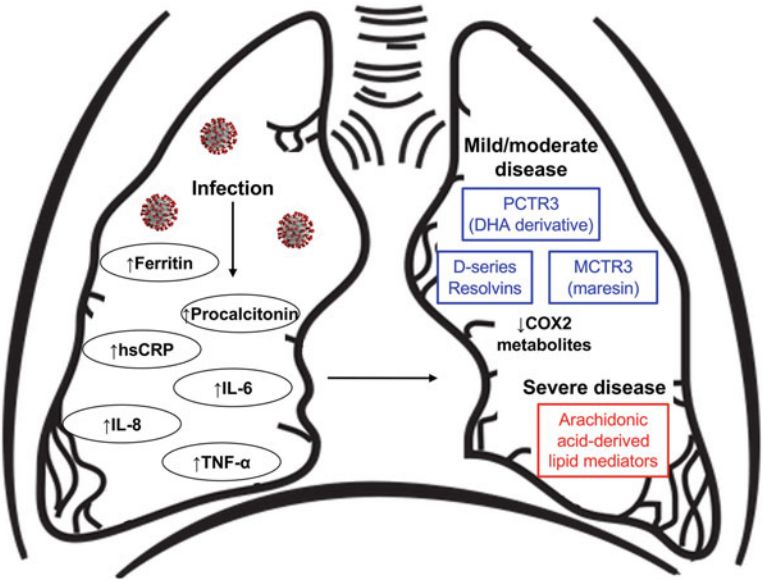

Fig. 3 Biomarkers implicated in COVID-19. Various studies have examined the inflammatory biomarkers and lipid mediators that characterize COVID-19 infection. Methodology may account for some differences between the findings, but several trends have emerged. Mild/moderate disease is characterized by increase in D-series resolvins, MCTR3, and PCTR3 with an overall decrease in COX2 metabolites. Severe disease is characterized by an increase in arachidonic acid-derived lipid mediators

can be clinically useful for evaluating both prognosis and therapeutic benefit. In addition to the syndromes and diseases described above, SPMs have shown promise as biomarkers for disease severity and degree of response to treatment in several other chronic illnesses.

SPMs have also been suggested as potential biomarkers in several chronic inflammatory diseases. Rheumatoid arthritis is a chronic and destructive inflammatory condition with systemic effects across several organ systems. Disease progression in rheumatoid arthritis has been positively correlated with serum concentrations of RvD1, RvD3, and MaR1 (Norling et al. 2018; Arnardottir et al. 2014; Jin et al. 2018). Synovial fluid samples of patients with rheumatoid arthritis revealed higher concentrations of all E- and D-series SPMs. SPM concentrations negatively correlated with disease severity as confirmed by a validated pain scale. Moreover, serum concentrations of D- and E-series SPMs negatively correlated with the erythrocyte sedimentation rate, a well-established marker of disease activity for rheumatoid arthritis (Barden et al. 2016). Researchers have also correlated synovial fluid phenotypes with SPM signatures and analyzed the response to disease-modifying antirheumatic drugs therapy within these subgroups (Gomez et al. 2020). They found that serum concentrations of D-series resolvins, specifically RvD4, were the most predictive of responsiveness to therapy.

Another chronic illness with characteristic SPM dysregulation is atherosclerosis. SPMs have been shown to have protective effects in atherosclerosis. Higher concentrations of RvD1 were associated with increased phagocytic uptake of apoptotic cells within atherosclerotic lesions which presumably is protective against plaque rupture and myocardial infarction or stroke (Viola et al. 2016). Serum concentrations of RvD1 were also lower among patients with atherosclerotic carotid artery disease when compared to healthy volunteers (Fredman et al. 2016). This finding was expanded upon in a later study. Patients with coronary artery disease had lower concentrations of all D-series resolvins when compared against healthy volunteers (Colas et al. 2018). These findings also support the use of n-3 PUFA supplementation for the treatment of coronary artery disease by the American Heart Association (Chaddha and Eagle 2015).

Similar findings are also seen in asthma, periodontal disease, and more (Dalli et al. 2022). Advances in analytic technology, especially high-resolution LC/MS, have allowed for swifter and more accurate quantitation of small biomolecules like SPMs. This is even more impressive because of the relatively low serum concentrations of these lipid mediators. Advances in these technologies have allowed for characterization of SPM profiles among various chronic diseases. As this technology improves and turnaround time for results shortens, SPM characteristics become an increasingly viable method for prognostication and therapeutic surveillance for critical illness.

Conclusions

This chapter introduces the concept of specialized pro-resolving mediators as the drivers of inflammatory resolution by highlighting their specific roles in pathologies of critical illness. Resolution and recovery from inflammation is an active process with unique cardinal signs that run counter to those of pro-inflammation. While pro-inflammation is characterized by the familiar *rubor*, *calor*, *tumor*, *dolor*, and *functio laesa*, the cellular signs of inflammation resolution are posited as removal, restoration, regeneration, remission, and relief (Basil and Levy 2016). Underpinning all of this is the idea that recovery is pressured by a drive toward homeostasis. Recovery from tissue insult via inflammation can only be achieved when pro-inflammatory processes work in tandem with inflammation resolution. All the SPMs mentioned in this chapter either enhance the pro-inflammatory process or drive the inflammatory milieu towards resolution.

Probably the biggest barrier of the application of these findings to clinical use is technological. There are no single mediators that can discriminate between moderate and severe COVID-19 or between COVID-19 and other diseases. Lipid mediators are highly variable within populations, and while there are underlying patterns, individuals may vary considerably within that pattern. Identification of a "signature" requires analysis of hundreds of lipids in a large number of subjects, coupled with advanced statistical techniques and machine learning. A workable lipidomic "signature" for critical illness and injury would be able to identify the disease process, give

some indication of prognosis, and suggest appropriate treatments. To date, only one group has proposed a lipidomic signature that can discriminate between severe and critical COVID-19, and it is not clear how that would drive clinical decision-making. Further, the signature was developed using just 38 patients. A great deal more work must be done before lipidomics can be applied to bring personalized medicine to critical illness, but that outcome is at least visible on the horizon.

Similarly, development of SPMs as therapies for critical illness is visible on the horizon (Hammock et al. 2020; Panigrahy et al. 2020). SPMs have potent anti-inflammatory, pro-resolving, and pro-repair properties in animal and human cell culture models. Based on the different SPM signatures in different critical illnesses, it seems probable that SPM therapies will also be specifically targeted toward distinct conditions. Since SPMs are endogenously produced, it is likely that therapeutic supplementation with SPMs will be well-tolerated. SPMs are also amenable to chemical modification to increase their potency and half-life or change their receptor selectivity. SPMs represent the next frontier in the diagnosis and treatment of acute and chronic inflammation and critical illness and injury (Table 1).

Mini-Dictionary of Terms

Specialized Pro-resolving Mediator

- Any of a group of PUFA lipid biomolecules derived from arachidonic acid, docosahexaenoic acid, or eicosapentaenoic acid and comprised by resolvins, protectins, maresins, and lipoxins. These molecules are powerful cellular effectors with actions on every cellular member of the immune system.

Key Facts

Key Facts of Inflammation Resolution

- A coordinated and complex process aimed at returning tissue integrity and function following tissue injury and the resultant inflammatory process. Inflammation resolution is mediated by several cellular systems including immune cells, extracellular matrices, parenchymal cells, and endothelial cells.

Key Facts of Sepsis

- A pathologic condition characterized by a dysregulated (and usually inordinate) immune response to an infectious insult. While there are several definitions of sepsis that vary from society to society, in this chapter, we regard sepsis characterized by a systemic inflammatory response syndrome in the presence of an identified infectious insult.

Table 1 Studies characterizing specialized pro-resolving mediators in critical illness or in animal models of acute inflammation

Compound	Function
EPA and DHA	Ratio of n-6 PUFAs to n-3 PUFAs was increased in patients with sepsis (Calder 2006; Duan et al. 2014)
	High ratio of n-6 PUFAs to n-3 PUFAs was associated with increased risk of death from sepsis (Mecatti et al. 2018)
	Supplementation may be associated with improved PaO_2 to FiO_2 ratio in ARDS (Langlois et al. 2018)
	Decreased levels of n-3 PUFAs in blood or a higher n-6:n-3 ratio were associated with severity and mortality in COVID-19 (Mazidimoradi et al. 2022, Sertoglu et al. 2022, Sun et al. 2022)
	Dietary supplementation with DHA and EPA during enteric feeding improved survival of COVID-19 patients (Doaei et al. 2021)
Lipoxins	Administration of lipoxin to a mouse model of cystic fibrosis was shown to upregulate monocyte ingestion of apoptotic neutrophils (Karp et al. 2004)
	15-epi-LXA4 restored impaired function of human neutrophils ex vivo and reduced lung inflammation in a mouse ARDS model (Sekheri et al. 2020)
	LXA4 reduced lung inflammation in a rat model of acid-induced ALI (Vadász et al. 2007)
	Increased LXA4 and LXA5 was associated with severe COVID-19 (Schwarz et al. 2020)
E-series Resolvins	RvD5 and RvE2 were increased in plasma from ARDS survivors compared to nonsurvivors (Dalli et al. 2017)
	RvE1 reduced lung inflammation in a rat model of ARDS (Luo et al. 2022)
	RvE4, RvD1, and RvD3 were included in a set of seven lipids that discriminated between severe and critical COVID-19 (Palmas et al. 2021)
D-series resolvins	Administration of RvD1 or RvD2 to PMNs from sepsis patients partially restored normal phagocytic function (Jundi et al. 2021)
	Receptors for RvD1 and RvD2 are increased three- to four-fold on PMNs and monocytes from patients with sepsis (Jundi et al. 2021)
	Addition of RvD1, RvD2, and MaR1 suppressed production of pro-inflammatory cytokines in LPS-stimulated human macrophages (Gu et al. 2015)
	RvD1 and RCTR1 inhibited lung inflammation in rodent models of ARDS (Zhang et al. 2019, Yang et al. 2021)
	17-epi-RvD1 restored impaired function of human neutrophils ex vivo and reduced lung inflammation in a mouse ARDS model (Sekheri et al. 2020)
	RvD1 blocked transmigration of human PMNs in a flow chamber and reduced the levels of pro-inflammatory mediators in neutrophil exudates in a mouse model of sepsis (Norling et al. 2018)
	RvD1 attenuated stretch-induced EMT in human bronchial cells (Yang et al. 2019)
	Increased RvD1 and 17-HDHA were found in serum and plasma from COVID-19 patients (Regidor et al. 2021)
	Increased RvD1, RvD2, RvD3, and RvD4 were associated with severe COVID-19 (Schwarz et al. 2020)
	RvD2 and RvD5 were decreased in serum from severe COVID-19 patients, but was increased by treatment with dexamethasone (Koenis et al. 2021)

(continued)

Table 1 (continued)

Compound	Function
	Increased RvD1 and 17-HDHA were found in BAL fluid from intubated COVID-19 patients (Archambault et al. 2021)
	RvE4, RvD1, and RvD3 were included in a set of 7 lipids that discriminated between severe and critical COVID-19 (Palmas et al. 2021)
	RvD3 partially reversed deficits in phagocytosis in PMNs from COVID-19 patients (Koenis et al. 2021)
Maresins	Addition of RvD1, RvD2, and MaR1 suppressed production of pro-inflammatory cytokines IL-1β and TNF-α in vitro (Gu et al. 2015)
	Increased MCTRs were found in plasma from COVID-19 patients (Koenis et al. 2021)
	MCTR3 partially reversed deficits in phagocytosis in PMNs from COVID-19 patients (Koenis et al. 2021)
Protectins	PDX was associated with development of ARDS (Dalli et al. 2017). Elevated PDX is also associated with survival in a mouse model of sepsis (Xia et al. 2020)
	PD1 inhibited replication of influenza A virus in human alveolar epithelial cells (Morita et al. 2013)
	Increased PCTRs were found in plasma from COVID-19 patients (Koenis et al. 2021)
	PCTR3 partially reversed deficits in phagocytosis in PMNs from COVID-19 patients (Koenis et al. 2021)

Key Facts of Acute Respiratory Distress Syndrome

- A pathologic condition with multiple causes, including either direct tissue injury or indirect injury related to a systemic inflammatory response that results in a constellation of radiologic and clinical findings. These include diffuse radiographic infiltrates consistent with noncardiogenic pulmonary edema as well as the need for mechanical ventilatory support. These findings are necessarily accompanied by severe hypoxemia.

Key Facts of N-3/N-6 PUFA Ratio

- The numerical values of 3 and 6 denote the location of the first double bond on each molecule with respect to the methyl end of the fatty acids. Many pro-inflammatory mediators are derived from n-6 PUFAs including AA, while most SPMs are derived from the n-3 PUFAs DHA and EPA (with the exception of lipoxins, which are derived from AA). The n-3/n-6 PUFA ratio is a broad indicator of whether an inflammatory response is in a pro-inflammatory phase (high n-6) or a pro-resolving phase (high n-3).

Summary Points

1. Much of critical illness pathogenesis is due to excessive and unregulated inflammation.
2. Inflammation is a dichotomous process in which pro-inflammatory pro--resolving processes are coordinated and work together.
3. Inflammation resolution is the process of restoring tissue integrity and function while minimizing damage done by the immune system following tissue insult, by either injury or infection.
4. Specialized pro-resolving mediators are lipid biomolecules that enhance some pro-inflammatory immune functions but mostly orchestrate and effect immune cells to carry out actions of inflammation resolution.
5. Proinflammatory lipid molecules are largely derived from n-6 PUFAs like arachidonic acid. These include the eicosanoids: prostaglandins, leukotrienes, and thromboxanes.
6. Proresolving mediators are largely derived from n-3 PUFAs like DHA and EPA and include the resolvins, protectins, and maresins. Lipoxins are a class of SPM derived from the n-6 PUFA, AA.
7. Dysregulation of SPMs and inflammation resolution contributes to critical illnesses like sepsis and ARDS.
8. SPMs enhance the immune response to infection, and serum SPM concentrations increase as disease severity intensifies.
9. SPMs pressure immune cells to promote restoration of tissue integrity in ARDS. SPM concentrations also increase as ARDS severity intensifies.
10. COVID-19 is characterized by an exaggerated pro-inflammatory cascade and dysregulated inflammation resolution.
11. Increased risk of infection and worse outcomes in COVID-19 are characterized by distinct lipid profiles in blood, immune cells, and BAL fluid.
12. Unique lipid signatures can be identified for various diseases and, as such, may shed light on both prognosis and therapeutic benefit.
13. Many animal and cell culture studies have demonstrated beneficial effects of the addition of SPMs to inflammatory models, suggesting that SPMs have human clinical therapeutic application.

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Part VII

Physical Platforms and Physiology

Optic Nerve Sheath Diameter as a Biological Marker and Its Radiological Evaluation in Brain Injury

45

Gokcen Yildiz and Yasemin Kayadibi

Contents

Abstract

High intracranial pressure (>20 mmHg) is one of the most influential factors in primary and secondary brain injury mortality and morbidity. Insertion of an intraventricular catheter is the gold standard method in intracranial pressure (ICP) monitoring. However, it has disadvantages such as being invasive, having complications such as haemorrhage and infection, requiring an experienced team, and could not be applied to every patient. Therefore, the need for easy-to-apply,

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reproducible, and inexpensive methods in clinical practice has arisen. The optic nerve is an extension of the central nervous system and is covered by the meninges. The potential space between the meninges and the nerve is filled with cerebrospinal fluid, so an increase in ICP causes distention of the optic nerve sheath diameter. In this chapter, we will discuss the optic nerve sheath diameter (ONSD) as a biological marker in neuromonitoring and its radiological evaluation in brain injury.

Keywords

Central nervous system · Computed tomography · Intracranial pressure · Intraventricular catheter · Magnetic resonance imaging · Neuromonitoring · Optical nerve sheath diameter · Trauma · Ultrasonography

Abbreviations

CBF	cerebral blood flow
CPP	cerebral perfusion pressure
CSF	cerebrospinal fluid
CT	computed tomography
DPOAEs	distortion-product otoacoustic emissions
GCS	Glaskow Coma Scoring
ICP	intracranial pressure
MAP	mean arterial pressure
MRI	magnetic resonance imaging
ONSD	optical nerve sheath diameter
TBI	traumatic brain injuries
USG	ultrasonography

Introduction

Central nervous system injuries may be the outcome of a primary pathology or may fellow a secondary injury. Primary injuries can arise due to cerebrovascular (ischemic or hemorrhagic) or traumatic (blunt or penetrating) events (Kaye Jr. 2011; Coronado et al. 2012; Raboel et al. 2012). In these patients, the purpose of neurological intensive care is to control the effect of the first injury and minimize secondary brain damage (Rosner et al. 1995). According to the Traumatic Coma Data Bank and International Data Bank, the effective parameters in this secondary injury are hypotension, hypoxia, and increased intracranial pressure (ICP) (Foulkes et al. 1991; McHugh et al. 2007). Regardless of the cause of secondary damage, the results manifest as changes in blood perfusion, oxygenation, and metabolic parameters of the brain (Plum and Posner 1980). Therefore, neuromonitoring is necessary to capture this secondary damage at the earliest stage and arrange the necessary treatment (Lane et al. 2000; Mauritz et al. 2008; Haddad and Arabi 2012).

Due to its rigid structure, the skull can compensate for an intracranial volume increase (mass, hematoma, swelling due to cerebral edema, etc.) up to a certain amount. As a result of a volume increase that exceeds compensation capacity, ICP begins to increase (Rosner et al. 1995). The methods used in monitoring ICP are classified into invasive and non-invasive methods (Mauritz et al. 2008; Raboel et al. 2012). Although invasive methods, particularly intraventricular catheterization, provide direct monitoring and controlling of the elevated ICP, it has its unique complications, and it is not readily available in every center, and in some cases, it is not cost-effective (Mayhall et al. 1984; Ghajar 1995a; Holloway et al. 1996; Raboel et al. 2012). For these reasons, alternative methods of ICP monitoring with lower complication rates and lower costs are favored by clinicians; hence in the last decade, the usage of non-invasive measurement technics is increased. (Bullock et al. 1996; Brain Trauma Foundation et al. 2007). Distortion-product otoacoustic emissions (DPOAEs) based on the relationship between intracochlear pressure and ICP, transcranial doppler based on the study of the speed of blood cells in the large caliber arteries of the Willis polygon, and optic nerve sheath measurements are among the non-invasive methods used in this last decade (Büki et al. 2002; Edouard et al. 2005; Brain Trauma Foundation et al. 2007; Raboel et al. 2012; Padayachy 2016).

The optic nerve is an extension of the white matter of the central nervous system. The intraorbital section of the nerve is surrounded by dura matter and cerebrospinal fluid (CSF) (Killer et al. 2003; Francois et al. 2011; Sekhon et al. 2014). The CSF space surrounding the optic nerve is connected to the intracranial subarachnoid spaces. Thus, an increase in intracranial pressure is transmitted to the subarachnoid space surrounding the optic nerve causing distension of the ONSD. The fact that ONSD can be examined and measured quantitatively in vivo by radiological imaging methods and quantitative measurement enhanced the relevance of this phenomenon in the current medical practice (Hansen et al. 1994; Geeraerts et al. 2007; Tayal et al. 2007; Tayal et al. 2007; Geeraerts et al. 2008; Moretti et al. 2009; Bäuerle and Nedelmann 2011; Padayachy 2016; Yazar 2019; Liu et al. 2020; Kayadibi et al. 2020). This chapter will discuss the optic nerve sheath diameter (ONSD) as a biological marker in neuromonitoring and its radiological evaluation in brain injury.

Intracranial Pressure in Traumatic Brain Injury

Traumatic brain injury is one of the leading causes of death and disability and accounts for most ER visits (Finkelstein et al. 2006; Coronado et al. 2012; Taylor et al. 2017). One of the main objectives of intensive care in severe head trauma is to prevent the increase of ICP, which leads to secondary brain damage, maintain brain blood flow, and keep oxygenation and blood pressure at an optimal level. Particularly in the first 48 hours after trauma, high ICP is related to increased mortality and poor survival rates (Bullock et al. 1996; Brain Trauma Foundation et al. 2007). In order to achieve success with the management of these patients, high ICP should be detected as quickly as possible and followed up. Clinicians need to determine the

underlying cause and try to restore the pressure (Brain Trauma Foundation et al. 2007; Badri et al. 2012).

Glasgow Coma Scoring (GCS) is used for the first 48 hours to determine the severity of traumatic brain injury. GCS classifies traumatic brain injuries (TBI) as mild (14–15), moderate (9–13), or severe (<8) (Teasdale and Jennett 1974; Foulkes et al. 1991). Computed tomography (CT) is the imaging method that should be performed as soon as possible in acute head trauma to determine patients who need lifesaving surgical interventions. Non-contrast head CT scan shows skull fractures, intracranial hematoma, and cerebral edema. According to current guidelines, CT imaging should be performed on every patient with GKS <14 (6–8) to verify the requirement of neurosurgical intervention (Servadei et al. 2000; Brain Trauma Foundation et al. 2007; Thomas et al. 2010; Tasker 2014; Liu et al. 2020); if a mass effect caused by hematoma, contusion, or edema was detected with CT in a case with GSC < 8, the patient should be monitored for ICP (Servadei et al. 2000; Schreiber et al. 2002; Thomas et al. 2010; Badri et al. 2012).

Pathophysiology

The average value of ICP is 15 mmHg ≤ in adults; an intracranial pressure greater than or equal to 20 mmHg is called intracranial hypertension. In adults, intracranial structures are protected by a stiff and rigid skull. This rigid structure can maintain a volume that can range from 1400 to 1700 mL. 80% of this volume comprises brain parenchyma, 10% cerebrospinal fluid, and 10% blood elements (Kaye Jr. 2011; Raboel et al. 2012). Due to the constant rigid structure of the skull, any presence of intracranial pathology (infection, tumor, hematoma) that will increase the intracranial volume can only be compensated up to a certain amount. When this compensable amount is exceeded, one of the components (brain, blood or CSF) must be relocated to keep the ICP stable. This displacement is within a certain limit; the ICP begins to increase again when this limit is exceeded. This mechanism is also known as the Monro-Kellie doctrine (Monro 1783; Kellie 1824; Mokri 2001). Intracranial compliance refers to the relationship between a change in intracranial volume and the resultant change in ICP. Intracranial compliance has a dynamic nature; in normal conditions, CSF and blood displacement provide high intracranial compliance so that only minimal changes are seen in ICP values. However, further increases in volume result in low compliance, and pressure increases abruptly. In the first step of the compliance mechanism, CSF is displaced to the thecal sac, vascular structures are contracted, and CSF volume is reduced by extracranial drainage. The amount of change in this volume and the rate of change also affect ICP. With the rapid volume change, the change in ICP will be high; with slow changes, the change in ICP will also be small. As ICP continues to increase, cerebral blood flow (CBF) decreases because of arteriolar vasoconstriction (Lassen and Agnoli 1972; Strandgaard et al. 1984; Strandgaard and Paulson 1989; Rosner et al. 1995; Mokri 2001).

Clinical Findings

Among the global symptoms of the increase in ICP, pain develops due to stimulating the pain fibers in the fifth cranial nerve and blood vessels. Changes in consciousness and vomiting may occur due to pressure on the reticular formation of the midbrain. Downward displacement of the brainstem stretches the sixth cranial nerve and causes papilledema. With increases in intracranial pressure, the Cushing response begins with a rise in systolic blood pressure, bradycardia, and irregular breathing pattern. Pressure gradient may result in subfalsine central transtentorial, uncal, upward cerebellar, tonsilar, and transcalvarial herniations (Plum and Posner 1980; Dinallo and Waseem 2021).

ICP Monitoring

ICP monitoring is crucial to keep the perfusion and oxygenation of the brain at the optimum level. To determine intracranial blood flow, ICP and average arterial blood pressure should be measured. Cerebral perfusion pressure (CPP) is an essential determinant of CBF, and it is defined by the formula: CPP = mean arterial pressure (MAP) − ICP. Measuring these two parameters in closed head trauma increases patient survival (Rosner et al. 1995; Lane et al. 2000; Bulger et al. 2002). Clinical suspicion for intracranial hypertension should be raised based on clinical findings combined with the patient's history and imaging results. Clinical suspicion of ICP increase under aggressive medical treatment and GKS <8 are indications of invasive ICP monitoring (Marik et al. 2002).

Invasive Methods

The "gold standard" for monitoring ICP is the intraventricular catheter (IVC) that is placed into the cerebral ventricles; this procedure involves invasive craniotomy and associated risks such as infection, hemorrhage, and tissue lesions. The intracranial pressure can also be monitored with devices placed in the subarachnoid, subdural, or epidural spaces or the brain parenchyma (Ghajar 1995a; Brain Trauma Foundation et al. 2007). The major complication of catheter monitoring is infection (20%) (Mayhall et al. 1984; Holloway et al. 1996; Holloway et al. 1996; Raboel et al. 2012). The risk of bacterial colonization increases significantly from 5 days of monitoring (Rickert and Sinson 2003). Hemorrhagic complications such as bleeding are seen in 2% of patients, reaching up to 5.8% in cases with coagulopathy or thrombocytopenia. Hematomas are observed on the insertion path of the ICP sensor in 1.1% of cases for intraventricular catheters and in 1.1% to 5.8% for intraparenchymal sensors. These hematomas require surgical drainage in 0.5% and 2% to 3.4% of cases, respectively (Rickert and Sinson 2003; Dubourg et al. 2011). Another disadvantage of this method is catheter insertion difficulty when the ventricles are small or compressed due to pressure caused by brain edema and trauma

(Ghajar 1995b). Measuring the opening pressure with a lumbar puncture is another method of invasive monitoring. It is the simplest and most frequently used method, but it can also lead to misinterpretations and herniation (Luerssen 1997; Raboel et al. 2012).

Non-invasive Methods

Non-invasive methods are also used in ICP measurement (Raboel et al. 2012). Transcranial Doppler measures blood flow rate from proximal brain circulation. Characteristic patterns in blood flow waves show the resistance to flow, which in turn reflects ICP changes. However, this method is poorly predictive for ICP (Edouard et al. 2005). Tissue resonance analysis is an experimental ultrasonography (USG)-based imaging method (Michaeli and Rappaport 2002). Intraocular pressure measurement with an optical tonometer is another applicable method, but it cannot be used in patients with oculofacial trauma or glaucoma (Lashutka et al. 2004). Measuring tympanic membrane displacement with DPOAEs is another method that can be used for ICP measurement (Reid et al. 1989).

Optical nerve sheath diameter (ONSD) can provide information about ICP and can be measured with imaging modalities [USG, CT, or magnetic resonance imaging (MRI)] (Raboel et al. 2012). Studies have shown that ONSD is correlated with ICP. Compared to other non-invasive methods, measuring ONSD has higher accuracy and reproducibility in ICP prediction (Moretti et al. 2009; Raboel et al. 2012; Hassen et al. 2015). However, the average ONSD measurement and the cut-off value are uncertain. The change of ONSD from 5 mm to 6 mm (measured 3 mm behind the globe) may discriminate patients with intracranial hemorrhage and traumatic brain injury and indicate intracranial hypertension. Mainly, user-dependent factors contribute to the wide variation in the exact cut-off value, which constitutes the most critical limitation of the method (Soldatos et al. 2008; Moretti et al. 2009; Dubourg et al. 2011; Hassen et al. 2015; Padayachy 2016).

Optical Nerve Sheath Diameter

Although intraventricular devices give more accurate results in ICP monitoring, the invasiveness, high risk of infection, and bleeding are the disadvantages. In addition, standard invasive tools of ICP monitoring require an experienced surgical team and expensive equipment and hence cannot be used in every trauma center (Lane et al. 2000; Brain Trauma Foundation et al. 2007).

Alternative measurement methods to predict ICP increase in clinical practice are investigated. Among these methods, the ONSD is the most studied. In the literature, it is reported that the sensitivity of ONSD in estimating ICP ranges from 36% to 100%, and its specificity ranges from 38% to 100% (Hansen et al. 1994; Geeraerts et al. 2007; Tayal et al. 2007; Bäuerle and Nedelmann 2011; Strumwasser et al. 2011;

Dubourg et al. 2011; Amini et al. 2013; Hassen et al. 2015; Padayachy 2016) (19,26,30–33).

The optic nerve is formed by the extension of the white matter of the telencephalon towards the optic canal during the embryological period (Killer et al. 2003; Hassen et al. 2015). The optical nerve sheath surrounds the optic nerve as a continuation of the meninx; CSF surrounds the potential gap between the sheath and the nerve. (Fig. 1) CSF in the nerve sheath merges with the subarachnoid cavity at the level chiasmatic cistern. ICP transmits into the sheath through this subarachnoid cavity. This relationship of ONSD with the intracranial cavity and the subarachnoid cavity was first mentioned by Hayreh et al. in 1964 (Hayreh 1964). Twenty-nine years later, Hansen and Helmke found a direct correlation between ICP and ONSD, suggesting that the increase in ONSD could be a biological biomarker of the change in ICP (Hansen et al. 1994; Hansen and Helmke 1996). Moretti et al. proved that ONSD changes in real time with ICP (Moretti et al. 2009). P.del.Saz-Saucedo et al. found that ONSD decreased simultaneously when they performed lumbar puncture for therapeutic purposes, so an effective and rapid variable could be used for direct follow-up of ICP (del Saz-Saucedo et al. 2016). However, it was also stated in the same study that ONSD might not change due to the compartmentation of the periodic subarachnoid cavity in patients with idiopathic intracranial hypertension. Therefore, it should be kept in mind that the degree to which the optic nerve sheath expands may differ in some patients (del Saz-Saucedo et al. 2016). Besides trauma, sedation, diabetic ketoacidosis, and hydrocephalus may also play a role on ICP, causing an increase in OSND. Optic atrophy, thyroid ophthalmopathy, and orbital injuries may cause erroneous measurements (Choi et al. 2015; Min et al. 2015; Padayachy et al. 2016; Padayachy 2016).

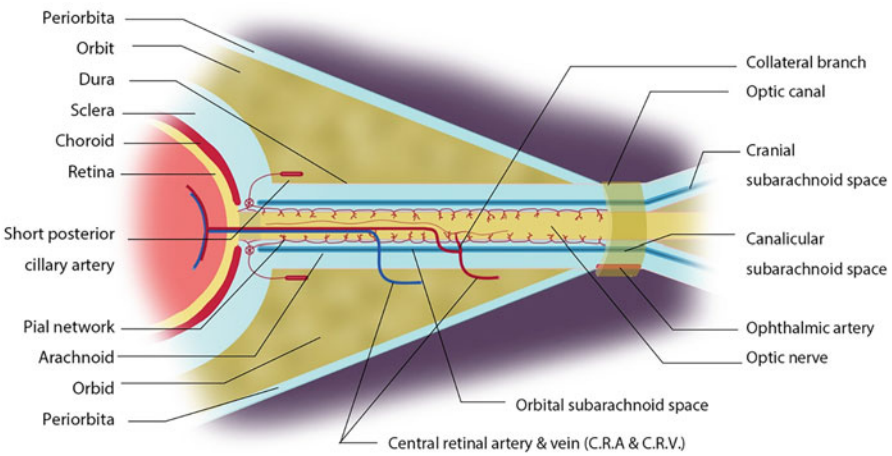

Fig. 1 Schematic drawing of the optic nerve showing the retrobulbar area, the nerve, the sheath, and the potential gap

The optic nerve is a 5 cm long tubular structure consisting of orbital (bulbar + intraorbital segment) and intracanalicular parts. The largest segment of the orbital part is the bulbar segment close to the ocular globe (Killer et al. 2003). Trabecula and septa are located between the optic nerve and the meninx. The amount of these structures varies according to the segments of the optic nerve. The bulbar segment includes only trabecula, while in the intraorbital segment septa and pillars; in the canalicular part, trabecules and pillars are located (Killer et al. 2003).

In a postmortem study, liquid gelatin was applied to the orbital perineural subarachnoid cavity of 54 human optic nerve specimens without any previous treatment to determine the optimal measurement segment for the orbital nerve sheath. According to this study, the widest diameter was obtained in the 3 mm posterior of the globe. The maximum value was obtained by liquid gelatin injection as 6.5 mm, and no correlation was found between the baseline diameter and the amount of expansion. Heterogenous distribution of arachnoid trabecular fibers may explain the difference in expansion capability of each nerve segment (Hansen and Helmke 1996). Although with this study the measurement of OSND at 3 mm behind the globe is considered as the standard method, some authors argue that the nerve is more fixated at 10 mm posterior to the globe and that the sheath will show more uniform expansion at this level, so the measurement should be done from that segment (Liu et al. 2020).

The ONSD does not differ significantly between the sexes, the right and left eye, for transverse and longitudinal measurement plans, different practitioners, body mass index, and different positions (Ballantyne 2002; Romagnuolo et al. 2005; Bäuerle et al. 2012; Sekhon et al. 2014; Hassen et al. 2015; Goeres et al. 2016; Yazar 2019). ONSD measurement is also an ICP-related biological marker in the pediatric age group (Ballantyne et al. 1999; Shofty et al. 2012; Padayachy et al. 2016). In our study, in which we separated pediatric patients by age range, we found that ONSD increases up to 6 years of age and remains stable (Ballantyne et al. 1999; Shofty et al. 2012; Kayadibi et al. 2020) (Fig. 2).

It is a non-invasive method for predicting ICP increase and can be easily measured with high accuracy by radiological methods. This features makes OSND a potential biological marker. Radiological imaging methods used for OSND measurement include USG, CT, or MRI (Tayal et al. 2007; Geeraerts et al. 2008; Soldatos et al. 2008; Dubourg et al. 2011; Shofty et al. 2012; Sekhon et al. 2014; Das et al. 2017).

ONSD Measurement Methods

Ultrasonography

B-mode USG was first used by Hansen et al. in 1994 to show its optical nerve sheath (Hansen et al. 1994). The intraorbital part of the optic nerve can be easily evaluated sonographically (Killer et al. 2003; Francois et al. 2011). In the sonographic examination, the patient lies in the supine position, the eyes are closed, the linear

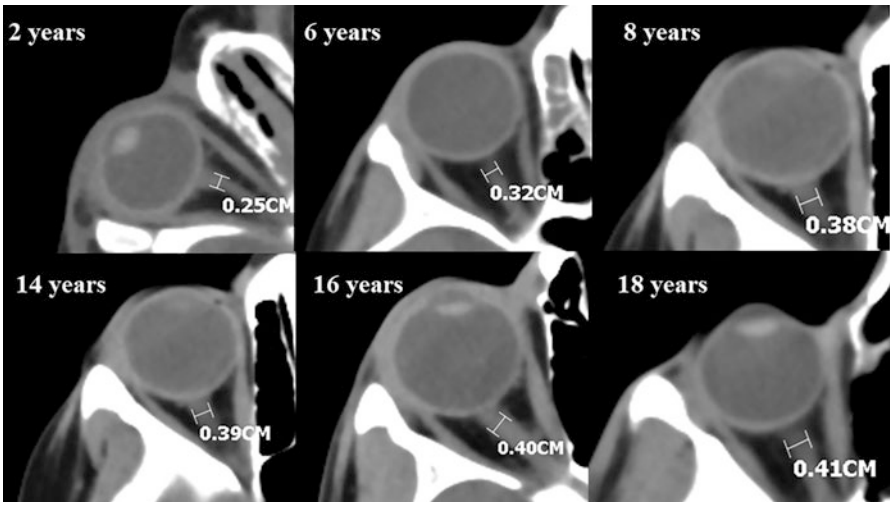

Fig. 2 Enlargement of ONSD with age (ONSD: optic nerve shealth diameter)

superficial probe is gently placed on the eyelid, and care is taken to ensure that the optical nerve vision plan is perpendicular position (Tayal et al. 2007) (Fig. 3). The optic nerve is monitored in the form of a hypoechoic tubular structure in hyperechoic retrobulbar adipose tissue. The recommended distance for measurement is from the bulbar segment from the 3 mm posterior to the globe (Geeraerts et al. 2007; Soldatos et al. 2008). The optic nerve is seen as a hypoechoic linear structure in the sheath by USG. The gap between the sheath and the nerve represents the subarachnoid cavity and has a trabeculated hyperechoic appearance (Fig. 4). ONSD measurement by USG is highly specific (85–100%) and sensitive (90–95%) in predicting the increased ICP (Blaivas et al. 2003; Geeraerts et al. 2007; Tayal et al. 2007; Dubourg et al. 2011; Ohle et al. 2015). The average ONSD in normal adults in USG is 3.68 mm, and an expansion of up to 7.5 mm is possible; increased ICP and normal ICP range from 4.53 to 5.9 mm, commonly accepted as 5 mm (Hansen et al. 1994; Geeraerts et al. 2007; Soldatos et al. 2008; Rajajee et al. 2011; Amini et al. 2013; Goeres et al. 2016; Tarzamni et al. 2016). ONSD measurement with USG is the most preferred radiological imaging method in clinical practice because it is both inexpensive, non-invasive, fast, and easy to learn even by inexperienced hands and does not require mobilizing the patient and can be done at the bedside (Tayal et al. 2007; Potgieter et al. 2011; Toit et al. 2015).

Computed Tomography

CT is the most frequent and the preferred imaging method in severe head traumas due to its readiness, availability in most centers, and providing lifesaving information about intracranial pathologies (subdural, subarachnoid, intraparenchymal

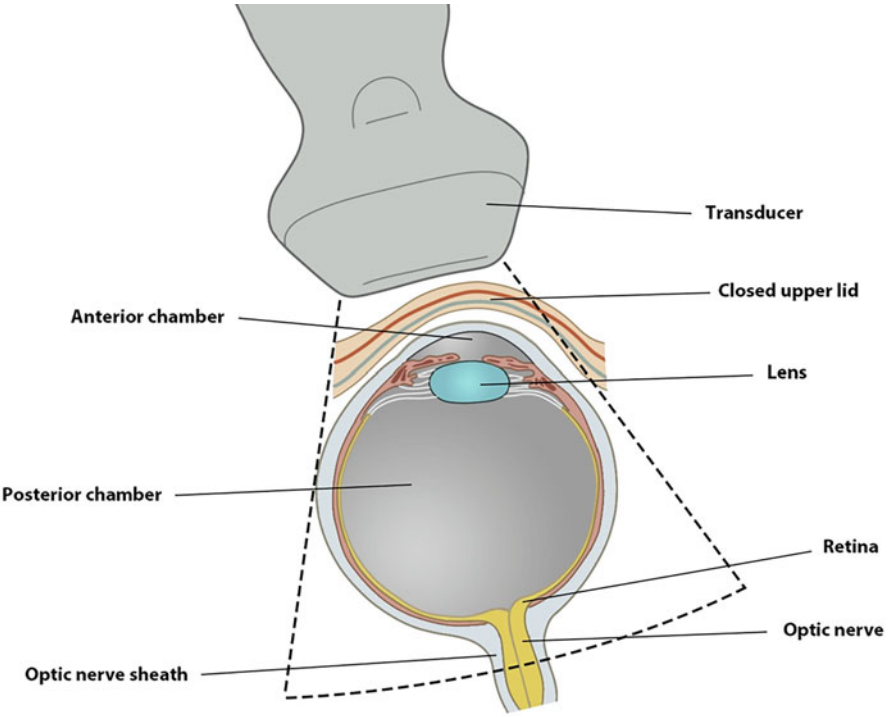

Fig. 3 Schematic drawing of the ocular sonography: the ultrasound probe, the ocular globe, the optic nerve, and the sheath

haemorrhage, fracture, and herniation). It also directs the clinician on the severity of head trauma and whether urgent surgery is required (Tasker 2014; Waqas et al. 2016). In CT, the shift in midline structures, hydrocephalus, sulcal effacement, the collapse of ventricles, and cisterna compression indicate increased ICP (Ohle et al. 2015; Bekerman et al. 2016; Waqas et al. 2016).

Although it is a preferred method for non-invasive and providing more information about the intracranial pathology, it has some disadvantages, such as the need for patient mobilization, time loss, and radiation exposure.

According to the meta-analysis of Ohle et al., ONSD measurement from CT images can detect increased ICP earlier more accurately than developing secondary findings (Sekhon et al. 2014; Ohle et al. 2015). The Rotterdam CT scoring system, based on CT images to predict the severity and prognosis of traumatic brain injury, is correlated with ONSD in adult patients, and ONSD greater than 5.8 mm is related to severe traumatic brain injury (Waqas et al. 2016) (Fig. 5).We have also shown the positive correlation of the Rotterdam scoring system with the ONSD measurement from CT images in predicting the prognosis of pediatric traumatic brain injuries (Kayadibi et al. 2020). In our study, cut-off values according to age were 4.40 mm (66.7% sensitivity, 95.4% specificity) in the 0–3 age range, 4.45 mm (100%

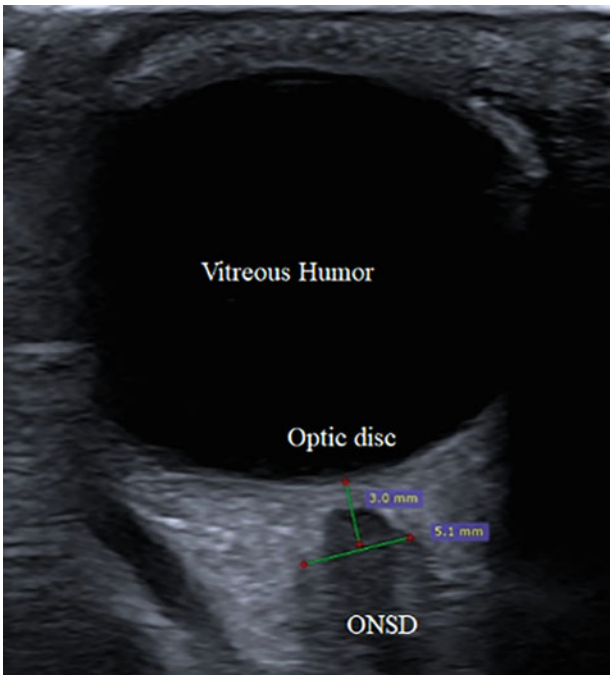

Fig. 4 Measurement of ONSD by ultrasonography at approximately 3 mm depth behind the globe. (ONSD: optic nerve shealth diameter)

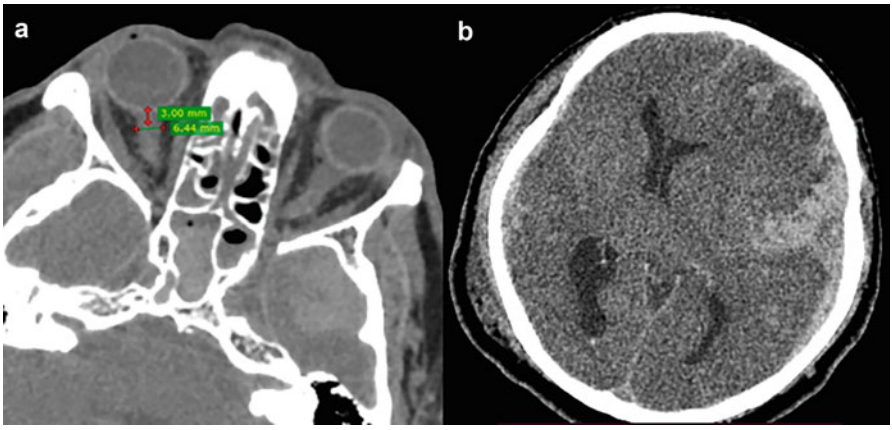

Fig. 5 A 75-year-old male patient after a traffic accident; increased ONSD (6.4 mm) (**a**), intraparenchymal subdural-subraracnoid hemorrhage in left frontoparetal region, shift in midline structures (**b**). (ONSD: optic nerve shealth diameter)

sensitivity, 87.7% specificity) in the 3–6 age range, 4.25 mm (100% sensitivity, 81.2% specificity) in the 6–12 age range, and 4.45 mm (100% sensitivity, 91%

specificity) in the 12–18 age range (Kayadibi et al. 2020). Lui and his colleagues also concluded that ONSD is a predictive parameter used to make decisions about the patient's surgical necessity. In this study, the performance of the cut-off value above 5.09 in predicting the surgical necessity has 85.7% sensitivity and 87.3% specificity, 78.7% PPV, and 91.8% NPV, and the performance of the cut-off value above 4.99 mm in predicting increased ICP has 68.75% sensitivity and 94.75% specificity (Liu et al. 2020). According to Sekhon et al., this cut-off has a value of 6 mm (97% sensitivity; 42% specificity) (Sekhon et al. 2014).

On the other hand, Vaiman et al. have determined the cut-off value as 5.5 mm (83% sensitivity; 94% specificity) (Vaiman et al. 2016). The study of Jenjitranant and his colleagues compared the ONSD measurement in CT and in the US and found that the diameters measured on CT are wider than sonographic measurements (Jenjitranant et al. 2020). The reason for these different measurement results may be the position and measuring distance from the orbital. In some publications, measurements were made from 3 mm posterior to the orbit, in some from 8 to 10 mm posterior, and in others from the level where it crosses the ophthalmic artery (Vaiman et al. 2016). Non-simultaneous ICP and ONSD measurements may also be the reason for the difference between studies. Another possible reason is that increased ICP was measured directly with the intraventricular device in some publications and evaluated based on CT findings in others (Liu et al. 2020; Jenjitranant et al. 2020; Lee et al. 2020). Although publications state that the chantomeatal line should be perpendicular to the stretcher to optimally display the optic nerve during CT shooting, most patients may not have been able to provide an appropriate conscious state during the CT exam (Liu et al. 2020).

Magnetic Resonance Imaging

Like CT, cranial MRI is a cross-sectional radiological imaging modality used for ONSD measurement (Raboel et al. 2012). In T2 images, the hypointense optic nerve is surrounded by hyperintense CSF cover (Fig. 6). Measurements of ONSD in T2-weighted images have been shown to correlate with ICP values in severe traumatic brain injuries (Geeraerts et al. 2008). MRI is a more advanced method with high spatial resolution, particularly for cranial imaging, compared to USG and CT. However, the patient needs to stay in the device for a longer period. It is not possible for every patient to make an optimal measurement during this period due to the artefacts of eye movements. In addition, the fact that MRI is not the modality of the first choice in acute traumatic patients and that patients who require ICP monitoring are usually connected to mechanical ventilators limits the use of MRI. In the study of Shirodkar et al., there is an acceptable agreement between the measurements obtained from the USG and MR. However, there are also studies reporting that the mean measurements obtained from MR (5.3–5.7 mm) are higher than the values measured in USG (3.2–4.9 mm) (Steinborn et al. 2012; Shirodkar et al. 2015; Patterson et al. 2018). Indeed, in Kimberly et al., the cut-off value of ONSD found for increased ICP is 5.82 mm (sensitivity of 90%, specificity of 92%),

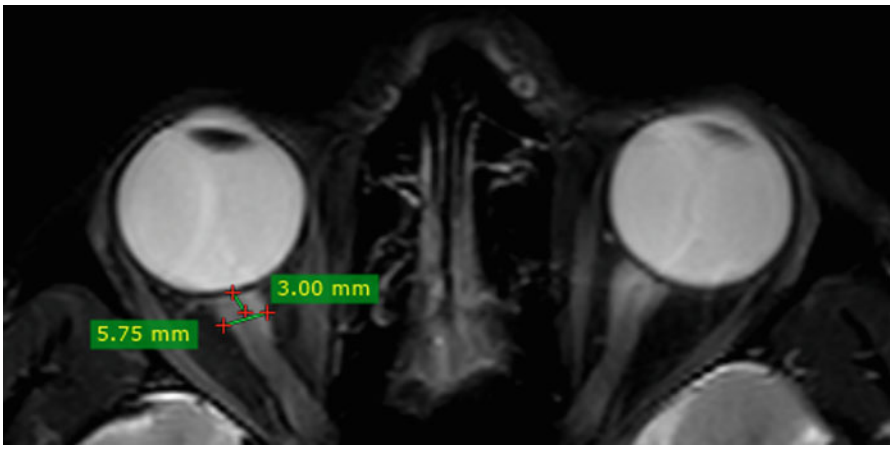

Fig. 6 Measurement of ONSD on T2-weighted magnetic resonanace imaging at approximately 3 mm depth behind the globe. (ONSD: optic nerve shealth diameter)

which is higher than the cut-off value (most of them around 5 mm) found in most sonographic studies (Kimberly and Noble 2008).

Applications to Prognosis

In summary, USG, CT, and MRI are radiological methods frequently used to evaluate ONSD in clinical practice and emergency units. The USG is the preferred imaging method that is easy to use, and measurements can be made ready at the bedside and during patient transportation with portable devices. USG exams of ONSD are beneficial in the follow-up of trauma patients as they can be repeated multiple times when necessary (Wang et al. 2019). On the other hand, interobserver disagreement between USG measurements has been reported in some studies (Hassen et al. 2015; Oberfoell et al. 2017). ONSD can be evaluated more objectively on CT and MRI. In addition, CT and MRI are usually necessary to define the cause of ICP increase (hemorrhage, effacement in basal cisterns, diffuse sulcal effacement, midline shift, herniation, hydrocephalus) (Tayal et al. 2007; Raboel et al. 2012; Jenjitranant et al. 2020). A major disadvantage of both CT and MRI is that they both require patient transport, which may increase the life risk of critically ill patients, and cross-sectional methods cannot be used for continuous follow-up (Beckmann et al. 2004). However, trauma patients admitted to the emergency department must have at least one CT exam for their initial management. For this reason, it is a helpful method in the investigation of brain edema and secondary findings of ICP increase, in the detection of patients who will require catheter placement, or in the evaluation of ICP in patients when invasive methods are contraindicated.

Conclusion

Increased ICP plays a significant role in secondary damage to the central nervous system in traumatic and non-traumatic brain injury. The gold standard in ICP monitoring is intraventricular catheterization. However, the fact that it is invasive has serious complications, and the fact that it may not be applied in every center has led clinicians to search for alternative methods. ONSD is a promising biomarker in the evaluation of ICP due to its direct relation with the subarachnoid space. This method is a proper additional technique to existing gold standard invasive methods. ONSD measurement can be instrumental in the triage of the patient at the first admission and the decision of surgical intervention, during patient transport, and in predicting the increase in ICP in cases where invasive methods cannot be applied.

Mini-Dictionary of Terms

Distortion-product otoacoustic emissions (DPOAEs): DPOAEs represent a type of emission that is present in essentially all normal ears.

The Glasgow Coma Scale (GCS): GCS is a clinical scale used to reliably measure a person's level of consciousness after a brain injury.

Willis polygon: Willis polygon is a circulatory anastomosis of the brain.

Monro-Kellie doctrine: The pressure-volume relationship between ICP, the volume of CSF, blood and brain tissue, and cerebral perfusion pressure is known as the Monro–Kellie doctrine.

Intracranial pressure: Intracranial pressure (ICP) is the pressure exerted by fluids such as cerebrospinal fluid (CSF) inside the skull and on the brain tissue.

Cushing response: Cushing is a physiological nervous system response to increased intracranial pressure (ICP) that results in Cushing's triad of increased blood pressure, irregular breathing, and bradycardia.

Key Facts of ONSD

- The optic nerve is the extension of the white matter of the telencephalon.
- CSF surrounds the potential gap between the optical nerve sheath and the optic nerve.
- ONSD measurement in ICP monitoring is gaining importance in the literature as it is a non-invasive, easily applicable, and reproducible method.
- The sensitivity of ONSD in estimating ICP ranges from 36% to 100%, and its specificity ranges from 38% to 100%.
- Radiologically, USG, CT, and MRI can be used in the measurement of ONSD.

Summary Points

- High intracranial pressure (>20 mmHg) is significant in brain injury.
- ICP monitoring is essential for patients with suspected ICP increases.
- Intraventricular catheter placement is the gold standard for ICP monitoring.
- The increase in ICP also causes an increase in ONSD.
- ONSD is a potential biomarker that can be used to predict ICP increase.

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Neural Activity and Oscillations as Biological Markers in Traumatic Brain Injury

46

Benjamin T. Dunkley

Contents

Abstract

Mild traumatic brain injury (mTBI) – or concussion as it is oftentimes known – is a common type of acquired brain injury that generally has a favorable prognosis. However, a significant minority of cases present with persistent postconcussive symptoms that can reduce quality of life through functional impairment. Structural brain imaging has shown to be insufficient for the scope of the problem, now

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labeled a "silent epidemic," and there are increasingly concerns about the number of accumulated head knocks during sport culminating in neurodegenerative disease, such as chronic traumatic encephalopathy (CTE). The advent of powerful new approaches to understanding brain function, such as magnetoencephalography (MEG), has shown promise in revealing the electrophysiological disturbances and functional consequence that mTBI can cause. In this chapter, we review some of the literature that shows MEG can image this "invisible injury" and potentially offer a rapid diagnostic tool for evaluating "subtle" brain injuries.

Keywords

Mild traumatic brain injury · Concussion · Neural oscillations · Brain waves · Brain rhythms · Brain imaging · Neuroimaging · Magnetoencephalography

Abbreviations

ADHD	Attention-deficit/Hyperactivity disorder
CTE	Chronic traumatic encephalopathy
DAI	Diffuse axonal injury
DMN	Default mode network
DTI	Diffusion tensor imaging
DWI	Diffusion-weighted imaging
EEG	Electroencephalography
FC	Functional connectivity
fMRI	Functional magnetic resonance imaging
GCS	Glasgow Coma Scale
LoC	Loss of consciousness
MEG	Magnetoencephalography
mTBI	Mild traumatic brain injury
OPM	Optically pumped magnetometry
PPCS	Persistent post-concussive symptoms
PTA	Post-traumatic amnesia
PTSD	Post-traumatic stress disorder

Introduction

Traumatic brain injuries (TBIs) are a common form of trauma, and the vast majority of TBIs consist of mild traumatic brain injury (mTBI) – more commonly referred to as a concussion (Alexander 1995) – with around 75–80% of those presenting to emergency departments with a head injury being classified as an mTBI. mTBI is increasingly a public health concern and is especially prominent in subgroups such as those in contact sports (Rabinowitz et al. 2014) and the military (O'Neil et al. 2013), where such head injuries are common. Nevertheless, the greatest incidence rates occur in young children (Barlow et al. 2010) and seniors (Mosenthal et al. 2004), despite the public perception of this injury being primarily of young adult

males, who are prone to risk-taking behaviors. Such injury can occur through numerous routes, including falls, assault, motor vehicle accidents, sports collisions, and blast exposure, but the common mechanism is one of injury evoked through force and energy transfer to the brain through movement-related acceleration-deceleration (Funk et al. 2007). A head impact is not required for an mTBI – they can occur through whiplash-type events where the motion of the brain inside the skill is enough to cause a concussion (Elkin et al. 2016).

Symptoms include somatic, cognitive, and emotional, as well as physical and behavioral change (Arciniegas et al. 2005). The current definition of an mTBI is that of a *transient impairment to mental functioning* – with most cases spontaneously resolving within 3 months (McCrory et al. 2017). However, a significant minority (around 20–30% in some populations) continue to experience long-term, chronic difficulties (Cooper et al. 2015), known as persistent post-concussive symptoms (PPCS). These can adversely impact quality of life and result in losses of productivity – yet despite the broad constellation of symptoms that points to neuropathological change and functional dysregulation, conventional brain imaging (e.g., structural MRI) techniques cannot reliably diagnose or prognosticate an mTBI (Shin et al. 2017). For this, new and emerging techniques that measure neural activity, such as magnetoencephalography (MEG), show promise (Hari 2011).

Signs, Symptoms, and Sequelae of mTBI

The most common signs and symptoms of mTBI include a constellation of somatic, cognitive, behavioral, and emotional impairments that occurs in the immediate post-injury acute phase. These commonly include dizziness, headache, nausea, blurred vision, slurred speech, disorientation, and vestibular problems (McCrory et al. 2017), as well as cognitive issues such as poor memory, confusion, and attentional and language difficulties (Bleiberg et al. 2004). These occur after a head injury and may or may not be present with loss consciousness (LoC) and/or changes in levels of consciousness (defined by the Glasgow Coma Scale; GCS), and post-traumatic amnesia (PTA). These scales provide definitive thresholding criteria for a "mild" TBI (Ruff et al. 2009), which, if exceeded, meet the definition for a "moderate" TBI – these include LoC for longer than 30 min, PTA for greater than 24 h, and GCS lower than 13.

The prognosis for an mTBI is good, with spontaneous resolution of signs and symptoms occurring within a two-week period for the majority of cases, and most injuries resolving completely within 3 months – however, a significant minority do continue to suffer from persistent post-concussive symptoms (PPCS) beyond this 3-month mark, with some presenting with long-term functional impairment that is often treatment resistant (Bigler 2008). These issues are compounded by preexisting risk factors that serve to maintain symptoms, and the interaction with psychosocial influences that may make recovery difficult (Rickards et al. 2022). Moreover, these issues can interact with prior or emergent psychiatric disorders that overlap in symptomatology with PPCS, including common comorbidities such as anxiety, depression, and attention-deficit/hyperactivity disorder (ADHD), as well as related

disorders that result from psychological trauma including post-traumatic stress disorder (PTSD) (Minen et al. 2016).

mTBI commonly presents with significant secondary cognitive and behavioral sequelae that can make day-to-day life problematic, and adjustment issues can compound these difficulties. Cognitive impairments that are most reported include attentional difficulties (e.g., "feeling in a fog"), slowing of information processing speed, lack of concentration, mental inflexibility, and working memory deficits. These higher-level cognitive difficulties are also often accompanied by lower-level perceptual dysfunction that can include visual and auditory hypersensitivities, double or blurred vision/convergence and accommodation issues, vestibular and balance difficulties, disorientation, and gait and coordination difficulty (Akin et al. 2017; Capó-Aponte et al. 2012; Thiagarajan et al. 2011). Sleep disturbance is a particularly common symptom of mTBI (Jaffee et al. 2015), even when individuals report daytime fatigue and tiredness.

The Biomechanical Forces and Neurochemical Consequence Serving mTBI

The mechanisms that can cause mTBI are broad, in that injury can be sustained either through contact forces directly impacting the head (e.g., a fall and striking ones' head on the pavement, or being hit in the head by an object such as a ball), or mediated by force transfer from bodily impact that transmits energy to the brain (e.g., a whiplash through a motor vehicle collision where the head does not necessarily impact a surface). The former often results in a local impact injury to the brain, known as a coup, and a concomitant conter-coup injury on the other side of the brain, induced through translational force application. Rotational injuries can likewise occur. mTBIs in the military are common – including those induced through blast-wave exposure, where fast-moving waves of compressed air travel rapidly from the site of an explosion, followed by a subsequent low-pressure wave. While there are differences in the exact biological mechanism of injury between direct impact, acceleration-deceleration injury, and blast exposure, they all result in transient compression, rotational, stress, and strain tensile force being applied to brain. These forces disrupt vasculature and ultimately impact neuronal functioning. Commonly, mTBI occurs with both a focal and/or diffuse component, with the morphological characteristics of the brain predisposing areas to being more susceptible to injury than others.

Diffuse axonal injury (DAI), which refers to white matter damage through rotational shearing and stretching forces (Inglese et al. 2005), is especially pernicious, and results in deafferentation and neuronal dysconnectivity. Axonal injury generated by biomechanical shifts include axonal deformation, stretching, straining, and shearing, particularly at the grey-white matter boundary, where the stress-strain characteristics at the boundary make neurons susceptible to physical forces – physical forces which alter the cellular membrane permeability and lead to a "biochemical cascade" and metabolic crisis energy (MacFarlane and Glenn 2015). Initially,

neuronal injury results in cellular membrane damage, leading to ionic dysregulation and a flood of excitatory neurotransmitter release, with this excitotoxic shock potentiating a positive feedback loop of further intra- and extracellular ionic disruption (Giza and Hovda 2014). This increased metabolic demand occurs simultaneously to vascular insult which reduces cerebral blood flow – this results in a brain energy crisis, due to disequilibrium in cellular energy demand and energy supply. This has a knock-on effect of neurotransmitter dysregulation with numerous neurotransmitter systems impacted by mTBI, including those that are important for the regulation of emotions, arousal, and mood. Secondary injury also results from reactive edema, neuronal swelling, and eventual demyelination through inflammation and oxidative stress (Signoretti et al. 2011).

Animal models of mTBI have proven to be incredibly informative when it comes to understanding the pathophysiology of concussion – however, animal models, especially rodents, lack the same physical properties and biomechanics that the human brain would endured during mTBI. Therefore, in humans, the application of noninvasive neuroimaging is required to understand the microstructural, local, and network level effects that occur after an mTBI in vivo.

Invisible Fingerprinting of the Brain: Magnetoencephalography

Despite their ubiquity and importance in understanding the structural consequence of moderate and severe brain injury, conventional structural brain imaging is blind to the dynamic functional repertoire of neural activity that is intrinsically impaired after an mTBI. Clinical MRI and CT of mTBI is unremarkable, with no distinctive neuroradiological abnormalities – if any brain injuries are noted, these are premorbid, incidental, or mean that the classification moves to a "moderate" or "severe" traumatic brain injury. This makes traditional neuroradiology insufficient for the scope of the problem in diagnosing an mTBI, or prognosticating outcome, or managing chronic, long-term PPCS. Advances in MRI such as diffusion-weighted imaging (DWI) and diffusion tensor imaging (DTI) were heralded as key to understanding mTBI and specifically DAI, but even diffusion-based methods provide limited sensitivity and specificity for identifying injury in individuals – for example, a recent study showed that just 23% of high-level athletes in the acute phase of injury showed axonal or vascular injury (Zimmerman et al. 2021). Complementary functional brain imaging approaches have played an important role in understanding mTBI, including those based on hemodynamics such as functional MRI, and electrophysiological techniques such as electroencephalography (EEG), and magnetoencephalography (MEG). The latter has been around since the 1960s, and in a clinical context has historically been used in assessment of epilepsy and presurgical function mapping of eloquent cortex – only recently has it been applied for other uses in understanding neurological and psychiatric morbidity (Uhlhaas et al. 2017).

MEG is a functional brain imaging technique based on electrophysiological principles that measures neural activity (Baillet 2017; Gross 2019; Uhlhaas et al. 2017). It is completely noninvasive and comprises highly sensitive magnetometers

placed in a helmet array, often in the order of hundreds of sensors, to measure the extracranial magnetic fields generated by minute electrical currents in the brain. These neuronal electrochemical currents generate an associated magnetic field that passes through intermediary tissue layers to reach the sensors placed around the head. This magnetic field measurement is a direct index of neural activity, representing the summated and synchronous excitatory postsynaptic dendritic potentials of co-aligned pyramidal cells in the grey matter. MEG can measure the temporality of neural activity in the order of milliseconds, and with a spatial resolution approaching millimeters, and close to that of fMRI (Barratt et al. 2018). There are certain technical challenges posed in recording these weak magnetic fields, which require the use of magnetically shielded rooms, reference sensors to account for environmental/ambient noise, and software-based methods for artifact removal (Oostenveld et al. 2011). The fields generated by electrochemical activity is many orders of magnitude smaller than the earth's magnetic field, and even that of standard electrical equipment – nevertheless, despite these technical challenges, MEG provides an extremely sensitive way to measure neural activity noninvasively in humans (Dunkley et al. 2013).

The neural activity measured by MEG tends to have characteristic rhythmic properties, colloquially known as "brain waves" – these periodic fluctuations in activity are referred to as *neural oscillations*. Rhythmic neuronal excitability is driven by stochastic processes in groups – or ensembles – of neurons. Neural oscillations have spectral properties, comprising of specific frequency bands that index the physiological properties of population-level neuronal activity, even at the level of specific cortical layers (Bonaiuto et al. 2018). These frequencies cover the spectrum of very slow to extremely fast, and span delta (1–3 Hz), through theta (3–7 Hz), alpha (8–12 Hz), beta (15–25 Hz), gamma (30–80 Hz), and high gamma (+80 Hz) ranges. The temporal sensitivity of MEG and the physics of magnetic field propagation mean that it is an ideally suited technology to image this broad frequency spectrum of neural activity, and particularly higher frequencies, like gamma, to which EEG is easily contaminated by muscle artifacts which lie in the same frequency range (Muthukumaraswamy 2013).

Neural oscillations are a functional mechanism to process and move information (Fries 2005), and form dynamical circuits – commonly known as functional connectivity (Brookes et al. 2011b). Functional connectivity is the broad term given to measures of brain network communication and was popularized by early fMRI studies (van den Heuvel and Hulshoff Pol 2010), yet neural oscillations are only directly measurable using electrophysiological modalities such as EEG and MEG. Neural oscillations are critical to the coordination and integration of information that travels between functionally specialized areas (Ward 2003). This mechanism evolved to organize brain circuits spontaneously and dynamically to support goal-directed cognition and behavior needed for everyday interaction within one's environment. Mental states and behaviors are coded by the different frequencies of oscillations, which vary systemically across different brain regions and in response to cognitive demands, with circuits functionally operating at different frequencies, occurring across local and global (i.e., brain wide) spatial scales. In essence, neural

oscillations and the frequencies at which they operate are a multiplexing method for the brain to process information. The increased temporal sensitivity of these methods allows for exploration of the dynamics of neural activity at behaviorally relevant time scales and provides an understanding of the mechanisms of maladaptive brain function that can result from injury and disease. Behavioral and cognitive pathology, in part, reflects dysfunctional brain circuits, and MEG represents a potent tool for evaluating function in those circuits. Neurophysiological studies have been less common than the use of fMRI in mild traumatic brain injury, yet they reveal important functional abnormalities to which other more common methods are blind.

Revealing the "Spectral Fingerprints" of mTBI with MEG

MEG has been used to study mTBI in two contexts: that of spontaneous or intrinsic brain function – putatively called the "resting state" – which is essentially "task free," and during task-based paradigms, that probe psychological processes, such as memory or attention, for example. In the latter, many trials are averaged over time to extract event-related fields related to stimulus (Gross et al. 2013). The following overview divides the reported results up by "resting-state" studies first, and then task-based paradigms.

Resting-State Studies

In resting-state MEG studies of mTBI, the most frequently reported localized abnormality in spontaneous brain activity is a potentiation in "source magnitude" or neuronal oscillatory power in the slow-wave/low-frequency range, comprising delta (1–3 Hz) and theta (3–7 Hz) activity (Huang et al. 2009; Safar et al. 2021; Zhang et al. 2021). This means that the relative contribution of these low-frequency oscillations to the measured signal is increased in power, referenced against other frequencies of brain activity. These low-frequency abnormalities have been reported in numerous studies, across children (Huang et al. 2020; Safar et al. 2021) and adults in nonblast (Dunkley et al. 2015; Huang et al. 2012; Kaltiainen et al. 2018) and blast-related mTBI (Huang et al. 2012, 2016).

Early studies of DTI and MEG showed that the locus of pathological slow-wave abnormalities occurred in proximity to white matter microstructure injury, below the grey matter surface (Huang et al. 2009). Similar to other functional neuroimaging studies that show the most common site of localized dysfunction, cortical areas such as frontal, temporal, and parietal cortices are prone to injury and show abnormal delta generation (Antonakakis et al. 2017; Li et al. 2015), although across studies the areas showing dysregulation are variable. These putative markers of pathological neural slowing are thought to reflect deafferentation, demyelination, and white-grey matter boundary shearing – importantly, the magnitude of these low-frequency oscillopathies in frontal cortices correlate with the subjective severity of post-concussive symptoms (Huang et al. 2014) and broader cognitive impairment

(Swan et al. 2015). Alterations in the theta range have also been noted in mTBI, that in a minority of participants persists at follow-up, longitudinally in parallel with symptoms (Kaltiainen et al. 2018).

Recent evidence also suggests mTBI disrupts faster neural rhythms, including those in the alpha and beta range (Safar et al. 2021; Zhang et al. 2020a), as well as high-frequency oscillations in the gamma range (Huang et al. 2019). Alpha activity was shown to be impacted by mTBI in adults (Antonakakis et al. 2016; Li et al. 2015; Misic et al. 2016; Popescu et al. 2016) and children (Safar et al. 2021). Other papers have shown that not only raw oscillatory power is affected by mTBI, but also neural complexity and multiscale entropy, across frequency bands, across multiple brain regions and systems (Misic et al. 2016). Recently, deficits in beta activity in the post-acute period have also been noted, particularly within frontal cortices (Zhang et al. 2020a). Fast frequency activity, such as gamma – a brain rhythm that can only be reliable revealed noninvasively in humans via MEG (Hoogenboom et al. 2006, 2010) – is also known to be altered. In a group of military personnel and veterans with PPCS after mTBI, elevated gamma activity has been observed (Huang et al. 2019). Gamma oscillations are implicated in the excitation and inhibition (Muthukumaraswamy et al. 2009), and these markers are thought to be due to disinhibition as a result of excitotoxicity, peri-neuronal net degradation, and thalamo-cortical circuits (Swan et al. 2015).

Moving beyond regional and localized measures of neural disruption – indexed via altered spectral power profiles – MEG also affords us a way to measure functional connectivity across distributed brain systems and circuits (Brookes et al. 2011a). This is calculated through the statistical relationship between neural dynamics in distinct brain areas, and the interactions between these areas is mediated through modulations in their phase and amplitude relationships. While outside the scope of this chapter, functional connectivity (FC) in MEG (and EEG) can be defined by multiple measures that capture complementary aspects of neural functioning (Engel et al. 2013), including amplitude-based measures (e.g., amplitude envelope correlations, coherence etc) and phase-angle-based synchronization FC measures (e.g., phase locking value, phase lag index, weighted phase lag index, etc.) – for a review see (Colclough et al. 2016; Palva et al. 2018; Palva and Palva 2012). Applications of FC reveal cross-spectral and multinetwork alterations in mTBI. Amplitude envelope correlation (AEC) measures of FC are positively correlated with the blood-oxygen-level-dependent (BOLD) effect in functional MRI and are thought to mirror the same kind of neural processes (Brookes et al. 2011b). Application of AEC measures show that low-frequency connectivity (delta through alpha) is increased in mTBI in frontotemporal, parietal, and subcortical areas (Dunkley et al. 2015) and the default mode network (DMN) (Dunkley et al. 2018), and these alterations correlate with the total symptom burden and subjective severity. Conversely, reductions in higher-frequency connectivity in the beta range was also observed in this same group (Zhang et al. 2020a). A follow-up study to the latter also showed that this reduced connectivity in the beta range was driven by deficits in the coincidence of transient beta burst phenomenon (Rier et al. 2021).

Complementary measures of FC can also be derived from phase-based measures, including phase angle synchronization – a key mechanism in the "*communication-through-coherence*" hypothesis (Fries 2015) which posits that phase alignment in the ongoing fluctuations in excitability between ensembles of neurons mediates information transfer between areas – in other words, when two groups of neurons align in their periodic fluctuations, spike information traveling between groups is more readily integrated than when the areas are out of phase. This phenomenon, which relies on the preservation of structural connectivity between areas, is known to be affected by mTBI. Numerous studies have shown that phase synchronization is impacted by injury. Alpha oscillatory synchronization can discriminate individuals with a high degree of accuracy (Vakorin et al. 2016). Further evidence points to deficits in phase-based connectivity, across multiple-frequency bands, including delta, alpha, and beta, prominently within the DMN (Alhourani et al. 2016).

Several other studies also examine measures of neural activity, such as signal complexity and entropy (Antonakakis et al. 2017; Dimitriadis et al. 2015), as well as cross-frequency coupling, including beta-gamma frequency bands (Antonakakis et al. 2016), and altered topological properties of these networks such as randomness/small-worldness, hubness/rich club dynamics, efficiency, and complexity (Antonakakis et al. 2017, 2020; Rowland et al. 2018). Presenting a simplified view across studies is difficult given their often-contrasting findings, heterogeneous study groups, differences in time since injury and mechanism, and not to mention the vast array of alternative MEG approaches to analyzing neural activity. However, it is quite clear from these results that low frequency increases in power and altered network functioning that span across multiple frequency bands are a common theme of injury – and that many of these observations can be explained by the dysregulated hyperexcitability of glutamatergic pyramidal cells, and disinhibition by alterations in GABAergic interneuron input. The combination of regional, localized measures of neural activity (e.g., source power and complexity) and measures of network connectivity and interaction that serve neural communication (e.g., phase synchronization) offer powerful complimentary approaches that can reveal the focal and distributed nature of concussive brain injuries (Peitz et al. 2021).

Task-Based Studies

While "resting-state" paradigms are powerful approaches to understanding intrinsic and spontaneous brain functioning, they tell us little about the relationship between specific cognitive and/or behavioral deficits and disrupted functional processing in neural circuits. By applying task-based protocols that are run in the scanner, we can link real-time neural activation to task performance in mTBI.

A number of different paradigms have been applied to the study of mTBI using MEG, including that of executive functioning, such as cognitive flexibility, working memory, and visuospatial attention, as well as other domains of psychological and behavioral processing such as auditory perception, object recognition, and motor execution. For cognitive flexibility – sometimes referred to as mental flexibility or

cognitive control – two studies on the same group have examined set-shifting, using two different MEG approaches – one that examined event-related, evoked fields (da Costa et al. 2014), analogous to evoked potentials, and another that investigated neural synchrony to probe functional connectivity (Pang et al. 2016). These studies found that performance in extradimensional set shifts (i.e., more difficult flexibility demands) was reduced in mTBI, and that this was subserved by atypical spatiotemporal profiles of evoked activity in executive function areas, and reduced neural synchronization across multiple brain regions, when compared with controls.

In working memory tasks that require sustained visuospatial attention, evoked hippocampal and occipitoparietal cortex activity was dysregulated and lateralized in mTBI (Shah-Basak et al. 2017). Another study showed that reduced alpha and beta oscillations in frontal and memory-related networks were reduced in mTBI, correlated with task performance, and were related to symptoms of PTSD (Popescu et al. 2016, 2020). In a task examining motor network functioning during a simple button press task, mTBI exhibits deficits in coincident beta bursting, a core feature of motor execution (Rier et al. 2021). In another visual attention task, it was found that those with mTBI require additional spatial cues to perform a comparable level to controls in an attention network test, and that this was concomitant with reduced P300m-evoked amplitude (Petley et al. 2018). In a visual-oculomotor tracking task, those with chronic mTBI show deficits in pursuit behavior and that this was associated with dysregulated alpha, beta, and gamma activity in frontoparietal networks involved in attentional and oculomotor control (Diwakar et al. 2015). In a picture naming task, those with mTBI were found to have difficulties with word finding and that this was the result of disrupted activity in a left lateralized language network, which also included associated areas (Popescu et al. 2017). In an auditory processing task that taps vigilance/sustained attention, alpha activity was altered in the left temporoparietal cortex, and that this activity negatively correlated with a cognitive flexibility task (Kaltiainen et al. 2019).

Differentiating "Invisible Injuries": Disentangling mTBI from PTSD

mTBI is a common occurrence in the military – not only from war zone deployment and combat, but also during training and preparation exercises. Similarly, post-traumatic stress disorder (PTSD) is also relatively common in the military, given operational stress and traumatic exposure. Unsurprisingly, the two can co-occur, simultaneously, but also separately – someone might have an mTBI and another PTSD – but a problem occurs when distinguishing the two conditions, which often have overlapping symptom profiles, as well as neurocognitive sequalae that can make a differential diagnosis difficult. This is likely due to the susceptibility of multiple brain regions and systems being at risk for injury from biomechanical trauma, and these vulnerable circuits are those that often exhibit maladaptive functioning in PTSD (Dunkley et al. 2020; Lanius et al. 2005). These include

frontotemporal areas, as well as the hippocampus and amygdala, which are susceptible to the impact of chronic stress on their functioning (Kim et al. 2015; Roozendaal et al. 2009).

To date, differentiating the neural substrates of symptoms and the cognitive, behavioral, and emotional effects of PTSD and mTBI has proven challenging (Wilde et al., 2015) – it is important that the two are distinguished at the level of neurobiological systems, as the treatments can differ drastically, and oftentimes establishing an effective treatment regimen can take months, if not years. Nevertheless, recent studies have shown that MEG holds promise in disentangling the neurophysiological signatures of PTSD and mTBI, with the application of machine learning routines, multivariate statistics, and classification algorithms that can reliably stratify the disorders and even identify individual cases with a high degree of accuracy (Misic et al. 2016; Rowland et al. 2017; Spadoni et al. 2018; Zhang et al. 2021).

Future Directions and Applications

Technological developments in MEG hardware and "big data" analytics are also showing great promise in the field of mTBI research. Optically pumped magnetometers (OPM), a new generation of room temperature MEG sensors that no longer require super colling via liquid cryogen (Boto et al. 2016, 2018; Hill et al. 2019, 2020), have seen the entry price point and maintenance costs of MEG systems drastically reduced, integrated with the latest in-field suppression technologies, and allow the OPM MEG sensor arrays to be worn on the head and close to the scalp. In contrast to this, traditional cryogenic MEG systems using superconducting quantum interference device (SQUID) technology must be placed approximately ten centimeters away from the scalp due to the need to be bathed in liquid helium.

The benefits of this evolution of the technology are manifold but include: 1. greatly increased signal-to-noise ratio due to a smaller distance between the brain and sensors; 2. head movement tolerance that allows for the development of naturalistic/ecologically valid paradigms that closely mimic "real-world" scenarios; 3. improved compliance in young children who are otherwise difficult to scan without sedation; and 4. smaller overall footprint of the magnetically shielded room and system that would allow hardware to be installed outside of hospitals, research institutes, and universities, and inside of clinics and rehabilitation centers. Moreover, with the advent of cloud servers, high-performance computing, "big data" analytics, machine learning, and pattern classification algorithms (Zhang et al. 2020b, 2021), MEG/OPM data in mTBI have untapped potential in supporting the rapid diagnosis of brain injuries, and the future identification of the most effective treatment pathway. Integrated with longitudinal assessments, these data will provide clinicians with additional information to be able to determine when it is appropriate to "return to work/play/deployment."

Conclusion

mTBI generally has a positive prognosis in individuals, but the scale of the persistent problems certainly means that these invisible injuries are a "silent epidemic." For a significant minority, the long hard road to recovery after an mTBI can be difficult, and medicolegal recognition required for compensation has meant that adequate care, rehabilitation, and support is in short simple. Nevertheless, advanced brain imaging techniques like MEG are allowing to understand the disrupted neural circuits that can underlie the physical signs and symptoms of PPCS, as well as the neurocognitive difficulties, and these potent new approaches will lead to faster routes to diagnosis, prognosis, and identifying the most effective treatment options.

Applications to Prognosis and Conditions

In this chapter, neural oscillations imaged via magnetoencephalography for biomarker discovery in mild traumatic brain injury were reviewed. Disruptions to these brain rhythms reflect microstructural alterations to neurons, their connections, axonal damage, white matter impairment, inflammation, inhibition/excitation, and dysregulated functioning as a result of injury. Currently, evidence suggests these may provide reliable discriminative markers for acute-subacute injury, and possibly the identification of lingering brain injury in persistent post-concussive symptoms – however, their prognostic value is largely unknown. To answer this question, large-scale, multisite, longitudinal studies are required. We do know that repetitive mTBI is increasingly thought to be a potent risk factor for chronic traumatic encephalopathy (CTE) and other neurodegenerative diseases, such as Parkinson's, Alzheimer's, and related dementias (Gardner and Yaffe 2015; Gavett et al. 2010; Zetterberg et al. 2013). Nevertheless, we know that there is some overlap in the biomarkers described here in mTBI (such as neural slowing in the delta-theta range, hyperexcitability-based on gamma activity, and altered connectivity from dysregulated neural synchrony) with MEG studies of neurodegenerative disease and mild cognitive impairment (Pusil et al. 2019), Alzheimer's (Gouw et al. 2021; Koelewijn et al. 2019), and Parkinson's (Boon et al. 2019) – such approaches described in this chapter and those papers can also be used to study CTE. Examining a combination of these groups longitudinally with MEG, and imaging the progression of mTBI and PPCS, repetitive mTBI, and development to CTE and dementias, should uncover the prognostic value of the approaches described to predicting later life outcome and progression to more severe neurological conditions.

Mini-Dictionary of Terms

Mild Traumatic Brain Injury/Concussion

These are acquired brain injuries from physical force and energy transfer to the brain that results in a transient impairment to the function of the brain and one's mental state. Nevertheless, despite a generally good prognosis, chronic lingering issues can be quite common and are termed persistent post-concussive symptoms.

Neural Oscillations/Brain Waves/Brain Rhythms

Neural oscillations are the rhythmic changes in ongoing neural activity that underlie our mental, cognitive, and behavioral states. They have been noted to occur at the microscale, including individual neurons, through to neural groups and circuits, to the macroscale that encompasses brain-wide networks.

Magnetoencephalography

MEG is an electrophysiological/functional brain imaging that has traditionally been used clinically for epilepsy source localization and functional brain mapping for presurgical evaluations. It has also been used as a cognitive neuroscience tool and is increasingly being used for exploration of other indications in psychiatry and neurology, such as PTSD and mTBI.

Key Facts of Neural Activity and Oscillations as Biological Markers in Traumatic Brain Injury

Mild Traumatic Brain Injury

mTBI is brain injury from physical forces resulting in a transient impairment to mental functioning.

Incidence is thought to be about 3.5 per 1000 people, but this differs by population, with athletes and military members showing higher rates – rates are highest in young children and the elderly however.

Signs and symptoms include loss of consciousness, amnesia, headaches, difficulties with balance, vision, and coordination, cognitive issues and sleep disturbances.

Prognosis is good with most symptoms resolving within 2 weeks – however, a significant minority, up to 30% in some cases, continue to suffer from persistent post-concussive symptoms.

Summary Points

Mild traumatic brain injury and concussion are increasingly common forms of acquired brain injury, especially in the very young and elderly.

Despite a generally favorable outcome, around 15–30% of those with an mTBI will suffer from persistent symptoms.

Symptom profiles are varied and heterogenous, but can be physical, cognitive, behavioral, and emotional in nature.

Treatment is difficult and conventional brain imaging (e.g., CT & MRI) does not reveal structural signs of brain injury and is insufficient for the scope of the problem.

Emerging techniques such as magnetencephalography reveal abnormalities after mTBI in neural activity – known as neural oscillations.

Neural oscillations are markers of neural functioning and are a mechanism for information processing in the brain and a way to image persistent dysregulation in neural circuits.

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Viscoelastic Hemostatic Assays in the Management of the Trauma Patient

47

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Abstract

Viscoelastic hemostatic assays (VHAs) have been proposed as an alternative strategy for coagulopathy monitoring, transfusion, and resuscitation guidance in critically injured and ill patients. Viscoelastic monitoring (VEM) assays provide information to assess platelet function, rate of clot formation, clot strength, stability, and dissolution in a short amount of time and while taking into account a whole blood sample. Several systems are available on the market. In this chapter, the basis of hemostasis and coagulopathy will be reviewed in a trauma patient, most commonly utilized viscoelastic monitoring systems are introduced, and the value of viscoelastic monitoring in care for the injured and bleeding patient is discussed.

Keywords

Viscoelastic hemostatic assays · TEG · ROTEM · Coagulopathy · Trauma · TIC hemorrhage · Transfusion

Abbreviations

ACT	Activated clotting time
APC	Activated protein C
CA5	Clot amplitude at 5 minutes
CCT	Conventional coagulation test
CFT	Clot formation time
CT	Clotting time
ISS	Injury severity scale
LI	Lysis index
LY30	Lysis fraction at 30 min
MA	Maximal amplitude
MCF	Maximal clot firmness
MTP	Massive transfusion protocol
R	Reaction time
ROTEM	Rotational thromboelastometry
TEG	Thromboelastography
TIC	Trauma-induced coagulopathy
TQIP	Trauma Quality Improvement Program
VEM	Viscoelastic monitoring
VHA	Viscoelastic hemostatic assay

Introduction

Correction of coagulopathy is essential to minimizing blood loss and preventing subsequent hemodynamic and inflammatory complications seen in trauma and in critically ill patients. Various resuscitation strategies have been proposed to make the

process timely and appropriate. The choice of resuscitation fluid, blood product ratios to administer, and tests to monitor the progress of correction are all aimed at achieving this goal. Traditionally, there is preference for empiric blood component transfusion in a balanced 1:1:1 ratio (RBCs/FFP/platelets) (Holcomb et al. 2015). Recently, resuscitation with whole blood has returned to prominence in the field and trauma centers (Jackson et al. 2020). The response to resuscitation can be assessed through conventional lab tests (CCTs) such as PT/INR, PTT, platelet count, and fibrinogen levels. There are, however, problems with using conventional coagulation tests to monitor evolving coagulopathy. These tests alone and as a whole fail to accurately portray the clinical picture and the severity of the injured and bleeding patient in the moment. The conventional tests can have a significant turnaround time of at least 40–60 min (Toulon et al. 2009; Khalaf-Adeli et al. 2019). Furthermore, one particular test, a simple platelet count, can identify thrombocytopenia but does not identify platelet dysfunction. Additionally, these conventional tests are plasma-based and do not account for the interaction of all key players involved in achieving hemostasis in vivo.

Ideally, a point-of-care test that assesses clotting characteristics within a whole blood sample in an environment that closely resembles that of an injured vessel, with a rapid turnaround time, can guide the resuscitation process more effectively. Viscoelastic monitoring (VEM) assays monitor kinetics of clot formation and break-down, with results that can be available as early as 5 min. As such, VEM assays have been suggested as an alternate strategy to conventional coagulation testing despite missing components outside of factors found in whole blood that effect coagulation. Although not perfect, VEM assays are slowly being incorporated into transfusion algorithms across various specialties and are becoming more prevalent as a guiding method for trauma resuscitation (Bugaev et al. 2020).

Viscoelastic monitoring was first described in 1948, by Dr. Hellmut Hartert, while studying the dynamics of a blood clot formation (Hartert 1948; Hans and Besser 2016). Since then, the method has evolved and been adapted for use in cardiac surgery, transplantation, and trauma resuscitation – where major blood loss is most frequently seen. VEM assays provide information to assess platelet function, rate of clot formation, clot strength, stability, and dissolution. In this chapter, the basis of hemostasis and coagulopathy will be reviewed in order to understand the value of viscoelastic monitoring in care for the injured and bleeding patient.

Trauma-Induced Coagulopathy

Understanding the utility of viscoelastic monitoring starts with review of coagulopathy in a bleeding patient, as viscoelastic hemostatic assays (VHAs) attempt to provide guidance on targeting each component of the coagulopathy for transfusion of blood products. For the trauma patient, the basis for coagulopathy is complex and multifactorial. The more injured the patient is based on their Injury Severity Score (ISS), the more likely the development of coagulopathy and related mortality (Niles et al. 2008). Mortality is significantly increased with early coagulopathy in trauma (MacLeod et al. 2003). Direct, ongoing blood loss leads to

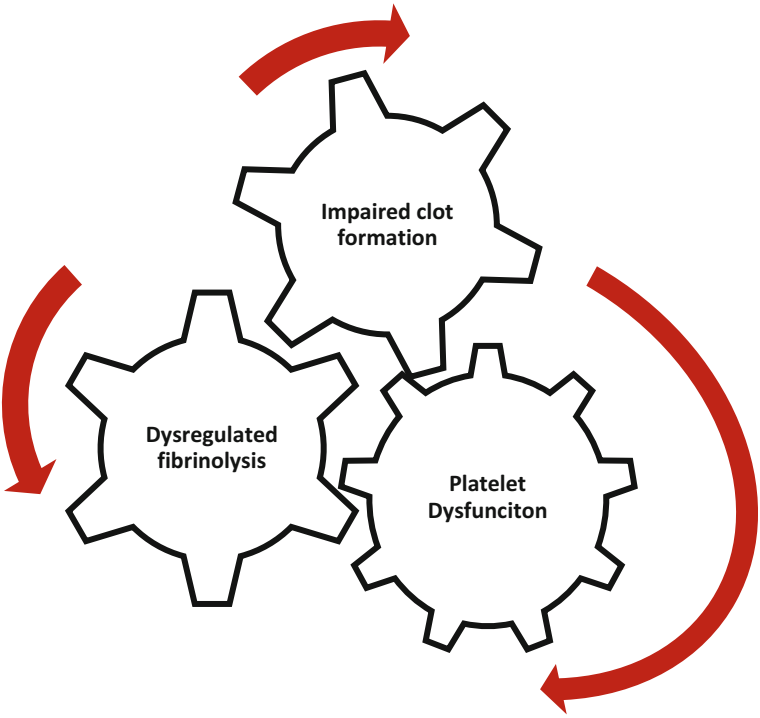

Fig. 1 Components of trauma-induced coagulopathy

innate consumption of clotting factors as the body attempts to control exsanguination. Thus, it is reasonable to assume that the primary goal of trauma resuscitation is stopping this blood loss. While the trauma team focuses on identifying and directly stopping the source of hemorrhage, hemostatic function post-trauma continues to progress towards worsening and ongoing coagulopathy (Fig. 1). It is absolutely necessary to halt or reverse this pathophysiologic process.

Impaired Clot Formation, Dysregulated Fibrinolysis, and Platelet Dysfunction

First, clot formation itself is significantly impaired by the previously mentioned consumption and dysfunction in its key coagulation factors. Excessive activated protein C (APC) is released in an attempt to balance the increased coagulation and inflammation related to major injury (Maegele et al. 2014). Consequently, APC serves to slow down clot formation by inactivating clotting factors Va and VIIIa (Moore and Moore 2020). Damaged endothelium releases glycocalyx glycosaminoglycans which has "heparin-like" activity and leads to endogenous heparinization and blood thinning. Excessive fluid resuscitation exacerbates coagulopathy by diluting the key factors in hemostasis.

Secondly, clot dissolution is impaired via dysregulated fibrinolysis. Fibrinolysis dysregulation is described on a spectrum in trauma-induced coagulopathy (TIC). Hyperfibrinolysis at initial stages leads to premature clot breakdown or dissolution, whereas a phenomenon known as "fibrinolysis shutdown" at later stages of trauma recovery is implicated in increased thrombotic sequelae (Duque et al. 2021). In either situation, the opposite effect of what is wanted leads to pathology – ongoing bleeding or unregulated thrombosis.

Finally, platelet dysfunction also has its role in TIC. Injury-related inflammation renders platelets inactive and unable to aggregate into platelet/fibrin plugs in order to achieve hemostasis (Maegele et al. 2014; Moore and Moore 2020; Duque et al. 2021).

Acidosis, Hypothermia, and Hypocalcemia

It is imperative to note several other additive factors leading to TIC resistant to simple correction with blood products. Acidosis from decreased perfusion and ongoing crystalloid resuscitation, as well as hypothermia, that is associated with hemorrhagic shock, are all implicated in coagulopathy (Townsend et al. 2017). Although controversial, it is believed that certain enzymes of the coagulation cascade require an optimum pH in order to function (Martini 2009; De Robertis et al. 2015). It is not uncommon to see pH drops down to 7.0 in critically injured patients. It is proposed that acidosis impairs propagation of thrombin generation as well as leads to decreased fibrinogen availability by accelerating its degradation (Martini 2009). On the other hand, correction of acidosis alone cannot reverse coagulopathy. Thus, some believe that the coagulation cascade is relatively resistant to pH changes and it is a combination of factors, such as acidosis and hypothermia, that actually leads to the propagation of coagulopathy (Gissel et al. 2016).

Hypothermia also inhibits optimal enzyme activity, propagating coagulopathy by rendering the clotting cascade dysfunctional. In fact, conventional coagulation assessment is carried out on blood samples only after those are warmed to 37 degrees Celsius. Hypothermia leads to decreased platelet aggregation and fibrinogen synthesis (De Robertis et al. 2015).

Finally, hypocalcemia is often observed in trauma patients requiring substantial blood product resuscitation or activation of institutional massive transfusion protocols (Giancarelli et al. 2016). The calcium ion is clotting factor IV, responsible for activation of platelet aggregation and several clotting factors, including factor XIII, which is responsible for stabilizing the fibrin clot (Singh et al. 2019; Barmore and Burns 2021). Low calcium levels will result in further dysfunction of the entire clotting cascade and prevent hemostasis.

Simultaneous consideration of these contributive factors as well as judicious, and appropriately guided use of resuscitation products is vital in the care of a trauma patient. VEM assays provide direction on the imbalance that occurs with TIC, pointing the provider to what part of the clotting mechanism is impaired – clot formation, dysregulated fibrinolysis, or platelet dysfunction.

How Viscoelastic Monitoring Assays Work

Currently, the most common VEM systems available are thromboelastography (TEG, Haemonetics) and thromboelastometry (ROTEM, Tem International GmbH) and, most recently, the Quantra Hemostasis Analyzer (Hemosonics) (Faraoni and Dinardo 2021). This review will focus on the first two technologies given their longer clinical utilization. Traditional systems measure blood's viscoelasticity utilizing rotational clot properties, while newer technologies look at clotting indirectly by measuring blood's harmonic resonance as it transitions from liquid to clot via sonography (Hartmann et al. 2020; Faraoni and Dinardo 2021). TEG and ROTEM are similar but not interchangeable (Sankarankutty et al. 2012; Rizoli et al. 2016; Sakai 2019).Taking advantage of the viscoelastic property of blood, traditional TEG 5000 and ROTEM delta monitor clot formation, maturation, and dissolution as a reflection of the increasing shear modulus of blood when it turns from liquid to gel (Carll and Wool 2020). Shear modulus is the tendency to resist deformation and is affected by the composition, interaction, and function of clot components – fibrin, platelets, and thrombin (Carll and Wool 2020). The majority of clot strength is likely provided by platelets rather than fibrin (Kornblith et al. 2014). Only whole blood samples are used for VMAs allowing for the evaluation of function of all blood components as they interact during hemostasis.

Assessment of shear modulus is approached indirectly in traditional viscoelastic monitoring assays, by measuring rotational clot properties. TEG and ROTEM differ in the reagents used and nomenclature of its analysis; thus they are not completely interchangeable. Neither is believed to be superior in its results (Rizoli et al. 2016). The mechanism of TEG is closest to the original method first described by Dr. Hartrett. A sample of whole blood is placed into a cylindrical cup with a pin suspended in the center on a torsion wire (Fig. 2). The cup slowly rotates in alternating directions with pauses in between, simulating venous flow. As clot forms in the cup, it eventually starts rotating the pin with itself, and the rotational force on the pin in the center of the growing clot is measured and transduced as a reading (Fig. 3). At the beginning, when the blood sample is completely liquid, the reading demonstrates zero deflection. It reaches max deflection at maximum clot strength and slowly returns to zero with onset of clot dissolution or fibrinolysis.

In the ROTEM system, the blood sample is placed in a similar cup and pin mechanism, except for the cup is held stationary while the pin rotates until the developing clot starts restricting its movement (Fig. 2). The restriction of pin movement is inversely proportional to clot strength (Hartmann et al. 2020) and is measured and transduced into a tracing similar to a TEG reading (Fig. 3). Nomenclature for the tracing results of each method will be defined in the analysis section of this chapter.

Both viscoelastic monitoring methods have undergone modifications since they first were introduced. These modifications have improved analysis and allowed for point-of-care use in locations such as the trauma bay. The newest version is TEG 6S, which is able to run several different assays on a blood sample simultaneously. In TEG 6S, blood is exposed to a specific sound frequency. As it undergoes transition

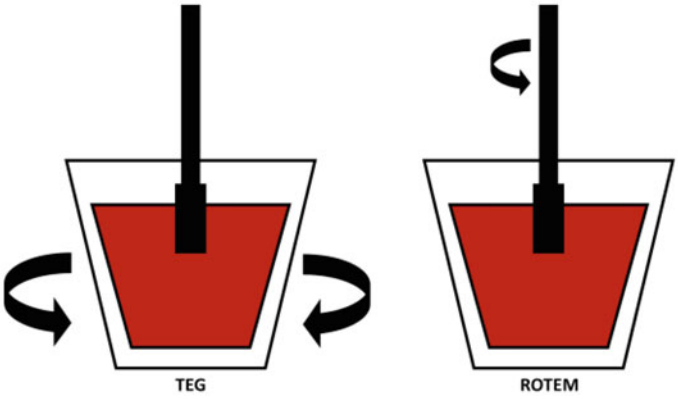

Fig. 2 Types of viscoelastic monitoring mechanisms. In the TEG mechanism, a sample of whole blood is placed into a cup with a pin sensor suspended in the center of the sample. The cup rotates back and forth simulating venous flow as clot forms. In the ROTEM mechanism, the blood sample is also loaded in a similar way. The cup remains stationary, but the pin rotates in the center.

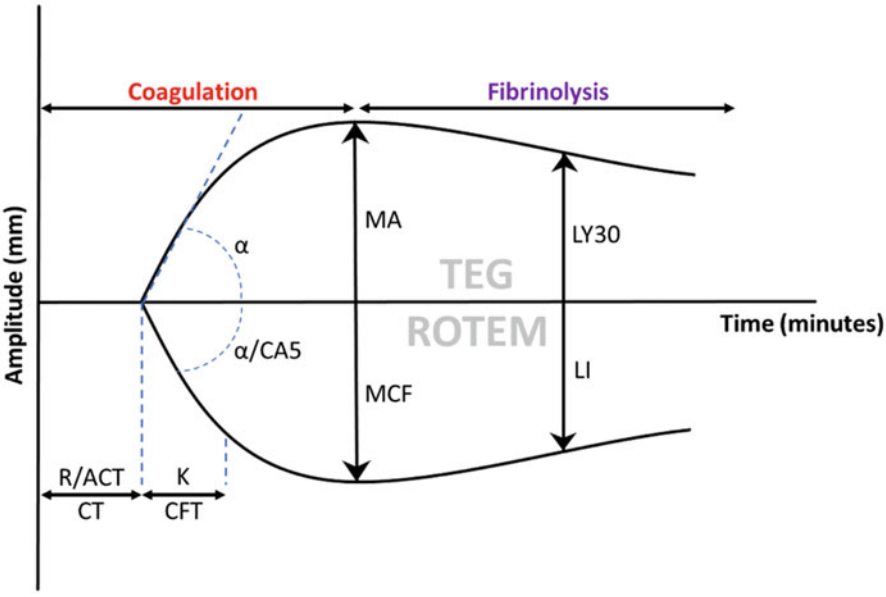

Fig. 3 Viscoelastic tracing as seen for each monitoring method. TEG tracing is depicted on the top and ROTEM on the bottom of the chart. Clot initiation is measured by R/ACT (activated clotting time) in TEG and CT (clotting time) in ROTEM. K time and alpha angle for TEG and CFT (clotting factor time) and alpha/CA5 (clot amplitude at 5 min) are measurements of clot kinetics. MA (TEG; maximum amplitude) and MCF (ROTEM; maximal clot firmness) measure peak clot formation and strength. LY30 (TEG; lysis fraction at 30 min) and LI (ROTEM; lysis index) are measures of fibrinolysis.

from liquid to gel, its increasing resonant frequency is measured, then translated, and reported in a similar tracing. TEG 6S utilizes harmonic resonance for indirect measurement of clot kinetics.

A variety of assays have been developed for each viscoelastic monitoring system (Table 1). The assays are not interchangeable, but the rationale behind each is similar. Intrinsic clotting cascade is activated with either kaolin in TEG or ellagic acid in ROTEM. Heparinase can be added to evaluate the effect of the inhibition of heparin in patients on long-term anticoagulation (i.e., ECMO patients) (Dias et al. 2017). Addition of tissue factor will activate the extrinsic clotting cascade and lead to faster clotting analysis, as with rapid TEG (rTEG). rTEG can be completed within 15 min as compared to conventional TEG that can take up to 30 min for complete results (Da Luz et al. 2013). Isolated fibrinogen contribution to clot strength/stability can be tested by utilizing assays that include the addition of platelet inhibitors (abciximab versus cytochalasin) (Schlimp et al. 2014). Another modification with the TEG 6S system allows for more specific evaluation of platelet function called platelet mapping. Platelet mapping is meant to evaluate clotting kinetics in patients on antiplatelet therapy (Ranucci and Baryshnikova 2020). To isolate platelet function for analysis, clotting is compared simultaneously between three differentially activated blood samples; one activated with kaolin to evaluate full clot activation, one with reptilase and factor XIIIa meant to evaluate clot without platelet activation at all, and one with reptilase, factor XIIIa, and a platelet activator such as arachidonic acid or ADP. The results of this test are not completely validated but available for use in cardiac patients and can be a valuable adjunct in perioperative assessment for cardiac surgery patients on antiplatelet therapy (Cattano et al. 2013).

Understanding VMA Results as They Reflect the Clotting Process

Viscoelastic monitoring tracings assist the clinician with indirect analysis of a patient's clotting factor function, fibrinolysis, and platelet function. VM tracings for TEG and ROTEM are similar (Fig. 3). For TEG, the tracing represents the change

Table 1 Standard reagents and assays for each monitoring system

	TEG		ROTEM	
Features	Assay	Reagents	Assay	Reagents
Activates intrinsic clotting cascade	CK	Kaolin	INTEM	Ellagic acid
Patient on chronic anticoagulation?	CKH	Kaolin + heparinase	HEPTEM	Ellagic acid + heparinase
Faster analysis ≤15 min	Rapid TEG (rTEG)	Kaolin + tissue factor	EXTEM	Tissue factor + heparinase
In vitro inhibition of fibrinolysis			APTEM[a]	EXTEM + Aprotinin/ tranexamic acid
Isolate fibrinogen function	Functional fibrinogen (FF)	Tissue factor + abciximab	FIBTEM	EXTEM + cytochalasin

[a]Results are compared to EXTEM for evaluation of fibrinolysis

Table 2 Comparison of TEG and ROTEM parameters, primary blood component responsible, and proposed treatment if there is a derangement

Measurement	TEG	ROTEM	Blood components	Treatment
Time to initiation of clot formation (2 mm deflection above baseline)	R	CT	Coagulation factors	Fresh frozen plasma
Time to achievement of certain clot firmness (2 to 20 mm deflection)	K	CFT	Fibrinogen	Cryoprecipitate
Rate of clot formation or strengthening (angle)	α	α/CA5	Fibrinogen	Cryoprecipitate
Time at maximum clot strength	MA	MCF	Platelets	Platelets
Degradation of clot or lysis at 30 min after max (% amplitude)	LY 30	LI	Plasmin	Antifibrinolytics (i.e., tranexamic acid)

in maximal oscillating torque of the suspended pin, while for ROTEM, the tracing follows change in extent of oscillation of the rotating pin (Carll and Wool 2020; Faraoni and Dinardo 2021). Parameters derived from these tracings allow characterization of clot initiation, propagation, and dissolution overtime. Table 2 lists comparable parameters in both systems.

Time to initial clot formation, as detected by the time of initial tracing deflection from zero, is reported as the R-time in TEG, ACT in rapid TEG, and clotting time (CT) in ROTEM. Clot initiation is primarily a reflection of the function of clotting factors and the presence of anticoagulation in blood.

Clot propagation or rate of clot development is reported as alpha angle for both systems. Clot propagation is reflective mainly of fibrinogen deposition.

Maximal clot strength is achieved at maximal amplitude (MA) for TEG and maximal clot firmness (MCF) for ROTEM. Platelet function is primarily responsible for this variable.

The final parameter for each system reflects fibrinolysis, Ly30 for TEG, and lysis index (LI) for ROTEM and is controlled by plasmin levels. It is derived as a percent decrease from maximal clot strength, MA for TEG or percent of MCF remaining for ROTEM at 30 minutes.

The presence of anticoagulation will augment the timing of all parameters.

VEM-Guided Trauma Resuscitation

Traditionally, TIC is defined by an INR greater than 1.2, but likely is more predictive of mortality and associated complications above 1.5 (Peltan et al. 2015). While conventional coagulation tests can recognize global coagulopathy, their values do not specify which component of the clotting mechanism is dysfunctional or depleted and requires repletion. Viscoelastic hemostatic assays, on the other hand, attempt to provide a goal-directed transfusion strategy for management of bleeding trauma patients. This allows for guided transfusion of appropriate blood products and

Table 3 VMA-guided transfusion triggers as recommended by ACS trauma quality improvement program massive transfusion guidelines

Product for Transfusion	Standard TEG	Rapid TEG	ROTEM
Plasma	R-value >9 min	ACT >128 s	CT EXTEM>100 s CT INTEM>230 s
Plasma/ cryoprecipitate	K-time > 4 min	K-time > 2.5 min	MCF FIBTEM <8 mm
Cryoprecipitate/ plasma	Alpha-angle <60 deg	Alpha-angle <60 deg	
Platelets	MA <55 mm	MA <55 mm	MCF EXTEM <45 mm MCF FIBTEM >10 mm
Anti-fibrinolytics	LY30 > 7.5%	LY >3%	LI EXTEM >15%

diminishes unnecessary product resuscitation. Table 3 provides the American College of Surgeons Trauma Quality Improvement Program (ACS**TQIP (ACS Best Practices Guidelines)**) recommendations for transfusion triggers for a patient undergoing massive transfusion if VHAs are available.

Currently, massive transfusion protocols use various criteria to identify trauma patients who may require large amounts of blood products. These criteria include blood pressure, blood pressure changes, heart rate, and blood pH among others (Semon and Cheatham 2014). In particular, hypotension tends to be a late finding in adults and even later finding in the pediatric patient. So rTEG's rapid identification of patients requiring transfusion can be extremely useful (Cotton et al. 2011; Vogel et al. 2013). Furthermore, as discussed above, they utilize various ratios of blood products, most commonly 1:1:1 across various traumatic injuries. This one-size-fits-all approach can likely be improved upon and optimized for different clinical presentations and patients. While it does not produce a complete picture of coagulopathy, rTEG allows for a more comprehensive picture than CCTs, and there is some evidence that it can improve outcomes for patients undergoing MTP (Tapia et al. 2013).

Limitations

Although VHAs are being adopted into trauma transfusion algorithms, there is still insufficient literature-based evidence to support their benefit or superiority to conventional tests (CCTs). A Cochrane review from 2015 attempted to determine the benefit of VHAs over CCTs in diagnosis of TIC but was only able to identify three studies that compared the diagnostic accuracy of VHAs to CCTs (all studies used ROTEM, the primary VHA method). The review was unable to establish diagnostic superiority between the methods (Hunt et al. 2015). To address this gap in literature, a large, multi-institutional randomized controlled trial has just concluded in Europe. **iTACTIC** compared outcomes in trauma patients whose MTP was guided either by

Table 4 iTACTIC algorithm-recommended transfusion triggers (Baksaas-Aasen et al. 2021)

Recommended blood component for transfusion	TEG	ROTEM
Plasma (4 units)	rTEG MA $\geq$ 65 mm AND ACT >120 s	EXTEM CA5 $\geq$ 40 mm AND CT > 80 s
Cryoprecipitate (equivalent of 4 g of fibrinogen)	FF TEG MA < 20 mm	FIBTEM CAS < 10 mm
Platelets (1 pack of platelets)	rTEG MA – FFTEG MA < 45 mm	EXTEM CA5 – FIBTEM CA5 < 30 mm
Antifibrinolytics (1 g TXA)	rTEG LY30 > 10%	EXTEM LI < 85%

CCTs or VHAs, with Table 4 showing the transfusion algorithm triggers used (Baksaas-Aasen et al. 2021). No difference in mortality or other outcomes were identified between the two groups. The VHA group received more transfusion interventions. This is in contrast to the reported decrease in transfusion reported in cardiac surgery and liver transplantation with VHA-guided transfusions (Wang et al. 2010; Meco et al. 2020). VHAs, however, were not obtained in the CCT group, and therefore, the comparison is not clearly similar. Yet, it can be inferred that CCTs may miss certain coagulation deficits that VHAs are able to identify. The value to correcting these specific deficits is yet to be defined. Interestingly, a subgroup of patients with severe TBI had lower 28-day mortality when their MTP was augmented with VHAs versus CCTs (Baksaas-Aasen et al. 2021). Perhaps, this was a chance finding, but according to the authors, this provides another niche for future research given early-guided coagulation correction may decrease brain injury mortality (van Gent et al. 2020).

Apart from lack of clear superiority of VHAs, there are other limitations to consider. The growing number of assays and methods for running VHAs adds difficulty to operation and interpretation of the results. As previously noted, although both TEG and ROTEM measure clot kinetics, their nomenclature varies. Additionally, results will vary depending on the type of blood sample used, and variation in results have even been reported based on gender, age, presence of anticoagulant or antiplatelet therapy, and alcohol use (Da Luz et al. 2013). Results from VHAs are potentially available quicker than CCTs, but this is highly dependent on the type of assay run, type of blood sample (preferably native, non-citrated, within 4 min of collection), and operator/machine readiness (Da Luz et al. 2013; Curry et al. 2018). Furthermore, the reported benefit of identifying platelet dysfunction or fibrinolysis dysregulation is often only available with a specific assay. Thus, efficacy and utility of VHAs are highly dependent on the resources available at an institution.

While VHAs aim to evaluate clot formation in a more comprehensive manner than CCTs (use of a whole blood sample), the mechanism behind its data is far from the original in vivo environment of a damaged blood vessel. The VHAs do not account for all of the systemic influence of inflammatory signaling. Finally, the proposed transfusion thresholds vary among VHA-guided algorithms. It may be obvious what blood products need to be transfused when VHA's variable levels are significantly different compared to normal; it may be difficult to justify treatment/

transfusion when the change is minor (i.e., within 1–5 values). Further studies are needed to identify clear, universal thresholds for transfusion. While VHAs have the potential to provide a variety of information to help guide resuscitation and reverse coagulopathy in trauma, its use alone is not yet validated, and there is still no universal algorithm or resource availability.

Predicting Hypercoagulability

Apart from the apparent need for management of coagulopathy to control bleeding in the initial stages following a trauma, another role is emerging for VHAs monitoring later in the recovery timeline. Patients surviving major trauma have up to 58% chance of developing venous thromboembolism (VTE) (Yumoto et al. 2017). Observations have been made showing correlation between VHA results and their ability to predict ongoing and future hypercoagulability. TEG MA $>$ 65 has been found to be an independent predictor of pulmonary embolism (PE) in adults (Cotton et al. 2012). Use of VHAs to predict VTE risk may be appropriate in certain patient populations. It is important to note, however, VHAs have not been proven to be useful in monitoring anticoagulation efficacy. Nor are they able to identify patients who are on chronic antiplatelet therapy either.

Future Directions

Timely and appropriate management of trauma-induced coagulopathy can significantly improve a severely injured patient's survival. Viscoelastic hemostatic assays have proven beneficial in decreasing transfusion requirements and improving outcomes in cardiac surgery and liver transplantation. Similar benefits have been expected in trauma, but are still being studied. With their potential for quicker results, expanded analysis of clot kinetics, and availability as a point of care test, VHAs are becoming a prominent option to guide trauma resuscitation. Furthermore, VHAs can provide information to guide transfusion requirements, predicting value of transfusion of specific blood products. While promising, VHAs have not been proven superior to conventional coagulopathy testing and cannot be depended on alone for resuscitation of a patient after major trauma (Wikkelsø et al. 2016). Additionally, the wide variety of testing platforms and available assays complicates the ability to create a universal protocol/algorithm to prove utility of VHA-guided trauma resuscitation. With such current understanding, VHAs should be considered as another tool or piece of puzzle for the trauma team to incorporate into their kit when available when managing a trauma patient. This diagnostic medium is still in its infancy in trauma patients. However, with future research, these tests could represent a transition to a more personalized, patient-centric method of resuscitation which could optimize treatment within the wide range of traumatic injuries for diverse populations.

Applications to Other Diseases or Conditions

In this chapter, we review the utility of viscoelastic monitoring (VEM) for management of coagulopathy, transfusion, and resuscitation in a traumatically injured adult patient. VEM assays provide an alternative to conventional coagulation tests, with the advantage of quicker results and with the ability to provide specific information regarding clot formation and fibrinolysis. This coagulopathy monitoring method has not only proved valuable in the trauma bay, but also intraoperative and ICU monitoring of the transplant and cardiothoracic patient (Khalaf-Adeli et al. 2019; Meco et al. 2020; Rali et al. 2020; Selby 2020). Additionally, within recent years, use of VEM has been expanded to the pediatric patient population (Phillips et al. 2021).

Mini-dictionary of Terms

- **K/CFT:** Time to achievement of certain clot firmness (from 2 mm to 20 mm amplitude deflection) on the viscoelastic monitoring tracing, reflective of fibrinogen function.
- **LY 30/LI:** Percent of clot lysis 30 min after maximum clot strength is reached, related to the efficiency of fibrinolysis.
- **MA/MCF:** Time when clot reaches its maximum strength and maximum amplitude, reflective of platelet function.
- **R/CT:** Measurement in VHAs, time to initial significant clot formation (2 mm deflection in amplitude on the viscoelastic monitoring tracing), reflective of clotting factor function.
- **Viscoelasticity:** The property of blood, exhibiting both viscous and elastic characteristics, as it is undergoing rotational deformation, mimicking the clotting process, measured by TEG and ROTEM.
- **α:** Rate of clot formation after initiation, reflective of fibrinogen function.

Key Facts of Trauma-Induced Coagulopathy (TIC)

- Early coagulopathy in trauma is related to significant mortality.
- Severity of injury as measured by ISS is associated with increased risk of coagulopathy development.
- Traditionally defined as meaningful when INR >1.2, TIC has multifactorial pathogenesis and lacks an all-encompassing definition to guide timely correction at bedside.
- TIC is driven by impaired clotting factor function, dysregulated fibrinolysis, and platelet dysfunction.
- Additionally, factors such as acidosis and hypothermia contribute to exacerbating trauma-induced coagulopathy.

Key Facts of Viscoelastic Hemostatic Assays

- VEM assays provide information to assess platelet function, rate of clot formation, clot strength, stability, and dissolution.
- Assessment of shear modulus is approached indirectly in traditional viscoelastic monitoring assays like TEG and ROTEM, by measuring rotational clot properties.
- Only whole blood samples are used for VMAs allowing for the evaluation of function of all blood components as they interact during hemostasis, unfortunately not accounting for the in vivo endothelial environment.
- Time to initial clot formation, as detected by the time of initial tracing deflection from zero to 2 mm, is reported as the R-time in TEG, ACT in rapid TEG, and clotting time (CT) in ROTEM.
- Rate of clot development is reported as alpha angle or CA5.
- Maximal clot strength is measured at maximal amplitude (MA) for TEG and maximal clot firmness (MCF) for ROTEM.
- Ly30 for TEG and lysis index (LI) for ROTEM is reflective of fibrinolysis, reported as a percent decrease from MA for TEG or percent of MCF remaining for ROTEM at 30 min.

Summary Points

- VEM assays are being incorporated into transfusion algorithms across various specialties and are becoming more prevalent as a guiding method for trauma resuscitation.
- Viscoelastic monitoring tracings provide indirect analysis of a patient's clotting factor function, fibrinolysis, and platelet function.
- Traditional VEM systems measure blood's viscoelasticity utilizing rotational clot properties, while newer technologies look at clotting indirectly by measuring blood's harmonic resonance as it transitions from liquid to clot via sonography.
- A variety of assays have been developed for each viscoelastic monitoring system, with goal of targeting specific components of blood and their function in coagulation.
- Viscoelastic hemostatic assays have proven beneficial in decreasing transfusion requirements and improving outcomes in cardiac surgery and liver transplantation and the potential to provide similar results in the trauma population.

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Biomarkers of Cardiac Stretch in Critical Illness: A Narrative Review

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Dilip Jayasimhan and Robert J. Hancox

Contents

Abstract

Biomarkers of cardiac stretch, particularly natriuretic peptides, are a fundamental component in the diagnosis and management of cardiac dysfunction in many clinical settings. The utility of these cardiac biomarkers have become of interest in critically ill and could play a role in predicting adverse outcomes and prognosticating in a number of cardiac and extracardiac conditions in intensive care. In this chapter, we review the evidence for the clinical utility of biomarkers of cardiac stretch in different critical illness syndromes. We also review the potential

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mechanisms that may explain the association between elevated biomarkers of cardiac stretch and adverse outcomes.

Keywords

Biomarkers of cardiac stretch · Natriuretic peptides · Critical illness · Cardiac surgery · ARDS · Sepsis · Trauma · Prognostication · Predictive utility

Abbreviations

ANP	Atrial natriuretic peptide
ARDS	Acute respiratory distress syndrome
BNP	Brain natriuretic peptide
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
EuroSCORE	European System for Cardiac Operative Risk Evaluation
ICU	Intensive care unit
MR-proANP	Midregional pro-atrial natriuretic peptide
NT-proANP	N terminal pro-atrial natriuretic peptide
NT-proBNP	N terminal pro-brain natriuretic peptide
pg/ml	Picograms per milliliter
RAAS	Renin-angiotensin-aldosterone system
RV	Right ventricle
SARI	Severe acute respiratory infection
TBI	Traumatic brain injury
VA ECMO	Venoarterial extracorporeal membrane oxygenation
VV ECMO	Venovenous extracorporeal membrane oxygenation

Introduction

The use of biomarkers in human disease has been vital in advancing diagnostic and treatment paradigms. Biomarkers in critical illness have been slow to gain traction due to the varied conditions causing critical illness and clinical syndromes such as sepsis and acute respiratory distress syndrome that are heterogeneous in presentation and response to treatment (Vincent et al. 2020). Identifying patients with a high baseline risk of mortality may be useful in clinical management and prognostic enrichment in clinical research. A single biomarker that is clinically important, easily obtainable, and able to provide prognostic value remains elusive.

Biomarkers of cardiac stretch offer promising results in prognostication of patients with critical illness presenting with cardiopulmonary disease and multi-system clinical syndromes such as sepsis. Initially used in the diagnosis and prognosis of heart failure, their applications have expanded to many other disease states that have flow-on effects on cardiorespiratory function.

This chapter aims to review the utility of biomarkers of cardiac stretch known as natriuretic peptides, their pathogenesis in health and disease, and their application in prognostication of critically ill patients.

Biomarkers of Cardiac Stretch

Biomarkers of cardiac stretch and their natriuretic properties were initially discovered in the 1980s with atrial natriuretic peptide, followed by B-type natriuretic peptide not long after (Potter et al. 2009). ANP is released from the atrial granules in response to the atrial wall stretch caused by intravascular volume. Its role in plasma volume regulation is through three mechanisms: increased renal salt and water excretion, vasodilation, and increased vascular permeability (Curry 2005).

BNP initially sequenced and purified from extracts of the porcine brain was found in greater concentrations in the ventricles of both animals and humans. Unlike ANP, which is stored in granules, BNP is transcribed in response to cardiac stretch associated with volume overload. The prohormone Pro-BNP is released in response to volume overload and cleaved to BNP and the biologically inactive NT-proBNP. Studies have also shown BNP to be released from the pulmonary vasculature in response to stress and hypoxic pulmonary vasoconstriction (Cargill and Lipworth 1995). BNP release also seems to be associated with pulmonary leakage (Bayes-Genis et al. 2004). Much like ANP and other natriuretic peptides, BNP's role is in natriuresis, peripheral vasodilatation, and inhibition of the renin-angiotensin-aldosterone system (Weber and Hamm 2006).

Natriuretic Peptides in Diagnosis and Prognosis of Heart Failure

These peptides can be measured in circulating serum using multiple different assays. Given the role natriuretic peptides have in response to cardiac stretch, congestion, and volume overload, they have been studied as a biomarker to both diagnose and prognosticate in heart failure. A systematic review and meta-analysis has shown MRproANP, BNP, and NT-proBNP to have a sensitivity of 95%, 95%, and 99%, respectively, in the diagnosis of heart failure in an acute care setting (Roberts et al. 2015). Plasma BNP and NT-proBNP have also shown prognostic value in patients with stable chronic heart failure (Oremus et al. 2014) and acute decompensated heart failure (Santaguida et al. 2014). In multivariable models, BNP and NT-proBNP are the strongest independent predictor of death or adverse cardiovascular events (Doust et al. 2005). The ADHERE registry study showed an increasing risk of mortality in each quartile of BNP levels for patients admitted with acute decompensated heart failure. These findings were independent of other clinical and laboratory variables (Fonarow et al. 2007). As for NT-proBNP, another study showed that for every doubling of NT-proBNP, the hazard ratio for death increases by a factor of 1.56

(Schou et al. 2007). These studies have led to the widespread use of BNP and NT-proBNP in the assessment and management of patients with heart failure in both the acute and outpatient setting.

The elevation in natriuretic peptides in heart failure, their prognostic significance, and their interaction in opposition to RAAS has led to an interest in targets for treatment in patients with heart failure. This culminated in the use of neprilysin inhibitors in heart failure, and the PARADIGM-HF randomized controlled trial using this in combination with an angiotensin-II receptor blocker (McMurray et al. 2014). A cut-off value of $\geq$150 pg/ml for BNP or $\geq$ 600 pg/ml for NT-proBNP levels was part of the inclusion criteria for this landmark study.

Natriuretic Peptides in Multisystem Critical Illness

Sepsis

Sepsis is defined as life-threatening organ dysfunction brought on by a dysregulated host response to infection (Singer et al. 2016). It is a leading cause of morbidity and mortality among ICU patients and is heterogeneous in its presentations and phenotypes. Circulatory failure with or without cardiac dysfunction is commonly observed in patients with sepsis and is associated with higher mortality rates. Natriuretic peptides such as BNP and NT-proBNP have been assessed for their prognostic utility in these patients. A systematic review of 12 studies showed elevated natriuretic peptides to be associated with an increased odds of mortality (OR 8.96 CI 4.99–21.58) (Wang et al. 2012). There was variation in the timing of measurement and cut-offs used between the studies included. A similar meta-analysis performed in 2020 analyzed 35 studies with 3508 patients and found a cut-off of 622 pg/ml for BNP and 4000 pg/ml for NT-pro-BNP having the highest discrimination for mortality (Vallabhajosyula et al. 2020). Aside from predicting mortality, an elevated BNP and NT-proBNP are also predictive of the need for renal replacement therapy in those with sepsis and predict longer-term physical disability and loss of muscle strength. Serial measurements of BNP have been shown to predict the resolution of septic shock and favorable outcomes in those who have a rapid fall in values. However, the indication of a serial decline in natriuretic peptides is not a consistent finding among studies. In a retrospective analysis of the ALBIOS trial assessing the use of albumin in sepsis, a rise in BNP in the intervention arm was predictive of favorable outcomes (Masson et al. 2016). Other studies assessing the utility of natriuretic peptides in guiding treatment include a reanalysis of the LEOPARDS trial evaluating the use of levosimendan in septic shock (Antcliffe et al. 2019). A prespecified subgroup analysis showed that levosimendan did not improve outcomes such as mortality or improvement in sequential organ failure assessment scores in patients with an elevated baseline NT-proBNP.

Although the cause of elevation in natriuretic peptides in patients with sepsis has been hypothesized to be due to significant congestion and septic cardiomyopathy, studies have not consistently shown a strong correlation between hemodynamic and echocardiographic markers of LV congestion and dysfunction and BNP levels (Papanikolaou et al. 2014). A study showed that peak ANP and BNP levels correlate strongly with the degree of left ventricular dysfunction in patients with sepsis (Witthaut et al. 2003), while other studies have shown BNP levels were elevated in patients without echocardiographic evidence of left ventricular dysfunction and correlates with the degree of inflammation as measured by C-reactive protein (Shor et al. 2006). There is also a strong correlation between the need for vasopressor and peak dose with BNP levels, suggesting BNP release may be multifactorial and related to the neurohormonal compensatory mechanisms involved in septic shock (Papanikolaou et al. 2014).

Major Trauma

Although direct cardiac trauma is not a prevalent finding among patients admitted to the ICU following major trauma, cardiac dysfunction and elevated biomarkers of cardiac stretch such as natriuretic peptides have been noted in these patients. A small study showed that in patients with major trauma, NT-proBNP and NT-proANP levels were significantly higher in those with multiple organ dysfunction compared to those without multiple organ dysfunction (Li et al. 2015). There was also a significant negative correlation between natriuretic peptide levels with cardiac indices up to 72 hours after the injury. NT-proBNP and NT-proANP were superior in diagnosing multiple organ dysfunction syndrome compared to other biomarkers such as CRP and white blood cell count. Findings of an elevated BNP in trauma patients is common but does not always correlate with left ventricular dysfunction and may represent release of BNP in the brain following injury. Another study showed elevated BNP levels in patients without heart failure and did not correlate with echocardiographic findings of left ventricular systolic dysfunction (Stewart et al. 2007). Although findings between natriuretic peptide levels and echocardiographic findings have not been consistently correlated, natriuretic peptides may still indicate the presence of subclinical heart failure or impaired diastolic function. Zapata and colleagues showed patients with elevated NT-proBNP had higher prevalence of LV diastolic dysfunction in the form of impaired relaxation, pseudonormal, or restrictive patterns of diastology (Zapata et al. 2014). In terms of the ability of natriuretic peptides in predicting mortality, dynamic changes in NT-proBNP over 7 days in patients with major trauma predicted in-hospital mortality (Qian et al. 2015).

Overall, the evidence supporting the use of natriuretic peptides in predicting adverse outcomes in patients with major trauma is promising but remains limited.

Natriuretic Peptides in Cardiac Critical Illness

Cardiac Surgery

Although postoperative morbidity and mortality of patients undergoing cardiac surgery has improved over the decades, there remains a group of patients at risk of adverse outcomes. The use of preoperative risk scores attempts to identify this subgroup of patient but do not account for changes in the immediate postoperative period and may be imprecise in certain scenarios. A biomarker such as natriuretic peptides is attractive in being able to identify patients at risk of adverse outcomes and its utility has been assessed in multiple studies. A number of studies have shown both preoperative and immediate postoperative NT-proBNP levels are predictive of adverse outcomes post cardiac surgery (Mahla et al. 2007; Abdel-Aleem et al. 2021).

Whether natriuretic peptide levels improve prognostication above preoperative risk scores remains debated. This may differ based on the natriuretic peptide test being assessed. Studies have shown that preoperative NT-proBNP predicts adverse outcomes independent of baseline risk scores such as the European System for Cardiac Operative Risk Evaluation (EuroSCORE) and the Parsonnet Score (Cuthbertson et al. 2009; Brynildsen et al. 2018) and have areas under the receiver operating characteristic curve predicting adverse outcomes that are comparable to such scores (Elíasdóttir et al. 2008). An analysis of RIPOSTE database showed that preoperative BNP levels do not improve prognostication above EuroSCORE II in patients undergoing cardiac surgery (Suc et al. 2020).

NT-proBNP levels may have value in predicting specific postoperative adverse events. Postoperative atrial fibrillation is associated with increased mortality in patients undergoing cardiac surgery (Eikelboom et al. 2021), and immediate postoperative prophylaxis may be associated with better outcomes (Burgess et al. 2006). However these medications are also associated with adverse effects such as hypotension and bradycardia. Hence, identification of high-risk patients is of great value, and natriuretic peptides may have a role in this function (Samy et al. 2012). Preoperative NT-proBNP levels also predict the incidence of postoperative acute kidney injury (Wang et al. 2021) and correlates with cerebral oxygen saturations (Mukaida et al. 2017) in this group of patients.

In summary, NT-proBNP levels are a good predictor of general and specific postoperative complications post cardiac surgery and may have value in addition to traditional preoperative risks scores.

Cardiac Arrest and Cardiogenic Shock

Natriuretic peptides have a role in the management and prognostication of patients post cardiac arrest and cardiogenic shock. Several studies have shown that BNP and NT-proBNP predict mortality up to 30 days or hospital discharge (Nagao et al. 2007; Platek et al. 2015; Smit et al. 2015) as well as time to shock resolution (Langeland et al. 2022) in patients who have been admitted following a cardiac arrest. Whether

these findings are independent of other baseline variables remains unknown as a study in 2015 showed that although elevated baseline NT-proBNP levels are associated with an increased risk of death at 30 days, this association is not significant when adjusting for other factors such as age, creatinine, and presence of ventricular fibrillation as the primary rhythm (Aarsetøy et al. 2020).

In patients with cardiogenic shock, natriuretic peptides are an attractive marker for prognostication. NT-proBNP levels predict 30-day mortality in patients admitted with cardiogenic shock following acute myocardial infarction (Jarai et al. 2009; Sharma et al. 2020). BNP levels may have a role in predicting no-reflow phenomena post revascularization in patients with acute myocardial infarction (Diao et al. 2021). In patients with cardiogenic shock weaned off extracorporeal membrane oxygenation, BNP levels predicted 6-month mortality (Kim et al. 2020). BNP may also be useful in ruling out cardiogenic shock in patients presenting with undifferentiated shock admitted to ICU (Tung et al. 2004). In patients with acute pulmonary edema, NT-proBNP levels predict failure of noninvasive ventilation and the need for endotracheal intubation (Luo et al. 2017).

In summary, natriuretic peptides show promise in prognostication of patients post cardiac arrest and cardiogenic shock although the current evidence base consists of small observational studies. Areas of future research include large prospective studies to confirm current findings, as well as the use of natriuretic peptides to guide management.

Natriuretic Peptides in Respiratory Critical Illness

SARI, ARDS, and Mechanical Ventilation

With the advent of the coronavirus disease (COVID-19) pandemic, there has been great interest in the prognostic value of natriuretic peptides in predicting outcomes among patients with severe COVID-19. A systematic review and meta-analysis of 18,856 patients admitted with COVID-19 showed BNP or NT-proBNP levels were significantly higher in non-survivors than in survivors (Zinellu et al. 2021). There was however significant heterogeneity observed between studies included. NT-proBNP levels correlate with acute cor pulmonale, pulmonary hypertension, and right ventricular-pulmonary arterial uncoupling in patients with COVID-19 (D'Alto et al. 2020; Tuo et al. 2021; Norderfeldt et al. 2021) which may explain the underlying pathophysiology for these levels and their association with mortality.

The prognostic value of natriuretic peptides is not limited to COVID-19-associated ARDS. In patients requiring VV ECMO, NT-proBNP independently predicts survival in COVID-19 (Zayat et al. 2021) and non-COVID-19 (Kaestner et al. 2018) related severe respiratory failure. A meta-analysis of patients admitted with ARDS showed that elevated levels of BNP or NT-proBNP were associated with 8.98 increased odds of death up to 60 days (Jayasimhan et al. 2021). Studies included however used different cut-off values to define an elevated level. Natriuretic peptides

are also associated with increased mortality in patients with community-acquired pneumonia (Chang et al. 2013; Akpınar et al. 2019; Huang et al. 2021).

In patients weaning from mechanical ventilation, natriuretic peptides may have a role in predicting weaning failure. A meta-analysis showed that BNP or NT-proBNP levels measured prior to or after a spontaneous breathing trial in patients being weaned from mechanical ventilation showed good sensitivity and specificity in predicting failure to wean from mechanical ventilation (Liu et al. 2021). However, studies included had different cut-offs for BNP and NT-proBNP levels, and there was significant heterogeneity between studies included. In patients who pass a spontaneous breathing trial, another meta-analysis showed the difference in BNP or NT-proBNP levels before and after a spontaneous breathing trial still reliably predicted failure to wean from mechanical ventilation suggesting it adds predictive value in addition to a spontaneous breathing trial (Deschamps et al. 2020).

Pulmonary Vascular Disease

Natriuretic peptides have shown great prognostic and predictive value in patients with pulmonary vascular diseases. In patients with acute pulmonary embolism, meta-analyses have shown that elevated NT-proBNP levels predict all-cause mortality and serious adverse events including the need for thrombolysis (Sanchez et al. 2008; Lega et al. 2009). A small single-center study showed that NT-proBNP along with imaging features predict the need for surgical embolectomy among patients with high-risk pulmonary embolism necessitating VA ECMO (Ghoreishi et al. 2020). Current guidelines recommend the use of NT-proBNP in risk-stratifying stable patients with acute PE as high levels are indicative of RV dysfunction and may require close monitoring and consideration for thrombolysis if there is evidence of shock (Konstantinides et al. 2020).

Natriuretic peptides have also become a crucial biomarker in the assessment and management of patients with pulmonary arterial hypertension. Current guidelines incorporate NT-proBNP levels in risk scores that predict medium- to long-term mortality and help determine response to treatment with pulmonary vasodilator therapy, as well as the need for treatment escalation (Chin et al. 2019). In a single-center study, NT-proBNP levels in addition to other parameters have shown prognostic value in predicting mortality in patients with decompensated pulmonary arterial hypertension admitted to the intensive care unit (Garcia et al. 2021).

In pulmonary vascular diseases, elevations in natriuretic peptides likely represent the degree of right ventricular-pulmonary arterial decoupling and right ventricular dysfunction which is strongly associated with mortality (Gan et al. 2006).

In summary, natriuretic peptides play a crucial role in the short- and long-term prognostication as well as ongoing management of patients presenting with critical illness associated with pulmonary vascular diseases.

Airways Disease

In patients with airways disease such as chronic obstructive pulmonary disease, biomarkers of cardiac stretch such as natriuretic peptides have prognostic value in patients experiencing exacerbations (MacDonald et al. 2016). Multiple cohort studies have shown natriuretic peptides increase during exacerbations, and the degree of elevations correlate with risk of mortality in the short and long term (Chang et al. 2011; Gale et al. 2011; Høiseth et al. 2012). Elevated natriuretic peptides also predict adverse outcomes such as the need for admission to the ICU (Stolz et al. 2008). The mechanism for elevation in natriuretic peptides is likely multifactorial due to a combination of a high prevalence of cardiac comorbidities in patients with COPD, inflammatory response predisposing to ischemic heart disease, and pulmonary hypertension with right ventricular dysfunction due to chronic lung disease.

In patients requiring lung resection, perioperative BNP levels predict both short- and long-term complications postoperatively. Two studies showed that an increase in BNP levels postoperatively predict cardiopulmonary complications in the early postoperative period (Cagini et al. 2014; Lafferty et al. 2020). Elevated preoperative BNP levels also predict the likelihood of new postoperative atrial fibrillation in those undergoing thoracic surgery (Nojiri et al. 2010; Amar et al. 2012). In terms of long-term outcomes, preoperative BNP levels predict deterioration in functional capacity post lung resection (Young et al. 2019). These studies did not however control for other common predictors of postoperative adverse outcomes such as age, comorbidities, preoperative lung function, or peak oxygen consumption obtained through cardiopulmonary exercise testing.

Natriuretic Peptides in Non-cardiorespiratory Illness

The prognostic utility natriuretic peptides have been assessed in non-cardiorespiratory critical illness. In renal disease, it is hypothesized that elevations of natriuretic peptides may be indicative of vascular congestion and may play a role in predicting the need for renal replacement therapy. Studies assessing this association have shown natriuretic peptide levels are predictive of the need for renal replacement therapy in critically ill patients (Chou et al. 2015). Improvements in natriuretic peptide levels in patients receiving renal replacement predict renal recovery and ability to wean from renal replacement therapy (Han et al. 2016; Fiorentino et al. 2019). As natriuretic peptides predict postoperative atrial fibrillation and mortality following cardiac surgery, the association is likely present in those undergoing non-cardiac surgery as well (Chong et al. 2010; Álvarez Zurro et al. 2016; Chokengarmwong et al. 2017). In burns patients, a combination of NT-proBNP with procalcitonin predicted catheter-related blood stream infection (Zhou et al. 2018) and death (Lindahl et al. 2013).

In patients with traumatic brain injury, natriuretic peptides rapidly rise following injury, and high levels are associated with higher severity of TBI, elevated intracranial pressures, cerebral vasospasm, and a higher risk of death (Sviri et al. 2003, 2006;

Wu et al. 2011). In acute ischemic stroke and subarachnoid hemorrhage, these associations are independent of age, illness severity scores, and cardiac imaging findings (Maruyama et al. 2017; McAteer et al. 2017).

The mechanism for elevations in natriuretic peptide in non-cardiorespiratory critical illness likely relates to the prevalence of cardiorespiratory comorbidity in patients experiencing critical illness as well as the sequelae these illnesses have on the cardiorespiratory system. In renal disease, a rise in natriuretic peptides may represent both an increased vascular congestion, neurohormonal stress response, as well as a lack of clearance of these peptides due to a drop in glomerular filtration rate (Honore et al. 2020). In patients with neurological injury, natriuretic peptides may rise as a result of local release of natriuretic peptides found in brain tissue (Ru et al. 2021) or neurocardiac interactions resulting in stunned and stretched myocardium due to sympathetic hyperactivity (Kolin and Norris 1984; Oppenheimer 1994).

Mini Dictionary of Terms

Airways disease: disease of the respiratory system characterized by chronic airflow limitation as defined by clinical features and spirometric evidence of airflow limitation.

Natriuretic peptides: Neurohormonal peptides released in response to cardiac stretch that activate compensatory mechanisms inclusive but not limited to natriuresis.

Sepsis: Life-threatening organ dysfunction brought on by a dysregulated host response to infection.

VV ECMO: A form of life support that provides oxygenation of the blood outside the body.

VA ECMO: A form of life support that provides blood flow outside the body to supplement or replace native cardiac output.

Key Facts of Natriuretic Peptides

- Natriuretic peptides are protein molecules released predominantly from cardiac tissue in response to cardiac stretch.
- Natriuretic peptides are also found to be released in lung tissue in response to hypoxic pulmonary vasoconstriction and brain tissue in response to injury in smaller amounts.
- Circulating levels of natriuretic peptides can be measured and correlate with adverse outcomes and mortality in patients with heart failure.
- Elevated natriuretic peptide levels are also noted in patients with critical illness with or without clinical evidence of heart failure or fluid overload.

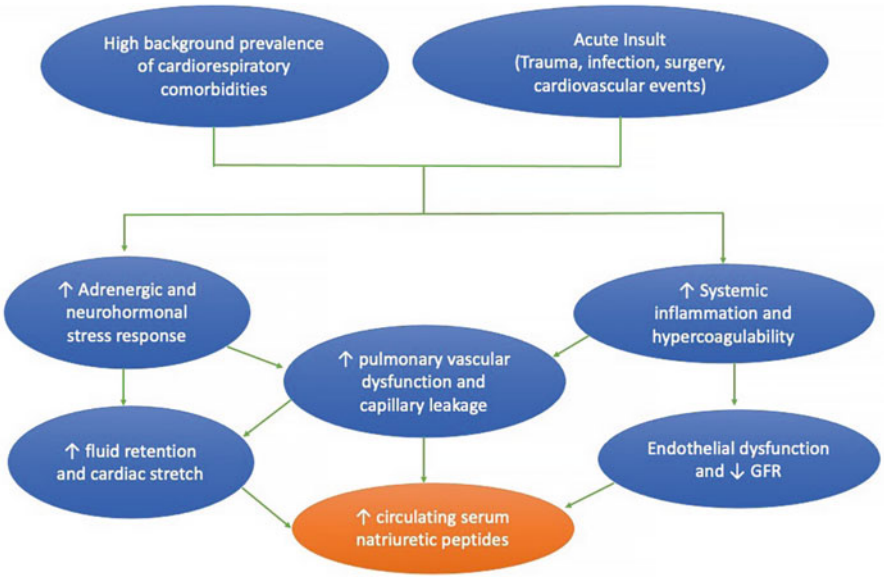

Fig. 1 Relationship between critical illness and circulating natriuretic peptides

Summary Points

- Critically ill patients with elevated natriuretic peptides are more likely to experience adverse outcomes and mortality compared to those with normal levels of circulating natriuretic peptides.
- This correlation is seen in common multisystem critical illness syndromes such as sepsis and trauma, cardiac conditions, as well as extracardiac conditions.
- The mechanism that underlies this association is unclear and likely multifactorial (Fig. 1).

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Cerebrospinal Fluid as a Platform for Biomarker Identification in Traumatic Brain Injury

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Proteomics and Other Protein Assays

Eric P. Thelin and Caroline Lindblad

Contents

Abstract

Traumatic brain injury (TBI) is a common cause for mortality and disability following severe head injury as well as milder concussive head injury. Despite high incidence and prevalence, no pharmacological treatments currently exist for TBI. Current research efforts aim to utilize existing pathophysiological data to

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derive therapeutic agents that can be utilized clinically. Of interest in this process are biofluid protein biomarkers of tissue fate – i.e., proteins that convey information that can be used as a surrogate for, e.g., injury severity or that can aid in patient prognostication. Traditionally, blood has been the biofluid of choice because of its accessibility. Yet, when it comes to the central nervous system, the biofluids in closer proximity with the brain are likely to be superior. As such, cerebrospinal fluid (CSF) is interesting, as it can be accessed either through an external ventricular drain in severely injured patients, or through a lumbar puncture for patients experiencing long-term sequelae following milder injuries. CSF naturally contains proteins, of likely importance following TBI. Numerous such proteins have been shown to be amenable to analysis using either exploratory or hypothesis-driven quantitative proteomic techniques. This has led to the discovery of important prognostic proteins following TBI, currently pointing toward neuroinflammation as a driver of cellular injury in the aftermath of TBI of potential importance both for development of therapeutics and for stratification of the individual patient. This chapter summarizes the field of protein biomarkers of tissue fate in CSF following predominantly severe TBI.

Keywords

Traumatic brain injury · Biomarker · Biofluid protein biomarker of tissue fate · Protein · Cerebrospinal fluid · Blood-brain barrier disruption · Neuroinflammation · Outcome · Proteomic techniques · Mass spectrometry · Enzyme-linked immunosorbent assay (ELISA) · Single-molecule array (SIMOA) · Proximity extension assay · Suspension bead antibody array

Abbreviations

AQP	Aquaporin
BBB	Blood-Brain Barrier
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
ECF	Extracellular Fluid
ELISA	Enzyme-Linked Immunosorbent Assay
EVD	External Ventricular Drain
GCS	Glasgow Coma Scale
GFAP	Glial Fibrillary Acidic Protein
GOS	Glasgow Outcome Scale
IL	Interleukin
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LP	Lumbar Punction
MS	Mass Spectrometry
M/Z	Mass/Charge Ratio
NCCU	Neuro-Critical Care Unit
NFL	Neurofilament Light
NSE	Neuron-Specific Enolase

PCR	Polymerase Chain Reaction
Q_A	Albumin Quotient
SBDP	Spectrin Breakdown Product
SIMOA	Single-Molecule Array
TBI	Traumatic Brain Injury
UCH-L1	Ubiquitin C-Terminal Hydrolase-L1

Introduction

A traumatic brain injury (TBI) entails "an alteration in brain function, or other evidence of brain pathology caused by an external force" (Menon et al. 2010). This broad definition includes patients who have sustained a concussion as well as patients unconscious at the scene of accident warranting neuro-critical care unit (NCCU) treatment for their injuries. This spectrum of disease severities is one contributing factor to TBI heterogeneity, warranting subgroup classification using the Glasgow Coma Scale (GCS) assessment of consciousness into mild, moderate, and severe injuries (Teasdale and Jennett 1974). In 2016, 27 million people suffered a TBI globally (James et al. 2019), of which more than 80% of all cases were expected to constitute a mild TBI (Levin and Diaz-Arrastia 2015). Yet, even when restricting the discussion to severe TBI, it is among the most common causes for death and disability (Jennett 1996). In fact, 37% of injury-related deaths in Europe can be attributed to TBI (Maas et al. 2017).

Worldwide, more than 55 million people live in the aftermath of a TBI (James et al. 2019). Among patients sustaining a severe TBI, mean mortality is ~35% (Stein et al. 2010). Among mild injuries, data is suggesting that as many as 10–30% of patients face post-concussive syndrome (Hou et al. 2012; Levin and Diaz-Arrastia 2015). Alarmingly, repetitive mild TBI has also been linked to chronic traumatic encephalopathy, a neurodegenerative tauopathy (Levin and Diaz-Arrastia 2015). In spite of this, as of today no approved treatment targeting the underlying mechanisms of injury exists for TBI.

Medical management in TBI strives to counteract the deleterious consequences of the disease, using pathophysiological knowledge of the natural course of the disorder. The trauma itself elicits a primary injury, for which the gold standard diagnostic modality following clinical assessment is neuroradiology, in the emergency setting computerized tomography (Czeiter et al. 2020; Huie et al. 2021). In more severe forms of TBI, disease progression is dominated by numerous cellular injury processes that ensue the primary trauma. These comprise among else neuroinflammation, edema, excitotoxicity, and blood-brain barrier (BBB) disruption (Kumar and Loane 2012). These processes risk to converge into a so-called secondary insult. Left untreated, a secondary insult can lead to a secondary ischemic brain injury (Jones et al. 1994). An emerging research field that shows promise across this pathophysiological panorama are biomarkers (Huie et al. 2021), which could be used to target one or several of the specific disease characteristics following TBI, thus ultimately enabling personalized TBI management (Czeiter et al. 2020; Mondello et al. 2021).

Within the TBI context, biomarkers from different sources can be used for diverse purposes. Among high-throughput biomarker techniques, enriched proteins of tissue fate have proven valuable to assess in biofluids (Manley et al. 2010; Huie et al. 2021). Historically, the biofluid of choice has been blood, either its serum or plasma fraction. Even though the accessibility of blood is an important benefit, blood also holds important limitations in the context of TBI biomarker research. Generally, blood biomarkers are subjected to marker-specific biophysical (Dadas et al. 2016) and kinetic properties (Thelin et al. 2017b), as well as marker-unique clearance (Dadas et al. 2016). Specifically for TBI, the common biomarkers S100B, glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), ubiquitin C-terminal hydrolase-L1 (UCH-L1), neurofilament light (NFL), and tau have been shown not to correspond to pathoanatomical or specific structural brain injury when assessed in blood, but more with lesion severity (Whitehouse et al. 2022a, b). This is likely a consequence of an important covariance between the different biomarkers (Thelin et al. 2019; Whitehouse et al. 2022a), as well as the inherent complexity of TBI with multiple concurrent lesions or perhaps an impaired cerebral perfusion not flushing out the protein from brain to blood. Moreover, blood in itself necessitates the release or clearance of the TBI biomarker from brain to blood, which makes the biomarker vulnerable to individual factors affecting this clearance such as patient age, BBB disruption, and extracranial injury, as well as the temporal profile of the biomarker in relation to the natural course of the trauma (Whitehouse et al. 2022b). All of these features potentially hamper incremental clinical utilization of blood biomarkers following TBI, which despite these limitations could prove highly beneficial throughout the course of the disease, perhaps particularly among severely injured patients, for whom the TBI evolves longitudinally with potentially critical implications for long-term functional outcome (Dadas et al. 2018).

An appealing option in selected circumstances is to use cerebrospinal fluid (CSF) instead of blood for biofluid protein biomarker discovery. Through its anatomical location, CSF is a superior biofluid for portrayal of intracranial conditions (Bogoslovsky et al. 2016; Agoston et al. 2017). As the characterization of the human brain proteome is emerging and expanding (HPA 2005), and numerous proteomic techniques are available with the possibility of multiplexing, CSF constitutes an important avenue for future protein biomarker discovery. In addition, CSF could be utilized clinically under defined circumstances and thereby possibly extend our current knowledge on pathophysiology following TBI. Below, the promise of CSF as a platform for protein biomarker discovery is expanded upon, with a special focus on the severe TBI context.

Current State of Proteomic Biomarker Research Following Traumatic Brain Injury

As early as 2001, biomarkers were identified to benefit from a common terminology. Here, it was suggested that a biomarker should be defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”

(Atkinson et al. 2001). Further initiatives such as the BEST resource (FDA-NIH Biomarker Working Group 2016) have elaborated on these definitions. In accordance with later definitions, biomarker characteristics entail a spectrum of features that can stretch from tissue *in situ* findings to results from imaging, and to fluid biomarkers (FDA-NIH Biomarker Working Group 2016; Robb et al. 2016). Given these broad definitions, biomarkers can naturally serve different purposes in different contexts (Atkinson et al. 2001). Considering the vast heterogeneity of clinical TBI, it is not surprising that different biomarkers can be utilized for discrepant purposes – stretching from emergency settings in mild TBI where easily sampled S100B in blood can aid when selecting patients for neuroradiology (Unden et al. 2013) to autoantibody detection conferring information about neuro-critical care unit pathophysiology and long-term sequelae (Needham et al. 2021). Common for most TBI-settings is the need for easily accessed and evaluated biomarkers. As such, biofluid biomarkers offer a broad versatility and richness in detail, as they theoretically offer the possibility to measure highly specific elements of the injured central nervous system (CNS). In fact, different groups have described different ways in which fluid biomarkers can be utilized following TBI (Table 1). Notably, biomarkers can serve important purposes across all aspects of the natural course of TBI, indicating that numerous different biomarkers are sought after in TBI.

Previous work has tried to limit the otherwise seemingly infinite number of possible TBI biomarkers by stipulating quality criteria that biomarkers should fulfill in order to be of interest. First and foremost, the fluid biomarker of interest needs to confer some type of information relevant to the intended patient metric – such as risk stratification or outcome (Thelin et al. 2017a). Next, the intended biomarker needs to be of intracerebral origin (Kleindienst et al. 2007) or else fulfill both high sensitivity and specificity for TBI (Thelin et al. 2017a). Within the CNS, the biomarker/protein of interest preferably should be as passive as possible, thus not affect the course of disease or take part in otherwise important physiological processes (Kleindienst et al.

Table 1 Fluid biomarkers and their utility following traumatic brain injury

Setting	Fluid biomarker utilization	References
Emergency department (mild TBI)	Decision-making on the need for neuroimaging	Unden et al. (2013), Bogoslovsky et al. (2016)
Neuro-critical care unit	Identify different types of structural injuries	Whitehouse et al. (2022a)
Neuro-critical care unit	Identify patients facing an increased risk of secondary injuries	Thelin et al. (2014a), Bogoslovsky et al. (2016)
Emergency setting, neuro-critical care	Outcome prediction	Marmarou et al. (2007), Perel et al. (2008), Bogoslovsky et al. (2016)
Emergency department/ Rehabilitation facility	Information/Stratification on long-term sequelae, counseling	Friberg and Thelin (n.d.), Bogoslovsky et al. (2016)

Various applications of biomarkers across the spectrum of TBI severities and timepoints ensuing injury. References are not all encompassing, but rather examples
TBI traumatic brain injury

2007; Thelin et al. 2017a) but rather be an exclusive injury marker. A commonly utilized biomarker in acute TBI is the astrocytic protein S100B, sampled in blood. This biomarker has been associated with long-term outcome following severe TBI (Thelin et al. 2013), secondary insults in the neuro-critical care unit (Thelin et al. 2014a), and has been included in Scandinavian mild TBI guidelines to discriminate between patients who need to undertake a computerized tomography (Unden et al. 2013). Importantly, S100B is well characterized with regard to half-life (Thelin et al. 2017b), BBB passage (Blyth et al. 2009; Lindblad et al. 2020), extracranial sources (Thelin et al. 2017a), and time frames of extracranial influence to peripherally derived blood levels (Thelin et al. 2016). Other tentative biomarkers following TBI, such as NSE, have less well-characterized kinetic properties (Thelin et al. 2017b), and not an as well-established BBB passage as S100B (Lindblad et al. 2020).

Even though unrestricted BBB passage (Kleindienst et al. 2007) and well-portrayed kinetic properties (Thelin et al. 2017a) are qualities historically sought after in blood biomarkers, blood biomarkers in themselves hold limitations. As highlighted above, blood biomarkers must be assessed with consideration to all aspects associated with movement from the CNS to the systemic compartment, including BBB passage, degradation ensued by elimination, and lower concentration of a particular biomarker in the systemic circulation (Bogoslovsky et al. 2016). In selected situations, particularly following severe TBI, interest has therefore increased to instead use CNS biofluids for biomarker detection and evaluation. The CNS biofluids entail CSF and brain extracellular fluid (ECF). Of these, CSF exists in the larger volumes and confer global CNS information. Although considered to be the superior biofluid for CNS portrayal (Bogoslovsky et al. 2016; Agoston et al. 2017), there are numerous limitations surrounding CSF primarily regarding its accessibility. Yet, the utilization of CSF rather than blood might represent a paradigm shift in which tools for TBI study will grow increasingly complex and broaden our view of what constitutes a biomarker following TBI.

Biological and Methodological Aspects of Cerebrospinal Fluid

The rationale for undertaking fluid biomarker studies in CSF is anatomically motivated because of the proximity between CSF and the brain parenchyma (Hühmer et al. 2006; Santacruz et al. 2021). In addition, intracranial biofluids – notably CSF and brain ECF – equilibrates (Hühmer et al. 2006), thus making CSF an excellent depiction of intracranial status. CSF has thus become a valuable target for studies of brain pathophysiology. In fact, some has even suggested CSF to be the superior biofluid for CNS biomarker studies (Bogoslovsky et al. 2016). Below, CSF is discussed first from a physiological perspective and then in relation to TBI proteomics, with a special section on proteomic assays and technical considerations.

Cerebrospinal Fluid Physiology

CSF is composed of water, proteins, neurotransmitters, ions, and glucose (Wichmann et al. 2022). As ionic balance is different in CSF than blood (Sakka et al. 2011) and since CSF contains a maximum of 5 cells/mL (Sakka et al. 2011), it is reasonable to state that CSF is more complex than a mere blood ultrafiltrate. Total protein concentration in CSF is 0.2–0.8 mg/mL, which is less than 1% of the serum protein concentration (Kroksveen et al. 2010). Of this, 20% of peptides in CSF are brain derived (Reiber 2003). In this context, *brain derived* primarily indicates the relative protein enrichment in CSF compared with blood, rather than brain specificity (Reiber 2003). Yet, it is important to acknowledge that the human CSF proteome is still incompletely characterized. Following the first attempts around the years 2000 stretching to 2015, the number of known CSF proteins has grown from ~20 to >3000 reflecting the complexity of this biofluid (Macron et al. 2018).

Synthesis of the ~150 mL of CSF that resides in the adult human CNS (Brinker et al. 2014) is widely recognized to be exerted from the choroid plexus (Wichmann et al. 2022) across the ventricular system of the CNS (Sakka et al. 2011) (Fig. 1). Here, ultrafiltration of blood together with secretion across the choroidal epithelium (Brinker et al. 2014) is imperative for CSF production. In addition, CSF production is dependent on movement of Na^+ transcellularly together with Cl^- and HCO_3^- and water. Whereas water moves from blood to ventricles via transportation through aquaporin-(AQP-)1, Na^+ transport is enabled by a Na^+/K^+/ATPase pump (Wichmann et al. 2022). Moreover, current data support CSF production from structures discrepant from the choroid plexus, e.g., ependyma and brain parenchyma (Brinker et al. 2014). CSF production speed is 0.3–0.4 mL/min (Brinker et al. 2014), yielding a CSF turnover time of four times per day (Sakka et al. 2011; Wichmann et al. 2022), although some have speculated that this is an underestimate (Proulx 2021).

CSF was historically believed to circulate exclusively from the center of synthesis within the lateral and third ventricles and then through the cerebral aqueduct to the fourth ventricle and lastly entering the subarachnoid space (Fig. 1) (Czosnyka et al. 2004). Today this convective or directional flow is appreciated to be accompanied by other CSF driving forces, including, e.g., arterial pulsations but also possibly the so-called glymphatic system (Proulx 2021; Wichmann et al. 2022). The latter was recently described as an intracranial system for perivascular and transparenchymal solute and fluid clearance (Iliff et al. 2012). CSF flow ultimately leads to CSF reabsorption, a field still surrounded by controversies. In fact, previous theories about CSF reabsorption through arachnoid villi have been questioned because of their lack of physiological data (Proulx 2021). Although this is still a matter of investigation, CSF absorption in addition seems to occur through perineural drainage and via lymphatic vessels (Louveau et al. 2017; Proulx 2021). The latter was evoked in recent works (Aspelund et al. 2015; Louveau et al. 2015). In summary, an overarching knowledge of CSF biology is imperative for understanding of CSF proteomics, as CSF physiology heavily influences interpretation of CSF protein levels.

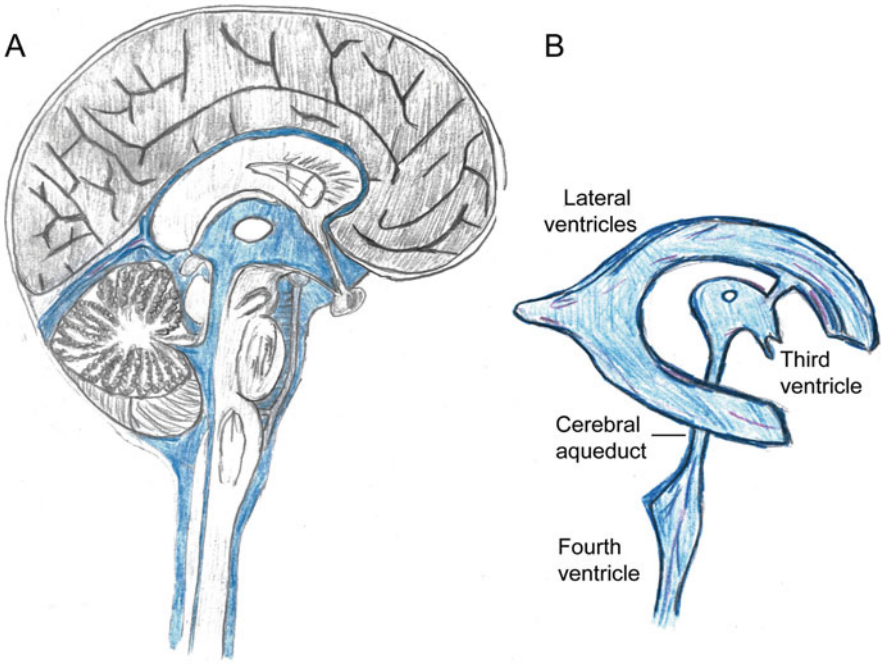

Fig. 1 Cerebrospinal fluid anatomy and physiology. Cerebrospinal fluid (CSF) acts as a protective shield for the central nervous system (**A**). CSF is produced in the choroid plexus of the lateral ventricles, and flows from the supratentorial ventricles through the cerebral aqueduct, and the fourth ventricle (**B**). A lot of controversy still surrounds the mechanisms for CSF reabsorption

Specific Methodological Considerations in Cerebrospinal Fluid Proteomics

CSF sampling, processing, and analysis warrant particular considerations with regard to CNS biology, and CSF characteristics. As CSF samples can only be obtained through invasive measures, sampling strategies and procedural recommendations for CSF collection have been suggested by the Biospecimens and Biomarkers Working Group in TBI research (Manley et al. 2010). For TBI patients, CSF is commonly collected through an external ventricular drain (EVD), where a ventriculostomy catheter resides in the supratentorial lateral or third ventricle. In other clinical settings, lumbar punction (LP) enables collection of CSF from the lumbar spine level. Both types of sampling procedures theoretically infer a risk of infection (Korinek et al. 2005) at each sampling time point. In order to enable repeated sampling with high time resolution while not exposing the study subject to an unethical risk of infection, one can, for example, collect CSF continuously through an enclosed system (e.g., LiquoGuard®), which has been successfully done (Thelin et al. 2014b; Lindblad et al. 2020). Independent of CSF sampling method, protein concentration gradients along the rostro-caudal axis need to be

considered. This is important as ventricular albumin is lower than lumbar albumin (Weisner and Bernhardt 1978), indicating that protein concentration varies along the rostro-caudal axis and thus could hamper interpretation. Moreover, for EVDs and LiquoGuard® like systems, CSF drainage can be either intermittent or continuous. This also presumably affects CSF protein concentration (Shore et al. 2004), of importance in longitudinal designs.

Other considerations with regard to CSF analysis pertain to the CNS barriers, comprising the BBB, the arachnoid barrier, and the blood-CSF barrier (Liddelow 2011). Following a TBI, CNS barriers break, both because of the trauma itself and because of subsequent cellular injury mechanisms (Chodobski et al. 2011). The gold standard metric for assessment of the extent of BBB injury is the albumin quotient Q_A (Tibbling et al. 1977). Importantly, hemorrhage into CSF such as, e.g., intraventricular hemorrhage (Bellander et al. 2011), could influence CNS albumin content and thus lead to an erroneous estimation of Q_A. In addition, Q_A is age dependent (Tibbling et al. 1977), which is an important limitation in CSF studies on adult study subjects. Similarly, CSF flow is also age dependent, leading to a clear age dependency in CSF protein concentration (Kroksveen et al. 2010).

Finally, it is important to mention control subjects in the context of CSF proteomic studies. Explorative proteomic efforts in CSF are likely to yield quantitative data on proteins previously unknown or proteins currently insufficiently characterized. This means that normal protein CSF concentration or reference interval will be unknown. For this purpose and for more complex analyses, such as outcome prognostication or predictive modeling, control subjects are a necessity. In TBI, researchers have suggested utilization of normal-pressure hydrocephalus patients or CSF samples from patients with unruptured aneurysms undergoing planned surgical interventions (Manley et al. 2010). Yet other research efforts in CSF neuroproteomics have utilized CSF samples from otherwise healthy individuals (Isung et al. 2021), showing both the ethical and practical feasibility of CSF sampling from healthy subjects. Of note, and if possible, age matching should be carefully sought after, because of its important confounding effects as highlighted above.

Proteomic Methods for Biomarker Discovery in CSF

Even though *proteomics* refers to big-scale protein analysis covering an entire organism (Matthiesen 2013), it is here used to denote different types of protein assays. Below follows a non-exhaustive delineation of proteomic techniques that can be used for CSF biomarker studies. For a more complete portrayal including commercial options, the reader is referred to Carlyle and colleagues (Carlyle et al. 2018). We focus on translational techniques that we believe can move the clinical biomarker field forward. Methodological distinctions and their respective strengths and limitations of the various methods are summarized in Table 2.

For translational biomarker research, the proteomic method of choice unexpectedly depends on the scientific question. As recognized above, the CSF proteome is still incompletely characterized (Macron et al. 2018). This means that the quest for

Table 2 Scientific considerations between different types of proteomic assays

Technique	Bias	Multiplex	Sensitivity	Else
Non-antibody-based techniques				
Mass spectrometry	None	>1000	Low	Versatile
Antibody-based techniques				
ELISA	Yes	Cumbersome	High	Gold standard
SIMOA	Yes	<10 analytes	High	Ultrasensitive
OLink®	Yes	>100	High	Reduces the risk for antibody cross-reactivity
Suspension bead antibody array	Yes	>100	High	Facilitated through, e.g., the Human Protein Atlas effort

Overview of various proteomic methodological characteristics. Above, various method-unique features are non-exhaustively described. The study aim influences the choice of method to employ. *ELISA* enzyme-linked immunosorbent assay, *SIMOA* single-molecule array

CSF biomarkers can either be focused on the discovery of yet unknown proteins, or the characterization of proteins already detected. In order to discover new protein biomarkers in CSF, hypothesis-free techniques such as mass spectrometry (MS) (Carlyle et al. 2018) are probably superior. Among mass spectrometry techniques, in particular bottom-up pipelines enabled through liquid chromatography tandem mass spectrometry (LC-MS/MS), have gained popularity (Xie et al. 2011). Even though these techniques have a vast inherent versatility (Matthiesen 2013), there are common elements across all variants. These entail sample preprocessing, generation of a peptide solution which is prefractionated and later separated using the peptides mass/charge ratio (m/z), ultimately yielding an intensity curve as a function of m/z. This can be further used for protein identification, quantitation, and delineation of posttranslational modifications (Matthiesen 2013). The major advantage of this approach is that it is unbiased (Freeman and Hemby 2004). This however comes at the expense of low sensitivity. It is well known that mass spectrometry commonly fails to detect low-abundance proteins (Kingsmore 2006), even though sample preprocessing such as prefractionation and protein-depletion techniques strive to enrich the concentration of low-abundant proteins and thus increase the overall method sensitivity (Davidsson and Sjögren 2005; Carlyle et al. 2018).

In stark contrast to the MS-based techniques stand hypothesis-driven techniques. Although biased by definition, these techniques have the potential to provide higher sensitivity than MS. Among available techniques, many are antibody based (Fig. 2). A single-plex example of the antibody-based concept is enzyme-linked immunosorbent assay (ELISA) (Fig. 2A) (Engvall and Perlmann 1971), which still today constitutes the gold standard for protein detection (Kingsmore 2006). Today, sandwich ELISA is most common. Here, a protein antigen is immobilized onto a capture antibody, followed by complex formation with a detection antibody. The whole complex is detected using either a secondary antibody, or through light emission such as chemiluminescence, or fluorescence (Van Gool et al. 2020). ELISA is a sensitive method, which can detect protein concentrations at the pg/mL level

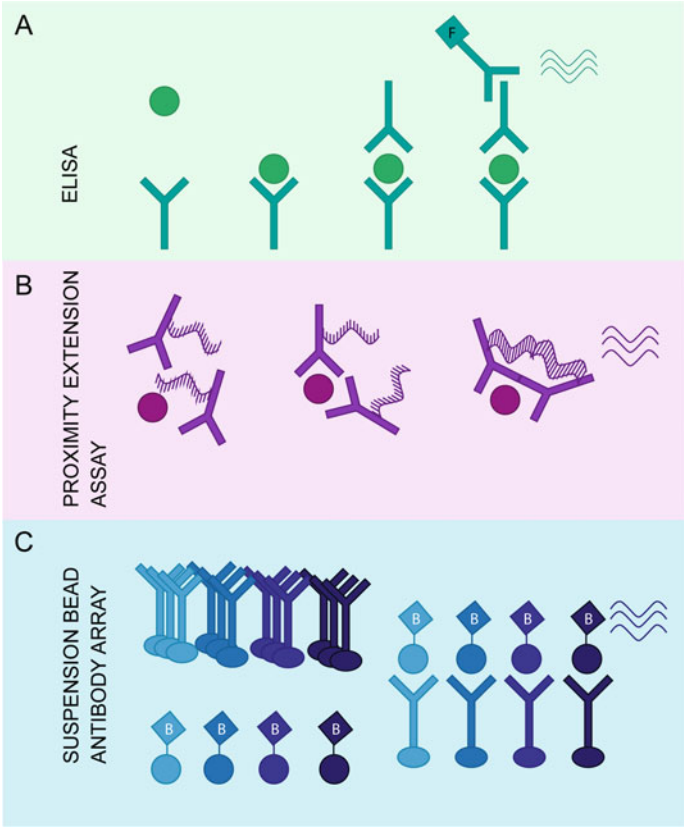

Fig. 2 Antibody-based proteomic techniques. The gold standard antibody-based techniques is enzyme-linked immunosorbent assay (ELISA) (**A**), reliant upon protein binding to antibodies. Commonly, a sandwich technique is employed, whereafter the protein-antibody complex is detected through light emission, for example, using a third antibody with a fluorescent protein attached to it. An alternative to ELISA is a so-called proximity extension–based assay (**B**), such as employed by, e.g., OLink®. Here, each antibody is tagged with DNA and upon antibody binding to the same protein, annealing occurs which can be captured through a polymerase chain reaction–based readout. Another technique enabled also through the Human Protein Atlas Project and color-coded beads is called suspension bead antibody arrays (**C**). Here, each antibody is attached to a color-coded bead. In parallel, proteins in suspension are tagged to biotin. Biotin-tagged proteins are then captured by antibody-bead complexes. The median fluorescent intensity of each protein per bead type can be detected using a flow cytometer. Suspension bead antibody arrays and proximity extension–based assays allow for a large extent of multiplexing in contrast to ELISA. *B* biotin, *ELISA* enzyme-linked immunosorbent assay, *F* fluorescent protein

(Kingsmore 2006; Carlyle et al. 2018). Ultrasensitive variants of ELISA, such as the single-molecule arrays (SIMOAs), have recently been developed (Rissin et al. 2010), and successfully applied to CSF (Andersson et al. 2020). Both these techniques thus offer high sensitivity, but with limited capability of multiplexing. A multiplex-compatible technique which also claims to reduce the risk for antibody

cross-reactivity is OLink® (Fig. 2B) (Carlyle et al. 2018). OLink® entails a so-called proximity extension assay, where antibodies are coupled to DNA strands. In order to detect a protein, DNA strands must anneal to one another, which only occurs upon binding to the same protein target (Lundberg et al. 2011). Final readout is obtained through a polymerase chain reaction (PCR). The OLink® system is theoretically advantageous because of its capability for multiplexing (Assarsson et al. 2014). In addition, it is also feasible for analysis of CSF samples (Isung et al. 2021).

Another interesting technique which utilizes antibodies and holds great capacity for multiplexing is antibody suspension bead arrays (Fig. 2C) (Schwenk et al. 2008), where beads are used instead of a planar array (Drobin et al. 2013). Antibody suspension bead arrays allow protein detection at concentrations ranges similar to ELISA while being able to multiplex several hundreds of proteins at the same time (Drobin et al. 2013). Importantly, this method also allows for high-throughput analysis of the number of subject samples (Kingsmore 2006; Drobin et al. 2013). This feature is due to the technique of using color-coded beads, as reviewed in Neiman (Neiman 2013), currently commercialized through, e.g., Luminex Corporation. In brief, by dual color coding of microbeads using differential ratio of color dye per bead, a vast number of beads with a unique color identifier can be generated and subsequently identified using a flow cytometer (Kettman et al. 1998; Neiman 2013). In antibody suspension bead arrays, unique antibodies are immobilized onto each color-coded bead and thus work as capture reagents (Schwenk et al. 2008). A plethora of antibodies can be found through the tour de force effort to characterize the human proteome, namely the *Human Protein Atlas* (HPA 2005; Uhlen et al. 2015). For the assay to quantify protein concentrations, biotinylated antigens (proteins) are mixed in suspension with antibody-coupled beads upon which fluorescent readout allows quantification of median fluorescent intensity per bead identity in each sample (Drobin et al. 2013). Importantly, this method has been shown to be feasible for CSF in addition to serum (Pin et al. 2019), with the important additional advantage of only requiring very small sample volumes.

Taken together, there are numerous versatile methodological options for the researcher inclined to undertake CSF proteomic studies. Ultimately, the choice of method depends on the intended study question. For the researcher undertaking hypothesis-generating work, mass spectrometry is likely the most eligible method moving forward (Kingsmore 2006), whereas more sensitive methods compatible with multiplexing such as OLink® and antibody suspension bead arrays are suitable for further pathway analysis and validation experiments of mass spectrometry. For the final steps of biomarker validation, concentration measurements, and implementation, single-plex techniques, preferable in the ultrasensitive format such as SIMOA, are suitable.

Cerebrospinal Fluid Protein Biomarkers Show Promise Across Traumatic Brain Injury Studies

Previous and future work pertaining to CSF biomarkers following TBI stretch across a broad domain. It is widely recognized that the field of mild TBI lacks biomarkers, of importance for diagnostics of post-concussive syndrome, and chronic traumatic

encephalopathy (Zetterberg and Blennow 2016). One might argue that in the emergency setting of mild TBI, blood biomarkers are preferable, as they can be obtained instead of neuroradiology. Meanwhile, it is controversial to undertake CSF sampling without preceding neuroradiology when there is a history of head trauma as a lesion with mass effect should be ruled out before lumbar puncture is attempted in this patient group. Thus, CSF biomarkers in the mild TBI emergency setting seems impractical. In contrast, for follow-up visits, or as part of routine investigation among patients with persisting symptoms post-TBI, CSF sampling seems both safe and eligible. Long-term follow-up of neurodegenerative sequelae following severe TBI has been the scope of recent biomarker studies in brain ECF and blood (Graham et al. 2021) and prospective CSF biomarker studies are under way (Friberg and Thelin n.d.). CSF studies in both the acute and subacute phases following severe TBI have already been undertaken for proteomic biomarker purposes (Connor et al. 2017).

Of particular interest in the setting of severe TBI is outcome prognostication, recently investigated in relation to CSF biomarkers in a systematic review (Santacruz et al. 2021). This review covered both traumatic and nontraumatic acute brain injuries. In total, $n = 27$ CSF biomarkers were assessed and included: caspase-1, -3, -9, cytochrome C, sFas, apoptosis-associated speck-like protein containing a caspase recruitment domain, S100B, brain-derived neurotrophic factor, ubiquitin carboxy-terminal hydrolase L1, microtubule-associated protein 2, alpha-2 spectrin, spectrin breakdown products (SBDP120, -145, -150), myelin basic protein, peroxiredoxin IV, matrix metalloproteinase-2, -9, neuron-specific enolase, amyloid-β 42, alpha-synuclein, Nacht leucine-rich repeat protein-1, glial fibrillary acidic protein, c-tau, sulfonylurea receptor-1, and apolipoprotein E. In total, TBI studies demonstrating an association between a CSF biomarker and outcome included in total $n = 1345$ patients, whereas studies with no outcome association entailed $n = 254$ patients. As some CSF biomarkers were the scope of multiple studies that reached discrepant results, some studies within this systematic review may be hampered with power issues (Santacruz et al. 2021). Another potential explanation for discrepant results upon study of similar biomarkers is temporal trajectories. As TBI by no means is a stationary disorder, previous work has emphasized the importance of longitudinal investigation (Agoston et al. 2017). One study employing a uniquely high sampling resolution illustrates this (Lindblad et al. 2020). Here, CSF samples were obtained through an enclosed system, thus allowing 6- to 12-hour epochs of CSF sampling. Notably, S100B, NSE, and Q_A can be seen to decay longitudinally (Fig. 3A, B, C). In this study, this high sampling resolution enabled deduction of CSF biomarker clearance to blood, of possible importance when utilizing blood-based biomarkers.

Another research approach to CSF biomarkers following TBI is pathophysiology-oriented biomarkers, such as shown above for Q_A (Lindblad et al. 2020), representing a quantitative BBB disruption biomarker. In fact, Q_A was recently shown to be an independent outcome predictor following severe TBI (Lindblad et al. 2021), which is novel and important. This finding was expanded upon in one of the largest proteomic efforts seen following severe TBI, recruiting in total $n = 186$ severe TBI patients, of whom $n = 90$ had CSF samples taken. Here, $n = 114$ out of in

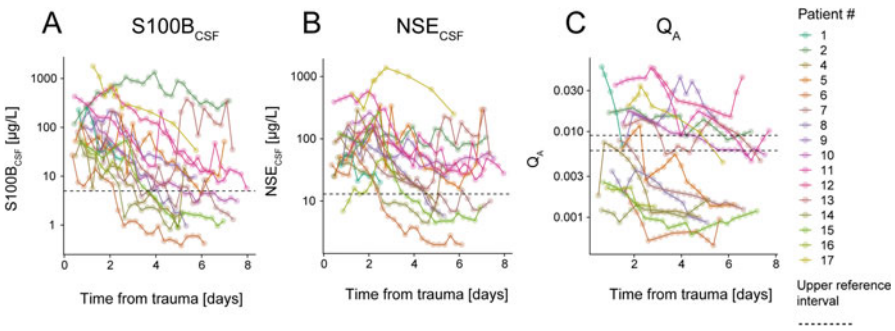

Fig. 3 High-resolution longitudinal studies of CSF biomarkers. Following severe TBI, S100B (**A**), NSE (**B**), and Q_A (**C**) were measured longitudinally with a uniquely high sampling resolution enabled through a closed CSF collection system. Interestingly, all biomarkers were seen to diminish longitudinally. In this study, the deduced longitudinal trajectory could be used to model how CSF proteins are cleared to peripheral blood. *CSF* cerebrospinal fluid, *NSE* neuron-specific enolase, Q_A albumin quotient, *TBI* traumatic brain injury. (Modified from Lindblad et al. (2020) and reprinted under the terms of the Creative Commons License (http://creativecommons.org/licenses/by/4.0))

total $n = 177$ CNS-enriched proteins correlated with Q_A (Fig. 4A). Importantly, protein levels clustered both depending on Q_A and on functional outcome (Glasgow Outcome Scale, GOS), indicating the importance of BBB disruption for long-term functional outcome (Fig. 4B). When these proteins were systematized into function-relevant pathways, inflammatory pathways emerged as particularly important (Fig. 4C) (Lindblad et al. 2021). This is well in line with current ideas of BBB disruption-induced triggering of innate neuroinflammatory mechanisms within the CNS while allowing for peripheral immune cell recruitment (Kumar and Loane 2012). The importance of CSF biomarkers in post-TBI neuroinflammation was systematically reviewed recently (Zeiler et al. 2017). Similarly to other CSF biomarker reviews, different included studies showed conflicting results, possibly because of inherent design issues. Among the neuroinflammatory factors studied, cytokines overall seemed to represent the largest body of literature and in particular tumor necrosis factor and the interleukins (IL-)-1β, IL-1ra, IL-6, IL-8, and IL-10 (Zeiler et al. 2017). Other promising neuroinflammatory mediators following TBI are factors belonging to the complement system (Kossmann et al. 1997; Stahel et al. 2001; Bellander et al. 2011). Interestingly, complement also stands out in recent TBI efforts, both in the experimental milieu (Alawieh et al. 2018) and among human study subjects (Lindblad et al. 2021).

Taken together, CSF holds the capacity for various biomarker discoveries in the context of acute severe TBI, as well as in the follow-up and chronic period following mild, moderate, and severe TBI. The CSF biomarker quest should ultimately be guided by the study question, but currently explored fields of study concern pathophysiology-guided biomarkers, and outcome-guided biomarkers.

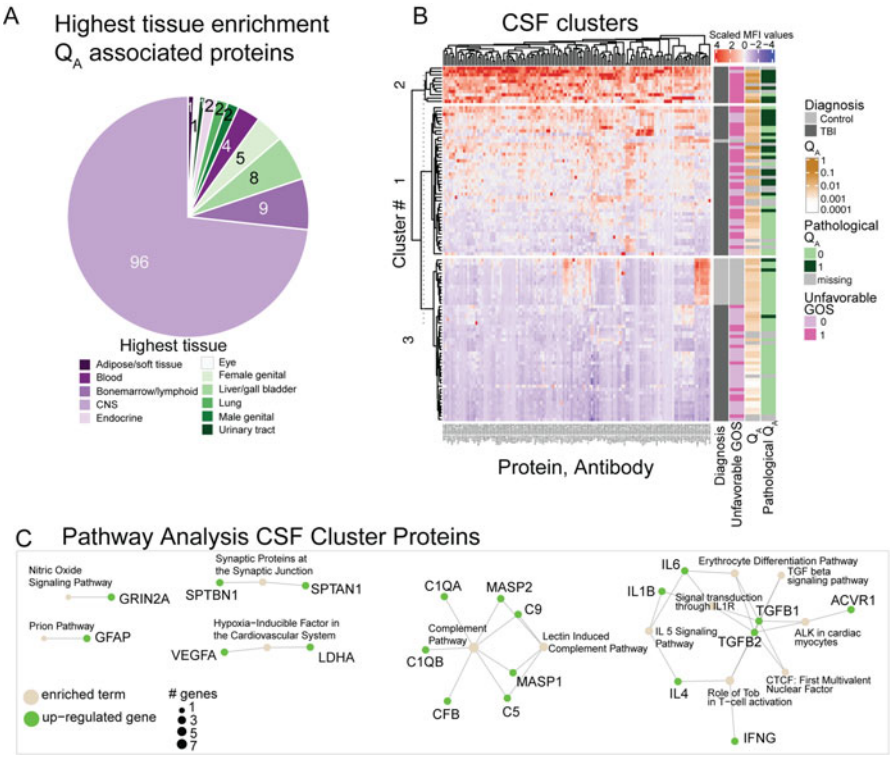

Fig. 4 Neuroinflammatory proteins in CSF are important outcome predictors following severe TBI. In one of the so far largest proteomic efforts following severe TBI, neuroinflammatory proteins in CSF were highly correlated with the extent of BBB disruption, assessed through the albumin quotient Q_A (**A**). Upon cluster analysis, CSF protein levels clustered based on long-term functional outcome (**B**). When systematizing these results in pathway analyses, inflammatory pathways seemed to be implied (**C**). *BBB* blood-brain barrier, *CSF* cerebrospinal fluid, Q_A albumin quotient, *TBI* traumatic brain injury. (Modified from Lindblad et al. (2021) and reprinted under the terms of the Creative Commons License (http://creativecommons.org/licenses/by/4.0/))

Discussion and Conclusions

This book chapter compiles and contextualizes our current understanding of proteomic studies in CSF, as well as their application within the field of TBI research. Overall, CSF biomarkers constitute a field of yet incompletely explored potential following TBI. Onward, CSF protein biomarker research following TBI face opportunities and challenges, discussed below.

As highlighted above, proteomic CSF biomarker studies warrant specific considerations. Sampling can be made from ventricular or lumbar CSF. As the albumin concentration in lumbar CSF is ~2.5 times that of ventricular CSF (Weisner and Bernhardt 1978), caution should be paid when comparing the two. Some authors

discourage comparison between lumbar and ventricular CSF (Manley et al. 2010). One might argue that this is study dependent. In fact, it might be superior to utilize a healthy control group with lumbar CSF samples than a diseased control group such as normal pressure hydrocephalus patients, recognized to have disease-specific CSF biomarker patterns (Lukkarinen et al. 2022).

If decided upon, CSF sampling ought to be done longitudinally (Agoston et al. 2017). Longitudinal sampling at a high resolution has the potential to reveal TBI dynamics, as has been done by some authors (Lindblad et al. 2020). Depending on the study time line and design, intense high-resolution sampling for a brief time might be as suitable as less frequent sampling for a longer period of time. In all situations, the risk of infection must be considered (Korinek et al. 2005), and measures taken to avoid it. There are clinical situations in which CSF is difficult or too cumbersome to acquiesce, e.g., when the patient does not have any EVD but rather an intracranial pressure monitor. In these instances, it would be valuable to utilize another neuromonitoring modality that gives access to CNS extracellular fluid. One such alternative could be cerebral microdialysis, which advantageously can be employed through a triple-lumen cranial access device. Current work supports the usage of cerebral microdialysis for neuroinflammatory protein studies (Helmy et al. 2009; Zeiler et al. 2017). Current restrictions for the usage of cerebral microdialysis includes the cerebral microdialysis catheter probe size (Helmy et al. 2009), but also catheter placement (Zeiler et al. 2017). Importantly, although cerebral microdialysis proteomics would theoretically pose an alternative to CSF proteomics, CSF is a global metric of CNS state, whereas cerebral microdialysis is local, thus making cerebral microdialysis less practical.

Lastly, CSF that has been acquired from the TBI patient needs to be analyzed. The ultimate choice of proteomic platform depends on study aim. As the CSF proteome is still incompletely characterized, unbiased hypothesis-free techniques such as mass spectrometry (Carlyle et al. 2018) offer the highest theoretical versatility for CSF biomarker discovery. However, for protein validation, increased sample size, and eventual clinical implementation, other techniques such as multiplex/single-plex antibody assays are likely more suitable (Kingsmore 2006). Eligible protein targets to examine include both proteins previously associated with clinical outcome (Santacruz et al. 2021), but also neuroinflammatory proteins (Zeiler et al. 2017). Certain types of antibody arrays allow for simultaneous evaluation of both pathway-specific and neuroinflammatory CSF proteins (Lindblad et al. 2021), which allowed for association between different cellular mechanisms following severe TBI, of importance for long-term functional outcome. In conclusion, CSF protein biomarkers entail a vast field of proteins, techniques, and hypotheses of large possible impact across the whole field of TBI studies.

Applications to Prognosis, Other Diseases, or Conditions

In certain TBI conditions, such as long after the injury occurred (>10 years), it might be difficult to detect alterations in the blood compartment. However, presumably, alterations might still be detectable in the CSF compartment due to its proximity to

injured CNS structures. This will be further explored in the ongoing study LONG-TBI (long-term follow-up in severe TBI, NCT05235802) where different metabolic-, lipidomic-, proteomic-, and auto-antibody profiling will be conducted across the blood and CSF compartments in order to discern any compartment-specific differences to structural injury and long-term functional outcomes. There are currently no gold standard fluid biomarkers of chronic TBI symptomatology and CSF sampling is a tentative venue for improved diagnostics.

Mini-Dictionary of Terms

Traumatic brain injury: A traumatically inflicted injury to the brain, typically caused by either external blunt or penetrating violence, or else by the brain's movement within the cranium because of external energy.

Cerebrospinal fluid: The clear fluid that surrounds the entire central nervous system that contains among else proteins, but to a lower extent than blood. Cerebrospinal fluid is likely together with brain extracellular fluid, the best source of central nervous system fluid protein biomarkers.

Blood-brain barrier: One of the selective barriers surrounding the central nervous system. Under homeostasis, the blood-brain barrier is impermeable to albumin. Following trauma, the blood-brain barrier breaks and the extent of injury is estimated as the quotient of albumin in CSF compared with blood.

Proteomics: Common denomination for protein assessments across the entire organism. Here, the term is used to portray various protein assessment techniques of relevance for the study of CSF protein biomarkers.

Neuroinflammation: The common term used to describe inflammatory events exerted both within the CNS and from the periphery into the CNS following (traumatic) CNS insults. Neuroinflammation usually encompasses both innate and adaptive mechanisms.

Key Facts

- Traumatic brain injury (TBI) is a heterogeneous and common condition, for which no pharmacological treatment currently exists.
- Current efforts in TBI strive to improve long-term outcome through multimodal monitoring and physiological optimization – a process in which fluid protein biomarkers can serve utile – especially in cerebrospinal fluid (CSF).
- CSF sampling and protein quantification warrant particular considerations, including sampling location (ventricular/lumbar), frequency of sampling, risk for infection, and presumptuous blood-brain barrier (BBB) injury.
- Among available proteomic techniques, mass spectrometry is a hypothesis-free method of lower sensitivity, whereas antibody-based methods are biased by definition but achieve a higher sensitivity.

- Previously, $n = 27$ CSF biomarkers of various types have been assessed following TBI and associated to, e.g., long-term prognosis.
- Recently, the so far largest proteomic effort following severe TBI and CSF proteomic assays were conducted and demonstrated the importance of BBB disruption and neuroinflammation following injury. Both BBB disruption and neuroinflammation seem to be important targets for for future therapeutic discoveries in TBI.

Summary Points

- Traumatic brain injury (TBI) is a heterogeneous and common condition, afflicting approximately 27 million people worldwide in 2016 (James et al. 2019), of which the vast majority were believed to be mild injuries (Levin and Diaz-Arrastia 2015).
- No pharmacological treatment exists for TBI as of today.
- Current clinical and research efforts in TBI are directed toward improving long-term prognosis through multimodal monitoring and physiological optimization – a process in which fluid protein biomarkers of tissue fate can serve highly utile.
- Cerebrospinal fluid (CSF) biomarkers have gained interest since CSF is one of the biofluids in closest proximity with the brain and therefore likely reflects the intracranial milieu.
- CSF is likely not yet fully characterized, but definitely contains proteins, although to a lower extent than blood (Kroksveen et al. 2010).
- CSF sampling and protein quantification warrant particular considerations, including sampling location (ventricular/lumbar), frequency of sampling, risk for infection, and presumptuous blood-brain barrier injury.
- Among available proteomic techniques, mass spectrometry is a hypothesis-free method theoretically allowing for the discovery of an infinite number of proteins. In reality, mass spectrometry is usually insufficient to discern proteins across a broad dynamic range, yielding it impractical. Instead, antibody-based techniques have gained popularity as a more refined, although hypothesis-driven/biased technique.
- Around $n = 27$ CSF biomarkers have been assessed following TBI with regard to their outcome predictive capability, and include among else proteins of apoptosis, structural proteins, neuroinflammatory proteins, and proteins associated with neurodegenerative conditions.
- The so far largest proteomic effort following severe TBI and CSF proteomic assays included $n = 90$ TBI patients with CSF samples. Here, numerous proteins were characterized with regard to their relationship with blood-brain barrier (BBB) disruption and outcome. Interestingly, BBB disruption seemed to be of importance for long-term prognosis. Among proteins that correlated strongly with BBB disruption, numerous proteins were neuroinflammatory. Some of these pertained to the complement system, which has been shown to be important in previous work.

Cross-References

- A Synopsis of Routine Blood Biomarkers in Trauma, Injury Critical Care and Recovery: General Overview
- Measures of Classical and Alternative Complement Function in Serum as Markers in Critical Care
- S100B As a Biomarker in Traumatic Brain Injury

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Part VIII

Scoring Systems, Models, and Indirect Measures

The APACHE II Scoring Systems and the ICU 50

Raghav Yelamanchi

Contents

Abstract

Medical care has evolved over the decades. Intensive care unit (ICU) care is the backbone for the improvement in the outcome parameters of patients across all specialties of medicine and surgery. Scoring systems are one of the widely used tools in medical practice to diagnose, stage, treat, and prognosticate patients. They help in deciding the nature and degree of intervention that is best for the

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patient. The acceptance of newer methods and technologies into the ICU is based on the results of statistically sound clinical studies. Many of these clinical studies will not be possible without the use of these scoring systems for sample selection and also to evaluate outcomes. So, the scoring systems are part and parcel of the ICU setup, and they have become inevitable in the present-day medical care.

Acute Physiological and Chronic Health Evaluation Index II (APACHE II) score is one of the widely used scoring systems in ICUs across the world. It has evolved from the APACHE I score and further modified into APACHE III and APACHE IV. One must be aware of the various pitfalls while calculating the APACHE II score and it uses. Apart from the APACHE II score, other scoring systems like Simplified Acute Physiological Score, Mortality Prediction Model, Pediatric Index of Mortality, Sequential Organ Failure Assessment score, etc. are used. In the setting of trauma, several scoring systems are used such as Revised Trauma Score (RTS), Injury Severity Score (ISS), Trauma Revised Injury Severity Score (TRISS), Penetrating Abdominal Trauma Index (PATI), etc. One must also be aware of how to choose a scoring system. Use of preprinted charts and online calculators in smart phones will make the calculation process easy. One must be wise enough to interpret the data from scoring systems to make clinical decisions as no scoring system is 100 percent perfect.

Keywords

Scoring systems · APACHE II score · SAPS II · APACHE III · APACHE IV · ICU scores · Mortality prediction · Mortality Prediction Model · Pediatric Index of Mortality · SOFA · Modified Marshall Score · Audit and research

Abbreviations

APACHE	Acute Physiological and Chronic Health Evaluation Index
AUROC	Area under the ROC curve
GCS	Glasgow Coma Score
ICU	Intensive care unit
ISS	Injury Severity Score
MPM	Mortality Prediction Model
PATI	Penetrating Abdominal Trauma Index
PIM	Pediatric Index of Mortality
ROC	Receiver operating characteristic
RTS	Revised Trauma Score
SAPS	Simplified Acute Physiological Score
SIRS	Systemic Inflammatory Response Syndrome
SMR	Standardized morality ratio
SOFA	Sepsis-related Organ Failure Assessment
TRISS	Trauma Revised Injury Severity Score

Introduction

Medical care has evolved over the decades. Many illnesses which were once considered to be death sentences are being cured. New innovations in science and better understanding of the physiology of the human body are responsible for this tremendous feat. Among many of these game changing practices, the intensive care unit (ICU) care is the backbone for the improvement in the outcome parameters of patients across all specialties of medicine and surgery. The initial assessment and prediction of outcomes of patients admitted to the ICU was earlier done subjectively by the expert clinicians. With improvement of the medical and nursing care, availability of newer investigations and medical devices and better understanding of the pathophysiology of the critical illness, there is a need to develop tools for the objective assessment of patient outcomes.

Scoring systems are one of the widely used tools in medical practice to assess the probability of diagnosis of a disease, stage, treat, and prognosticate patients. The scoring systems are based on multiple parameters determining the patients' outcomes ranging from demographic details, physiological parameters, and treatment parameters. As clinicians understand the pathophysiology of the diseases better, these scoring systems have been modified and revamped with new parameters which improve the efficacy but at the same time make these scoring systems more complex to use. One must remember that these scoring systems have limitations and clinical decisions by expert clinicians are always the best in the context of dilemma.

Characteristics of an Ideal Scoring System

- Sensitive and specific for the desired outcome prediction.
- Easy to use.
- It should be based on few parameters.
- Outcome prediction should be fast.
- Should not be complex to understand and use such that even para-medics can use with ease.
- It should be reproducible and widely applicable.

Why Do We Need Scoring Systems in ICU?

Most of the ICU patients are very ill, and the mortality rate in ICU is quite high for the same reason. The common question which clinicians encounter while explaining about the condition of the patient to their kin is "How bad is our patient and what the chance of recovery is?" In this medico legal era, these scoring systems provide objective evidence about the condition of a patient. The critical care physicians often experience dilemma to decide the best treatment for a patient. The scoring systems come handy to some extent in such situations. They help in deciding the nature and degree of intervention that is best for the patient at that point of time. As the scoring

systems are objective, they help to avoid biases in clinical decisions made on the basis of instincts of clinicians.

The acceptance of newer methods and technologies into the ICU is based on the results of statistically sound clinical studies. Many of these clinical studies will not be possible without the use of these scoring systems for sample selection and also to evaluate outcomes. Scoring systems help to compare like patients with like in clinical studies. Scoring systems in ICU can help in conducting multinational multicenter studies with ease. So, the scoring systems are part and parcel of the ICU setup, and they have become inevitable in the present-day medical care. The present chapter discusses about the Acute Physiological and Chronic Health Evaluation Index II (APACHE II) in detail and also about few other important scoring systems used in the ICU.

Evolution of the APACHE Scores

The APACHE I score was first developed by Knaus WA et al. in 1981. It was based on physiological variables and was used to measure the severity of illness. The APACHE I score was modified to include more variables which lead to the APACHE II score in the year 1985 (Knaus et al. 1985). APACHE II score is the most widely used scoring system in the ICU setting and also in the management of critically ill patients. It consists of age, 12 physiological variables, and chronic health points. The various variables are listed in Tables 1, 2, and 3. The total score is the sum of the score of individual variables. The score ranges from 0 to 71. Knaus WA et al. excluded patients below 16 years, burns, coronary artery disease patients, and those with ICU stay less than 8 hours while introducing the APACHE II score. However, later studies proved that APACHE II score could be used in pediatrics, burns, and cardiovascular patients though the number of studies is limited (Novac et al. 2014; Chhangani et al. 2015).

Pitfalls While Calculating APACHE II Score

One must be well aware of the APACHE II score and use a preprinted chart for accurate calculation. The following are the common mistakes which clinicians commit during calculation (Polderman et al. 2001):

1. Chronic health points: While calculating chronic health points, one must strictly adhere to the description given in the score. One must note that mild and moderate systemic illnesses such as New York Heart Association grades I, II, and III, mild chronic obstructive lung disease, compensated cirrhosis without portal hypertension, chronic kidney disease not requiring dialysis, etc. are not given any points.
2. Considering inconsistent single abnormal values of physiological variables such as heart rate, blood pressure will derive a false score. As the physiological

Table 1 Physiological variables of APACHE II score

a: Acute physiology score (12 variables)	High abnormal rage					Low abnormal range			
Physiological variables	+4	+3	+2	+1	0	+1	+2	+3	+4
Temperature – Rectal (°C)	≥41	39–40.9		38.5–38.9	36–38.4	34–35.9	32–33.9	30–31.9	≤29.0
Mean arterial pressure (mm hg)	≥160	130–159	110–129		70–109		50–69		≤49
Heart rate-ventricular response	≥180	140–179	110–139		70–109		55–69	40–54	≤39
Respiratory rate non-ventilated or ventilated	≥50	35–49		25–34	12–24	10–11	6–9		≤5
Oxygen: A – a DO or PaO_2 (mm hg) $FiO^2 \geq 0.5$ record A – aDO2 $FiO_2 < 0.5$ record only PaO_2	≥500	350–499	200–349		<200 $PO_2 > 70$	$PO_2$61–70		$PO_2$55–60	$PO_2 < 55$
Arterial ph	≥7.7	7.6–7.69		7.5–7.59	7.33–7.49		7.25–7.32	7.15–7.24	<7.15
Serum hCO_3 – Only if no ABGs	≥52	41.5–1.9		32–40.9	23–31.9		18–21.9	15–17.9	<15
Serum sodium (mmol/I)	180	160–179	155–159	50–154	130–149		120–129	111–119	≤110
Serum potassium (mmol/I)	≥7	6–6.9		5.5–5.9	3.5–5.4	3–3.4	2.5–2.9		<2.5
Serum creatinine (umol/l)	≥350	200–340	150–190		60–140		<60		
Hematocrit (%)	≥60		50–50.9	46–49.9	30–45.9		20–29.9		<20
White blood cell court (x1000 /mm^3)	≥40		20–39.9	15–19.9	30–14.9		1–2.9		<1
Glasgow coma score (GCS)	Score = 15 minus actual GCS								

Table 2 Chronic health points of APACHE II score

History	Points for elective surgery	Points for emergency surgery and nonoperative patients
Liver: Biopsy-proven cirrhosis and documented portal hypertension or prior episodes of hepatic failure	2	5
Cardiovascular NYhA class IV	2	5
Respiratory, e.g., severe COPD, hypercapnia, home O_2 pulmonary hypertension	2	5
Renal chronic dialysis	2	5
Immunocompromised	2	5

Table 3 Points for age for APACHE II score

Age in years	Points
≥ 44	0
45–54	2
55–64	3
65–74	5
≥ 75	6

parameters of a patient are subjected to minute-to-minute variations, the values taken from the monitors should be consistent and in line with the observed trend. This ensures erroneous higher and lower values do not skew the total score.

3. When to calculate? APACHE II score is an admission score and should be calculated within 24 hours of admission to ICU. Common errors include considering the pre-resuscitation parameters recorded in the emergency room before initial resuscitation for calculation of the score. Similarly, in surgical patients, parameters recorded in the operating room should not be considered due to the physiological alterations during surgery.
4. Few studies have shown that APACHE II score calculated at admission is better and less laborious than calculating the worst 24 hours APACHE II score as originally described (Ho et al. 2006).
5. A common mistake clinicians commit is to repeat the score calculation at regular intervals in the ICU to assess the improvement of the patient. One must remember that APACHE II score is an admission score, and recalculating the score at regular intervals in the ICU may not be useful.
6. While calculating the score, rectal temperature should be used as it is validated in various studies.
7. Acute kidney injury: Only acute elevations of serum creatinine should be given double points. Points should not be erroneously doubled for chronically elevated serum creatinine. Clinical judgment is necessary to attribute whether the elevation is because of acute kidney injury or not.

8. Calculating Glasgow Coma Score (GCS): Sedation or anesthesia-induced low GCS should be disregarded. While poisoning induced, metabolic and drug-induced low GCS is given points.
9. Calculating (A -a) O_2 gradient or PaO_2 (mm hg): FiO_2 value should be considered strictly while choosing either (A -a) O_2 gradient or PaO_2 (mm hg) for calculating the points. If $FiO_2 < 0.5$, use PaO_2. If $FiO_2 > 0.5$, use (A-a) O_2 gradient.
10. In case of unavailability of a few parameters required for the calculation of the score, they should not be considered normal while calculating the score.

Uses of APACHE II Score in the ICU

1. **Outcome prediction:** The main aim of the score when designed was to predict the outcomes of the severely ill patients, among which mortality is the primary outcome. APACHE II score correlates with the mortality of the patients in the ICU as per the original article (Knaus et al. 1985). The expected mortality calculated from the APACHE II is score is shown in Table 4. Later studies proved that APACHE II score overestimates the mortality of acutely ill patients (Sedloň et al. 2016). The graph between APACHE II score and predicted mortality is a sigmoid curve with a gradient as variables like need for emergency surgery are also included.

   The equation for predicting mortality using APACHE II score is

$$\frac{e^{logit}}{1 + e^{logit}}$$

$$where\ logit = -3.517 + (APACHE\ II) \times 0.146.$$

2. **Communication among medical professionals:** In the late twentieth century when the critical care was expanding and improving, there was a dire need for a common method of communication between intensivists. The GCS introduced by Jenett B and Teasdale G was a breakthrough in the field of neurotrauma. APACHE II is one such score which is widely used for communication regarding patients receiving critical care across various platforms. As it includes multiple

Table 4 Predicted mortality as per APACHE II score

0 to 4 points:	4% non-op, 1% post-op
5 to 9 points:	8% non-op, 3% post-op
10 to 14 points:	15% non-op, 7% post-op
15 to 19 points:	24% non-op, 12% post-op
20 to 24 points:	40% non-op, 30% post-op
25 to 29 points:	55% non-op, 35% post-op
30 to 34 points:	Approx 73% both
35 to 100 points:	85% non-op, 88% post-op

physiological variables, it is more reliable than any other single parameter used earlier.

3. **Allocation of resources:** Obviously, sicker patients need greater resources and care. They are expected to stay for a longer time and demand more care. APACHE II is one such score which can be used to predict the need for resources in the ICU and make appropriate arrangements of inventory beforehand. In the resource poor countries, there is always a disproportion between the available ICU beds and the patients needing them. Some institutes follow the principle of allocation of ICU beds for only salvageable patients based on the APACHE II score, even though it is ethically not appropriate to deny a patient of much needed care though the outcomes are expected to be poor. This is often called as the patient triage for ICU care. One must learn that none of the ICU scores are 100 percent specific!
4. **Clinical decision-making:** APACHE II score apart from predicting mortality is also proved to predict the expected severity of various clinical illnesses, thereby helping to decide the most appropriate treatment. For example, APACHE II score is highly validated for predicting severity, local complications, and length of hospital stay in acute pancreatitis. A score of greater than eight is highly suggestive of need for aggressive resuscitation and monitoring.

   Similarly for surgical patients, APACHE II score is predictive of various surgical complications in the postoperative period. In the operating room, it is used as a measure to decide the need for a damage control surgery or a definitive intervention. For example, APACHE II score can be used as a guide to predict the anastomotic leak and hence avoid anastomosis and prefer making a stoma in critically ill surgical patients.
5. **Clinical audit and research:** Audit refers to regular self-assessment and measuring one's performance against already set standards and institute corrective measures if the performance is falling short. Clinical research refers to testing new methods and technologies of clinical care for their effectiveness. Both audit and research require measuring tools to quantify how sick is a patient admitted to the ICU. APACHE II is one of the commonly used tools for this purpose. Measuring the impact of corrective measures and newer practices require scoring systems such as the APACHE II score.
6. **Others:** Nosocomial infections are a burden to the healthcare and add to the mortality and morbidity of the patients especially in the ICU setting. High APACHE II score was associated with nosocomial infections in a study (Suka et al. 2004).

APACHE III and APACHE IV

Knaus et al. in 1991 developed the APACHE III score as an improvement over the existing APACHE II score (Knaus et al. 1991). It is based on the data collected from 17,400 patients across 40 ICUs located in the United States (US). The total score ranges from 0 to 299. It is based on 17 physiological variables. The weightage for

several parameters has been modified, and the diseases included have been expanded. Further modifications of the APACHE III score resulted in versions like "i" and "j" APACHE III scores. The APACHE IV score was introduced in 2002 as a modification of APACHE III score. Several changes are added such as in the absence of any value of a parameter, "the day nearest to the moment of ICU admission" value could be used. A method for neurological evaluation of sedated patients was also added. These complex modifications of APACHE III and APACHE IV are based on the US population and not widely validated across the globe. At present, the only APACHE score that is being used widely across the globe is APACHE II.

Other Scoring Systems Used in ICU

Even though the APACHE II score is the most widely used prognostic score in the ICU, there are several other scoring systems also which are used in practice. Simplified Acute Physiological Score (SAPS) II is based on 12 physiological variables, age, chronic health points, and type of admission (Le Gall et al. 1993). SAPS III is an updated version of SAPS II score with inclusion of several other variables such as reason for ICU admission, surgical status of the patient, anatomical site of injury, presence of infection, and planned/unplanned admission. Even though SAPS III score is inferior to APACHE IV, it is applicable to a greater population across the world than APACHE IV.

The Mortality Prediction Model (MPM) score and several of its modifications are also used to predict the mortality in ICU patients. MPM score is relatively easy to calculate as the variables are binary. Sekulic AD et al. compared SAPS II score, APACHE II score, and MPM II score for predicting mortality in critically ill and found that MPM score has the highest discriminatory power among the three scores (Sekulic et al. 2015). The Pediatric Index of Mortality (PIM) scores is a set of scores specifically designed for predicting the mortality in patients younger than 16 years of age. The PIM score was introduced by Shann in 1997 and was updated in 2003. PIM consists of both clinical and laboratory values (Gandhi et al. 2013). Gandhi J et al. evaluated PIM II score in pediatric ICU for the prediction of mortality and found a significant association between PIM II score and mortality (Gandhi et al. 2013).

The Systemic Inflammatory Response Syndrome (SIRS) is the occurrence of any two or more of the following criteria: temperature $> 38.0\ ^{\circ}C$ or $< 36.0\ ^{\circ}C$, tachycardia >90 beats/minute, tachypnea >20 breaths/minute, leucocytosis $>12*10^9$/l, or leucopoenia $<4*10^9$/l (Bone et al. 1992; Dellinger et al. 2008). SIRS with any source of infection is defined as sepsis. Sepsis-related Organ Failure Assessment (SOFA) (latter renamed as Sequential Organ Failure Assessment score) was introduced by Vincent JL et al. (Vincent et al. 1998). It is used to assess the organ failure due to sepsis by grading the PaO_2/FiO_2, mean arterial blood pressure, and the use of inotropes, serum creatinine, serum bilirubin, GCS, and platelet count. A score of ≥ 2 is suggestive of organ failure. As per the Sepsis-3 guidelines, SOFA score is better than SIRS criteria for mortality prediction in ICU (Seymour et al. 2016). Quick SOFA (qSOFA) is a bedside score consisting of GCS, respiratory rate, and systolic

blood pressure as an easy alternative to SOFA score. qSOFA score predicts in hospital mortality outside ICU better than the SOFA or Systemic Inflammatory Response Syndrome (SIRS) score (Koch et al. 2020). The other score used for assessing the organ failure is modified Marshall Scoring system. It is based on PaO_2/FiO_2, systolic blood pressure, and serum creatinine. Falcão ALE et al. compared SAPS III, SOFA, and APACHE II score for predicting ICU deaths and found no difference between them (Falcão et al. 2019).

In the setting of trauma, several scoring systems are used such as Revised Trauma Score (RTS), Injury Severity Score (ISS), Trauma Revised Injury Severity Score (TRISS), Penetrating Abdominal Trauma Index (PATI), etc.

Evaluating a Scoring System

As newer scoring systems continue to be developed and evolve over time, one must know how to choose a best scoring system. It should be based on the following:

- **Discrimination:** It is the ability of scoring system to distinguish those with mortality from those who survive. It is measured by plotting a receiver operating characteristic (ROC) curve and calculating the area under the ROC curve (AUROC). If the AUROC is one, then it signifies a perfect scenario. Any AUROC above 0.7 is considered reasonable with values greater than 0.9 depicting excellent discriminatory power.
- **Calibration:** It is the measure of how close the predicted values are to the observed values. It is specific for a clinical situation and a geographic location.
- **Validity:** It is how well the designed scoring system works when reapplied on another set of patients and on a different population.

Standardized morality ratio (SMR) is defined as the ratio of observed deaths to predicted deaths and is used to compare the performance of different ICUs. The performance of a single ICU over a period of time can also be measured by using exponentially weighted moving averages and cumulative sum charts. There has been a move from using sensitivity, specificity, and AUROC to using decision-making analysis in the evaluation of the prediction scores (Vickers 2008).

Conclusion

Scoring systems in ICU are very useful to predict patient outcomes like mortality and to make clinical decisions. They play an important role in audit and research. One must be aware about the pitfalls while calculating the scoring systems. Use of preprinted charts and online calculators in smart phones will make the calculation process easy. One must be wise enough to interpret the data from scoring systems to make clinical decisions as no scoring system is 100 percent perfect. As our clinical knowledge evolves, these scoring systems also evolve.

Applications to Prognosis, Other Diseases, or Conditions

Apart from the originally intended use in predicting the mortality in the ICUs, APACHE II has evolved and has been validated for various other purposes and diseases. APACHE II score is used for evaluating quality of care and financial incentives to ICUs (Wagner and Draper 1984). It is also used as a criteria for admitting and referring patients in specific hospitals (Wagner and Draper 1984). In acute pancreatitis, APACHE II score is used to assess the severity of the disease and therefore help to guide management and treatment (Saneesh et al. 2021). APACHE II score is also used to assess the mortality and morbidity in acute ill surgical patients such as patients of perforation peritonitis (Yelamanchi et al. 2020). In surgical practice, APACHE II score also correlated with postoperative surgical complications and tissue flap failures (Grant et al. 2007). APACHE II score has also been validated in predicting outcomes in patients of acute neurological illness, heart failure, respiratory illness, liver cirrhosis, and renal failure.

Mini-Dictionary of Terms

Intensive care unit: An organized medical setup comprising of sophisticated life-saving equipment and monitors along with trained medical care personnel for treating the patients who are very sick and ill.

Clinical study: A process of accepting or refuting a hypothesis based on statistics.

Audit: Method of comparing self-reported outcomes with existing standards and implementing corrective measures.

Glasgow Coma score: A scoring system used to assess the severity of coma or neurological dysfunction. It is based on motor responsiveness, verbal performance, and eye-opening of the patient.

Serum creatinine: A biochemical test to measure the renal function.

PaO_2: Partial pressure of oxygen gas in the arterial blood.

FiO_2: Fraction of oxygen gas in the inspired air.

(A -a) O_2 gradient: Gradient of partial pressure of oxygen gas in the alveoli and arterial blood which signifies ventilation perfusion mismatch.

Key Facts of APACHE II Score Calculation

- There are many preprinted formats of APACHE II score sheets available online. Hospitals can design their formats based on local policies.
- A list of locally encountered diseases and their chronic health points should be prepared by expert physicians, which may be useful for the paramedical personnel while calculating the score.

- Workshops have to be conducted to familiarize the ICU staff with the scoring systems.
- If in doubt during score calculation, do not approximate and feel free to consult a senior.
- Scoring systems are just a guide. Clinically appropriate decisions have an upper hand over those derived from a scoring system in case of dilemma.

Summary Points

- The APACHE score was first developed by Knaus WA et al. in 1981.
- APACHE II score consists of age, 12 physiological variables, and chronic health points.
- One must use a preprinted chart for accurate calculation, and also be aware of the pitfalls while calculating the APACHE II score.
- The main aim of the APACHE score when designed was to predict the outcomes of the severely ill patients, among which mortality was the primary outcome.
- The graph between APACHE II score and predicted mortality is a sigmoid curve.
- APACHE II score is also used for many other purposes like communication among professionals, resource allocation, audit and research studies, etc. in the ICU.
- Apart from the ICU, APACHE II is also used for predicting outcomes in diseases like acute pancreatitis, acute surgical emergencies, etc.
- There are several newer modifications of APACHE score such as APACHE III and IV which need further modification.
- There are other scores like SAPS, MPM, PIM, SIRS, SOFA, and other disease-specific scores which are being used for outcome prediction.
- Scoring systems are evaluated on the basis of discrimination, calibration, and validity.

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The Score for Trauma Triage in Geriatric and Middle-Age (STTGMA): Utilizing Macroscopic Clinical Biomarkers to Guide Patient Care

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Abstract

Orthopedic trauma in the geriatric and middle-aged cohort is a significant challenge facing the medical community due to their complex physiologic profile which portends worse outcomes compared to their younger counterparts. As the population ages and medical advances keep patients alive longer, the frequency and burden of geriatric orthopedic trauma will continue to increase.

The Score for Traumatic Triage in Geriatric and Middle-Aged patients (STTGMA) was created as a risk assessment tool to augment the decision-making process for treatment of geriatric trauma. Utilizing macroscale clinical variables (which are real-world manifestations of microscopic biomarkers) shown to be predictors of mortality, STTGMA constructs a model to characterize a patient's short- and long-term mortality risk. On arrival to the hospital, a patient's baseline physiologic and injury status is used to create an objective numeric assessment of their perioperative mortality and morbidity risk, with certain thresholds prompting specialized medical consultations and pathways designed to optimize a patient's outcome.

The algorithm is designed to evolve to match changing healthcare environments and to provide modern assessments for each orthopedic trauma patient encountered. A patient's frailty, American Society of Anesthesiologists physical status classification system (ASA) score, and COVID-19 status on admission have all been added as risk factors for mortality.

Utilizing STTGMA to risk stratify patients, medical practitioners can better assess patient outcomes and perform comparative analysis among patients with various fracture types, including hip, femur, tibia, ankle, and humeral fractures. Because STTGMA utilizes macroscale clinical manifestation of the downstream effects of various biomarkers, it can provide an improved understanding of how various biomarkers interact on the microscopic scale.

In the future, STTGMA will continue to evolve to better incorporate macroscale clinical variables that reflect geriatric trauma patients' morbidity and mortality risk profile. If rapid tests are developed that are able to detect microscale biomarkers, then these variables could be incorporated into the STTGMA algorithm to improve the ability to risk stratify patients at the time of initial patient contact in the emergency department setting.

This chapter goes on to discuss the origins and evolution of the validated risk score STTGMA with further analysis-associated applications and impact on orthopedic care.

Keywords

Geriatric · Trauma · Hip · Fracture · Mortality · Morbidity · Risk tool

Abbreviations

ADL	Activities of daily living
AIS	Abbreviated Injury Scale
ASA	American Society of Anesthesiologists
AUROC	Area under receiver operating characteristic
CCI	Charlson Comorbidity Index
COVID-19	Coronavirus Disease of 2019
DEXA	Dual-energy X-ray absorptiometry
ED	Emergency Department
GCS	Glasgow Coma Scale
IADL	Instrumental activities of daily living
ISS	Injury Severity Scale
RBC	Red blood cell
ROC	Receiver operating characteristic
STTGMA	Score for traumatic triage in geriatric and middle aged
TRISS	Trauma Injury Severity Scale
WHO	World Health Organization

Introduction

As the population ages and life expectancy continues to increase over the last several decades, there is an increasingly urgent need to care for the geriatric population. The 2020 US Census showed that 22.9% of the US population is over the age of 60. This is a 4.7% increase since the 2010 census that had 18.2% of the US population over the age of 60 (US Census Bureau 2011; US Census Bureau 2019) (Fig. 1). Much of this increase is driven by the Baby Boomers born between 1946 and 1964. The current life expectancy in the United States is 78.54 years old (The World Bank 2020). By 2030, the US Census Bureau has projected that nearly one in five US residents will be over 65 years old. Additionally, by 2050, the percentage of the US population that is made up of older Americans is expected to double the 2010 fig. (40.2 million to 88.5 million) (Fig. 2) (Vincent and Velkoff 2010).

Internationally, the number of people over the age of 60 is expected to increase from about 900 million to roughly 2 billion between the years 2015 and 2050 (WHO 2021). This correlates to a proportional increase of roughly 12% to 22%. Just in the last two decades, the average life expectancy has risen by roughly 5 years from 67 to 72 years old. The population globally is getting older and aging at a faster rate than previously (WHO 2021). With increasing development, low- and middle-income countries are expected to experience the majority of this aging burden, with roughly 80% of individuals over the age of 60 expected to be living in low- and middle-

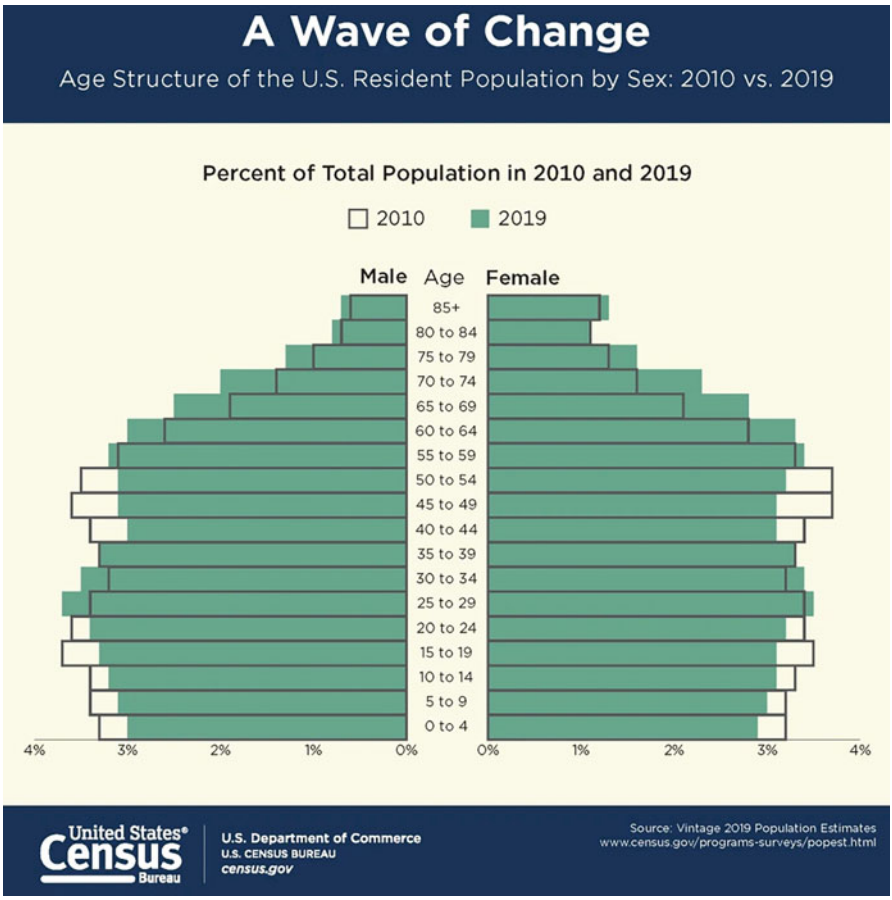

Fig. 1 A wave of change: Age structure of the US resident population by sex: 2010 vs 2019, from US Census Bureau. (From the Vintage 2019 Population Estimates, US Census Bureau (US Census Bureau 2020))

income countries in 2050 (Fig. 3) (United Nations et al. 2017; WHO 2021). Despite the increasing average life expectancy, this does not imply a longer period of "good health" in the global population (Beard et al. 2016). An aging population has also demonstrated the phenomenon of "multimorbidity" across the globe, with interactions between multiple ailments and disorders leading to a worse overall health status and potential need for higher level of care (Marengoni et al. 2011). No matter the location, an aging population presents unique health risks and health challenges.

In the United States, trauma is the leading cause of death for individuals less than 45 years old. As of 2019, for all age groups, trauma is the third leading cause of death in the United States (Kochanek et al. 2020). Based on data reported by the US National Trauma Databank (NTDB), from 2005 to 2015, there has been an increase

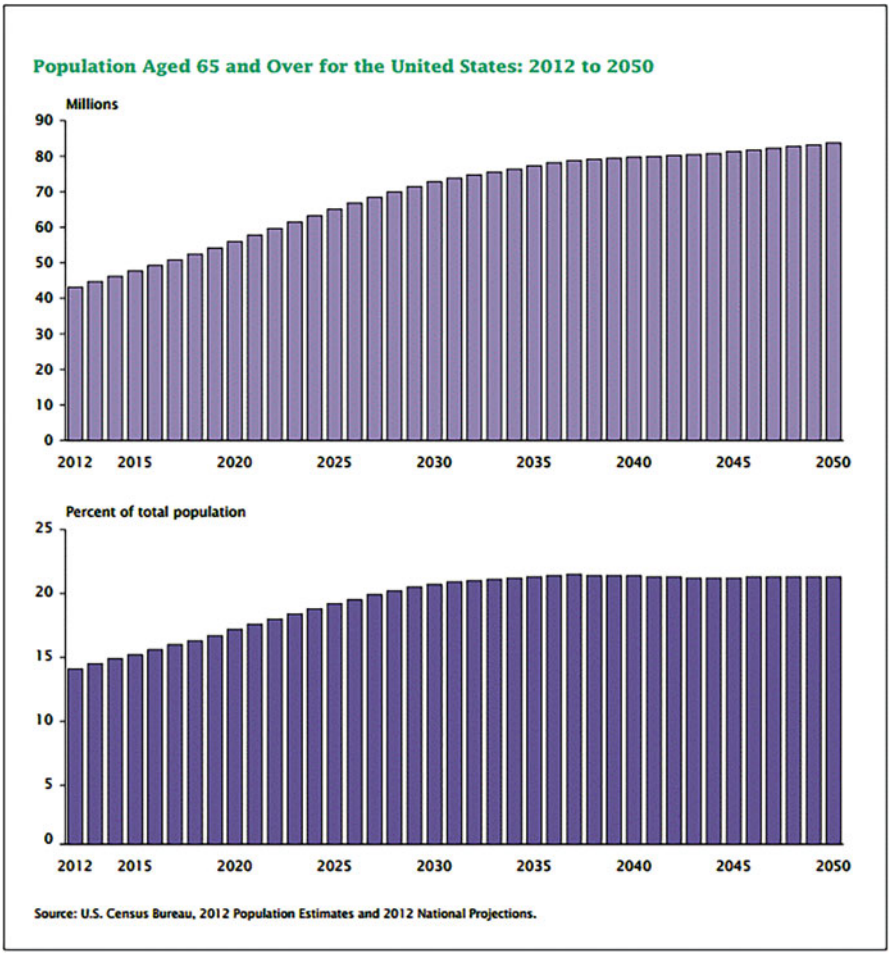

Fig. 2 Population aged 65 and over for the United States: 2012 to 2050. (From the US Census Bureau, 2012 Population Estimates and 2012 National Projections (Ortman et al. 2014))

in the percentage of geriatric traumas from 18% to 30% (NTDB 2020). Internationally, in low-income countries, trauma due to road injuries is the seventh leading cause of death. In middle-income countries and upper-middle income countries, trauma due to road injuries is the tenth leading cause of death (WHO 2020). With a higher volume of trauma patients, especially older patients who have various comorbidities, there is the need to develop tools to augment the treatment of these patients. In particular, risk assessment in these patients is important to develop strategies to optimize patient care and outcomes. The Score for Trauma Triage in the Geriatric and Middle-Aged (STTGMA) is a risk assessment tool that was developed to provide risk assessment in this aging trauma population.

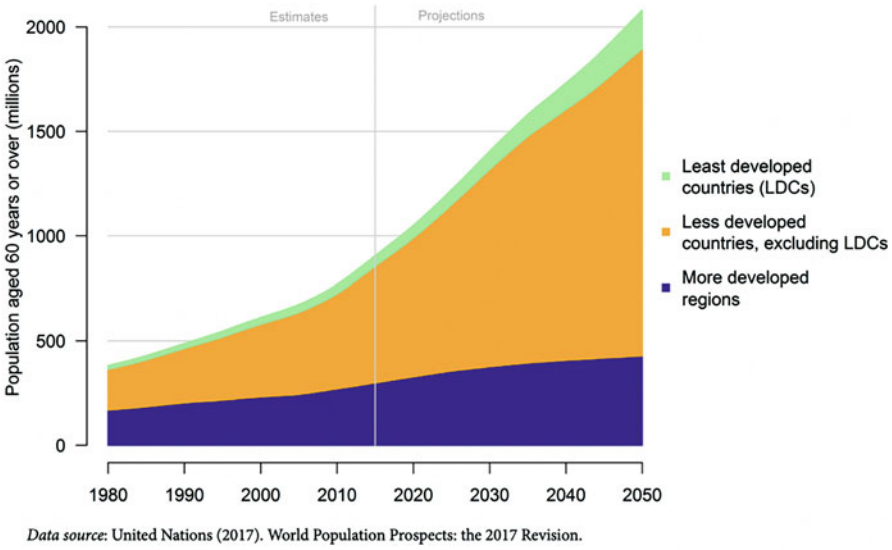

Fig. 3 Number of persons aged 60 years or over by development group, from 1980 to 2050. (From World Population Ageing 2017 Highlights, by the Department of Economic and Social Affairs, Population Division, © 2017 United Nations. Reprinted with the permission of the United Nations. (United Nations et al. 2017))

Middle-aged and geriatric trauma patients present with unique care challenges as they tend to have more comorbidities and require higher levels of care to return to their baseline after injury. This patient population often has worse functional recovery and requires earlier intervention compared to younger patients (Kelley-Quon et al. 2010; Grossman et al. 2012; Tillou et al. 2014). For older patients, the loss of mobility or a change in functional status due to injury makes completing activities of daily living (ADLs) challenging. It has been shown that geriatric patients who are compromised in their ability to complete ADLs after trauma have more difficulty remaining independent and are at higher risk for additional trauma. Many of these patients require placement in a rehabilitation facility and have higher rates of mortality (Tsuji et al. 1994; Gill et al. 1998). Understanding the characteristics of this patient population and why they are at higher risk for morbidity and mortality is important in meeting their healthcare needs and allocating the appropriate resources. The elements that lead to these higher risks are multifactorial and include understanding their preinjury functional status, comorbidities, socioeconomic status, demographics, and injury status upon presentation to the Emergency Department (ED) (Makary et al. 2010; Seematter-Bagnoud et al. 2013; Joseph et al. 2014).

In the process of developing STTGMA, our institution undertook an epidemiological study to better understand the age-related frequency, demographics, costs, and inpatient needs of the middle-aged and geriatric orthopedic trauma population. From 2014 to 2017, all patients >55 years of age who required orthopedic, trauma, and/or neurosurgery consultations at 3 hospitals within our academic medical institution were prospectively followed. This allowed for analysis of a cohort of

Table 1 Comparison of variables found to be independent predictors of mortality

	$STTGMA_{LE}$ OR (95% CI)	P-value	$STTGMA_{HE}$ OR (95% CI)	P-value
Age	1.05 (1.03–1.07)	<0.01	1.08 (1.03–1.17)	<0.01
CCI	1.28 (1.14–1.44)	<0.01	–	–
GCS	0.72 (0.69–0.76)	<0.01	0.69 (0.64–0.75)	<0.01
AIS-HN	1.67 (1.49–1.87)	<0.01	1.77 (1.44–2.21)	<0.01
AIS-CHS	1.52 (1.19–1.92)	<0.01	1.51 (1.20–1.90)	<0.01
AIS-EXT	–	–	1.59 (1.14–2.21)	<0.01

From Konda et al. (2016) with permission.
STTGMA score for trauma triage in the geriatric and middle-aged; *LE-GMTP* low-energy geriatric and middle-age trauma patient; *HE-GMTP* high-energy geriatric and middle age trauma patient; *CCI* Charlson comorbidity index; *GCS* Glasgow coma scale; AIS = abbreviated injury score, *HN* Head & Neck; *CHS* Chest; *EXT* extremity

3965 patients of whom 82% sustained low-energy trauma and 18% sustained high-energy trauma (Konda et al. 2020d). This study found that hospital complications, need for ICU care, length of stay, mortality, and cost of care increase with increasing age (Konda et al. 2020d). Another previous study at our institution reported that some of the factors that were predictive of inpatient mortality among the geriatric population included age, AIS Head/Neck, AIS Chest, and GCS score (Table 1) (Konda et al. 2016, 2017). Understanding these factors is important for care planning.

Despite a plethora of research on orthopedic trauma in the young adult population, a need for focused research in geriatric orthopedic trauma was needed. STTGMA focuses strictly on this population and makes the important distinction to discriminate between low and high-energy mechanisms of injury. This is important as the relevance of comorbidities on inpatient mortality has been shown to be different depending on whether a low or high-energy mechanism of injury was sustained. Low-energy patients are more at risk for inpatient mortality as a result of their comorbidities, while high-energy patients are more at risk as a result of their injury (Table 1). With this background framework in mind, the original STTGMA ($STTGMA_{ORIGINAL}$) was developed with the following variables, each with a different impact on inpatient mortality: age, preexisting conditions, preinjury ambulatory status, Glasgow Coma Scale (GCS), and finally anatomic injuries defined with the Abbreviated Injury Scale (AIS) for both the Head/Neck and Chest (AISH/N and AISC) (Konda et al. 2016).

While possibly not fitting of the traditional definition of a biomarker, many of the variables used in the calculation of STTGMA can be considered macroscopic clinical markers that are downstream effects of microscopic biomarkers that result from injury. Biomarkers, in the broadest sense, are considered to be markers or signs that are well characterized and can be predictive of future clinical outcomes. They are sometimes affiliated with treatments and associated with different populations and can be thought of as small as at the molecular level to as large as certain manifestations on a physical exam or a specific blood test result. The World Health Organization (WHO) has defined biomarkers as being inclusive of "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may

be functional and physiological, biochemical at the cellular level, or a molecular interaction" (WHO 1993). The variables used in STTGMA fall under this definition as this tool evaluates the clinical manifestations of a patient's physical, biological, and functional status to determine a clinical endpoint. Over many iterations and after being tested and validated on thousands of patients, STTGMA has been able to consistently and accurately predict the clinical outcome of inpatient mortality and correlate to development of post-injury complications and long-term functional outcome and mortality.

This chapter further explores the origins and development of the STTGMA score, the validation process, and further iterations that have strengthened the predictive value of STTGMA. Also, each variable in STTGMA is analyzed with respect to the macroscopic clinical manifestation it measures and the underlying physiologic process behind it. Each of these variables can be thought of as biomarkers at the macro level, the culmination of many micro variables that may lead to increased risk for inpatient mortality.

Origin of STTGMA

As described above, there was a need to study orthopedic trauma in the geriatric population and develop a validated geriatric and middle-aged risk assessment tool. Many of the preexisting injury severity indices used to characterize mortality risk in trauma were developed in a young adult cohort and have been extensively studied in a predominantly young population (Baker et al. 1974; Osler et al. 1997). In comparison to the relatively healthy young adult trauma population, the geriatric population has different physiologic responses to trauma due to their increased burden of comorbidities. In addition, many of the existing tools failed to take into account the importance of mechanism of injury within the older trauma population (Konda et al. 2016). Prior studies combined both low-energy and high-energy mechanisms when evaluating older patients leading to skewed and poor predictive capabilities (Jacobs et al. 2003; Konda et al. 2014, 2015). Thus, a novel score to calculate risk of inpatient mortality in low-energy and high-energy geriatric and middle-aged patients (Score for Trauma Triage in Geriatric and Middle-Age, STTGMA) was developed.

STTGMA stratifies patients based upon energy imparted by the mechanism of injury: high energy or low energy. High energy is defined as falls from height $\geq$2 stairs, motor vehicle or motorcycle accidents, and pedestrian struck accidents. Low energy is defined as falls from a height of $<$2 stairs. Patients are considered middle-aged between 55 to 64 years of age and considered to be geriatric if they are 65 years of age or older. The risk model was developed based on the experiences of one level 1 trauma center that has a high volume of middle-aged and geriatric patients and then subsequently validated in over 100,000 patients culled from the National Trauma Databank (Konda et al. 2016). The overall purpose of STTGMA was to develop a predictive tool that assists in clinical decision-making early in a geriatric trauma patient's hospital course (Konda et al. 2016).

How STTGMA Was Created

Backwards stepwise multivariate binomial logistic regression analysis was used to create a model with the dependent variable of inpatient mortality (dichotomous outcome; No = 0 Yes = 1) and over 30+ independent variables. Independent variables were chosen to represent broad clinical indicators of physiologic status, comorbidity burden, and anatomic injuries. Through backwards stepwise regression, variables that did not meet the threshold for inclusion into the model ($p < 0.20$) were sequentially excluded until only independent variables with $p < 0.05$ were remaining. The model with the highest area under receiver operating characteristic (AUROC) and the best calibration was chosen. This resulted in the final model including five independent variables: age; Charlson Comorbidity Index (CCI); Abbreviated Injury Scale (AIS) for both the Head/Neck and Chest and Extremities (AISH/N and AISC, AIS-EXT); and Glasgow Coma Scale (GCS) (Konda et al. 2016). These five variables were found to all be independent predictors of mortality in middle-aged and geriatric patients (Table 1). The initial STTGMA calculation is as follows:

$STTGMA_{LE}$

Probability of Inpatient Mortality = 1/ (1± Exp(−(−3.41±0.05*AGE±0.25*CCI-0.33*GCS ± 0.51*AISHN±0.42*AISCHS)))

$STTGMA_{HE}$

Probability of Inpatient Mortality = 1 / (1 ± Exp(−(− 8.69 ± 0.11*AGE-0.37*GCS ± 0.57*AIS_HN ± 0.41*AIS_ CHS ± 0.46*AIS_EXT)))

These models were validated using the National Trauma Databank (NTDB) Research Dataset with 59,965 low-energy and 97,034 high-energy geriatric and middle-age trauma patients (Konda et al. 2016). Additionally, the STTGMA model was compared to the Trauma and Injury Severity Score (TRISS), the most commonly used trauma mortality risk tool. Both $STTGMA_{LE}$ and $STTGMA_{HE}$ were found to have significantly greater predictive capacity compared to TRISS (Table 2). The validation of this model on the NTBD dataset and the ability to outperform TRISS show the utility of the tool to predict inpatient mortality. The clinical variables used in $STTGMA_{LE}$ and $STTGMA_{HE}$ are macro-level manifestations of an array of biomarkers that reflect a patient's physiological response to injury and which have been shown to accurately predict a patient's risk of mortality.

An example of the predictive ability of STTGMA can be found in a recent article published in the Journal of Orthopedic Trauma by Konda et al. (2020c). The addition of a patient's COVID-19 status allowed for their STTGMA score to be updated rapidly to account for the changing medical landscape during the COVID-19 pandemic. Patients stratified to the low-risk STTGMA quartile had a mortality rate of 0.6%, whereas patients stratified to the high-risk STTGMA quartile had a mortality rate of 5.6% (Konda et al. 2020c). This difference was shown to be significant with a p-value of <0.01, highlighting STTGMA's ability to provide physicians with a tangible risk assessment score to help triage trauma patients and allow for more informed decisions in a patient's care.

Table 2 Difference in STTGMA variables between the NTDB and the Carolinas cohorts and comparison of STTGMA versus TRISS in the NTDB $STTGMA_{LE}$

$STTGMA_{LE}$	NTDB (N = 59,965)	Carolinas (N = 2387)	
	Mean ± 1 SD	Mean ± 1 SD	P-value
Age	74.7 ± 10.6	75.9 ± 11.1	<0.01
GCS	14.26 ± 2.34	14.12 ± 2.50	<0.01
AIS Chest	0.48 ± 1.09	0.20 ± 0.72	<0.01
AIS head and neck	1.58 ± 1.98	1.64 ± 1.86	0.13
CCI	0.66 ± 1.17	0.97 ± 1.39	<0.01
$STTGMA_{LE}$ (AUROC)	0.83		<0.01
TRISS (AUROC)	0.80		
$STTGMA_{HE}$	NTDB (N = 97,034)	Carolinas (N = 1434)	
	Mean ± 1 SD	Mean ± 1 SD	P-value
Age	67.9 ± 9.4	66.7 ± 9.2	<0.01
GCS	14.06 ± 2.91	14.09 2.85	0.70
AIS Chest	1.37 ± 1.61	1.03 ± 1.44	<0.01
AIS head and neck	1.54 ± 2.0	0.85 ± 1.5	<0.01
AIS extremities	1.38 ± 1.20	0.93 ± 1.22	<0.01
$STTGMA_{HE}$ (AUROC)	0.86		<0.01
TRISS (AUROC)	0.85		

From Konda et al. (2016) with permission.
$STTGMA_{HE}$ Score for trauma triage in the geriatric and middle-aged – High energy; *$STTGMA_{LE}$* Score for trauma triage in the geriatric and middle-aced – Low energy; *TRISS* Trauma score-injury severity score; *NTDB* National Trauma Databank; *GCS* Glasgow coma scale; *AIS* abbreviated injury scale; *CCI* Charlson comorbidity index; *SD* standard deviation

Evolution of STTGMA

As the medical landscape has evolved over time, so too has STTGMA. The following section delineates the timeline of modifications to STTMGA since its inception. Modifications to STTGMA are achieved through additional permutations that input new biomarkers into the risk modeling algorithm and then compare the new models to prior models.

Frailty Factor

Beginning in 2017, STTGMA tested the use of clinical manifestation of frailty in the risk algorithm. Frailty is defined as a clinical syndrome resulting in an individual's decreased physiologic reserve and increased susceptibility to disability in the presence of various stressors such as illness or trauma (Fried et al. 2001). The biomarkers of frailty that were tested in the algorithm were as follows:

1. Preinjury assistive device use (disability).
2. Independent ambulatory status (functional independence).
3. Albumin level (nutrition).

1486 patients involved in orthopedic or neurosurgical traumatic consultations between September 2014 and September 2016 were included in the analysis. Successive binary logistic regression analyses were applied to determine if addition of the frailty variables listed above would improve the ability of STTGMA to predict mortality. Interestingly, there was no significant improvement in the predictive capacity of $STTGMA_{frailty}$ compared to $STTGMA_{original\ LE\ or\ HE}$ (Figs. 4 and 5). The conclusion from this study is that the original STTGMA score appropriately

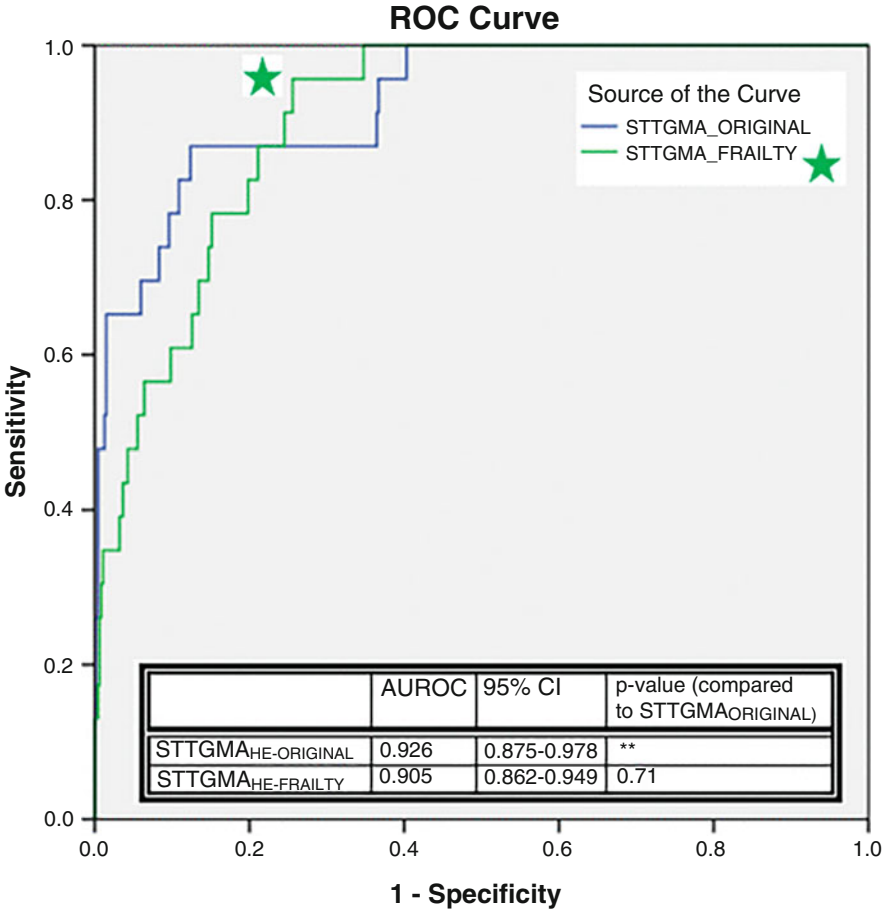

Fig. 4 The ROC curves for $STTGMA_{HE\text{-}ORIGINAL}$ and $STTGMA_{HE\text{-}FRAILTY}$ and comparison of AUROC for 2 models; $STTGMA_{HE\text{-}ORIGINAL}$, high-energy score for trauma triage in the geriatric and middle-aged; $STTGMA_{HE\text{-}FRAILTY}$, high-energy score for trauma triage in the geriatric and middle-aged with additional frailty variables (Konda et al. 2017). (From Konda et al. (2017) with permission)

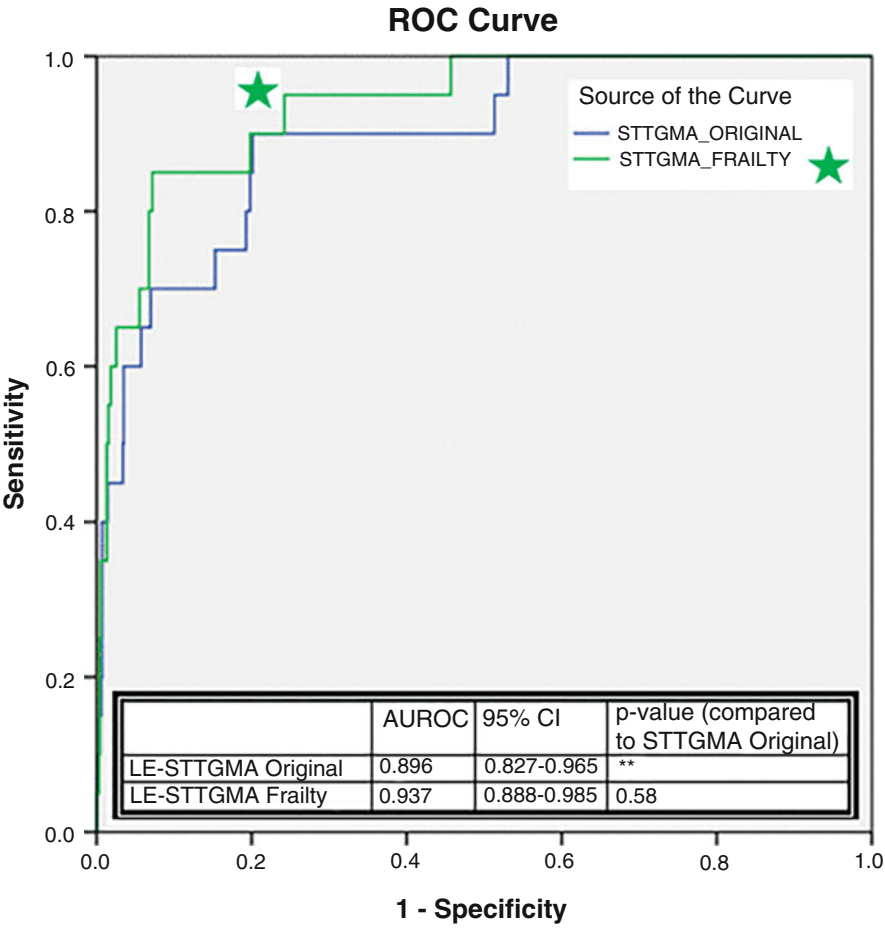

Fig. 5 The ROC curves for $STTGMA_{LE\text{-}ORIGINAL}$ and $STTGMA_{LE\text{-}FRAILTY}$ and comparison of AUROC for two models. AUROC indicates area under the receiver operating characteristic curves; $STTGMA_{LE\text{-}ORIGINAL}$, low-energy score for trauma triage in the geriatric and middle-aged; $STTGMALE_{FRAILTY}$, low-energy score for trauma triage in the geriatric and middle-aged with additional frailty variables (Konda et al. 2017). (From Konda et al. (2017) with permission)

accounts for a patient's frailty at the time of injury and the original clinical biomarkers are sufficient to predict mortality (Konda et al. 2017).

COVID-19 Factor

The spring of 2020 changed the world. The onset of the COVID-19 pandemic stressed healthcare systems across the globe and altered the face of medicine. The COVID-19 virus represented a new entity that was directly affecting patient mortality and was previously unaccounted for in risk modeling algorithms. To accommodate for this unexpected risk factor, a patient's COVID status on admission was

added to the STTGMA score model. The COVID-19 cohort of hip fracture patients included 136 patients treated during the initial pandemic outbreak between February 1, 2020 and April 15, 2020. A comparison cohort of 1278 hip fracture patients treated between October 2014 and January 31, 2020, was used as the control group. Compared to $STTGMA_{ORIGINAL}$, $STTGMA_{COVID}$ was able to triage 100% of COVID-19-related inpatient and 30-day mortalities into the highest-risk quartile (Fig. 6). This demonstrated the ability of the STTGMA tool to adapt to new risk factors and appropriately assess patient outcome (Konda et al. 2020c).

ASA Score

The most recent STTGMA addition occurred in January 2021 with the inclusion of a patient's ASA physical status classification system score. The ASA Physical Status Classification System is used to assess a patient's pre-anesthesia medical comorbidities to assess fitness for surgery. The ASA score ranges from 1 (healthy patient) to 6 (brain dead patient), and each ASA category describes a broad swath of clinical manifestations of a patient's disease burden (Table 3). It historically has been used to stratify a patient's perioperative risk (Daabiss 2011). To better assess how the ASA score could be used within the STTGMA tool, 1332 patients aged 55 and older with a hip fracture sustained via a low-energy mechanism between October 2014 and February 2020 were included for analysis. A new logistic regression model termed $STTGMA_{ASA}$ was created which was compared against the ability of $STTGMA_{ORIGINAL}$ to triage inpatient mortality. Both models included the original clinical biomarkers of age, CCI, GCS, AIS-Ext, AIS-CHEST, and AIS-HN. $STTGMA_{ASA}$ included the additional clinical biomarker of ASA score. $STTGMA_{ASA}$ was found to have a significantly better ability to predict inpatient mortality compared to $STTGMA_{original}$ and also was better able to stratify patient hospital quality measures (length of stay, complication risk, readmission risk, and discharge disposition) (Fig. 7) (Konda et al. 2021d).

STTGMA Has Wide Applicability

STTGMA is a validated geriatric trauma risk prediction tool. This section highlights its application to further assess many aspects of orthopedic trauma care.

STTGMA Application for Various Fracture Patterns

Fracture Locations

While the origin of STTGMA derived from general trauma cases that included both orthopedic and non-orthopedic traumatic injuries and subsequent studies focused primarily on traumatic hip fracture patients, this is not the limit of its scope. Multiple studies have demonstrated the ability of STTGMA to risk stratify patients with

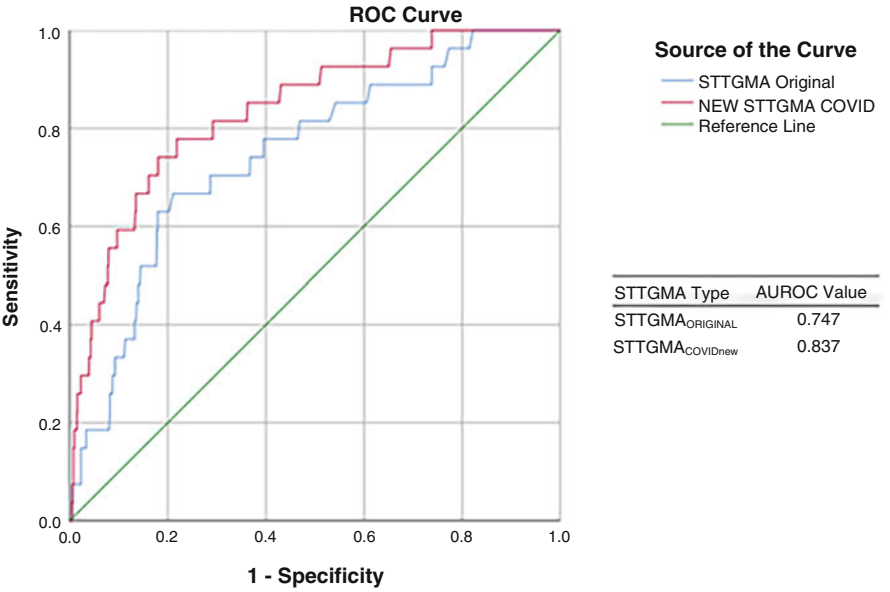

Fig. 6 ROC curves generated from $STTGMA_{ORIGINAL}$ and $STTGMA_{COVIDnew}$ with the area under each operator curve (AUROC) (Konda et al. 2020c). (From Konda et al. (2020c) with permission)

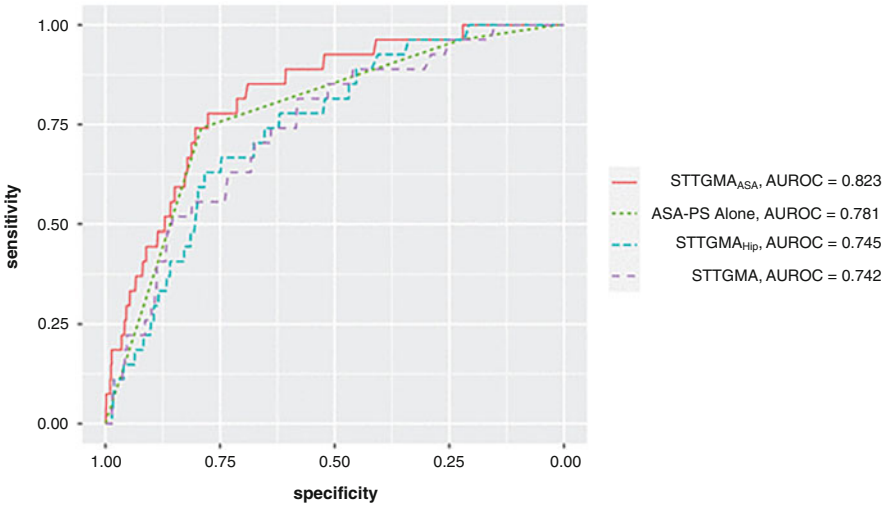

Fig. 7 ROC curves generated from $STTGMA_{ASA}$, ASA-PS alone, $STTGMA_{Hip}$, and STTGMA risk scores with the AUROC (Konda et al. 2021d). (From Konda et al. (2021d) with permission)

Table 3 ASAPS physical status classification system level descriptions

American Society of Anesthesiologists Physical Status Classification System (ASAPS)	Description
Level	
ASA 1	A normal healthy patient. Example: Fit, nonobese (BMI under 30), a nonsmoking patient with good exercise tolerance
ASA 2	A patient with mild systemic disease. Example: Patient with no functional limitations and a well-controlled disease (e.g., treated hypertension, obesity with BMI under 35, frequent social drinker, or cigarette smoker)
ASA 3	A patient with a severe systemic disease that is not life-threatening. Example: Patient with some functional limitation due to disease (e.g., poorly treated hypertension or diabetes, morbid obesity, chronic renal failure, a bronchospastic disease with intermittent exacerbation, stable angina, implanted pacemaker)
ASA 4	A patient with a severe systemic disease that is **a constant threat to life**. Example: Patient with functional limitation from severe, life-threatening disease (e.g., unstable angina, poorly controlled COPD, symptomatic CHF, recent (less than three months ago) myocardial infarction or stroke
ASA 5	A moribund patient who is not expected to survive without the operation. The patient is not expected to survive beyond the next 24 hours without surgery—Examples: Ruptured abdominal aortic aneurysm, massive trauma, and extensive intracranial hemorrhage with mass effect
ASA 6	A brain-dead patient whose organs are being removed with the intention of transplanting them into another patient

From the American Society of Anesthesiologists (From Doyle et al. 2021 with permission)

specific orthopedic injuries including ankle fractures, distal radius fractures, tibial shaft and plateau fractures, proximal and mid-shaft humerus fractures, and hip and femur fractures (Konda et al. 2018a, 2021a, 2020b; Lott et al. 2019; Adenikinju et al. 2021).

For each fracture pattern, STTGMA reliably risk stratifies a patient's hospital quality measures (length of stay, complication risk, discharge disposition, readmission status) and associated direct hospital costs during admission. Stratifying patients based on their admission STTGMA scores provides care providers with the ability to improve a patient's index admission outcomes while simultaneously reducing cost for both the patient and the hospital. This ultimately results in improved value per episode of patient care.

STTGMA Application for Inpatient Admission

The clinical biomarkers that STTGMA utilizes have been demonstrated to apply to a wide range of clinical scenarios that occur during inpatient hospitalization.

Delays to Surgery

The standard of care for most hip fracture patients remains operative fixation, especially in the geriatric population (Parker and Johansen 2006). Widespread comorbidities and a higher risk of mortality in this population reinforce the need for timely intervention after the injury occurs. Recent studies discuss the temporal urgency of hip fracture care, notably that each successive day post-injury increases a patient's complication rate, with a delay in surgery 2 or more days resulting in an increased mortality rate (Ryan et al. 2015). Due to this, a study in 2020 sought to determine whether STTGMA could predict a patient's time to surgery, risk factors for a delay in time to surgery, and 30-day mortality risk. Six hundred eleven patients with operative hip fractures met inclusion criteria and were reviewed further. On admission, each patient was stratified into 1 of 4 risk quartiles based on STTGMA score. Results found that median time to surgery and delay to surgery (defined as operative fixation >48 hours post admission) both have significant association with STTGMA stratification. Patients in the high-risk quartile experienced 6.6% higher rate of 30-day mortality compared to the lowest-risk quartile, demonstrating STTGMA's utility in assessing 30-day mortality risk. Similarly, STTGMA can identify operative hip fracture patients who will likely have a delay in time to surgery so that early action can be taken to target these patients and adequately optimize them so that operative fixation may proceed in a timely fashion (Konda et al. 2020a).

Effective Timing of Blood Transfusions in Hip Fracture Patients

Geriatric and middle-aged hip fracture patients often experience significant morbidity and mortality following the inciting injury and subsequent admission. Cardiac, pulmonary, and renal complications are common, while significant acute blood loss associated with the injury and surgery similarly also occurs often (Sheehan et al. 2019). Anemia secondary to this acute blood loss episode remains the most common complications associated with the perioperative period, occurring in 25–50% of hip fractures (Carpintero et al. 2014)**.** Anemia (a deficiency in red blood cells/hemoglobin) in a patient correlates with many adverse outcomes following hip fracture, including longer admissions with worse outcomes and higher rates of readmission or mortality (Sim et al. 2018). Red blood cell count is a form of microscopic biomarker for anemia.

A study in 2021 sought to test whether transfusion timing correlated with outcomes in hip fracture patients. Previous studies have reported that postoperative blood transfusion leads to increased complication risk and mortality (Smeets and

Verbruggen 2018). Patients were separated into one of three cohorts depending on transfusion time: preoperative, intraoperative, or postoperative. The three cohorts were matched based on STTGMA, sex, and procedure type. Outcomes were compared both before and after propensity matching. The study results revealed that after propensity matching there were comparable outcomes among hip fracture patients who received preoperative blood transfusions as compared to intraoperative or postoperative blood transfusions (Parola et al. 2021). Therefore, patients with low red blood cell counts should be transfused whether it is in the pre-, intra-, or postoperative period without fear of worsening their clinical outcome. Management of anemia with timely blood transfusion perioperatively connects the micro marker entity of RBC count with the clinical sequelae of a patient's outcome postoperatively. STTGMA offers one method to bridge this gap between microscopic biomarkers and observable macroscopic clinical outcomes.

STTGMA Application after Discharge

The relevance of STTGMA does not end once a patient leaves the hospital. The following section highlights the various aspects of a patient's post inpatient hospitalization period where STTGMA has been shown to be predictive and capable of effective intervention.

Discharge Location

STTGMA has been shown to predict discharge location once a patient is ready to leave the hospital. Since STTGMA may be calculated on admission, there is strong incentive to utilize this score to expedite decision-making early on in the admission to provide patients with the best outcomes. When assessing a group of 408 low-energy hip fractures that were stratified into risk groups based on their respective STTGMA score, there was no difference in readmission rate seen within each cohort with regard to their discharge location. However, upon further analysis with a focus on individual discharge locations and their respective risk scoring, it was shown that STTGMA could accurately risk stratify these patients based on admission. Namely, low-risk patients were deemed fit to be discharged home without close observation (only 3.5% required readmission), and moderate–high-risk patients were deemed necessary to have close outpatient observation whether at home or in a post acute-care facility due to higher readmission rates (24.5% requiring readmission). Therefore, STTGMA can be used to guide clinical care and resource allocation for patients who are discharged home, reducing costs by limiting risk of readmission (Konda et al. 2018b).

Post-Discharge Fall Risk

Apart from readmission, STTGMA has been applied to assess other post-discharge outcomes, notably a patient's risk of fall, dislocation, or fracture following femoral

neck fracture surgery. 401 patients who underwent hip arthroplasty surgery after femoral neck fracture were reviewed for incidents after discharge, including falls, secondary fractures, and prosthetic dislocations. After stratification by STTGMA, the low-risk group had 201 patients, while the high-risk group had 200. It was shown that the high-risk cohort had significantly more falls after discharge (1.54 times more likely, 24.5% vs. 15.9%) which also occurred sooner after discharge (5.7 days earlier on average), thus validating STTGMA's predictive ability for post-discharge falls and further demonstrating how the macroscopic clinical biomarkers used by the STTGMA tool are able to capture clinical outcomes (Konda et al. 2021b).

Loss of Ambulatory Level and ADLs at 1 Year

The associated loss in functional status following an operative hip fracture is well documented in the literature. Many studies have highlighted the impact of a patient's age, preinjury functional status, and associated comorbidities on this functional decline (Dyer et al. 2016). However, STTGMA provided the first validated score with the ability to predict this loss of ambulatory function. A cohort of 556 patients with operative hip fracture fixation were included in the study with 268 patients or families responding. 184 were living at the time. Further analysis demonstrated that those with higher STTGMA scores were older, had more comorbidities, required more assistance for day-to-day activities, and had a more significant impact on their ADLs. This may be expected as the macroscopic biomarkers included in STTGMA are a reflection of these factors. These same patients with higher STTGMA scores were more likely to continue needing an assistive device for ambulation, in contrast to patients with lower STTGMA scores that were more likely to never require an assistive device. Finally, patients stratified into the highest-risk group were 1.5× more likely to have a resultant impairment in their functional status postoperatively. This prediction provides a basis to target patients at high risk for loss of ambulatory independence so that timely and effective intervention can occur via early coordination with the proper consultations and rehabilitation services (Konda et al. 2021c).

Conclusion

STTGMA is a mainstay in our institution's standard of care for orthopedic trauma. Each patient older than 55 receives a score on arrival when presenting with acute trauma. Their presentation is met by a validated risk assessment tool, converting macroscopic clinical biomarkers into a calculated score to help improve management and expedite their admission. A patient's STTGMA score indicates if they require earlier surgery and a rapid discharge to the appropriate location based on their injury and functional status.

As demonstrated by the multiple iterations since its inception, STTGMA is a dynamic tool that can adapt with the current medical environment. Future developments for this score include the addition of a variable that assesses a patient's bone quality on admission and a further focus on the biomarkers associated with pathology

leading to fracture such as osteoporosis or osteopenia. Seasonality of the patient's injury also represents a future variable addition in hopes of correlating STTGMA epidemiologically to the world at large, stratifying patient risk based on time of year and common injuries based on season. Finally, the COVID-19 pandemic continues to impact the world, shaping healthcare reform on an international scale. The addition of a patient's COVID vaccination status as a variable will further allow healthcare institutions to provide patients with the best orthopedic trauma care amidst the backdrop of the pandemic. STTGMA represents the summation and weighting of many patient variables combined to assess inpatient mortality risk, bridging the gap between the micro and macro biomarkers to better inform our understanding and management of the downstream clinical manifestations seen in acute orthopedic trauma.

Additional Applications

Applications to a Healthcare Landscape Increasing in Cost

This chapter highlights the medical benefits of STTGMA, with brief mentions of its associated cost applications. A reduction in cost with similar outcomes or an improved outcome at similar cost both demonstrate the possible outcomes of a value-based care model. Thus, providing the patient with the most benefit at the least cost to the hospital and provider is very important. STTGMA gives a framework to standardize our care in the field of orthopedics, allowing for continued improvement in management to cut out unnecessary interventions and start patients on their path to recovery in a safe manner promptly.

Applications to Other Diseases or Conditions

In this chapter, we review the details of a validated inpatient mortality risk assessment score (STTGMA) and its utility in the field of orthopedics. In particular, STTGMA is focused on improving outcomes while reducing complication risk and overall medical cost. As demonstrated in studies that have taken place since STTGMA's inception, it is possible that it can be applied to many other topics that have not yet been studied. This includes alternative fracture patterns and anatomic locations, aspects of a patient's inpatient, and post-discharge experiences. In addition, as seen with the development of $STTGMA_{COVID}$, STTGMA has the flexibility to adapt to address additional disease processes. For example, if there were to be another pandemic in the future, STTGMA could be adapted to include a weighting factor for the potential patient impact of that illness.

Application to Orthopedic Biomarkers

This chapter focuses on the downstream clinical manifestations of biomarkers. However, just as STTGMA has been shown to be predictive for many of these

manifestations, the retrograde application of STTGMA also has promise, demonstrated by the inclusion of albumin representing nutritional status as an initial component of STTGMA. Inclusion of biomarkers for osteoporosis (vitamin D, calcium) and subsequent clinical manifestations (DEXA scan results) offer up future possibilities for STTGMA's application elsewhere in the field.

Mini-Dictionary of Terms

- **Activity of Daily Living**. The tasks required for everyday life, including dressing, bathing, and feeding oneself.
- **Arthroplasty**. The surgical reconstruction or replacement of a joint in the body.
- **Comorbidity**. The presence of multiple disease entities in one patient at any given time.
- **Discharge**. Completion of a patient's hospitalization.
- **Distal** (Anatomically). Denoting a place further away from the center of the body (ankle vs knee).
- **Distal Radius**. Part of the forearm closest to the hand; fracture here can include part of wrist joint.
- **Femoral Neck**. Portion of the femur that is often fractured, found between the head of the femur (ball component of hip joint) and trochanteric region (bony prominence felt on outside of an individual's hip).
- **Geriatric**. Topics relating to older individuals.
- **Hemoglobin**. Protein in red blood cell that is responsible for binding oxygen and transporting it throughout the body. Gives RBCs their red coloration.
- **Humeral Shaft**. Middle portion of humerus bone.
- **Myocardial Infarction**. Alternative name for heart attack, incident where the heart tissue dies as a result of oxygen deprivation.
- **Orthopedics**. Surgical field dedicated to treatment of the musculoskeletal system.
- **Osteoporosis**. Medical condition where the bone is weak/fragile as a result of becoming thinner and less dense.
- **Perioperative**. Around the time of surgery.
- **Proximal** (Anatomically). Denoting a place closer to the center of the body (knee vs ankle).
- **Tibia Plateau**. Region of the tibia/shin bone that is closest to the knee, articulates as part of the knee joint.
- **Tibial Shaft**. Middle portion of tibia bone.

Key Facts of Hip Fractures

Hip fractures affect ~ 18% of woman and ~ 6% of men globally.
Global hip fracture numbers are expected to be 4.5 million annually by 2050 as a result of the aging population (Veronese and Maggi 2018).

Age-adjusted incidence of hip fractures in the USA shows a downward trend, although the exact cause of this decrease is unknown (Swayambunathan et al. 2020).

Eighty percent of hip fractures occur in women with an average age of 80 years (LeBlanc et al. 2014).

Fall-related injury is the most common cause of hip fractures globally.

Key Facts of Osteoporosis

It affects more than ten million people in the United States. Worldwide, roughly 200 million women have osteoporosis (Lane 2006).

Likelihood of developing osteoporosis is highest in Europe and North America (Lane 2006).

Risk factors include female gender, increasing age, history of calcium or vitamin D deficiency, and glucocorticoid usage.

Patients with osteoporosis have much higher risk of pathologic fracture or fragility fractures, many times impacting elderly patients with hip fractures following falls (Srivastava and Deal 2002).

Treatment options include load-bearing exercise, calcium and vitamin D supplementation, bisphosphonates, and selective estrogen receptor modulators (Raloxifene) (Srivastava and Deal 2002).

Key Facts of Abbreviated Injury Scale and Charlson Comorbidity Scale

Abbreviated Injury Scale is a subset of Injury Severity Score.

Both AIS and CCI are treated as ordinal variables meaning each jump is exponential not linear.

AIS score is 0–6 – based on the "threat to life" after an injury.

CCI score is 0–24 based on a patient's medical history/comorbidities.

AIS is used to assess the Head/Neck, Chest, and Extremity/Pelvis.

Summary Points

- *STTGMA is a validated risk assessment tool to predict a patient's risk of inpatient mortality.*
- *STTGMA combines factors of inpatient mortality (Age, GCS, CCI, AIS) through logistic regression to calculate an informative risk score on admission.*
- *STTGMA iterations over time incorporate new factors (COVID-19 status and ASA score) to better cope with the changing medical landscape.*
- *STTGMA includes cost as a variable to better provide value-based care in the setting of ongoing healthcare reform.*

- *STTGMA applies to fracture patterns and injuries throughout the body.*
- *STTGMA provides predictive capacity to both inpatient and post-discharge aspects of a patient's care plan.*
- *STTGMA offers an adaptive method to incorporate both micro (Lab Values, Vaccination/Disease Statuses) and macro (Clinical signs) biomarkers into a patient's management to influence their downstream clinical manifestations.*

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Body Mass as a Biomarker and Femoral Fracture

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Abstract

Body mass represents a wide range of components, from atoms to tissues or organs. In clinical practice, body composition is generally assessed at the molecular level (fat mass, fat-free mass, bone mineral density) and tissue level (skeletal muscle mass, adipose tissue) that are altered by aging and disease. Loss of bone mineral density with aging leads to osteopenia and osteoporosis, resulting in an increased risk of femoral fracture due to falls. Low body mass index, low skeletal muscle mass, and excessive fat accumulation in the visceral area may predict the risk of hip fracture. Both low body mass index and low skeletal muscle mass are associated with the risk of death, complications, and refracture. Conversely, the effect of body mass on functional recovery remains controversial. Body mass should be routinely assessed in patients with femoral fractures to estimate prognosis and correct altered body composition.

Keywords

Body mass · Body mass index · Skeletal muscle mass · Fat mass · Fat-free mass · Bone mineral density · Femoral fracture · Body cell mass · Mortality · Functional outcome · Complication

Abbreviation

ADL	activities of daily living
AKI	acute kidney injury
BCM	body cell mass
BI	Barthel Index
AWGS	Asian Working Group for Sarcopenia
BIA	bioelectrical impedance analysis
BMD	bone mineral density
BMI	body mass index
COPD	chronic obstructive pulmonary disease
CI	confidence interval
CT	computed tomography
DALYs	disability-adjusted life-years
DXA	dual X-ray absorptiometry
FIM	Functional Independence Measure
FFM	fat-free mass

FM	fat mass
FNIH	Foundation for National Institutes of Health
HHS	Harris Hip Score
HR	hazard ratio
LBM	lean body mass
MRI	magnetic resonance imaging
OR	odds ratio
PMS	Parker Mobility Scale
QOL	quality of life
RR	relative risk
SD	standard deviation
SMI	skeletal muscle mass index
TUG	Timed Up and Go test
YAM	young adult mean
WHO	World Health Organization

Introduction

Femoral fractures are the most common type of fracture in older adults and result in disabilities, poor quality of life (QOL), and worse survival (Solbakken et al. 2017; Papadimitriou et al. 2017). Globally, 2–574 per 100,000 women and 2–290 per 100,000 men experience femoral fracture per annum, with the highest and lowest incidence found in Denmark and Nigeria, respectively (Kanis et al. 2012). Older adults with hip fractures showed a 2.4–2.7 times lower survival rate than age- and sex-matched controls (Solbakken et al. 2017). In addition, disability-adjusted life-years (DALYs) were lost in 27/1000 persons due to hip fractures (Papadimitriou et al. 2017). It also increases the socioeconomic burden due to an increase in healthcare costs for acute care, rehabilitation, and long-term care (Veronese and Maggi 2018). Therefore, prevention and improvement of prognosis for femoral fractures are serious issues worldwide.

Low bone mineral density (BMD) and falls are the predominant etiologies of femoral fractures. These pathophysiologies are attributed to modifiable and unmodifiable factors. Known risk factors for low BMD are low BMI, low calcium intake, excessive alcohol consumption, low exposure to sunlight, inflammatory disease, and certain drugs such as corticosteroids (Veronese and Maggi 2018). Low body mass index (BMI) is affected by decreased BMD and other body components, such as muscle mass, fat mass (FM), and body fluid. Additionally, low BMI is associated with increased mortality, poor activities of daily living (ADL), and QOL in older adults after femoral fracture (Flodin et al. 2016; Larsen et al. 2015). It is assumed that body mass and its components potentially contribute to the onset and clinical course of femoral fractures.

In this chapter, we describe the components of body mass, its role in femoral fracture incidence, and the predictive ability of body mass.

Body Mass and Its Components

Body mass in humans reflects a wide range of compounds from elements to organisms, resulting from the accumulation of nutrients or other constraints. The primary sources of body mass are nutrients. The nutrient sources in humans shift during the life course, from circulating nutrients from the placenta to breast milk and various foods. As a result, the amount and components of nutrients, genetic determinants, and environmental factors contribute to an individual's body mass.

Five Level Model

The most common theory for describing and assessing body composition is the "five-level model": atomic, molecular, cellular, tissue-system, and whole-body levels (Wang et al. 1992). This model relies on the principle that the components of each layer are clearly defined, and the total mass of the parts is equal to the individual body weight.

The *atomic level* is the minute and fundamental level of the body composition. Of all components, more than 98% of the human body is composed of only six elements: oxygen, carbon, hydrogen, nitrogen, calcium, and phosphorus. Other dominant atoms include sodium, potassium, sulfur, chlorine, and magnesium. The elements in the human body can be assessed in vivo by instrumental methods such as neutron activation analysis (Wang et al. 1992); however, this procedure is not practical and is limited to research purposes. These elements combine to form molecules.

There are six critical components at the *molecular level*: water, lipids, proteins, carbohydrates, bone minerals, and soft tissue minerals (Going et al. 2014). The most affluent part of this level is water, with approximately 60% in an adult male. Body water is further subdivided into two major body fluid compartments: extracellular and intracellular fluid. Proteins are compounds composed of amino acids and nucleoproteins. Minerals account for 5.3% of the body mass, which are distributed mainly in bones and soft tissues (Wang et al. 1992). Of these, more than 80% of bone minerals are found in the skeleton. On the other hand, lipids are a group of insoluble chemical compounds classified into simple lipids, compound lipids, steroids, fatty acids, and terpenes (Wang et al. 1992). The amount of lipids is also referred to as FM, and the sum of the remaining components constructs fat-free mass (FFM) or lean body mass (LBM). In other words, a total amount of FM and FFM are equal to the individual body weight. The two parts are traditionally used to describe body composition in a very simple way by the two-compartment model. FFM approximates the amount of metabolizing tissue, and it is also affected by the fluid shift. Therefore, body composition assessment based on FFM should be carefully interpreted when the examinees have a derangement of fluid status, such as edema, pleural effusion, ascites, and dehydration.

At the *cellular level*, body mass is classified by cell type. Extracellular solids, extracellular fluid, and cell mass were the predominant components at this level.

Extracellular solids include collagen, reticular fibers, and elastic fibers (Wang et al. 1992). In comparison, the extracellular fluid is composed of intravascular fluid (blood) and interstitial substances. The cell mass is further subdivided into fat and body cell mass (BCM). BCM is an actively metabolizing cell and encompasses muscle mass and organs. On the other hand, fats is not the same as lipids. Triglycerides, phospholipids, and structural lipids are various types of extracted lipids. On the contrary, Fat is a non-metabolizing tissue that primarily consists of triglycerides (Going et al. 2014).

The *tissue-system level* is mainly composed of four components: muscular tissue, connective tissue, epithelial tissue, and nervous tissue (Wang et al. 1992). Among these, the central tissues for body composition assessment are the skeletal muscle, adipose tissue, and bone. Skeletal muscle mass, adipose tissue, and bone account for 40%, 21.6%, and 7.1% of body weight, respectively (Wang et al. 1992). Lipids are mainly present in adipose tissue but are also found in the liver, nerves, and skeletal muscles.

To evaluate body composition at the *whole-body level*, one can use the statue (height), body weight, segment length, limb circumference, and body surface area (Wang et al. 1992). Among these indicators, BMI is most widely used to assess individual body mass. It is calculated by body weight in kilograms divided by the square of height in meters. BMI is the simplest way to assess the risk of malnutrition (undernutrition and overnutrition). The reference values of BMI are <18.5 kg/m^2 for underweight, ≥ 25 kg/m^2 for overweight, and ≥ 30 kg/m^2 for obese (WHO 2000). However, an increase in BMI levels is associated with an increase in the risk for specific diseases, such as type II diabetes and cardiovascular disease, which may differ among races. Thus, race-specific cut-off values have been proposed for Asian patients (WHO Expert Consultation 2004).

Change in Body Composition Through Aging

By the end of puberty, height and body weight, which are vital determinants of body mass, increase dramatically. The peak height gain velocity occurs earlier in females (approximately 12 years) than in males (approximately 14 years) (Stang 2008). The increase in height is followed by weight gain. Growth in stature and body weight gain usually continue until the late teens in females and early twenties in males (Stang 2008).

After reaching a peak of physiological maturity, the body composition of an individual gradually changes with age. A study analyzing the data from 1069 Australian males revealed that lean mass and bone mineral content decreased, whereas body fat percentage increased with aging (Atlantis et al. 2008) (Fig. 1). Older adults lose muscle mass by approximately 40% at the age of 80s compared to those in their 20s, while muscle fibers are shifted during aging from type II to type I (Vincent et al. 2012). This physiological change in muscle mass and function leads to age-related sarcopenia (Cruz-Jentoft et al. 2019). Concurrently, body fat increases gradually throughout life. The expected increase rates in FM are approximately

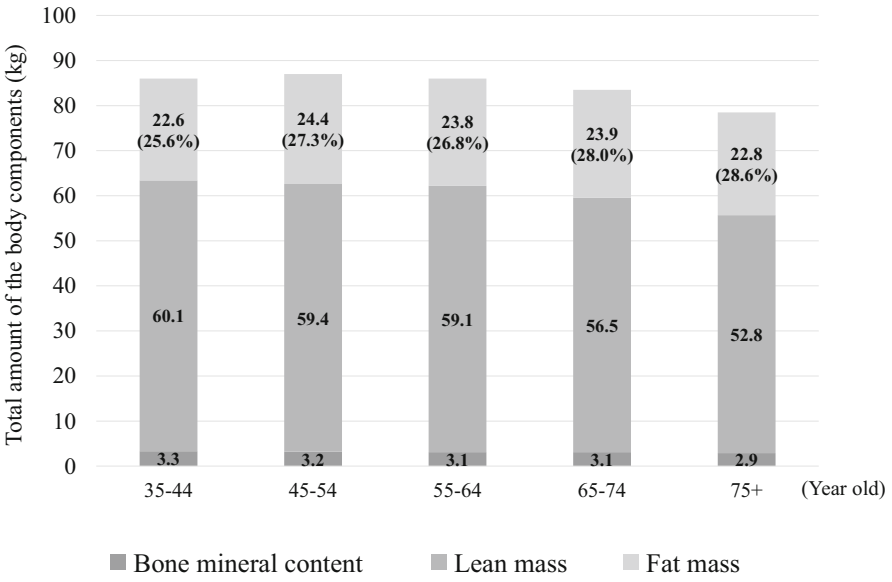

Fig. 1 Change in body composition with age (Atlantis et al. 2008)
Data from the study including 1069 Australian males showed that lean mass and bone mineral content decreased, whereas body fat percentage increased with aging

0.32–0.57 kg/year in females and 0.37–0.57 kg/ year in males (Going et al. 2014). Adipose tissues are distributed more to the abdomen than subcutaneously in older adults (Vincent et al. 2012). In addition, fat infiltration into muscles is more prevalent in seniors than in young adults and may result in ADL limitation and increased mortality (Akazawa et al. 2021, Miljkovic et al. 2015).

As with aging, BMD gradually declines. BMD in males decreased at approximately 0.3% per year by the age of 65, and its rate increased progressively by the age of 95 (Kim et al. 2018a). On the other hand, BMD in females declines by 1.0% per year at the age of 65 and remains until 75 years; after that, it drastically accelerates up to 3% per year at the age of 90 (Kim et al. 2018a). Other study suggested that the annual loss in BMD was 0.5–0.7% greater in women than in men aged 60 years and older (Daly et al. 2013). In older adults, BMI was significantly associated with lower BMD in the femoral neck and proximal femur (Wu and Du 2016). Low BMD at the spine and hip indicate an increased risk of vertebral and hip fractures (Marshall et al. 1996).

Body Mass as a Risk Factor of Hip Fracture

A decrease in BMD is one of the most important factors leading to the occurrence of femoral fractures. BMD indicates the amount of minerals per unit volume of bones. BMD is an indicator of osteopenia or osteoporosis. Osteopenia is defined as a BMD

Table 1 Diagnosis of osteoporosis

Status	Criteria
Normal	BMD is greater than −1.0 SD of YAM (T score ≧ −1)
Osteopenia	BMD is less than −1.0 SD and greater than −2.5 SD of YAM (−1 < T score > −2.5)
Osteoporosis	BMD is less than −2.5 SD of YAM (T score ≦ −2.5)
Severe osteoporosis	BMD is less than −2.5 SD of YAM And the presence of one or more fragility fractures

Abbreviation: *BMD* bone mineral density, *SD* standard deviation, *YAM* young adult mean

of −1.0 and − 2.5 standard deviation (SD) of young adult mean (YAM) (T-score), while osteoporosis is defined as a BMD of < −2.5 SD of YAM (WHO 1994; Kanis 2020). Severe osteoporosis is defined as a BMD of less than −2.5 SD of YAM and the presence of one or more fragility fractures (Table 1). Osteoporosis is a risk factor for the occurrence of femoral fractures and other fragility fractures (Seeman 2008). Females are at high risk of femoral fracture because BMD decreases with estrogen secretion reduction after menopause (Kanis 1994).

Muscle mass, which accounts for approximately 40% of the body mass, decreases with age (Janssen et al. 2000; Daly et al. 2013). In a study comparing the muscle mass of males and females aged 18–88 years old using magnetic resonance imaging (MRI), the muscle mass decreased after 50 years old, especially in the lower limb (Janssen et al. 2000). Loss of muscle mass affects the occurrence of femoral fractures independently of BMD (Malkov et al. 2015). Decreased subcutaneous fat (HR = 1.44; 95%CI = 1.02–2.02), thigh muscle attenuation measured by CT (HR = 1.40; 95%CI = 1.05–1.85), and appendicular lean mass by height squared (HR = 0.58; 95%CI = 0.36–0.91) are associated with the occurrence of femoral fracture in men. In females, decreased subcutaneous fat (HR = 1.39; 95% CI = 1.07–1.82) and cross-sectional muscle are associated with the occurrence of femoral fracture, which are measured using dual X-ray absorptiometry (DXA) (HR = 0.78; 95%CI = 0.62–0.97) (Malkov et al. 2015). Adjusted for age and sex in Japanese, patients with femoral fracture have a lower appendicular and leg skeletal muscle mass index (SMI) ($P < 0.001$) compared to older outpatients who did not have a hip fracture (Hida et al. 2013). The type II fibers of the vastus lateralis muscles with femoral fracture were atrophied (mean age 82 ± 1.5 years, 2609 ± 185 μm^2) compared to younger adults (20 ± 0.4 years, 4755 ± 335 μm^2) and healthy older adults (79 ± 1.7 years, 3723 ± 322 μm^2) (Kramer et al. 2017). In addition, female patients with femoral fractures had more intramuscular fat in the adductor, abductor, and flexor muscles compared to using quantitative computed tomography (CT) to healthy older adults (Lang et al. 2008). Thus, patients with femoral fractures have unique changes in both bone mass and muscle mass. Hence, prevention and treatment that focus on both are necessary.

The measurement of muscle mass in patients with femoral fractures has many problems. First, it is difficult to measure accurately the muscle mass after surgery

Table 2 Muscle mass measurement in patients with femoral fracture

Criteria	Muscle mass measurement site	Devise	Cut-off value	Study
AWGS 2019	Skeletal muscle mass of extremities	DXA	Men: SMI < 7.00 kg/m^2 Female: SMI < 5.40 kg/m^2	Iida et al. (2021)
FNIH	Lean soft tissue mass of upper and lower extremities	DXA	ALM-to-BMI ratio Men: <0.789 kg Female: <0.512 kg Crude ALM Male: <19.75 kg Female: <15.02 kg	Landi et al. (2017)
	Appendicular lean mass in unfractured leg × 2 + lean mass in arms	DXA		Di Monaco et al. (2006b, 2007, 2014)
	Unfractured leg × 2 + lean mass in arms	DXA		Visser et al. (2000)
	Total skeletal muscle area at L3 or L4 level	CT		Kim et al. (2018b) and Chang et al. (2018)

Abbreviations: *AWGS* Asian Working Group for Sarcopenia, *DXA* dual-energy X-ray absorptiometry, *SMI* skeletal muscle index, *ALM* appendicular lean mass, *FNIH* Foundation for the National Institutes of Health, *BMI* body mass index, *CT* computed tomography

(Table 2). DXA, which can measure both BMD and muscle mass, is often used to measure muscle mass in patients with femoral fractures (Di Monaco et al. 2006b, 2007; Landi et al. 2017; Iida et al. 2021). However, inserted implants overestimate the muscle mass of the fracture leg when performing DXA (Di Monaco et al. 2006b). The overestimation of muscle mass is also found in bioelectrical impedance analysis (BIA) due to altered electrical conductivity. Edema due to hypoalbuminemia, immobility, and swelling associated with fracture and surgery also causes overestimation of muscle mass. Several methods have been reported to avoid factors that inhibit accurate muscle mass measurement. Some studies have reported that corrected appendicular lean mass was calculated as a lean mass in the unfractured leg × 2 + lean mass in the arms (Visser et al. 2000; Di Monaco et al. 2006b, 2007, 2014). Other studies used the total cross-sectional area of the bilateral paraspinal, psoas, and abdominal wall muscles at the L3 (Kim et al. 2018b) or L4 level (Chang et al. 2018) measured using CT as muscle mass. To clarify the impact of muscle mass on clinical outcomes, it is necessary to develop a method to accurately measure muscle mass even after fracture or surgery. Ultrasound may solve these problems by measuring local muscle mass. Ultrasound is expected to be a new method for measuring muscle mass because it is simple, noninvasive, low cost, and mobile (Cruz-Jentoft et al. 2019); however, further studies on femoral fractures are needed. In addition, the definition of low muscle mass differs depending on the criteria, which makes

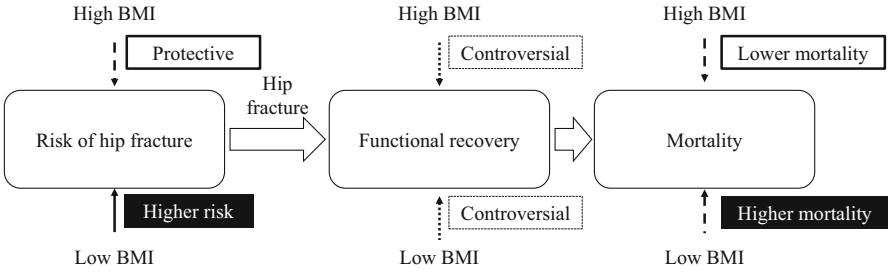

Fig. 2 Hypothesized effect of BMI on risk of hip fracture, functional recovery, and mortality in patients with hip fracture
High BMI may reduce the risk of hip fracture incidence and mortality. The effect of BMI on functional recovery is controversial

difficult to compare the impact of muscle mass on clinical outcomes (Table 2). Low muscle mass in the previous study was defined according to the Asian Working Group for Sarcopenia (AWGS) (Chen et al. 2020) and the Foundation for National Institutes of Health (FNIH) (Studenski et al. 2014). The loss of muscle mass is the main feature of sarcopenia. Low muscle mass should be defined according to the standardized criteria for sarcopenia.

BMI is one of the most easily obtained indicators that may predict the occurrence of femoral fractures. A low BMI is a risk factor for the onset of femoral fractures (Fig. 2). In the meta-analysis by De Laet et al., <20 kg/m^2 was approximately twice the risk of femoral fracture compared to >25 kg/m^2 (RR = 1.95; 95% CI = 1.71–2.22), and a BMI of 30 kg/m^2 was associated with a 17% reduction in hip fracture risk compared with a BMI of 25 kg/m^2 (RR = 0.83; 95% CI = 0.69–0.99) (De Laet et al. 2005). Low BMI ($<$ 22 kg/m^2) combined with cognitive impairment (Mini-Mental State Examination <21 points) increases the rate of femoral fracture more than four times compared with patients without cognitive impairment with the same BMI (34.6% vs. 8.7%) over 7 years (Alfaro-Acha et al. 2006). High BMI is a risk factor for chronic diseases, but it has been reported to protectively affect the occurrence of hip fracture (McTigue et al. 2006). Meanwhile, Rikkonen et al. reported that the risk of "early" femoral fracture aged 58–70 was higher in the obese and normal weight compared to the overweight female with HR of 2.3 (95%CI = 1.4–3.7) and 2.0 (95%CI = 1.3–3.1) using the WHO categories, while "late" femoral fracture in aged 70–83 was no difference between BMI categories (log rank: $p = 0.14$) (Rikkonen et al. 2021). Among all age groups, the incidence of femoral fracture in female aged 70–79 years was the most affected by BMI, with 21.0 fractures per 1000 person-years at <22 kg/m^2 compared to 8.5 fractures per 1000 person-years at ≥ 30 kg/m^2 (Søgaard et al. 2016). Meyer et al. reported that waist circumference (RR per 10 cm increase = 1.13; 95% CI = 1.04–1.23) and waist-to-hip ratio (RR per 0.1 unit increase = 1.14; 95% CI = 1.04–1.23), which are the commonly used surrogate marker of visceral fat, are correlated to increased risk of hip fracture in women but not in men

(Meyer et al. 2016). BMI and body fat are modifiable factors, and intervention for low BMI in older adults is necessary to prevent the occurrence of femoral fractures.

Body Mass and Mortality

BMI is associated with mortality in patients with femoral fractures. Several studies have reported that low BMI leads to higher mortality (Schaller et al. 2012; Solbakken et al. 2017), whereas high BMI leads to higher survival rates (Karin et al. 2019; Hori et al. 2020). Vosoughi et al. reported that BMI at fracture onset was predictive of 3-month mortality (odds ratio [OR] = 0.88; 95%CI = 0.82–0.96) and 1-year mortality (OR = 0.90; 95%CI = 0.85–1.0) (Vosoughi et al. 2017). Meanwhile, a U-shaped association between BMI and mortality has been reported (Akinleye et al. 2018). On the other hand, BMI also reflects the nutritional status. Approximately 25% of femoral fracture patients were malnourished before fracture, and malnourished patients had significantly lower BMI (18.2 ± 2.4 kg/m^2) compared with the patients at risk of malnutrition (19.5 ± 2.5 kg/m^2) and well-nourished patients (23.4 ± 2.7 kg/m^2) (Inoue et al. 2017). Malnutrition leads to increased mortality (Foo et al. 2021). Avenell et al. reported in their meta-analysis that oral nutritional supplements may reduce complications (RR = 0.71; 95% CI = 0.59–0.86) and unfavorable outcome (death or complications) (RR = 0.67; 95%CI = 0.51–0.89) in patients with femoral fracture (Avenell et al. 2016). Thus, nutritional intervention before and soon after surgery for femoral fractures is essential to improve clinical outcomes.

Low muscle mass is a risk factor for increased mortality. Low muscle mass (men, SMI <7.00 kg/m^2; women, SMI <5.40 kg/m^2 evaluated using DXA according to the AWGS 2019 criteria) was a risk factor for 1-year mortality (HR = 3.182; 95% CI = 1.097–9.226) (Iida et al. 2021). Meanwhile, sarcopenia defined by SMI at the level of the third lumbar (L3) vertebra (men, <42.2 cm^2/m^2; women, <33.9 cm^2/m^2) was not a risk factor for 1-year mortality but a risk factor for 5-year mortality (Kim et al. 2018b). The discrepancy between these results may be due to differences in the definition of low muscle mass among the studies.

Body Mass and Functional Status

The impact of muscle mass on functional status is controversial in patients with femoral fractures (Table 3). Functional recovery is one of the most important goals for patients with femoral fractures, and clarifying the relationship between body mass and functional recovery emphasizes the need for rehabilitation and nutritional therapy. Di Monaco et al. reported that corrected appendicular lean mass (lean mass in unfractured leg × 2 + lean mass in arms) upon admission was associated with Barthel Index (BI) score (r = 0.480; p = 0.013) and BI efficiency (r = 0.633; p = 0.001) after rehabilitation in male femoral fracture patients admitted to a rehabilitation hospital (Di Monaco et al. 2007). In addition, sarcopenia defined by

Table 3 Effect of muscle mass on functional outcomes in patients with femoral fracture

Effect of muscle mass on functional outcomes							
No association				Association			
Author	Sex	Setting	Outcomes	Author	Sex	Setting	Outcomes
Visser et al. (2000)	Female	From acute hospital to after 12 months	5 items mobility function	Di Monaco et al. (2007)	Male	Rehabilitation hospital	BI score after rehabilitation BI efficiency
Di Monaco et al. (2006b)	Female	Rehabilitation hospital	BI scores after rehabilitationBI scores change	Landi et al., 2017	Both	Rehabilitation hospital	BI at the discharge and after 3 months of follow-up
Di Monaco et al. (2014)	Female	Rehabilitation hospital	BI scores and TUG after rehabilitation BI effectiveness				
Shin et al. (2020)	Both		Harris hip score at last follow-up				

Abbreviations: *BI* Barthel index, *TUG* timed up and go test

low muscle mass alone (FNIH criteria) had a higher probability of incomplete functional recovery (OR = 3.07; 95%CI = 1.07–8.75) and lower BI scores at the time of discharge from the rehabilitation unit (69.2 versus 58.9%, respectively; $p < 0.001$) and after 3 months (90.9% versus 80.5%, respectively; $p = 0.02$) of follow-up compared to non-sarcopenic patients (Landi et al. 2017). Meanwhile, it was reported that corrected appendicular lean mass in female femoral fracture patients did not correlate with the BI score after rehabilitation or BI score change during hospitalization (Di Monaco et al. 2006b). Shin et al. reported that sarcopenia defined by low muscle mass alone was not associated with Harris Hip Score (HSS) (Shin et al. 2020). Grip strength upon admission was associated with BI scores ($r = 0.075$; $p = 0.41$) after rehabilitation, BI effectiveness ($r = 0.006$; $P = 0.53$), and Timed Up and Go test (TUG test) ($r = 0.05$; $p = 0.96$), but muscle mass was not associated with any outcome in patients with femoral fractures (Di Monaco et al. 2014). Changes in hand grip strength from the occurrence of femoral fracture after 12 months were significantly associated with recovery of mobility function, but changes in muscle mass were not associated with recovery of mobility function (Visser et al. 2000). Many studies have revealed that muscle strength can predict clinical outcomes, whereas muscle mass shows inconsistent results. Thus, muscle strength has tended to be highly prioritized in the diagnosis of sarcopenia (Cruz-Jentoft et al. 2019). The usefulness of muscle mass as a predictor of clinical outcomes needs to be carefully discussed in patients with femoral fractures.

It is controversial whether high or low BMI has a positive impact on functional status. We previously examined the association between BMI and functional recovery in 13,348 patients with acute hip fracture using nationwide administrative claims and discharge data (Nishioka et al. 2020) (Fig. 3). BMI was categorized into four groups based on the WHO recommendation for Asian population: underweight (<18.5 kg/m^2), normal (18.5–22.9 kg/m^2), overweight (23.0–27.4 kg/m^2), and obese (≥ 27.5 kg/m^2) (WHO Expert Consultation 2004). The results showed that being underweight (BMI <18.5 kg/m^2) was associated with a lower BI at discharge from acute care hospital (partial regression coefficients = −2.324; 95%CI = −3.538 to −1.109). Meanwhile, overweight and obese were related to higher BI at discharge (partial regression coefficients = 3.080 and 5.732; 95%CI = 1.709–4.451, 3.075–8.392, respectively). In addition, underweight patients have significantly higher odds of overall complications (OR = 1.195; 95%CI = 1.028–1.390) (Nishioka et al. 2020). Interestingly, the hip fracture patients with missing BMI values showed a strong association with lower BI at discharge, suggesting that patients whose body weight cannot be obtained may have a higher risk of poor ADL recovery (partial regression coefficient = −5.763; 95%CI = −7.611 to −3.915). Di Monaco et al. reported that BMI was associated with BI score after rehabilitation (partial regression coefficient = −0.162) and change in Barthel Index score during rehabilitation (partial regression coefficients = −0.513) (Di Monaco et al. 2006a). In addition, Sim et al. reported that low BMI (<18.5 kg/m^2) is not associated with Parker Motility Scale (PMS), HHS, and QOL (SF36) after 6 months (Sim et al. 2021). Meanwhile, we reported that BMI scores of the Mini Nutritional Assessment-Short Form upon discharge were not associated with Functional

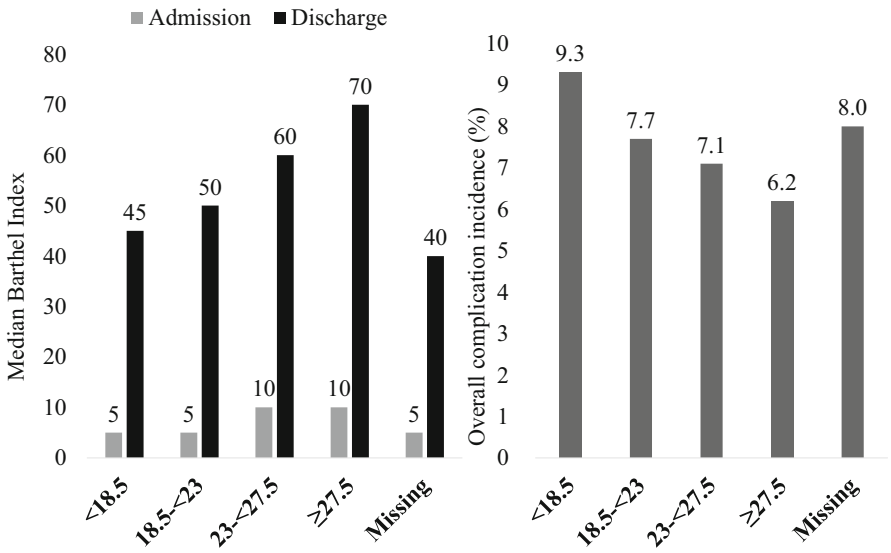

Fig. 3 Effects of body mass index on Barthel Index (left) and the incidence of overall complication (right) in 13,348 patients with acute hip fractures (Nishioka et al. 2020)
Highest median Barthel Index was found in obese (BMI < 27.5 kg/m^2) patients, whereas lowest Barthel Index was the patients with missing BMI, followed by the underweight (BMI < 18.5 kg/m^2) patients. Conversely, overall complication incidence was highest in underweight patients, while lowest incidence was obtained in obese patients.

Independence Measure (FIM), but weight loss scores were associated with FIM efficiency (standardized coefficient = 0.156) in patients with malnourished femoral fractures admitted to convalescent rehabilitation units (Nishioka et al. 2018). These results suggest the need for health management to prevent weight loss during hospitalization.

Body Mass and Complication and Secondary Fracture

The impact of BMI on the occurrence of complications depends on the type of complication. Acute renal failure and progressive renal insufficiency liner increased with increasing BMI, blood transfusion liner decreased with increasing BMI, and the relationship between superficial infection and BMI was bell-shaped (Akinleye et al. 2018). The risk of incident in acute kidney injury (AKI) was high in obese patients (BMI ≥30 kg/m^2), and short-term (6–30 days post-surgery) and long-term mortality (31–365 days post-surgery) was also high in patients with low BMI (<18.5 kg/m^2), regardless of the presence of AKI (Pedersen et al. 2017). It has also been reported that higher BMI is associated with an increased risk of periprosthetic joint infections (OR = 1.092; 95%CI = 1.002–1.189) (Zajonz et al. 2019). Secondary femoral

fracture is one of the most serious complications of primary hip fractures. It has been reported that patients who experience contralateral hip fracture have a significantly lower BMI than those who did not experience contralateral hip fracture (22.2 kg/m^2 versus 26.5 kg/m^2, $p = 0.01$) (Aurégan et al. 2017).

Conclusion

Measurement of body mass enables assessment of the alteration of various tissues, such as bone mass, muscle mass, FM, and organ tissues. Estimations of these quantities can predict the risk of fracture of the femur as well as the mortality, complications, and decreased functionality after hip fracture. In patients with hip fracture, body mass should be routinely assessed to estimate the risk of adverse outcomes and to regain appropriate body mass by treatment for modifiable risk factors with altered body composition.

Mini-Dictionary of Terms

ADL

ADL indicates the basic daily activities required for daily life. There are two types of ADL: basic ADL (BADL) and instrumental ADL (IADL). BADL refers to the basic activities of getting up, transferring, moving, eating, dressing, toileting, bathing, and dressing. IADL refers to complex ADLs, such as cleaning, cooking, laundry, and shopping.

AWGS

AWGS is an organization that makes statements on the definition of sarcopenia in the Asian population. To date, the AWGS has made statements regarding sarcopenia in 2014 and 2019.

BI

The BI is a rating scale for ADL. The BI consists of ten items (feeding, bathing, grooming, dressing, bowels, bladder, toilet use, transfers, mobility, and stairs). 0 point indicates fully dependency, and 100 indicates independent.

BIA

The BIA is based on the relationship between the volume of a conductor and its electrical resistance; muscles are the predominant conductor because skeletal muscle is the largest tissue in the body and rich in electrolytes and has low resistance.

DXA

DXA is a method that can separate adipose tissue, bone tissue, and lean tissue using X-ray absorption due to the attenuation of two X-ray energies.

FIM

The FIM is a scale for evaluating ADL, consisting of 13 motor items (eating, grooming, dressing upper and lower body, toileting, bladder and bowel management, bed, chair, or wheelchair transfer; toilet transfer; tub transfer; walk/wheelchair; stairs) and 5 cognitive items (comprehension, expression, social interaction, problem solving, memory). Each item is scored from 1 to 7, with a higher score indicating a high ADL.

HHS

The HHS is an evaluation scale for the hip joint and consists of pain (44 points), function (47 points), deformity (4 points), and range of motion (5 points). The maximum was 100 points, in which a higher point is indicated as better hip joint function.

Mini Mental State Examination

The Mini-Mental State Examination is a screening of cognitive function composed of 11 items, such as disorientation, calculation, object calling, and sentence recitation. A total score ranges 0 to 30 points, and 23 points or lower is a prediction for dementia.

Mini Nutritional Assessment-Short Form

The Mini Nutritional Assessment-Short Form is a screening tool for nutritional status consisting of the following six items: decline in food intake over the past 3 months, weight loss during the past 3 months, mobility, psychological stress or acute disease in the past 3 months, neuropsychological problems, and BMI or calf

circumference. A score of 12–14 indicates well-nourished, 8–11 indicates a risk of malnutrition, and 0–7 indicates malnutrition.

Muscle Fiber

The muscle fiber constitutes the muscle bundle, and the muscle bundle collectively constitutes the muscle. Type I fibers are slow-contracting, endurance muscle fibers, whereas type II fibers are fast-contracting, instantaneous muscle fibers that fatigue easily.

PMS

The PMS is a composite measure of a patient's mobility and gait function in a variety of situations, including indoors, outdoors, and shopping. Patients were assigned a score from 0 to 9.

Sarcopenia

Sarcopenia is a skeletal muscle disease characterized by loss of skeletal muscle mass and decreased muscle strength, which leads to falls, fractures, hospitalization, and death. Sarcopenia can be caused by aging, disease, insufficient food intake, and inactivity.

SMI

SMI is an indicator of whole-body muscle mass, and several definitions and equations exist. The most commonly used equation is dividing appendicular muscle mass (kg) by height in meters squared (m^2).

TUG Test

The TUG test is a physical function test. It measures the time it takes to stand up from a seated position in a chair with armrests, walk 3 m, turnaround, and sit down again.

YAM

YAM is the young adult mean BMD value. In Japan, YAM is 20–44 years old for the lumbar spine (L1-L4 or L2-L4) and 20–29 years for the proximal femur. This is used as the criterion for osteoporosis.

Applications to Other Diseases or Conditions

Low BMI is also known as a prognostic factor for other diseases such as end-stage renal disease, chronic obstructive pulmonary disease (COPD), stroke, and heart failure with reduced or preserved ejection fraction (Suzuki et al. 2020; Spella et al. 2017; Powell-Wiley et al. 2018). Decreased FFM and skeletal muscle mass might be a potential explanatory factor for these associations; higher FFM was significantly associated with the survival of COPD patients, but FM was not (Schols et al. 2005), and the prevalence of loss of muscle mass and muscle function (sarcopenia) in heart failure patients was 20% higher than in healthy individuals (Springer et al. 2017). Patients with sarcopenia after heart valve surgery showed significantly higher mortality than those without sarcopenia (Okamura et al. 2019). On the other hand, stroke patients with higher BMI showed increased mortality (Towfighi and Ovbiagele 2009) or no association with the risk of death (Dehlendorff et al. 2014). Conversely, the protective effect of obesity on functional recovery after stroke has been inconsistently reported (Nishioka et al. 2016; Kalichman et al. 2007). The amount of muscle mass can be explained by the protective effect of obesity, as sarcopenia and sarcopenia with obesity (i.e., sarcopenic obesity) were significantly associated with worse recovery of ADL in convalescent stroke patients (Matsushita et al. 2019; Matsushita et al. 2020).

Key Facts of Body Mass as a Biomarker and Femoral Fracture

- Body mass encompasses various tissues such as bone mass, muscle mass, fat mass, and organ tissues.
- BMI, BMD, and muscle mass might be a predictor of the onset of hip fractures in older adults.
- Higher mortality was found in hip fracture patients with low BMI and low muscle mass.
- There is inconsistent evidence for the association between reduced muscle mass and functional recovery from hip fractures.
- Limited evidence has shown that lower BMI is significantly associated with several complications, including acute renal failure and secondary fracture.

Summary Points

- At the molecular level, body mass is composed of water, lipids, proteins, carbohydrates, bone minerals, and soft tissue minerals. The lipid configures fat mass, whereas protein is a major component of muscle mass.
- Bone mineral density decreases with age, resulting in osteopenia and osteoporosis. This status increases the risk of hip fractures due to falls.

- Loss of muscle mass may also contribute to the increased risk of fracture of the femur, although the validity and feasibility of their measurements remain a problem.
- BMI was linearly associated with decreased mortality, with odds ratios of 0.88–0.90. The amount of muscle mass can explain this association.
- The predictive ability of muscle mass on functional outcome after hip fracture is discrepant among the studies; muscle function (e.g., muscle strength) might have a higher predictive ability than muscle mass.
- Linear associations have been reported between BMI and an increased risk of acute renal failure and lower requirement for blood transfusion.
- Higher BMI was associated with periprosthetic joint infections, while lower BMI was found in patients with secondary fractures than in those without.

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Scoring for Hemorrhage Severity in Traumatic Injury

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Abstract

Severity scores have long been used for the classification or stratification of patients for clinical care and in the evaluation of outcomes. Such scores find highest utility as a standardization instrument in the study of large cohorts of patients. The advent of pervasive tools and capabilities of Artificial Intelligence (AI) and Machine Learning (ML) increases the value of applying such scores to personalized bedside decisions regarding single patients, in which case the score has the data richness of a biomarker of disease. While not molecular biomarkers, such scores are often informed by molecular indicators of pathophysiology and find utility similar to that of traditional biomarkers. This chapter explores the use of severity scores such as the Injury Severity Score (ISS) and New Injury Severity Score (NISS), an internationally recognized scoring system which correlates with mortality, morbidity, and other measures of severity. We also highlight recently introduced scores that capture the rich pathophysiological data of the Emergency Department (ED) and the Intensive Care Unit (ICU).

Keywords

Trauma · Hemorrhage · Scoring · HISS · NISS

Abbreviations

AI	Artificial Intelligence
HISS	Hemorrhage Intensive Severity and Survivability Score
ML	Machine Learning
MODS	Multiple Organ Dysfunction Syndrome
MOF	Multiple Organ Failure
SOFA	Sequential Organ Failure Assessment
START	Simple Triage and Rapid Treatment

Introduction

Severity scoring systems are important adjunct clinical tools for rapidly assessing patient acuity, estimating prognostic outcomes and survivability, and guiding clinical decision-making and triage for critically ill patients. In this respect, while not molecular biomarkers in a traditional sense, severity scores have nonetheless emerged with the clinical significance of a biomarker. A variety of severity scores are widely used in the care of the traumatically injured patient, from the time of first encounter by emergency response healthcare professional through admission via a trauma center or emergency room onto the intensive care units. The aim of such use

is to provide for patient monitoring and the prediction of morbidity and mortality. Designed for straightforward and rapid manual calculation, these integer-based scoring systems are typically computed as the summation of component scores drawn from fixed thresholds of worst-value physiological measurements assessed over a given time frame.

The national trauma triage protocol provides guidelines in the form of the field triage decision scheme to identify the status of the trauma patient (Sasser et al. 2012). The decision scheme draws upon vital signs such as systolic blood pressure (Hypotension $<$90 mmHg) (Cocchi et al. 2007; Vandromme et al. 2010; Lin et al. 2012), abnormal respiratory rate ($<$10 or $>$29 breaths per minute) (Sasser et al. 2012), abnormal heart rate (Tachycardia $>$100 heart beats per minute) (Brasel et al. 2007), and the Glasgow coma scale ($\leq$13) (Teasdale and Jennett 1974, 1976). A commonly used algorithm for mass casualty triage in the USA is the Simple Triage and Rapid Treatment (START) (Benson et al. 1996; Garner et al. 2001; Jenkins et al. 2008; Sacco et al. 2005), which, under austere conditions, is used in conjunction with secondary triage for Secondary Assessment of Victim Endpoint (SAVE) (Benson et al. 1996). Both START and SAVE employ criteria such as respiratory rate, cognitive function (ability to listen and respond to commands), and radial pulse to stratify for triage. The Injury Severity Score (ISS) (Baker et al. 1974) is yet another scoring algorithm based on the Abbreviated Injury Scale (AIS) system which aggregates the assessed injury to six regions of the body and establishes correlations with mortality and morbidity.

A Multiple Organ Dysfunction Syndrome (MODS) prediction score in polytrauma patients was originally developed and reported by Marshall et al. in 1995, wherein a Sequential Organ Failure Assessment (SOFA) score (0–4) was applied following physiologic measurement of dysfunction in six organ systems: (i) respiratory function (PaO_2/FIO_2 ratio), (ii) renal function (serum creatinine), (iii) liver function (serum bilirubin), (iv) cardiovascular function (PAR), (v) hematologic (platelet count), and (vi) neurologic (Glasgow Coma Score) (Marshall et al. 1995). The input points are then totaled to achieve a score corresponding to the patient's ICU mortality %, hospital mortality %, and ICU stay. The MODS score easily and accurately identifies patients at risk for MODS postinjury. MODS may progress to multiple organ failure (MOF) and may be terminal. Progress along the MODS-MOF axis is often assessed by the aforementioned SOFA score (Rendy et al. 2017). Modern manifestations of the MODS score recommend the addition of plasma lactate LqSOFA (Rendy et al. 2017). Several variations in SOFA scoring have been added since its early development and reflect applicability to specific patient populations (e.g., CLIF-SOFA in patients with liver failure and SOFA-HM in patients with hematologic malignancies). The qSOFA (quick-SOFA) and LqSOFA (lactate quick-SOFA) were devised as simplified versions of the SOFA for easy use by most medical personnel in the emergency department (Kashyap et al. 2021). Changing paradigm in trauma care emphasizes understanding the pathophysiology of trauma during the "Golden Hour" through real-time physiologic status monitoring using implantable biochips (Guiseppi-Elie 2011; Gray et al. 2018; Rodrigues et al.

2020), the use of ML to fuse expert and physiological data, and artificial intelligence (AI) for predictive analytics to support more rigorous mortality prediction.

Severity Scores As Biomarkers

In the context of traumatic injuries, recent development of implantable sensing devices has enabled the generation of continuous and real-time physiological biomarkers which can improve precision, robustness, and early response of trauma-induced hemorrhage severity estimation. The intramuscularly implanted Physiologic Status Monitoring (PSM) Biochip is one such example that is capable of wirelessly monitoring glucose, lactate, pH, potassium, and oxygen tension (Guiseppi-Elie 2011, 2012). These forms of microanalytical data streams can enable the integration of molecular biomarkers of physiological stress into new forms of hemorrhage severity scoring systems which could enable earlier triage compared with global vital signs. The Hemorrhage Intensive Severity and Survivability Score (HISS) severity index has been proposed to integrate the five PSM-based physiochemical biomarkers from trauma victims into a hemorrhage severity scoring system (Bhat et al. 2020). Using synthetically generated Sensible Fictitious Rationalized Patient (SFRP) data and several individual expert scoring assessments, HISS was developed as a complementary scoring measure that is primarily focused on metabolic biomarkers as compared with traditional trauma-induced hemorrhage severity scores and groups victims as exhibiting low (0), guarded (1), elevated (2), high (3), or severe (4) triage severity (Bhat et al. 2020).

One hundred instances of SFRP data were digitally generated, and the HISS score was assigned by five clinically active physician experts (100[5]). Standard classifier algorithms, linear support vector machine (SVM-L), multiclass ensemble bagged decision tree (EBDT), artificial neural network with Bayesian regularization (ANN:BR), and possibility rule-based using function approximation (PRBF) were each separately evaluated for their potential to similarly classify and predict a HISS score. SVM-L, EBDT, ANN:BR, and PRBF generated score predictions with testing accuracies (majority vote) corresponding to 0.91 ± 0.06, 0.93 ± 0.04, 0.92 ± 0.07, and 0.92 ± 0.03, respectively, with no statistically significant difference ($p > 0.05$). Further analysis revealed that improved accuracies of 0.99 could be achieved with SFRP data sizes of 147 digital patients and seven clinical expert scores 147[7] (0.99) and that accuracies of 0.999 could be achieved with SFRP data sizes of 154 digital patients and nine clinical expert scores 154[9] (0.999). These studies also revealed that clinicians were highly self-consistent in their scoring but that they varied one from the other in their assessments.

As patient data collection becomes increasingly granular, as data curation becomes more democratic, and as AI techniques continue to evolve, there is great future opportunity for refining existing severity scoring systems and developing novel frameworks for more accurate, timely, and robust clinical decision support.

Patient Stratification

An archetypal example is the Sequential Organ Failure Assessment (SOFA) score, which assigns a score ranging from 0 to 4 to six bodily systems based on gross physiological variables corresponding to respiratory (PaO_2/FiO_2 ratio), cardiovascular (mean arterial pressure and vasopressors), liver (bilirubin), renal (creatinine and urine output), coagulation (platelet count), and neurological (Glasgow Coma Scale) systems (Vincent et al. 1996, 1998). An overall SOFA score is obtained by summing component-wise scores and is correlated with in-hospital mortality. Similarly, the Multiple Organ Dysfunction Score (MODS) is another example utilizing static physiological measurements from the same six bodily systems and is correlated with ICU length of stay, ICU mortality, and in-hospital mortality (Marshall et al. 1995). Several other ICU severity scores have been proposed and are widely used in clinical practice for rapid means of patient monitoring and acuity assessment, including Acute Physiologic Assessment and Chronic Health Evaluation (APACHE) (Larvin and Mcmahon 1989), Simplified Acute Physiology Score (SAPS) (Le Gall et al. 1993), Modified Early Warning Score (MEWS) (Burch et al. 2008), and Logistic Organ Dysfunction System (LODS) (Le Gall et al. 1996), among others. Formulated as rapid adjunct monitoring devices, all such severity scoring systems rely on integer component scores based on fixed physiological descriptor thresholds.

In the context of patients experiencing traumatic injuries, severity scoring systems are valuable instruments of triage for assessing hemorrhage severity and directing resources and care to maximize expected benefit and survivability. Similar to ICU severity scoring systems, trauma-focused severity scores are traditionally comprised of fixed thresholds of isolated physiological measurements and neurological assessments. Table 1 provides a comparison of the various metrics for the APACHE II, SAPS II, SOFA, LODS, and MEWS scoring systems.

Current guidelines for field triage of injured patients from the National Expert Panel on Field Triage provide recommendations for early identification of critically injured patients based on either abnormal cognition (Glasgow Coma Scale $\leq$ 13), hypotension (systolic blood pressure $<$ 90 mmHg), or abnormal respiratory rate ($<$10 or $>$29 breaths per minute or required mechanical ventilation) (Sasser et al. 2012). For mass casualty incidents, Simple Triage and Rapid Treatment (START) is the most commonly used triage algorithm in the United States and recommends immediate intervention for nonambulatory patients based on physiological indications of ambulatory status, respiratory rate, perfusion (including radial pulse presence and capillary refill time), and cognitive function by measure of command response (Benson et al. 1996). JumpSTART is a similar triage system for pediatric patients using modified physiological severity thresholds (Romig 2002). When resources are limited, Secondary Assessment of Victim Endpoint (SAVE) seeks to maximize utility following initial START triage by grouping patients by expected survivability and intervention benefit. The Injury Severity Score (ISS) is based on anatomical severity classifications from the Abbreviated Injury Scale (AIS) and is calculated based on worst-value AIS codes from six body regions (head or neck,

Table 1 Comparison of metrics for the APACHE II, SAPS II, SOFA, LODS, and MEWS scoring systems. Scores are utilized in various clinical settings, and interpretation of the score can be used in many cases to predict mortality rates using mathematical models. Each scoring system has been reviewed and applied to various cohorts, resulting in the ability to determine the relative sensitivity and specificity

	APACHE II	SAPS II	SOFA	LODS	MEWS
Situation for use	Newly admitted ICU patients	ICU patients	ICU patients	ICU/surgery patients	All hospitalized patients
Score range	0–71	0–163	0–24	0–22	0–14
Interpretation	>25: predicted mortality of 50% >35: predicted mortality 80%	>52: predicted mortality of 50% >64: predicted mortality of 75%	>10: predicted mortality of 50% >15: predicted mortality of 80%	>10: predicted mortality of 68.3% >15: predicted mortality of 94.6%	≥5: statistically increased likelihood of death or admission to ICU
Mortality Rate (MR)	$MR = -3.517 + 0.146 * APACHE + 0.603^{*} + diag.cat.wt.$ *only if postop surgery	$MR = \frac{e^x}{1+e^x}$ where $x = -7.7631 + 0.0737 * (SAPS) + 0.9971 * ln(SAPS + 1)$	No direct mathematical conversion to mortality rate	$MR = \frac{e^x}{1+e^x}$ where $x = -3.403 + 0.4173 * (LODS)$	No direct mathematical conversion to mortality rate
Sensitivity	54%	39.4%	9.5 or more: 81.2%	63.9%	4 or more: 75% 5 or more: 38%
Specificity	87%	95.6%	9.5 or more: 83.5%	70.4%	4 or more: 83% 5 or more: 89%
Source	Headley et al. (1992)	Capuzzo et al. (2000)	Wang et al. (2017)	Hu et al. (2021)	Gardner-Thorpe et al. (2006)

face, chest, abdominal or pelvic contents, extremities or pelvic girdle, and external) and establishes correlations with morbidity and mortality (Baker et al. 1974).

ML for Patient Stratification in Trauma

Technological advancements in statistics, computer science, and AI have created the opportunity for more precise and data-driven risk estimation and patient stratification approaches. While additive, rule-based frameworks used in traditional assessment and triage are simple to calculate and do not require advanced computational methods, they are limited in precision due to coarse physiological downsampling (e.g., worst value over a 24-h window) and fixed threshold cutoffs that may prove suboptimal across institutions or populations. ML presents an attractive opportunity for automatically learning stratifying features and combinations directly from more granular data – in essence, "letting the data speak for itself"(Obermeyer and Emanuel 2016).

Broadly speaking, supervised ML methods seek to learn discriminative functions of data that map a set of input features to one or more outputs. These models are developed by repeated iterations through a collection of labeled data samples, where internal data transformations are implicitly learned to extract feature combinations and representations that best separate the output classes. The predictions of some ML models are readily explainable (e.g., decision trees), while others are notoriously indecipherable and involve several nonlinear and complex feature transformations (e.g., deep neural networks).

In the context of healthcare and patient stratification, the supervised ML paradigm involves developing models to predict the occurrence of a patient outcome (e.g., inpatient mortality) based on sets of patient-level clinical descriptors derived from sociodemographic indicators, physiological measurements, laboratory tests, radiological images, clinical notes, and more. After passing a single data sample through the trained model, the resulting output is often interpreted as a risk probability. Methods such as the Youden Index (Fluss et al. 2005) can help determine an optimal risk threshold for stratifying patients into low and high-risk categories based on the estimated risk.

Many researchers have directly compared prognostic efficacy between existing severity scores and ML models. ML has been shown to outperform many traditional scoring systems including MEWS (Wu et al. 2021; Islam et al. 2019; Burdick et al. 2020; Ong et al. 2012; Kia et al. 2020) SAPS (Thorsen-Meyer et al. 2020; Hou et al. 2020; Kong et al. 2020; Zhai et al. 2020), APACHE (Kang et al. 2020; Hsieh et al. 2018), SOFA (Shickel et al. 2019; Barton et al. 2019; Su et al. 2021; Shimabukuro et al. 2017), and TRISS (Rau et al. 2019) for a variety of patient outcomes including in-hospital mortality, sepsis, mechanical ventilation requirement, and cardiac arrest. This list is nonexhaustive; the literature is abundant with examples demonstrating superior risk estimation and patient stratification performance of ML compared with existing severity scoring systems.

Although ML algorithms have shown superior predictive power compared with traditional severity scores, several important aspects limit wider adoption in healthcare settings, such as ethical considerations (Char et al. 2018; Hardt and Chin 2020) and issues surrounding bias, fairness, and health equity (Gianfrancesco et al. 2018; Mccradden et al. 2020; Hague 2019; Parikh et al. 2019; Panch et al. 2019; Rajkomar et al. 2018). Furthermore, many of the most accurate ML models for risk estimation implement deep learning algorithms (Lecun et al. 2015; Shickel et al. 2018), which successively build increasingly complex and abstract data representations from raw inputs. As a consequential trade-off, deep learning models are limited in their ability to justify and explain their predictions (Castelvecchi 2016), posing a significant challenge when used for patient health applications and clinical decision support (Miotto et al. 2018). Several recent designs of ML frameworks for patient risk estimation include specific aspects to address prediction explainability, such as attention mechanisms in the DeepSOFA score for mortality risk estimation (Shickel et al. 2019), or LIME in automated methods for detecting severe chest injury (Kulshrestha et al. 2021).

Artificial Intelligence (AI) for Predictive Analytics in Trauma

Predictive analytics encompasses a variety of mathematical techniques used to forecast the probability of a certain outcome given a set of prior variables. Complex statistical methods, largely based on regression, have been employed successfully in predicting morbidity and mortality in trauma patients (Clark et al. 2018; Eftekhar et al. 2005). However, these models often rely on a linear relationship between variables, a relationship that does not often reflect pathophysiology or clinical practice (Loftus et al. 2020; Bertsimas et al. 2018) and may be insensitive to temporal evolution of those variables. Instead, ML techniques represent complex relationships among variables and trace their interactions simultaneously. These methods are well-suited to morbidity and mortality prediction for trauma patients.

The first published use of an ML to predict outcomes in trauma patients was by Hadzikadic et al. in 1996 (Hadzikadic et al. 1996). Using an early version of classification clustering and concept formation, their model had equivalent performance to logistic regression in predicting mortality, though more successfully accounted for missing variables. Dybowski et al. followed closely using a combination of a genetic algorithm and a neural network, which outperformed logistic regression models in predicting mortality (Dybowski et al. 1996).

Studies published in the succeeding decades focused on mortality as the sole outcome. A variety of ML techniques were used to improve survival prediction in burn injury (Estahbanati and Bouduhi 2002; Patil et al. 2011; Stylianou et al. 2015), traumatic brain injury (Edwards et al. 1999; Rughani et al. 2010; Shi et al. 2013; Eftekhar et al. 2005; Hale et al. 2018), and pediatric trauma (Hale et al. 2018; Dirusso et al. 2002). A review by Liu et al. identified 65 studies using ML to predict outcomes after traumatic injury (Liu and Salinas 2017). Most of the studies used artificial neural networks, though decision trees, support vector machines, naïve

Bayes classifiers, K-nearest neighbors, and random forest techniques were also used. These strategies outperformed logistic regression in all but one study. Critically, only eight of the studies calibrated their data or reported a goodness of fit. When comparing the various techniques directly, neural networks had superior balanced accuracy (75.1%) and specificity (51.5%) relative to support vector machines, logistic regression, and TRISS (Rau et al. 2019).

Several authors began to examine other variables using ML algorithms. Niggli et al. created a visual analytics tool with IBM's WATSON to predict SIRS and sepsis in traumatic patients; this model has recently outperformed TRISS in predicting early death (Niggli et al. 2021a, b). A Bayesian belief system accurately predicted hospital acquired infection, length of stay, and wound healing in severely wounded soldiers (Stojadinovic et al. 2010). While more granular outcome predictions are few in trauma patients, there is a robust field of predictive analytics in critical illness. These technologies may eventually alert physicians to the early development of acute kidney injury, dementia, cardiovascular complications, need for mechanical ventilation, neurologic events, sepsis, venous thromboembolism, and wound infections (Adhikari et al. 2019; Davoudi et al. 2019; Bihorac et al. 2019).

In addition to individual patient outcomes, predictive analytics has also been applied to trauma systems. There are known, observable patterns in the rise and fall of trauma volume, and predicting these patterns may aid in resource allocation (Ali and Willett 2015; Johnson et al. 2020). Traditional statistical methods had been used previously to identify temporal associations with trauma volume and acuity (Vaziri et al. 2007; Stonko et al. 2018). Dennis et al. demonstrated how an artificial neural network can expand on temporal predictions and plot the interplay of time of day, temperature, and precipitation (Dennis et al. 2019). Stanko et al. identified methods that can be used to both optimize existing trauma centers and plot areas of need (Stonko et al. 2021). This technology is especially relevant in resource-limited settings or in developing trauma systems (Christie et al. 2018). The use of AI techniques in predictive analytics for trauma patients is an established but still burgeoning field. These models are well suited to assess the nonlinear and dynamic variables that impact outcomes in trauma. Figure 1 illustrates the four-layer architecture of an artificial neural network with inputs that include metabolite, molecular, and AIS scores as well as demographic data in predicting survival (Rau et al. 2019).

Challenges and Opportunities

The utility of severity scores has largely been limited to research applications and outcome predictions for large cohorts of patients (Lecky et al. 2014). Real-time, physiological data acquisition when combined with ML models can consolidate multiple parameters into a single score to aid in individual, bedside clinical decision-making. The Physiologic Status Monitoring Biochip (PSM-Biochip) developed by Guiseppi-Elie et al. (Guiseppi-Elie 2011) is an intramuscularly indwelling bio-SONDE that measures, monitors, and wirelessly transmits physicochemical information from within a victim of hemorrhaging trauma (Guiseppi-Elie 2011).

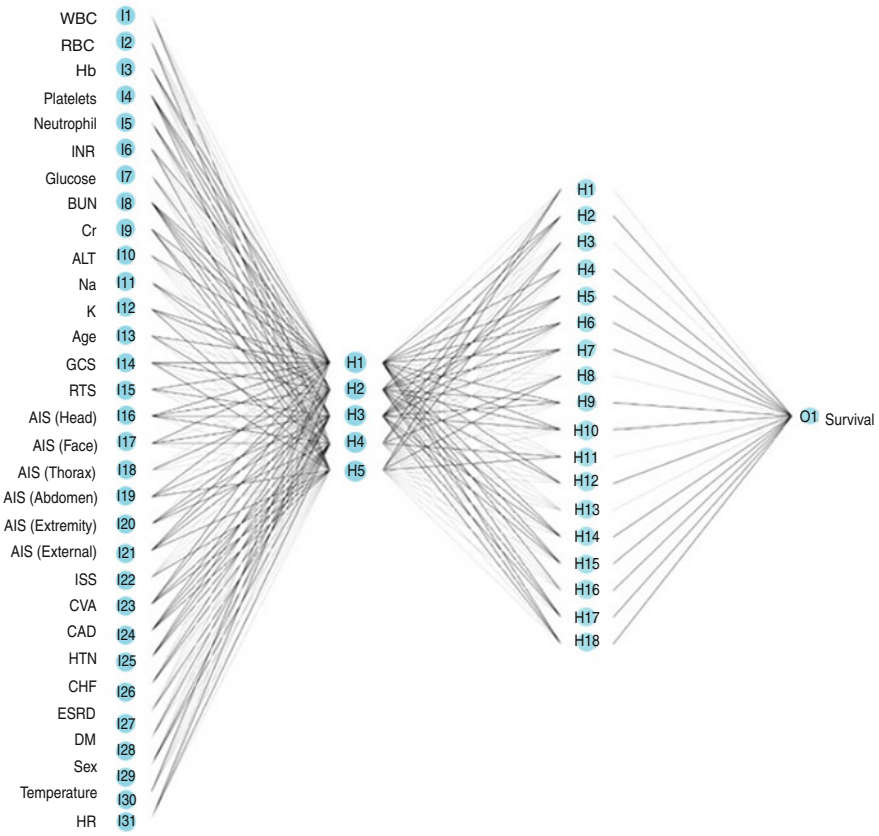

Fig. 1 Architecture of a 4-layer neural network used for survival prediction in trauma patients. (Reproduced with permission from Rau et al. 2019)

The bio-SONDE can acquire the relevant physiological data pertinent to hemorrhagic shock states and thus is a source of temporal data for subsequent fusion with expert data. The PSM-Biochip enables the continuous, real-time monitoring of the patient's physiological status via the key molecular indictors of glucose, lactate, pH, potassium, and oxygen tension. This system would potentially guide evidence-based decision-making (Bal et al. 2014) derived from the real-time pathophysiological profile of the patient (Bhat et al. 2020).

However, such indwelling systems are rich in challenges should they hope to one day achieve clinical practice. Implantable biochip systems continue to be plagued by many challenges including (i) the foreign body response (Wang et al. 2015), (ii) footprint (shape and size) appropriate for implantation with minimum trauma to the indwelling site, (iii) the power demands for long duration implantation (Amar et al. 2015; Zhao et al. 2020a), (iv) efficient multiplexing capabilities to accommodate multiple analytes (Dincer et al. 2017), and (v) the development of stable

biotransducers for the efficacious measurement of biochemical indicators (Aggas et al. 2020).

Navigating the clinical course of a trauma patient is driven both by objective measurements and subjective impressions. Clinical decisions can be immediate, for example, ventilator management, fluid and product resuscitation, or need for surgical intervention. They can also be intermediate and long term, for example, obtaining imaging and diagnostic studies; admission to an intensive care unit or ward; or prognostication of morbidity and mortality. Outside of research and quality improvement initiatives, current scoring systems mostly aid in the latter category and work within the strengths of ML (Husum and Strada 2002; Eid and Abu-Zidan 2015; Garner et al. 2001; Hou et al. 2020). These programs can make narrow decisions based on large amounts of previously learned training data without requiring much memory of previous events. One can imagine an electronic medical record that uses severity scores to recommend ICU admission. Similarly, in discussing prognosis with family members of the critically ill, real-time scoring can provide accurate mortality predictions which may aid in decisions to continue aggressive treatment or transition to comfort care (Hou et al. 2020; Ji et al. 2009).

Immediate decisions are often broader and rely on clinical variables that are not incorporated into the training models of ML algorithms. Nevertheless, there is much potential in this field. Physiologic data and appropriateness guidelines for CT imaging may be incorporated into a scoring system to avoid unnecessary scans (Kohli et al. 2015). Similarly, fluid balance, one of the most perplexing problems for trauma and critical care physicians, incorporates dozens of variables that a properly trained algorithm may excel at interpreting (Zhang et al. 2019). Thromboelastography, coupled with routine labs and pharmacologic and metabolic data, may help manage coagulopathy in medically complex patients (Hasegawa et al. 2020; Zhao et al. 2020b). Severity scoring may be similarly useful in triaging patients with ARDS to more appropriate ventilatory strategies (Hezarjaribi et al. 2018; Yu et al. 2019; Bakkes et al. 2020).

As research into the pathophysiology and individual-patient response to trauma increases in complexity, so will our need to develop tools that can synthesize large amounts of data in a timely, interpretable, and transparent fashion. Currently, most severity scoring systems are limited in their clinical use to predicting mortality, general morbidity, and length of stay (Lecky et al. 2014). Soon, however, real-time data acquisition and point-of-care severity scores generated by ML may help to identify patients who are veering off their expected clinical course and also help guide clinicians' decision-making.

Mini-Dictionary of Terms

Triage: The order given to individual patients for care among many patients or casualties based on the degrees of urgency associated with wounds or illness

Machine Learning (ML): Computer systems based on algorithms that employ mathematical and statistical models that are capable of learning and adapting to

patterns within data to analyze and draw inferences from said patterns without explicit external instructions

Artificial Intelligence (AI): Computer systems based on algorithms that employ mathematical and statistical models that are capable of learning and adapting to perform tasks normally associated with human intelligence, such as image and speech recognition, decision-making, and translation between languages

Patient Stratification: The placement of an individual patient into a category for appropriate care based on the degree of urgency associated with biomarker profiles and/or severity scores

Severity Score: Quantitative measures defined for predicting the mortality of patients by aggregating patient characteristics recorded during their ICU visits

Key Facts of Scoring for Hemorrhage Severity in Traumatic Injury

- The Abbreviated Injury Scale (AIS) was developed in 1969 to grade the severity of individual injuries and is the basis for the Injury Severity Score (ISS).
- Severity scoring is used in clinical trauma management, e.g., triage decision-making.
- Severity scoring, while based on multiple inputs, achieves the utility that equates to a biomarker.
- Physiologically relevant biomarkers may be fused to yield derivative scores.

Summary Points

- Severity scoring systems are important adjunct clinical tools for rapidly assessing patient acuity, estimating prognostic outcomes and survivability, and guiding clinical decision-making and triage for critically ill patients.
- Severity scoring, while based on multiple inputs, achieves clinical utility that equates to that of a biomarker.
- Machine Learning (ML) is well positioned to fuse bioanalytical, physiological, and clinical expert data into novel scoring indices to predict patient mortality.
- Artificial Intelligence (AI) is poised to have a dramatic impact on predictive analytics.
- Indwelling biochips and wearable health devices are a new source of real-time, multiparametric, physiologic data to support bedside clinical decision-making.

Cross-References

► Circulating Polyunsaturated Fatty Acids (PUFAs) as Biological Indicators in Trauma
► Glucose Variability Measures in Critical Care
► Prognostic Biomarkers to Predict Outcomes in Trauma

- ▶ The APACHE II Scoring Systems and the ICU
- ▶ The Score for Trauma Triage in Geriatric and Middle-Age (STTGMA): Utilizing Macroscopic Clinical Biomarkers to Guide Patient Care
- ▶ Tumor Necrosis Factor-Alpha (TNF-Alpha) as a Biomarker in Trauma and Critical Care

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Declarations Conflicts of Interest

A. G.-E. is founder and scientific director of ABTECH Scientific, Inc., manufacturer of microfabricated biochip devices.

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Modeling Orthopedic Injury and Its Impact: Biological Measures of Hypercoagulability and their Applications

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Kristen T. Carter and Matthew E. Kutcher

Contents

Abstract

Post-injury hypercoagulability is commonly seen in injured patients and is associated with an elevated risk of thromboembolic complications, morbidity, and mortality. Orthopedic injuries are common elements of polytrauma and are

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associated with both bleeding and thrombosis depending on fracture patterns. Animal models of orthopedic injury allow for standardization, control of heterogeneity and confounding, investigation of biochemical mechanisms, and testing of novel therapeutics. This chapter discusses the known impact of orthopedic injuries on coagulation, reviews existing animal models of orthopedic injury that assess markers of coagulation and fibrinolysis, and summarizes the advantages and disadvantages of different species used in orthopedic injury animal models.

Keywords

Trauma · Orthopedic injury · Hypercoagulability · Fibrinolysis · Preclinical animal models · Viscoelastic testing · Coagulation biomarkers

Abbreviations

A2AP	Alpha 2 antiplasmin
A2PI	Alpha 2 plasmin inhibitor
aPTT	Activated partial thromboplastin time
DVT	Deep venous thrombosis
IL-10	Interleukin-10
IL-6	Interleukin-6
INR	International normalized ratio
PAI-1	Plasminogen activator inhibitor 1
PE	Pulmonary embolism
PT	Prothrombin time
TEG	Thromboelastography
tPA	Tissue plasminogen activator
TUCA	Taurocholic acid
uPA	Urokinase
VET	Viscoelastic testing
VTE	Venous thromboembolism

Introduction

Injury is a leading cause of death worldwide. More than 85% of injured patients requiring hospital admission develop post-injury hypercoagulability, which has been linked to the development of venous thromboembolism (VTE) (Engelman et al. 1996; Brill et al. 2017). This risk is compounded in patients with orthopedic injuries, which are present in >45% of hospitalized trauma patients (White et al. 2014; Clement et al. 2013). The use of animal models gives investigators the ability to design experiments that mimic specific clinical scenarios, allows for control of confounding variables, and makes it possible to obtain pre-injury baseline measurements; none of which are possible in studies of human injury. Animal models further allow investigation of the mechanisms of post-trauma responses and assessment of possible biomarkers and treatment strategies that can be translated to human patients.

Hypercoagulability, Fibrinolysis, and Viscoelastic Testing in Trauma

Hypocoagulability has long been the primary focus of studies looking into coagulation derangements after traumatic injury, as uncontrolled hemorrhage is a major preventable cause of death after trauma (Moore et al. 2021). However, investigators have shown that a number of injured patients are actually in a hypercoagulable state on admission (Kaufmann et al. 1997). Additionally, those patients that present in a hypocoagulable state frequently transition into a hypercoagulable state within hours to days after their injury (Schreiber et al. 2005). This hypercoagulable state, whether delayed or present on admission, predisposes patients to the development of thromboembolic events (Brill et al. 2017).

Coagulopathy has traditionally been measured by "conventional" coagulation tests such as international normalized ratio (INR), prothrombin time (PT), and activated partial thromboplastin time (aPTT). These tests were initially developed to assess isolated clotting factor deficiencies or to monitor anticoagulant therapy but have been extrapolated to utilization in other clinical scenarios (Kaufmann et al. 1997; Mccully et al. 2013). However, INR and APTT are limited as they represent isolated in vitro, static, acellular assessments (Mccully et al. 2013; Kaufmann et al. 1997). Studies have found that overall these tests do detect hypercoagulability and do not predict thromboembolic events (Park et al. 2009).

Viscoelastic testing (VET) is a dynamic assay of clot formation and dissolution performed in whole blood which provides comprehensive multi-parameter analysis of clot initiation, propagation, stabilization, and dissolution. Thromboelastography (TEG) was introduced in 1948 and first became integrated into surgical management in the 1960s in liver transplants (Volod et al. 2022). TEG has been shown to successfully detect post-injury hypercoagulability, and this hypercoagulability is predictive of thromboembolic events in critically ill patients (Francis et al. 1994; Schreiber et al. 2005; Coleman et al. 2018; Kashuk et al. 2009; Park et al. 2009).

Based on VET profiles, investigators have shown that anywhere from 25%–65% of trauma patients are hypercoagulable on admission and approximately 85% of injured patients will eventually develop hypercoagulability (Kaufmann et al. 1997; Engelman et al. 1996; Branco et al. 2014). Deep venous thrombosis (DVT) rates as well as pulmonary embolism (PE) rates have been shown to be higher in patients with hypercoagulable TEG profiles (Geerts et al. 1994; Meissner et al. 2003; Brill et al. 2017). DVTs occurs in 10–80% of patients following trauma (Attia et al. 2001). PEs occurs in anywhere from 2–22% of trauma patients, and fatal PE is the third most common cause of death in patients who survive the first 24 hours (O'malley and Ross 1990). These rates remain as high as 28% even with adequate thromboprophylaxis and are significantly higher without it (Geerts et al. 1994; Van et al. 2009; Meissner et al. 2003). Furthermore, 30% of patients with a thromboembolic event will require readmission, at an estimated cost to the US healthcare system of 250 million dollars annually (Rattan et al. 2018).

In addition to hypo- and hypercoagulability, VET allows for analysis of fibrinolysis. Three fibrinolytic phenotypes have been characterized based on the percentage

of clot lysis at 30 minutes after reaching maximal clot strength (LY30): hyperfibrinolysis (LY30 >3%), physiologic fibrinolysis (LY30 0.81–2.9%), and fibrinolytic shutdown (LY30 0.0–0.8%) (Moore et al. 2014). Fibrinolytic shutdown is the most common phenotype after traumatic injury, occurring in 46–64% of patients within 12 hours after injury, and is associated with increased mortality (Moore et al. 2014; Moore et al. 2016; Meizoso et al. 2017). Mortality for both hypo- and hypercoagulability exceeds that of patients with physiologic fibrinolysis (Moore et al. 2014). Patients with hyperfibrinolysis most often die early and of exsanguination, while patients in shutdown die later and from multiple organ failure (Moore et al. 2014). While fibrinolytic shutdown has been associated with tissue injury in human and animal studies, risk factors that predispose to this phenotype are unclear (Moore et al. 2014; Moore et al. 2015). Furthermore, the potential for increased bleeding with the use of profibrinolytic agents to normalize shutdown have largely relegated these therapies to historical interest (Moore et al. 2019). Although the clinical relevance of hypercoagulability and fibrinolytic shutdown is profound, the exact timing and biochemical properties of these states are not well-characterized.

The Impact of Orthopedic Injury on Coagulation

The use of VET for the perioperative management of blood component therapy during orthopedic surgeries, such as spinal fusion, total hip arthroplasty, and total knee arthroplasty, has been explored in several studies (Froessler et al. 2015; Spiezia et al. 2016; Zhang et al. 2021; Hanke et al. 2020). However, VET use in the acute management of coagulation perturbations related to orthopedic injury itself is less well studied and has focused principally on management of pelvic fracture-associated hemorrhage (Mamczak et al. 2016; Bostian et al. 2022; Nelson et al. 2020). These studies highlight the importance of the fibrinolytic state as a key determinant of bleeding versus thrombotic risk in patients with pelvic fractures; while hyperfibrinolysis was associated with increased blood loss, transfusion requirements, and mortality, more than 50% of patients presented with fibrinolytic shutdown and an increased risk of DVT (Nelson et al. 2020; Bostian et al. 2022). Similar findings related to hypercoagulability and VTE risk have been identified in patients with hip and long bone fractures (Wilson et al. 2001; Liu et al. 2016; Tsantes et al. 2021). These studies parallel longitudinal studies of VET during the postoperative period after orthopedic surgery, which document the effects of perioperative conduct and thromboprophylaxis on long-term VTE risk (Wu et al. 2019; Li et al. 2020; Kohro et al. 1998; Yang et al. 2014; Klein et al. 2000; Oswald et al. 2015; Tsantes et al. 2021; Fan et al. 2022). Considering that nearly 50% of trauma patients present with some element of orthopedic injury, a deeper understanding of the utility of VET after acute fracture is likely to have significant clinical impact (Clement et al. 2013).

While many animal models and human studies focus on coagulation abnormalities during hemorrhage, only 30% of human trauma patients present with evidence of active hemorrhage and less than 10% present in true hemorrhagic shock (Sauaia

et al. 1995). Activation of the coagulation and fibrinolytic pathway by bony injury has been previously documented in humans, with the magnitude of the response related to the degree of injury (Robinson et al. 2001; Giannoudis et al. 2008). However, determining the effect of the fracture itself on coagulation is confounded by the separate perturbation of fracture fixation, with many studies focusing on pre- and post-intramedullary nailing coagulation assessment since comparing pre- and post-fracture is not feasible (Robinson et al. 2001; Lasanianos et al. 2010). Intramedullary nailing of long bone fractures has been referred to as a "second hit phenomenon" and has been found to exacerbate the initial trauma response, consumptive coagulopathy, resultant inflammation, and progression of organ failure and mortality after injury (Lasanianos et al. 2010; Morley et al. 2008). Studies have found that fracture fixation alone produces significant increases in PT, aPTT, prothrombin fragments, and D-dimer levels, and a decreases in fibrinogen levels, platelet reactivity, and platelet count (Robinson et al. 2001). Therefore, animal models which can facilitate baseline measurements, eliminate confounders, and test interventions are critical to achieving a deeper understanding of the effects of orthopeidc injury on coagulation.

Current Orthopedic Trauma Animal Models Including Coagulation Assays

Benefits of Using Animal Models

Human trauma studies are inherently challenging, as pre-injury baseline measurements are unobtainable and heterogeneity among injury patterns makes clinical inference difficult. Animal models are limited by inter-species differences, making translation to human clinical application challenging; but the benefit of pre-injury assessment, standardization, detailed mechanistic exploration, and therapeutic testing are appealing. Here we describe existing animal models of orthopedic injury and summarize their strengths and limitations in order to facilitate experimental planning as well as contextualization of results.

Overview

A majority of published animal orthopedic trauma models include orthopedic injury as an element of polytrauma, in combination with hemorrhagic shock and/or severe blunt injury (Tsukamoto and Pape 2009; Weber et al. 2019). These combined models make it difficult to ascertain the effect of the orthopedic injury alone; furthermore, many of these are motivated by the study of hemorrhage, leading to a focus on hypocoagulability rather than hypercoagulability. Additionally, requirements for fracture fixation in some models further obscure the effects of orthopedic injury by the addition of surgical injury. According to the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals," experiments involving animals with

Table 1 Existing animal models of orthopedic injury

	Author (Year)	Details of Model	Strain
Animal Swine	Stormann et al (2020)	Isolated femur fracture + fixation vs polytrauma (femur fracture with fixation, lung contusion, liver laceration, hemorrhagic shock, and resuscitation)	Yorkshire
Sheep	White et al (2006) Gray et al (2009)	Femur fracture + fixation with soft tissue injury Femur fracture +/- fixation (internal vs external) +/- soft tissue injury	Scottish black face sheep Gray-faced sheep
Rabbits	Williams et al (1990) Heim at al (1995) You et al (2022)	Bilateral femur fracture + fixation femur fracture +/ fixation (reamed vs unreamed nail) Traumatic knee intraarticular hemarthrosis with immobilization	New Zealand White rabbits Burgundy rabbits New Zealand White rabbits
Rats	Xiang et al (2010) Carter et al (2020) Hayakawa et al (2021)	Bilateral fibula fracture + soft tissue injury + bone homogenate Bilateral fibula fracture + soft tissue injury + bone homogenate Blunt trauma (via wheel with internal shelves)	Zucker rats Wistar rats Wistar rats
Mice	Kobbe at al (2008) Darwiche et al (2012)	bilateral femur fracture (no fixation) +/- soft tissue injury Pseudofracture - bilateral muscle crush injury + bone homogenate	C57/Bl6 mice N/A

long bone fracture should be treated with fractures fixation surgery (1966; Council 2011). Surgical fixation of fractures has been shown to exacerbate the initial trauma response in animals, following the well documented "second hit phenomenon" in humans (Lasanianos et al. 2010; Morley et al. 2008). Models focusing primarily on orthopedic injury or including a fracture-only subgroup are summarized in Table 1 and discussed in detail below.

Nonhuman Primates

Nonhuman primates are similar to humans with regards to physiology, immune function, and even genetics, which makes them ideal for investigation into disease mechanisms and post-trauma modeling and often allows them to serve as a true translational model (Schaub et al. 2017; Hauser 2005; Redl and Bahrami 2005). The majority of the trauma models in nonhuman primates focus on investigating mechanisms of trauma-induced coagulopathy (Schaub et al. 2017; Sheppard et al. 2019; Sheppard et al. 2018; Macko et al. 2017). Schaub et al. reported a model of polytraumatic hemorrhagic shock in rhesus macaques that involved a soft tissue injury, midline laparotomy, and femur fracture (Schaub et al. 2017). The authors identified that nonhuman primates demonstrate early platelet dysfunction after polytrauma similar to humans. Macko et al. used the same model to study the effect of tissue injury on fibrinolysis, in which animals underwent hemorrhagic shock alone, shock with soft tissue injury, or shock with soft tissue injury and concomitant femur fracture (Macko et al. 2017). The authors looked at lactate as well as several coagulation parameters (PT, aPTT, D-dimer, fibrinogen, antithrombin III, von Willebrand factor, and VET) and found that animals in hemorrhagic shock with

associated tissue injury had acute suppression in fibrinolysis. This study demonstrated that nonhuman primate models reflect the fibrinolytic shutdown response to traumatic injury in human patients.

Swine

The majority of orthopedic trauma models in swine are combined models with a component of hemorrhage and/or additional blunt traumatic injury (Hildebrand et al. 2013). Störmann et al. reported a model investigating inflammatory damage after trauma in swine with isolated femur fracture and fixation versus polytrauma (femur fracture with fixation, lung contusion, liver laceration, hemorrhagic shock, and resuscitation) (Störmann et al. 2020). The authors did not look specifically at coagulation parameters, but they did find that there were increased levels of pro-inflammatory markers in the liver and lungs of swine after injury in both groups as well as polymorphonuclear leukocyte infiltration in both the liver and lungs after 72 h. Histology showed tissue damage in the lungs after isolated femur fracture and in both the liver and lungs after polytrauma. Overall, isolated femur fracture induced inflammatory changes primarily in the lungs while polytrauma induced a stronger inflammatory response in the lungs as well as the liver. However, the swine in this study underwent immediate fixation, thus exposing them to the "second hit phenomenon" and making it difficult to quantify the effect of the fracture itself on the levels of pro-inflammatory markers. Horst et al used the same model, including post-injury fracture fixation, to look in more detail at inflammatory markers in local muscle tissue and the fracture hematoma at several time points after injury (up to 72 h). The authors found that there was an increase in pro-inflammatory markers over time in local muscle while in the fracture hematoma there was an initial increase followed by a decrease close to the initial measurement, and overall the levels were significantly higher in the fracture hematoma and in the femur fracture and fixation group. These findings could have implications on fracture healing after trauma though this was not fully explored in this study. These authors also did not look at coagulation parameters but the alterations in inflammatory markers could be associated with coagulation abnormalities, as demonstrated in other studies, and potentially could be investigated with this model (Horst et al. 2019).

Sheep

The majority of orthopedic models in sheep either involve fracture with subsequent fixation or include a component of hemorrhage and/or severe blunt trauma (Giannoudis et al. 2008; Hildebrand et al. 2005). White et al. reported a sheep model of reproducible high-energy fracture to the femur and tibia and injury to the surrounding soft tissues in Scottish Blackface sheep (White et al. 2006). Animals were divided into three groups: a sham group, fracture only group (bilateral femur and bilateral tibial fractures), and fracture and fixation group. A pneumatic actuator

with a cylindrical head was used to create high-energy bone and soft tissue injuries in the animals. The authors found that the coagulation system was activated after injury, seen by the consumption of fibrinogen and antithrombin III. This response was not seen in control animals and interestingly was not significantly different between fracture and fracture plus fixation groups.

The same model was used by Gray et al. to look at external stabilization as compared to intramedullary stabilization after femur fracture, though the model was performed in gray-faced sheep (Gray et al. 2009). The authors compared sham animals to fracture only, fracture and external fixation, or fracture and reamed intramedullary nailing. The authors reported an increased embolic load with intramedullary nailing. The authors also reported a coagulation response in all four groups, including sham animals, with prolonged aPTT and PT and decreased antithrombin III levels. This is incongruent with the findings of White et al., who in contrast reported coagulation differences between sham and injury (White et al. 2006). It is possible that the use of different strains of sheep may have contributed to these disparate results (Gray et al. 2009; White et al. 2006).

Rabbits

Williams et al. investigated the effect of isolated long bone fractures on pulmonary endothelial injury and fibrinolysis in New Zealand white rabbits (Williams et al. 1990). Their model involved bilateral femur fracture using a drop-weight method followed by fracture fixation. Similar to results reported in human studies, the authors found that plasminogen activator inhibitor-1 (PAI-1) levels were significantly elevated over control values at four and twelve hours after injury, suggesting fibrinolytic shutdown as a result of the injury and subsequent surgery. While this model was used to investigate abnormalities in fibrinolysis, it still involved a component of the "second hit phenomenon" as these rabbits underwent fracture fixation.

Heim et al. investigated the effect of reamed versus unreamed intramedullary nailing on pulmonary embolism in Burgundy rabbits (Heim et al. 1995). Though this model involved fracture fixation, one of the six groups studied underwent isolated femur fracture without fixation (and were sacrificed within 60 minutes of injury in order to not cause undue pain or suffering to the animal). This study included flow cytometry of platelet activation markers, antithrombin III activity, fibrinogen activity, and thromboplastic activity of bone marrow. Isolated femur fracture was used as the control group, so it is difficult to assess the impact of this injury alone on coagulation, but the authors did find that reamed nailing induces activation of platelets and plasma-based coagulation.

You et al. used an in vivo rabbit joint injury model to measure trauma-induced coagulopathy and to test the effect of a mast cell stabilizer as a potential therapeutic agent for joint contracture in New Zealand white rabbits (You et al. 2022). The authors created traumatic intra-articular hemarthrosis while maintaining joint integrity and subsequently immobilized the injured knee for 8 weeks using a Kirschner

wire. Serial VET measurements were performed to assess coagulation, with samples drawn after the acclimatization period, before surgery, and weekly until normalization to baseline. The therapeutic intervention of interest was given at three postoperative time points and compared to placebo injection of saline. The investigators found that one week after injury, the control animals who had received the injury along with saline injections all had a hypercoagulable TEG profile, but that it returned to baseline by five weeks after surgery.

Rats

Rodents have well-described cost advantages compared to swine, sheep, and non-human primates and are easier to house and handle. They have also been shown to have similar bone anatomy and physiology to humans making them an intriguing preclinical model for orthopedic injuries (Gunter and Dhand 2002). However, many of the models in rodents utilize a femur or tibia fracture, and as previously described these injuries require fixation (Schindeler et al. 2018). These models are therefore not only affected by the "second hit phenomenon" but require additional expertise to perform fixation (Claes et al. 2017). From a coagulation standpoint, rats are known to be more hypercoagulable than humans at baseline and are resistant to tissue plasminogen activator (tPA) mediated fibrinolysis (Moore et al. 2015). Therefore, modifications have to be made to samples to not only account for they native hypercoagulability but to allow changes in fibrinolysis to be detected by VET.

In order to modify VET assays for rodent hypercoagulability, Wohlauer et al. published a standardized technique to perform TEG in which whole blood samples were anticoagulated with 4% sodium citrate at a 1:10 dilution and run as a native TEG, with the omission of kaolin or tissue factor activation (Wohlauer et al. 2011). This modification provided values similar to those of kaolin-activated TEG results seen in clinical human use. Moore et al. subsequently published a standardized protocol of treating citrated whole rodent blood with a pro-fibrinolytic agent, taurocholic acid (TUCA), in order to allow for resolution of differences in fibrinolysis related to mechanism of injury (Moore et al. 2015). This study also looked at levels of PAI-1, an inhibitor of plasminogen activators tPA and urokinase (uPA), and a known biomarker of fibrinolytic shutdown (Yasar Yildiz et al. 2014; Moore et al. 2014). Studies in humans have shown that PAI-1 levels are 20 times higher in injured patients with fibrinolytic shutdown, although this phenomenon has also been shown to occur in the absence of elevated PAI-1 levels (Moore et al. 2014, 2017). In a model of hemorrhagic shock not including orthopedic injury, Moore et al. found undetectable levels of PAI-1 in injured and sham animals (Moore et al. 2015). The authors also identified angiostatin, a circulating degradation product of plasminogen by neutrophil-related proteases, as a possible biomarker (and potential mediator) of fibrinolysis, though it was only decreased following shock and not tissue injury.

Hayakawa et al. published on fibrinolytic activation in a model of severe blunt trauma in 2021 in Wistar rats (Hayakawa et al. 2021). The authors used a well-documented model for severe non-hemorrhagic blunt injury, inducing trauma using a

plastic wheel with internal shelves. The authors found that tPA levels were significantly increased immediately after injury and that the balance between tPA and PAI-1 tipped toward fibrinolytic activation. While levels of both these mediators increased gradually in various organs and in blood after trauma, PAI-1 levels increased exponentially, while tPA levels returned to baseline within 60 minutes of trauma. Fibrinolytic activation was only seen immediately after trauma and was quickly and intensely suppressed, similar to the transition from hypo- to hypercoagulability in humans seen after injury (Schreiber et al. 2005). In addition to PAI-1, the authors also measured soluble fibrin, plasminogen, and alpha 2 plasmin inhibitor (a2PI), which are also well-known markers of coagulation activation and fibrinolysis, respectively. They found that immediately after trauma the balance was in favor of coagulation activation and that while fibrinolytic activation occurred after trauma, it was quickly suppressed. Though this is a non-hemorrhagic trauma model, it is one of whole-body blunt trauma rather than an isolated long bone fracture, which makes identifying specific contributions of bony versus soft tissue injury difficult.

Xiang et al. developed a model of orthopedic injury in obesity that involved a combination of bilateral fibula fracture, soft tissue crush injury, and bone homogenate injection in Zucker rats (Xiang et al. 2010). The authors showed that this model recapitulated the remote organ pathology seen after injury (including acute lung and kidney injury and elevation in interleukin-6 (IL-6)). Specifically this model mimics the inflammatory response seen after bilateral femur fractures without requiring the "second hit" of fracture fixation (Mittwede et al. 2013, 2015). Fracture of the non-weight-bearing fibula in this model, as opposed to the femur, allows for post-injury mobility, and the lack of hemorrhage parallels a common human orthopedic injury pattern (Sauaia et al. 1995). The Zucker strain (a myogenic model of severe obesity due to a leptin receptor mutation) was used in this model to evaluate effects of obesity on inflammation (Kasiske et al. 1992). Obesity is known to produce a prothrombotic and impaired fibrinolytic state (Willenberg et al. 2010; Previtali et al. 2011). In human studies of coagulation after injury, obesity is an independent risk factor for post-injury VTE (Kornblith et al. 2015; Yang et al. 2012; Eichinger et al. 2008; Stein et al. 2005).

To mitigate the prothrombotic effects of obesity, our laboratory described a modification to the model developed by Xiang et al. using Wistar rats, the genetic precursor for Zucker rats, in order to evaluate the effects of bilateral hindlimb orthopedic injury on coagulation and fibrinolysis in non-obese rats (Xiang et al. 2010; Carter et al. 2020). This isolated orthopedic injury model was found to mimic the post-injury hypercoagulability seen in injured patients. Specifically, orthopedic injury induced overall VET-based hypercoagulability and impairment of fibrinolysis as measured by TUCA-augmented TEG LY30 (Carter et al. 2020). Additionally, fibrinolytic impairment correlated with significant injury-associated elevation of PAI-1. This is in line with human studies correlating elevated PAI-1 levels with fibrinolytic shutdown, although other work in rodent models has described low baseline expression of PAI-1 in plasma (Moore et al. 2015, 2014). Furthermore, fibrinolytic shutdown in injured humans has been shown to occur in the absence of

elevated PAI-1 and A2AP levels (Moore et al. 2017). This suggests that further rodent model research may help to identify additional mediators of fibrinolytic shutdown worth of investigation in this and other systems (Gould et al. 2015; Kutcher et al. 2019).

Mice

As with other animal models, long bone fractures in mice requires fixation in longitudinal survival models. In addition to the "second hit phenomenon" which can affect results, fracture fixation in mice can be complex and is not easily reproducible between technicians (Bonnarens and Einhorn 1984; Holstein et al. 2009; Manigrasso and O'connor 2004).

Kobbe et al. described a model of fracture-associated soft tissue injury in male C57/Bl6 mice (Kobbe et al. 2008). Mice sustained either severe soft tissue injury to both thighs, bilateral femur fracture without fixation and with minimal soft tissue injury, or a combination of both. The authors found that soft tissue injury and bilateral femur fractures induced a systemic inflammatory response, marked by elevations of IL-6 and interleukin 10 (IL-10) which was more pronounced after soft tissue injury. The combination model of soft tissue injury and bilateral femur fractures had a synergistic response and lead to alterations in liver function, suggesting the early development of organ dysfunction. Darwiche et al. reported on a pseudofracture model in mice (Darwiche et al. 2011). This model involved a bilateral muscle crush injury to the hindlimbs followed by injection of a bone homogenate solution into the injured muscles. Since this model does not involve a fracture, it has the advantage of post-injury mobility, facilitating long-term survival and analysis. The authors also reported a biphasic immune response, involving an early hyperinflammatory response with a peak at six hours and a delayed immunosuppressive response with a trough at 48 hours.

Summary of Coagulation Profiles in Animal Model Species

It has been well documented that significant differences exist between the coagulation systems of humans and experimental animals. Differences in "normal" and "baseline" parameters make direct comparisons in coagulation parameters and biomarkers difficult. Clinically relevant coagulation assays used in humans frequently need optimization to account for inherent differences in baseline coagulation that may not be appropriately detected by conventional human assay protocols. Stettler et al. looked at baseline coagulation differences between healthy humans, swine, and Sprague Dawley rats based on citrated native TEG within two hours of collection (Stettler et al. 2017). They found that both experimental animals were significantly hypercoagulable, with rapid clotting times and clot strengths nearly 50% stronger than humans. They also found that swine had more fibrinolysis than humans (at 3.3%) while rats had less (at 0.5%). In addition, the effects of hemorrhagic

Table 2 Coagulation parameter differences between experimental animal systems (as compared to humans)

Coagulation parameter	Nonhuman primate	Swine	Sheep	Rabbits	Rats	Mice
Viscoelastic assays	=	Hypercoagulable	=	=	Hypercoagulable	?
Fibrinolysis / plasmin generation	=	=	?	Highly variable	Impaired	?
Thrombin generation	=	Lower	=	Higher	Lower	=
Platelet count	=	Higher	Higher	=	Higher	=
PT	=	Longer	Longer	=	Longer	Strain dependent
aPTT	=	Shorter	=	Longer	Shorter	Strain dependent
Fibrinogen	=	Higher	=	=	=	?
A2AP	=	=	=	=	=	?
Antithrombin III	=	=	=	=	=	?

shock on non-coagulation-related inflammatory markers (such as lactate and succinate) vary widely between species, leading to variability between the severity of shock states induced by otherwise standard injuries that may have secondary implications for coagulation (Reisz et al. 2018). Species-specific coagulation profiles are summarized in Table 2 and discussed in detail below.

Large Animals

Rhesus macaques have largely similar baseline VET and fibrinolysis studies compared to humans. When comparing VET values between swine and humans, with or without exogenous activation, lysis parameters were similar in swine and humans, suggesting that swine models may be advantageous for evaluating the fibrinolytic pathway after injury (Siller-Matula et al. 2008). Sheep and humans had similar viscoelastic parameters with or without exogenous activation, though a 100 times lower dose of thrombin was required to shorten the clotting time compared to swine, rabbits, and rats (Siller-Matula et al. 2008). Endogenous thrombin potential was the same in both humans and sheep, suggesting sheep as potential models of thrombin generation (Siller-Matula et al. 2008).

Small Animals

Rabbits have a platelet count within normal human range, with platelets only slightly smaller than those of humans (Heim et al. 1995). When comparing VET values between rabbits and humans, maximum clot firmness, with or without thrombin stimulation, was similar, suggesting rabbit models are good options to evaluate platelet function (Siller-Matula et al. 2008).

Rats are hypercoagulable compared to humans. They have been shown to have a comparable PT, substantially shorter aPTT, comparable levels of fibrinogen, and higher platelet counts relative to their blood volume (Wohlauer et al. 2011). They are also inherently resistant to tPA-induced fibrinolysis, with rodents requiring a tenfold higher dose of exogenous tPA to elicit fibrinolysis in whole blood compared to humans (Moore et al. 2015). They have similar levels of a2AP and antithrombin III,

compared to humans (Karges et al. 1994; Singh et al. 2020; Lämmle and Griffin 1985). Coagulation factors 2, 5, 12, and 13 are elevated, while 8, 10, and 11 are reduced, in rats as compared to humans (Karges et al. 1994). Rats also have a significantly higher platelet count and are four times less responsive to thrombin (Wolfensohn and Maggie 2003). In comparison with VET values, the clotting time without thrombin stimulation in rats is three times longer than in humans, and TEG values show shorter clot formation and increased clot firmness in rats (Siller-Matula et al. 2008).

There are numerous strains of mice, which are appealing due to the ability to introduce genetic mutations to recapitulate specific diseases and conditions of interest, however studies looking at baseline coagulation factors have found significant differences depending on the strain of mouse. aPTT and PT are strain dependent (Emeis et al. 2007). Mouse fibrinogen chains are 70–90% identical to human chains; however, murine clots are relatively resistant to lysis by native plasminogen (Lijnen et al. 1994). Other markers of coagulation and coagulation factors are similar to humans, but baseline "normal" levels are also strain specific (Emeis et al. 2007). Despite the differences in baseline levels, there are standardized methods for analyzing blood clotting and coagulation factors in mice that help with reproducibility of these tests (Brake et al. 2019).

Considerations and Limitations of Specific Models for Future Studies

While not all of the models mentioned above investigated the effect of orthopedic injury on biomarkers of coagulation and fibrinolysis, it is likely that they can be repurposed in order to look at additional parameters as research into the underlying mechanisms of hypercoagulability and fibrinolytic shutdown continues. Beyond considerable variation in coagulation parameters between species, investigators must also take into account the strain of animal used in experimental models, especially those models with published "baseline" or "normal" levels of variables of interest. Inter-strain variability is relatively under-recognized compared to inter-species variability, presenting challenges to reproducibility and causal inference. Careful use of strain-, age-, gender-, and exposure-specific controls are key practices to help mitigate these challenges. Differences between experimental animal species relevant to orthopedic injury and coagulation are summarized in Table 3 and discussed in detail below.

It is important to consider the exact experimental protocol including animal handling and environmental parameters of the laboratory, collection of samples, experience of research personnel, and even the laboratory equipment used as minute differences in interventions or handling techniques can have a drastic impact on outcomes and can affect the reproducibility of published models and studies (Cho et al. 2009; Savage et al. 2005). Platelets in particular can be activated via contact activation, and even subtle technical differences such as direct large vessel or cardiac puncture blood draws versus in-dwelling catheter-facilitated blood draws can activate platelets and affect results (Wohlauer et al. 2011). Additionally, animal handling

Table 3 Factors to consider in selecting an experimental animal model of orthopedic injury

Small animals (mice, rats, rabbits)	
Mice	
Advantages	Low cost Easy to care for and house Rapid reproduction Availability of knockout and transgenic animals
Disadvantages	Significant variation in coagulation parameters between strains Difficult to instrument and operate More numerous genetic differences with humans Small blood volume for sampling Higher rates of bone healing and remodeling than humans
Rats	
Advantages	Low cost Easy to instrument and operate Easy to care for and house Rapid reproduction
Disadvantages	Fewer readily available knockout or transgenic animals More numerous genetic differences with humans Small blood volume for sampling Higher rates of bone healing and remodeling than humans
Rabbits	
Advantages	Moderate cost Easy to instrument and operate Easy to care for and house Rapid reproduction Moderate blood volume for sampling
Disadvantages	No knockout or transgenic animals Genetic differences with humans' Higher rates of bone healing and remodeling than humans

(continued)

Table 3 (continued)

Large animals (sheep, swine, nonhuman primates)	
Sheep	
Advantages	Easy to instrument and operate Large blood volume for sampling More physiologically relevant data Similar body weight to humans Similar rates of bone healing and remodeling to humans
Disadvantages	Higher cost More difficut to care for and house Individual variability Genetic differences with humans Slower reproduction
Swine	
Advantages	Easy to instrument and operate Large blood volume for sampling More physiologically relevant data Similar rates of bone healing and remodeling to humans
Disadvantages	Higher cost More difficut to care for and house Individual variability Genetic differences with humans Slower reproduction
Nonhuman primates	
Advantages	Easy to instrument and operate Large blood volume for sampling Most physiologically (and clinically) relevant data Can use human reagents Most genetically similar Similar rates of bone healing and remodeling to humans
Disadvantages	Highest cost Most difficult to care for and house Individual variability Slower reproduction Ethical considerations Theoretical risk of zoonotic disease transmission

and stress can affect coagulation parameters (Chohan et al. 1984; Carbone and Austin 2016). Chohan et al. reported that rats exposed to continuous excess noise had significantly prolonged bleeding times, a higher plasma fibrinogen content, and a shorter aPTT than unexposed animals (Chohan et al. 1984). Posttraumatic analgesia can also have an impact on results; buprenorphine has been shown to be associated with coagulopathy and increased plasma fibrinogen in healthy rats (Griffin et al. 2017).

When selecting an animal model for study or interpreting results, it is important to consider not only baseline physiologic characteristics and laboratory values of the animals compared to humans, but also the clinical question at hand and relative cost of the model. While swine have similar gastrointestinal and cardiovascular physiology to humans, they are more expensive and have a larger footprint in an animal facility (Tsukamoto and Pape 2009). Nonhuman primate models share similar physiologic responses to humans in terms of hemodynamic and immune responses to traumatic injury, and many reagents have adequate cross-reactivity to allow human agents to be used in studies; however, primate use requires significant expertise, infrastructure, and expense, making them relatively cost-ineffective for models of traumatic injury (Hauser 2005; Lomas-Niera et al. 2005; Redl and Bahrami 2005). Rodents have similar bone anatomy and physiology and are considerably less expensive than swine and nonhuman primates; however, they are considerably more hypercoagulable at baseline with reduced fibrinolytic activity requiring significant coagulation assay modifications in order to obtain human-relevant results (Gunter and Dhand 2002; Moore et al. 2015).

Conclusion

Numerous animal models are available for the study of coagulation abnormalities related to orthopedic injury; however, no single model provides perfect fidelity to human injury. Relatively few models incorporate isolated orthopedic injury, and fewer still look at the effects of orthopedic injury on coagulation response and fibrinolysis after injury. However, significant opportunities exist to adapt previously described models for the evaluation of coagulation processes and relevant biomarkers of hypercoagulability and fibrinolysis moving forward.

Applications to Prognosis, Other Diseases, or Conditions In this chapter, we explore the use of animal models in orthopedic trauma, focusing on biomarkers of coagulation and fibrinolysis. Traumatic injury frequently results in hypercoagulability, and this is compounded when an orthopedic injury is present (Engelman et al. 1996). Hypercoagulability has been linked to the development of thromboembolic events, increased morbidity and mortality, and increased costs to the healthcare system (Brill et al. 2017; Schreiber et al. 2005; O'malley and Ross 1990; Rattan et al. 2018). Animal models allow for pre-injury baseline measurements, standardization of injury patterns, and management of heterogeneity and confounding; none of these are possible in human studies. Thus animal models of

orthopedic injury may provide mechanistic insight, identify key biomarkers, and allow for testing of novel therapeutics for the management of orthopedic injury-related coagulation and fibrinolytic abnormalities seen in injured human patients. Insight gained from these animal models will facilitate advancements in the understanding and management of post-injury hypercoagulability in trauma patients, with broader application to other physiological and pathological non-traumatic hypercoagulable states.

Mini-Dictionary of Terms

- Hypercoagulability – An increased tendency for blood to clot, either due to inherited or acquired factors.
- Fibrinolysis – The enzymatic breakdown of fibrin bonds that stabilize blood clots, leading to clot dissolution.
- Viscoelastic testing – Whole blood coagulation assay allowing dynamic monitoring of clot initiation, propagation, and breakdown, including thromboelastography and thromboelastometry.
- Thromboembolic events – Pathological venous clot formation, occurring in the pulmonary artery, or in the deep veins of the abdomen or extremities.

Key Facts of Orthopedic Injury

Orthopedic injury is a common component of trauma, occurring in more than 45% of hospitalized injured patients.

Orthopedic injury can lead to either bleeding or thrombosis, depending on fracture patterns and other components of injury.

Orthopedic injuries frequently lead to hypercoagulability, placing patients at increased risk for thrombotic complications.

Coagulation effects of orthopedic injuries are difficult to study in injured patients, as fracture fixation and immobilization contribute to hypercoagulability after orthopedic injury.

Orthopedic injuries are often the result of blunt trauma such as a motorcycle crash or car crash.

Orthopedic injuries can occur by themselves or with other injuries such as bleeding or a head injury.

Key Facts of Animal Models

Human trauma is difficult to study, since injuries are unplanned, are heterogeneous, and occur in subjects with a variety of pre-existing medical conditions.

Animal models of injury have long been used to identify specific effects of standard injuries and to test potential therapies related to injury management.

Each species used in an animal injury model has aspects of its physiology and response to injury that can either increase or decrease clinical relevance to injured humans.

Many animal injury models primarily investigate effects of hemorrhagic shock; however, hemorrhagic shock is present in only 10% of injured human patients though hemorrhagic occurs in approximately 30%.

Key Facts of Hypercoagulability

Hypercoagulability is a common sequela of injury and predisposes to potentially fatal thrombotic complications during recovery from injury.

Hypercoagulability is not well-measured by standard laboratory coagulation tests but generally requires viscoelastic testing to diagnose.

Hypercoagulability can emerge from one or multiple components of clot formation, including abnormalities in coagulation factors, platelets, fibrinolytic mediators, and immune molecules.

Assessment of hypercoagulability requires different baseline ranges and assay modifications for different animal species.

Summary Points

- Hypercoagulability after orthopedic trauma is difficult to study in human patients.
- Animal models of injury allow for baseline measurements, standardization of injury patterns, and control of heterogeneity and confounding, as well as deeper probing of mechanisms and testing of therapeutics.
- Each animal injury model technique and background species affects the interpretation of results and clinical applicability to human injury.
- Orthopedic injuries have generally been seen to cause hypercoagulability and impairment in fibrinolysis.
- The specific mechanisms underlying hypercoagulability and fibrinolytic impairment after orthopedic injury are not clearly understood.

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Part IX

Resources

Recommended Resources for Biomarkers in Disease: Trauma, Injury, and Critical Care

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Contents

Abstract

It is extremely challenging to assess some victims of trauma, injury, or critical care. An accurate history may be difficult to obtain, and imaging may miss subtle damage to tissues. Visible signs may also be absent. Accurate tools for assessment are required. Biomarkers can be objectively measured and evaluated and are extremely useful in trauma, injury, or critical care. Biomarkers can define the presence and extent of tissue damage which can facilitate risk assessment and guide treatment regimes. Some biomarkers can also provide great insight into the

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pathophysiology of traumatic injuries. The application of biomarkers is actively being investigated for a variety of traumatic injuries and critical illnesses, and the understanding of this topic has advanced in recent years. However, keeping abreast of current information and recommendations is often difficult, so we have compiled resources recommended and used by active clinicians, practitioners, and researchers. This includes information on more than 100 regulatory bodies, societies, organizations, and other resources including over 20 books.

Keywords

Books · Evidence · Journals · Development · Professional societies · Regulatory bodies

Introduction

Assessing the victims of traumatic injury can be difficult. Patients are often unable or unwilling to provide a clear account of the precise events leading up to the traumatic injury. Those at greatest risk of trauma (for example, alcohol misusers, Rajendram and Preedy 2008) are particularly unlikely to be cooperative. Similarly, in critically ill patients, symptomology may be difficult to establish. While physical examination and detailed imaging modalities such as computed tomography can be used to assess the extent of traumatic injuries and disease processes, tissue damage that leads to loss of function can still be missed (Harntaweesup et al. 2022). Thus, accurate laboratory-based tools for the assessment of tissue damage are beneficial. Patients in critical care are especially vulnerable to both misdiagnosis and delayed diagnosis. This is especially important as mortality can be as high as 50%, 6 months after discharge from the intensive care unit (Detsky et al. 2017).

As characteristics that can be measured objectively, biomarkers can demonstrate physiology, pathophysiology, or responses to therapies (Atkinson et al. 2001). Biomarkers are, therefore, extremely useful in trauma and critical illness and indeed in other biomedical settings such as in toxicology (Rajendram et al. 2023b) and diabetes where end organ damage may arise (your reference in diabetes).

Biomarkers can measure tissue damage and patients' overall health after trauma or discharge from the hospital setting. Post-trauma events may be adaptive or develop into more pathological events necessitating intensive or critical care. Many biomarkers are organ specific. So, while creatine kinase isoenzyme MM activity can be used to detect muscular injury (Shehata et al. 2022), it does not reflect renal injury. On the other hand, more severe cases of skeletal muscle damage can lead on to rhabdomyolysis and consequently kidney damage (Torres et al. 2015). Essentially, injuries and critical illnesses vary in severity and can affect any organ directly or secondary to adverse clinical events. Thus, in the acute phase during or after trauma, injury, or critical illness several biomarkers may be required to provide as complete a picture as possible of the extent of the injury. Thus, there is no clear

consensus on the best biomarkers to use to identify the tissues damaged by a specific mechanism of injury.

Regardless, biomarkers are clinically useful tools that can facilitate risk assessment and treatment of traumatic injuries. Biomarkers can reduce the harmful effects of delayed detection of injury. Some biomarkers can also provide great insight into the underlying pathophysiological mechanisms of tissue damage induced by injury. Biomarkers established in preclinical studies are also important as they have the potential to lead to clinical usage or a greater understanding of mechanistic processes. Scoring systems, often based on circulating biomarker analytes, are also very useful in critical care medicine. The APACHE II Score, for example, uses measures of blood sodium, potassium, creatinine, hematocrit, and white blood cell count (ref).

The application of biomarkers is actively being investigated for traumatic injuries to a variety of organs. These include the brain (Dadas et al. 2018), spinal cord (Leister et al. 2020), heart (Keskpaik et al. 2020), kidneys (Saraç et al. 2021), muscle (Shehata et al. 2022), and numerous other tissues and pathometabolic events covered in Rajendram et al. (2023a). Of course, these are only examples, as there are even specialized journals devoted to biomarker discovery. As we have often stated before, experienced researchers, and clinicians, need to stay up-to-date, and this is fulfilled by professional societies and statutory bodies. Those embarking in the research fields of biomarker discovery, trauma, injury, or critical care often need a starting point upon which they can investigate these fields further. In the past 5 years, for example, there have been over 75,000 articles publications relating to biomarkers. We have therefore produced tables containing resources as recommended by active researchers and practitioners, which draws upon the insights acquired over many years. The list below acknowledges all the experts who helped to prepare these valuable resources.

Resources

Tables 1, 2, 3, 4, and 5 list the most up-to-date information on the regulatory bodies (Table 1), professional societies (Table 2), books (Table 3), emerging technologies, and platforms (Table 4), and other resources of interest (Table 5) that are relevant to an evidence-based approach to biomarkers of trauma, injury, postinjury scenarios, and critical illness. Some organizations are listed in more than one table as they occasionally fulfill multiple roles.

Other Resources

The Wellcome Collection (https://wellcomecollection.org/collections) and The British Library (https://www.bl.uk/) also list material on topics related to biomarkers or biomedical sciences. There are also specialist journals devoted to biomarkers which include the following examples (listed alphabetically):

Table 1 Regulatory bodies or organizations dealing with biomarkers, trauma, injury, or critical care

Regulatory body or organization	Web address
American Academy of Neurology	https://www.aan.com/
American Board of Toxicology (ABT)	http://www.abtox.org/
American College of Cardiology	https://www.acc.org/
American Public Health Association (APHA)	https://apha.org/
American Society for Surgery of the Hand (ASSH)	https://www.assh.org/hande/s/tetraplegia
American Spinal Injury Association (ASIA)	https://asia-spinalinjury.org/
Cardiovascular Research Institute Basel (CRIB)	http://www.crib-usb.ch/
Centers for Disease Control and Prevention	https://www.cdc.gov/
European Medicines Agency (EMA)	https://www.ema.europa.eu/en
European Paralympic Committee	https://www.europaralympic.org/
European Stroke Organization	https://eso-stroke.org/
Heart and Stroke Foundation of Canada	https://www.heartandstroke.ca/
Institute for Clinical and Economic Review (ICER)	https://icer.org/
International Agency for Research on Cancer (IARC), WHO	https://www.iarc.fr
International Organization for Standardization	https://www.iso.org/home.html
National Health Service (NHS)	https://www.nhs.uk/
National Health Surveillance Agency (ANVISA)	https://www.gov.br/anvisa/pt-br
National Institute of Health and Care Excellence (NICE)	https://www.nice.org.uk/
National Institute of Aging (NIA)	https://www.nia.nih.gov/
National Institute On Alcohol Abuse And Alcoholism (NIAAA)	https://www.arcr.niaaa.nih.gov
National Institutes Of Health (NIH)	https://www.nih.gov
Pan American Health Organization (PAHO)	https://www.paho.org/en
Sepsis Alliance	https://www.sepsis.org
Swiss Heart Foundation	https://www.swissheart.ch/
United European Gastroenterology	https://ueg.eu/
United Nations Educational, Scientific and Cultural Organization (UNESCO)	https://www.unesco.org/en
United States Department Of Veterans Affairs	https://www.research.va.gov
US Food and Drug Administration	https://www.fda.gov/
World Health Organization (WHO)	https://www.who.int/

This table lists the regulatory bodies and organizations involved with biomedical health, biomarkers, or trauma and associated conditions. The links to biomarkers are indirect in that some of the site may describe the usage of biomarkers indirectly, for example, in connection with disease. The links were accurate at the time of going to press but may move or alter. In these cases, the use of the "Search" tabs should be explored at the parent address or site. In some cases, links direct the reader to pages related to biomarkers of trauma within parent sites. Some societies and organizations have a preference for shortened terms, such as acronyms and abbreviations. See also Table 2

Table 2 Professional societies or other organizations relevant To biomarkers, trauma, injury, or critical care

Society name	Web address
Academy of Spinal Cord Injury Professionals (ASCIP)	https://www.academyscipro.org/
Administration For Community Living	https://acl.gov/
American Academy of Physical Medicine and Rehabilitation (AAPM&R)	https://www.aapmr.org/
American Association for Clinical Chemistry	https://www.aacc.org/
American Association for Laboratory Animal Science	http://www.aalas.org/
American Association of Clinical Chemistry	https://www.aacc.org/
American Association Of Neurological Surgeons	https://www.aans.org/
American Chemical Society	https://www.acs.org/
American Congress of Rehabilitation Medicine (ACRM)	https://acrm.org/
American Diabetes Association	https://www.diabetes.org/
American Society for Mass Spectrometry	https://www.asms.org/
American Stroke Association	https://www.stroke.org/
Association for Clinical Biochemistry and Laboratory Medicine	https://www.acb.org.uk/
Australasian Association of Clinical Biochemists	https://www.aacb.asn.au/
BioMedAlliance (Biomedical Alliance in Europe)	https://www.biomedeurope.org/
Brain Injury Association Of America	https://www.biausa.org/
Brain Trauma Foundation	https://www.braintrauma.org/
Canadian Cardiovascular Society	https://ccs.ca/
Center Of Excellence For Medical Multimedia (CEMM)	https://tbi.cemmlibrary.org/
Clinicaltrials.Gov	https://clinicaltrials.gov
European Association for the Study of the Liver	https://easl.eu/
European Association of Nuclear Medicine	https://www.eanm.org/
European Federation of Clinical Chemistry and Laboratory Medicine	https://www.eflm.eu/site/
European Institute for Biomedical Imaging Research	https://www.eibir.org/
European Society of Cardiology	https://www.escardio.org/
European Society of Intensive Care Medicine	https://www.esicm.org/
Intensive Care Society	https://www.ics.ac.uk/Society/
International Association for Trauma Surgery and Intensive Care/	https://www.iatsic.org/
International Brain Injury Association	https://www.internationalbrain.org/
International Continence Society	https://www.ics.org
Neurocritical Care Society	https://www.neurocriticalcare.org/home
Society for Risk Analysis (SRA)	http://www.sra.org/
Society of Critical Care Medicine	https://www.sccm.org/Home
Society of Nuclear Medicine and Molecular Imaging	https://www.snmmi.org/

This table lists the professional societies involved with biomedical health, biomarkers, trauma, injury, or critical care. Some of the links have indirect reference to the use of biomarkers. The links were accurate at the time of going to press but may move or alter. In these cases, the use of the "Search" tabs should be explored at the parent address or site. In some cases, links direct the reader to pages related to biomarkers of trauma within parent sites. Some societies and organizations have a preference for shortened terms, such as acronyms and **abbreviations. See also** Table 1

Table 3 Books On biomarkers, trauma, injury, or critical care

Book title	Authors or editors	Publisher	Year of publication
Absolute Neurocritical Care Review	Levy Z	Springer	2017
Acute Ischemic Stroke	Park J	Springer	2017
Biomarkers in Drug Discovery and Development: A Handbook of Practice, Application, and Strategy, second Edition	Rahbari R	John Wiley & Sons	2020
Cardiovascular Manual (German)	Rickli H	Cantonal Hospital St. Gallen	2019
Cholestatic Liver Disease	Lindor KD, Talwalkar JA	Springer	2008
Continuous Glucose Monitoring	Jia W	Springer	2018
Drug Discovery Toxicology: From Target Assessment to Translational Biomarkers	Will Y	John Wiley & Sons	2016
Essentials of Neurocritical care	McLaughlin DC	Springer	2018
Introduction to Mass Spectrometry, fourth Edition	Watson J, Sparkman O	John Wiley and Sons Ltd	2007
Ischemic Stroke	D'Aliberti G, Longoni M, Motto C, Oppo V, Perini V, Valvassori L, Vidale S	Springer	2017
Liver: A Complete Book on Hepato-Pancreato-Biliary Diseases	Al Mahtab M, Rahman S	Elsevier	2009
Managing Diabetes and Hyperglycaemia in the Hospital Setting	Draznin B	American Diabetes Association	2016
Mass Spectrometry – Principles and Applications, third Edition	de Hoffman E, Stroobant V	John Wiley and Sons, Ltd	2007
Mass Spectrometry in Metabolomics	Raftery D	Springer	2014
MRI: The Basics	Hashemi R, Bradley W, Lisanti C	Philadelphia: Lippicott Williams and Wilkins	2010
Neurocritical Care	Kinoshita K	Springer	2019
Neurocritical Care Board Review, second Edition Questions and Answers	Asma Z, Pouya TF	Springer	2018
Stroke Biomarkers	Peplow P, Martinez B, Dambinova S	Springer	2020
Stroke Revisited: Diagnosis and Treatment of Ischemic Stroke	Lee SH	Springer	2017

(continued)

Table 3 (continued)

Book title	Authors or editors	Publisher	Year of publication
Stroke Revisited: Pathophysiology of Stroke – From Bench to Bedside	Lee SH	Springer	2020
Traumatic Brain Injury Pathobiology, Advanced Diagnostics and Acute Management	Gorbunov N, Long J	IntechOpen	2018
Traumatic Brain Injury: A Clinician's Guide to Diagnosis, Management, and Rehabilitation	Tsao JW	Springer	2019

This table lists books relevant to biomarkers, trauma, injury, or critical care

Table 4 Techniques and platforms related to biomarkers, trauma, injury, or critical care

Organization or company name	Web address
Biocept	https://biocept.com/
Biomarker Bay	https://biomarkerbay.com/
Biomarkers – ACROBiosystems	https://www.acrobiosystems.com/
Biomarkers Platform – A_IATRIS	http://www.aiatris.it/biomarkers
CORDIS – Biomarker Discovery and Validation	https://cordis.europa.eu/programme/id/H2020_IMI2-2020-23-03
Dexcom	https://www.dexcom.com/
Digital Biomarker Platform – Koneksa	https://www.koneksahealth.com/platform/
Medtronic	www.medtronicdiabetes.com/products/guardian-connect-continuous-glucose-monitoring-system
Upsurgeon	https://www.upsurgeon.com/?v=cd32106bcb6d

This table lists technologies or platforms relevant to biomarkers, trauma, injury, or critical care. Please note, occasionally the location of the websites or web address changes

Advances in Biomarker Sciences and Technology (https://www.sciencedirect.com/journal/advances-in-biomarker-sciences-and-technology/); Biomarker Insights (https://journals.sagepub.com/home/bmi);
Biomarker Research (https://biomarkerres.biomedcentral.com/); and
Biomarkers (https://www.tandfonline.com/journals/ibmk20).

Other chapters on resources relevant to trauma, injury, and critical care (recommended by authors and practitioners) may also be relevant to biomarkers of trauma. These include traumatic brain injury (Rajendram et al. 2022a) and spinal cord injury (Rajendram et al. 2022b).

Table 5 Other resources of interest or relevance for health care professionals or patients related to biomarkers, trauma, injury, or critical care

Name of resource or organization	Web address
Adapt Functional Movement Center	https://adaptmovement.org/
Administration For Community Living: Material on Traumatic Brain Injury	https://acl.gov/programs/post-injury-support/traumatic-brain-injury-tbi
After trauma	https://www.aftertrauma.org/
American Association Of Neurological Surgeons: Material on Traumatic Brain Injury	https://www.aans.org/Patients/Neurosurgical-Conditions-and-Treatments/Traumatic-Brain-Injury
BEST Resource: Harmonizing Biomarker Terminology	https://www.fda.gov/media/99221/download
Biologic Medicine Information Centre (BMICC): Urinary Protein Biomarker Database	Biologic Medicine Information Centre (BMICC): Urinary Protein Biomarker Database
Biomarkers Consortium Resources	https://fnih.org/what-we-do/biomarkers-consortium/about/resources
Biomarkers Database -Charles River	https://wwwapps.criver.com/BiomarkersDB/
Biomarkers Definition Working Group	https://doi.org/10.1067/mcp.2001.113989
Biomarkers Resources – Eurofins	https://www.eurofins.com/biopharma-services/bioanalysis/biomarker-testing-services/biomarkers-resources/
Brain and Spine Foundation	https://www.brainandspine.org.uk/
Centre for the Rehabilitation of the Paralysed (CRP) Bangladesh	https://www.crp-bangladesh.org/
Christopher and Dana Reeve Foundation	https://www.christopherreeve.org/
Clinical Practice Guidelines – European Society of Cardiology	https://www.escardio.org/Guidelines/Clinical-Practice-Guidelines
Diabetic Care – American Diabetes Association	https://care.diabetesjournals.org/content/40/4/509
Disabilities trust	https://www.thedtgroup.org/
Essentials of Neurocritical Care – Neurocritical Care Organization	https://www.neurocriticalcare.org/education/essentials
European Federation of Pharmaceutical Industries and Associations: Working with Patient Groups	https://www.efpia.eu/relationships-code/patient-organisations/
FDA-NIH Biomarker Working Group	https://www.fda.gov/about-fda/center-drug-evaluation-and-research-cder/fda-biomarkers-working-group
Guidance Document for Blood Glucose Monitoring Test Systems, US Food and Drug Administration	https://www.fda.gov/regulatory-information/search-fda-guidance-documents/blood-glucose-monitoring-test-systems-prescription-point-care-use
Guidelines and Position Statement Library – Canadian Cardiovascular Society	https://ccs.ca/guidelines-and-position-statement-library/
Guidelines On Intravenous Thrombolysis for Acute Ischaemic Stroke – European Stroke Organization	https://eso-stroke.org/intravenous-thrombolysis/

(continued)

Table 5 (continued)

Name of resource or organization	Web address
National Institute of Neurological Disorders and Stroke: Patient Organisations	https://www.ninds.nih.gov/Disorders/Support-Resources/Patient-Organizations
National Institute On Alcohol Abuse And Alcoholism (NIAAA): Material on Traumatic Brain Injury	https://www.arcr.niaaa.nih.gov/arcr392/article06.htm
National Institutes Of Health (NIH): Material on Traumatic Brain Injury	https://www.nih.gov/about-nih/what-we-do/nih-turning-discovery-into-health/traumatic-brain-injury-tbi
Sepsis – Centers for Disease Control and Prevention	https://www.cdc.gov/sepsis/index.html
Spinal Injuries Association	https://www.spinal.co.uk/
Surviving Sepsis Campaign	https://www.sccm.org/SurvivingSepsisCampaign/Home
The Patients Association	https://www.patients-association.org.uk/
United States Department Of Veterans Affairs: Material on Traumatic Brain Injury	https://www.research.va.gov/topics/tbi.cfm
Urinary Protein Biomarker Database (UPBD) – BMICC	http://upbd.bmicc.cn/biomarker/web/indexdb

This table lists other resources of interest or relevance to biomarkers, trauma, injury, or critical care. Please note, occasionally the location of the websites or web address changes

Other chapters on resources relevant to biomarkers (recommended by authors and practitioners) may also be relevant to biomarkers of trauma, injury, and critical care. These include nutrition and oxidative stress (Rajendram et al. 2020), general aspects of biomarkers (Rajendram et al. 2016c), biomarkers of cardiovascular disease (Rajendram et al. 2016b), biomarkers of renal disease (Rajendram et al. 2017), aging (Rajendram et al. 2021), and toxicology (Rajendram et al. 2023b).

This list of material in these tables is included to provide general information only. It does not constitute any recommendation or endorsement of the activities of these sites, facilities, or other resources listed in this chapter, by the authors or editors of this book.

Summary Points

Biomarkers are of great clinical significance in trauma and associated specialties including critical care medicine.

In injury, trauma, and critical care, organ damage may be direct or secondary.

Scoring systems, often based on circulating biomarker analytes, are also very useful in critical care medicine.

No single biomarker is applicable in all scenarios.

Several biomarkers must be used in any given situation.

This chapter lists resources relevant to the use of either biomarkers, trauma, injury, or critical care medicine.

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