Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care

Silvia De Rosa
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ISBN 978-3-031-18590-8 ISBN 978-3-031-18591-5 (eBook)
https://doi.org/10.1007/978-3-031-18591-5

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

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Endotoxin: Structure Source and Effects

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Alessandro Perrella, Novella Carannante, Nicolina Capoluongo, Annamaria Mascolo, and Annalisa Capuano

1.1 Endotoxin

The concept that endotoxin, an insoluble part of the bacterial cell, was a toxic substance able to evoke a typical picture of bacterial infection, even without the presence of living bacteria was introduced for the first time by Richard Pfeiffer in 1892 [1]. Subsequently, many years were needed to characterize the exact structure, function, and mechanism of action of endotoxin, nowadays recognized as lipopolysaccharide (LPS).

LPS is the major component of the cell wall of Gram-negative bacteria, recovering the 75% of the surface of the outer leaflet of the outer membrane of the cell wall. It is a glycolipid composed of a hydrophobic lipid part (lipid A) anchored in the outer leaflet and a hydrophilic polysaccharide part that extends outside the cell. The polysaccharide part is divided into two domains: the core region and the O antigen (also named O-chain). The O-chain is composed of several units of oligosaccharide and is tied to lipid A through the core region [2]. The main role of LPS molecules is to create a hydrophobic structure that results in a permeability barrier that protects bacteria from antimicrobial factors [3].

LPS is produced by most Gram-negative bacteria, with a few exceptions represented for example by Treponema pallidum [4]. Although the structure of LPS is

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_1.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_1

well conserved, differences can be observed among species of bacteria. For example, an LPS without the O-chain is produced by some species of Gram-negative bacteria and it is called as "rough" LPS, as opposed to a "smooth" LPS, which includes the O-chain [5, 6]. LPS is a component of the bacterial wall essential for survival in a hostile environment. Indeed, Gram-negative bacteria that lack LPS or have LPS without an O-chain are more sensitive to antibiotics and, in general, to the host's defense mechanisms [3].

Among LPS components, lipid A deserves particular attention, as it is responsible for activating the immune system and for inducing pyrogenic and toxic effects. The structure of lipid A can differ among Gram-negative bacteria in the number and the length of fatty acid chains attached and the presence or absence of phosphate groups or other residues [3]. Generally, in most cases, LPS is constituted by a diglucosamine backbone phosphorylated at positions 1 and 4 and acylated with 5 or 6 fatty acyl chains. The most present fatty acyl chain is the 3-hydroxy-tetra-decanoinc acid. Studies demonstrated that alterations of lipid A can cause alterations in its biological activities. Indeed, the variable structure of lipid A determines its stimulatory or inhibitory action. For example, lipid A with a diglucosamine backbone, two phosphates, and six fatty acyl chains, is best sensed by the host's complex of myeloid differentiation factor 2 and the toll-like receptor 4 (MD-2-TLR4) [7].

LPS in the cell membrane of anaerobic Bacteroidales, which are present in the commensal microbiota of the human gut, has an under-acylated (tetra- or penta-acyl) lipid A that is a potent TLR4 inhibitor. Consequently, by silencing the TLR4 pathway, it facilitates the host's tolerance of gut microbes [8]. However, it is unknown if this phenomenon has any effect on the progression of infection [9]. In fact, the lipid A structure of Pseudomonas aeruginosa but also of many other Gram-negative bacteria does not possess six fatty acyl chains [7]. Yersinia pestis instead is able to produce hexa-acyl LPS at 21–27 °C and tetra-acyl LPS at 37 °C, and thus it is able to escape the host's first-line defense in mammals. Moreover, a genetically modified strain of Yersinia pestis which produces hexa-acylated LPS at 37 °C appeared to be avirulent, as it is able to facilitate the early recognition of infection and the effective onset of immune signaling [10]. During chronic infection, modifications of LPS molecules are possible and happen to facilitate the evasion of host immune defense and biofilm adaptation [11].

Gram-negative bacteria are a major part of the gut microbiota and are a source of LPS [12]. Normally, minor amounts of LPS can move into the bloodstream with the potential of triggering an immune response. However, to protect the host from a noxious over-activation of the immune system, several mechanisms exist for detoxification and elimination of LPS [13]. Among them, there is the rapid sequestration of LPS by lipoproteins, mainly high-density lipoproteins (HDL) in cooperation with the phospholipid transfer protein (PLTP). Lipoproteins transport LPS to the liver, where it is inactivated by enzymes such as acyloxyacyl hydrolase and alkaline phosphatase and, then, excreted in the bile [13].

Another mechanism of detoxification relies on the binding of LPS to the small form of HDL (called HDL3), which is produced by intestinal epithelial cells. In

particular, HDL3 by binding the LPS binding protein (LBP) captures the LPS and forms the HDL3-LBP-LPS complex. This complex hides LPS from liver macrophages, and instead induces its inactivation by favoring the effect of the plasmatic enzyme acyloxyacyl hydrolase (AOAH), thus protecting the liver from inflammation and fibrosis that may develop in the course of chronic exposure to LPS [14].

These mechanisms of detoxification are insufficient in case of disruption of the intestinal barrier, and an increased quantity of endotoxin enters the bloodstream. This is likely when the intestinal epithelium, formed by only one layer of cells, is damaged by hypoperfusion, inflammation, or dysregulation of commensal flora, resulting in an increased gut-barrier permeability and LPS translocation into the blood [15–17].

1.2 Pathway of LPS

LPS can stimulate extracellular and intracellular pathways that lead to the activation of the immune response.

1.2.1 Toll-Like Receptor 4-Myeloid Differentiation Protein 2 (TLR4-MD-2) Pathway

The TLR4 is the main receptor for LPS and one of the pattern recognition receptors responsible for the early detection of microbes by the innate immune system. TLR4 is expressed on the surface of monocytes, neutrophils, macrophages, dendritic, and epithelial cells, as well as within endosomes, forming the front line of the host's defense mechanisms against Gram-negative bacteria.

LPS molecules in the bacterial cell wall and also soluble LPS-aggregates can bind the LBP that in turn forms a complex with either a soluble or membrane-bound cluster of differentiation-14 (CD14), which is subsequently transferred to the TLR4/MD-2 complex. This promotes the TLR4/MD-2 dimerization and then the activation of intracellular MyD88 (myeloid differentiation factor 88) pathway, which determines the early activation of nuclear factor κB (NFκB), leading mainly to the production of proinflammatory cytokines (TNF-α, IL1B, IL-6, IL12B), or the TRIF (Toll-like receptor domain adaptor inducing interferon-β) pathway, which, on the other hand, is involved in the late phase of transcriptional activation of cytokines (IL-10) and in the development of endotoxin tolerance [18, 19]. The hyperactivation of the immune system triggered by pathogens and the subsequent cytokine storm leads to organ damage, multiorgan failure, and death [20].

However, the progress in research on LPS recognition systems led to important discoveries of TLR4-independent pathways sensible to LPS that may also play a central role in the pathophysiology of infection and related mortality.

1.2.2 Transient Receptor Potential (TRP) Ion Channels

TRP ion channels are membrane-bound channels that act as cellular sensors of environmental and intracellular stimuli. LPS can bind TRP channels present in neurons and airway epithelial cells [21]. Specifically, the activation of the subtype TRPA1 channels in nociceptive neurons by the LPS induces pain during inflammation [22]. The activation of the TRPV4 channels in the airway epithelium instead boosts ciliary beat frequency and the production of bactericidal nitric oxide, which facilitates the pathogen clearance from the airways. TRP channels by recognizing LPS provide an immediate response to invading pathogens, which is faster and independent of the canonical TLR4 pathway [21].

1.2.3 Intracellular LPS Pathways

LPS can enter the cytosol as LPS/outer-membrane-vesicle (OMV)-high mobility-group-box-1 (HMGB1) complex internalized through the receptor for advanced glycation (RAGE). When LPS enters the cytoplasm of macrophages, as well as endothelial and epithelial cells, it is sensed by inflammatory caspases such as caspase-4/5 in humans. The activation of caspases plays a crucial role in intracellular pathogen detection and defense. Indeed, caspases can lead to the induction of pyroptosis, an inflammatory form of cell death. Moreover, activated caspases can cause pore formation in the cell membrane with subsequent cell lysis and release of proinflammatory cytokines (IL-1β and IL-18) [23]. Inflammasome activation and pyroptosis are important mechanisms of the innate immune response against pathogens that are able to invade the cytosol and have a major role in the pathophysiology of sepsis. Caspases such as caspase-11 is also responsible for bacterial clearance of Klebsiella pneumoniae and Acinetobacter baumannii, as well as Burkholderia lung infections [23]. Furthermore, caspases may be responsible for sensing penta-acylated LPS, which is not detected by TLR4 [24]. Caspase-mediated pyroptosis of endothelial cells has a fundamental role in the host's defense and immune surveillance functions of the microvasculature [25]. Finally, an over-activation of pyroptosis can cause excessive cell death and inflammation leading to organ failure and septic shock [26].

1.2.4 Endotoxic Shock and Organ Damage Caused by LPS

Endotoxic shock is a severe, generalized inflammatory response caused by high bloodstream levels of LPS. A large amount of LPS triggers an extensive, uncontrolled systemic inflammation that leads to multi-organ failure and death. Patients typically present with fever and refractory hypotension. Organ failure secondary to hypoperfusion is common and patients may have oliguria, lactic acidosis, acute alterations in mental status, and disseminated intravascular coagulation (DIC). The pathological modifications induced by endotoxin in several organs contribute to the fatal outcome and are shown in Fig. 1.1.

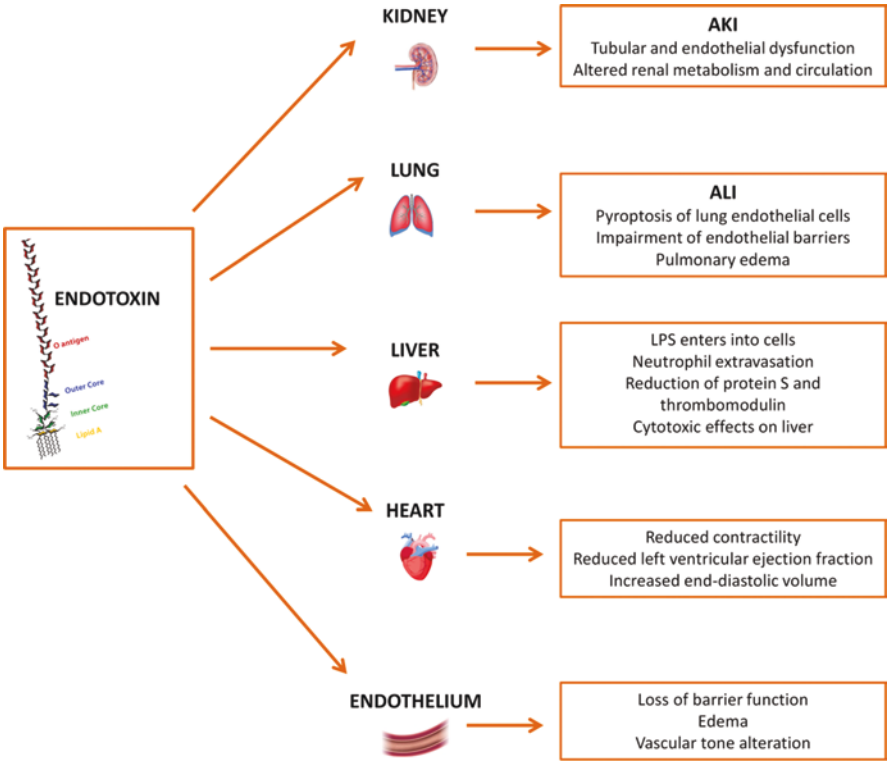

Fig. 1.1 Organ damage induced by lipopolysaccharide (LPS). *ALI* Acute lung injury, *AKI* Acute kidney injury

1.2.5 The Kidney

Acute kidney injury (AKI) is reported in at least 40–50% of patients with shock and is associated with significantly higher mortality [27, 28]. AKI is also characterized by metabolic and fluid abnormalities, which necessitate adjustments in volume and pharmacotherapy, most notably limiting antimicrobial choice. The pathophysiology of septic AKI is complex and, in addition to hypoperfusion, involves the interaction between vascular, tubular, and inflammatory factors. The exact mechanism underlying septic renal dysfunction is unknown, but experimental evidence is supporting the role of the TLR4, which is expressed in the kidney [29]. Specifically, TLR4 is located in the tubular epithelium, in the vascular endothelium and glomeruli. LPS is indeed filtered in renal glomeruli and internalized by S1 proximal tubules through TLR4 receptors. TLR4 activation causes the release of cytokine and chemokine; infiltration of leukocytes, which results in endothelial dysfunction; tubular dysfunction and altered renal metabolism and circulation [30]. In this way, there is a development of severe oxidative stress and damage also to the near S2 segments [30, 31]. Among other effects, TLR4 can directly block bicarbonate absorption in the medullary-thick ascending limb, reduce renal sodium, chloride, and glucose

transporters, induce luminal obstruction, and decrease tubular flow [30]. Other factors that contribute to septic AKI are endothelial activation and alterations to glomerular glycocalyx and the deposit of neutrophil extracellular traps (NETs) in the renal tissue [32, 33]. Direct renal damage by LPS can explain the occurrence of AKI, even when hemodynamic parameters are well-preserved [31]. In fact, it was shown that protocolized hemodynamic resuscitation did not influence either the development or the course of AKI in patients with septic shock [28]. As a result, the concept of acute tubular necrosis attributed to ischemia from hemodynamic changes in AKI was replaced by the theory of the interplay between inflammation, oxidative stress, and microvascular dysfunction [34].

1.2.6 The Lung

Histological alterations induced by LPS in the lungs are thickening of the septum, edema, congestion, and high leukocyte infiltration into the interstitium, which correlated with a significant increase in the serum concentrations of NETs and the extent of lung injury [33]. The inflammatory response is characterized by the release of prostaglandins, platelet-activating factors (PAF), leukotrienes, and thromboxanes, which can cause the respiratory distress syndrome by increasing the vascular permeability and contractions of smooth muscle cells in the lung. Lung injury was also attributed to the LPS-triggered pyroptosis of the endothelial cells. Specifically, LPS via caspase-4/5/11 mediated pyroptosis that led to disruption of the endothelial barrier resulting in pulmonary edema, the release of proinflammatory cytokines, fluid protein leakage, and a massive infiltration of leukocytes [25].

1.2.7 The Heart

TLR4 is also expressed in cardiomyocytes and its activation induces an inflammatory response with the production of cytokines and chemokines that have a negative effect on cardiac contractility [35]. LPS may trigger heart multiple caspase activation and cytochrome c release from the mitochondria causing myocardial cells apoptosis. Moreover, caspase-3 activation may also directly induce changes in calcium myofilament response, in troponin T cleavage, and in sarcomere disorganization, without determining death of myocardial cells [36]. In healthy volunteers, increased endotoxin levels resulted in a reduction of left ventricular ejection fraction and an increase of end-diastolic volume [37]. In the experimental model, the administration of LPS determined significant pathological changes such as myocardial bundles, congestion of capillaries with leukocytes attached to the endothelium, and histological changes of cardiomyocytes [33]. Other studies also indicated that LPS-associated cardiac dysfunction was also mediated by TLR4 activation [38].

1.2.8 The Liver

The liver is an important participant in the body's reaction to endotoxemia. Experimental studies demonstrated that LPS uses both TLR4 and caspase-11/gasdermin D pathways to induce the release of the nuclear protein high mobility group box 1 (HMGB1) from hepatocytes [39]. Complexes of HMGB1 and LPS are internalized via RAGE into the cytosol of macrophages and endothelial cells, where LPS activates caspase-11 and induces pyroptosis and cell death [40]. The intracellular effect of LPS is considered to play a central role in the pathogenesis of sepsis [23].

In the liver, LPS affects the architecture of the sinusoidal endothelium and blood flow velocities, which leads to extravasation of neutrophils, interaction of neutrophil and hepatocyte, decrease of protein S and thrombomodulin, which contributes to a procoagulant state and has a cytotoxic effect directly on hepatocytes [32]. Histological changes induced by LPS in the liver included enlarged sinusoids, increased volume of endothelial cells, high number of leukocytes in the lumen, hypertrophy and hyperplasia of Kupffer cell, along with the presence of leukocytes close to periportal areas and congestion of the central vein with swollen hepatocytes [33].

1.2.9 The Vascular Endothelium

Endothelial cell dysfunction is considered a key factor for the progression to organ failure [32]. The presence of LPS in the blood causes shedding of the glycocalyx lining of the vascular endothelium that leads to the loss-of-barrier function, the formation of edema, and the dysregulation of vascular tone [32].

The stimulation of endothelial cells with LPS determines the upregulation of several adhesion molecules (E-selectin, P-selectin, intercellular adhesion molecule-1, etc.), cytokines (IFN-α, INF-γ, IL-6), and chemokines (CCL2, CCL3, CCL5). Moreover, LPS decreases the expression of thrombomodulin, tissue-type plasminogen activator, and heparin, while increasing the expression of tissue factor (TF) and plasminogen activator inhibitor 1 (PAI-1) [36]. Moreover, LPS can induce the activation of the Hageman factor that stimulates the intrinsic pathway of coagulation that leads to the conversion of fibrinogen in fibrin. These effects, together with the activation of the extrinsic pathway, determine the shift of the hemostatic balance from an anticoagulant to a procoagulant state and induce endovascular thrombosis and the occurrence of DIC.

Furthermore, LPS can induce the release of nitric oxide (NO) and reactive oxygen species that cooperate in increasing endothelium damage and permeability. Endothelial damage determines the attachment of neutrophils, which further amplify the oxidative response. The activated Hageman factor can induce the stimulation of the kinins system by converting the pre-kallikrein into kallikrein that, in turn, catalyzes the conversion of kininogen into bradykinin, a vasoactive peptide that determines vasodilation and increases vascular permeability. LPS can also activate the complement cascade through the classic or alternative pathways, further

contributing to the increased permeability and chemotaxis of polymorphonuclear leukocytes. Finally, LPS can trigger caspase-dependent pyroptosis in endothelial cells resulting in the disruption of the endothelial barrier and fluid leakage [25].

1.3 Evaluation of Endotoxin-Induced Shock

There is no doubt that a clinical diagnosis of endotoxin-induced shock cannot be established by using only merely diagnostic tools, but it also needs the recognition of signs by clinicians. However, the prompt identification of clinical criteria to use in this setting has become over the years increasingly important since they have an impact on mortality and morbidity. In this context, the recognition of the stage from early inflammation to multi-organ dysfunction is fundamental.

Among clinical criteria, there is the use of Acute Physiologic Assessment and Chronic Health Evaluation (APACHE) score, Sequential Organ Failure Assessment (SOFA), and quick SOFA score. All scores evolved with the intent of finding an easily applicable scoring system to use in any clinical setting to predict the presence of shock, the risk of organ dysfunction, and the in-hospital mortality.

In case of a rapid identification of the source of infection, clinical investigations are individualized to the infected organ. On the contrary, in the absence of an apparent source, a time-sensitive search for infectious sources becomes a priority. Society guidelines endorse a routine collection of specimens from blood, sputum, urine, and any other wound for culture within the first hour of evaluation and before starting any antibiotic treatment [41].

Fundamental is the cardiovascular monitoring of patients with shock, who should be rapidly brought to a critical care area to assist, if necessary, the rapid resuscitation and optimal hemodynamic support. Continuous electrocardiographic monitoring and pulse oximetry are tools used in the management of critically ill patients. Monitoring venous oxygen saturation can give important information on the oxygen demand, especially in the early resuscitation phase of the shock therapy [42]. Indeed, a markedly low value of saturation indicates an imbalance in the oxygen supply/demand and likely indicates a need for augmenting global oxygen support.

Depending on the severity of endotoxin-induced shock, routine investigations can include the evaluation of indirect metabolic parameters to evaluate the extent of perfusion impairment and end-organ injury. The use of biomarkers is helpful for the diagnosis process. Among inflammatory biomarkers, there are procalcitonin, lactate, cytokines and chemokines, and C-reactive protein [43]. Lactate is currently the most commonly used metabolic parameter to monitor the effectiveness of resuscitation and cardiovascular support, since it can be indicative of tissue perfusion [42]. However, the other biomarkers are also essential to enhance lactate's effectiveness. Moreover, in a multi-marker panel, combinations of pro- and anti-inflammatory biomarkers may help to identify patients who are at major risk of developing severe shock and multi-organ dysfunction. However, one of the most significant direct parameters to assess the level of risk to develop a septic shock is related to the measurement of endotoxin activity assay. The Endotoxin Activity Assay (EAA) is a

useful test to risk stratify patients with severe sepsis and assess for Gram-negative infection evolution being assessed on a large multicenter study (Medic-study), demonstrating usefulness in flowing-up disease evolution in critically ill patients [44–46].

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Pathophysiology of Endotoxic Shock

2

Marta Pillitteri, Etrusca Brogi, Chiara Piagnani, and Francesco Forfori

2.1 Introduction

Endotoxin, also known as lipopolysaccharide (LPS), is an amphiphilic molecule consisting of a hydrophilic polysaccharide part and a covalently bound hydrophobic lipid component, called lipid A, which is responsible for the toxic effects of LPS. Endotoxin is a constitutive component of the Gram-negative bacterial cell wall, acting as a barrier with the function of protection of the bacterial cell. Thus, LPS assumes an important role in sepsis induced by Gram-negative bacteria and its release causes the activation of the immune system. Whereas Gram-positive bacterial pathogens remain the most common cause of septic shock in intensive care, the knowledge of pathophysiology of endotoxic shock is necessary to understand its clinical manifestations and its role in multiorgan dysfunction for an early diagnosis and treatment.

2.2 Endotoxin Effects and Interaction with Immune System

The first line of immune response to the host against pathogens is represented by the recognition of pathogen structures, called pathogen-associated molecular patterns (PAMPs), by pattern recognition receptors (PRRs) [1]. PRRs are expressed on the surface of immune cells. The linkage between PAMPs and PRRs triggers cellular

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_2.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_2

pathways which induce the production of inflammation mediators. Even more, PRRs can recognize damage-associated molecular patterns (DAMPs), circulating necrotic cell fragments of the pathogens. The recognition of PAMPs and DAMPs triggers leukocyte activation and the inflammatory response.

LPS is recognized as PAMPs, consequently, the recognition of the LPS by PRRs active anti-inflammatory response [2]. Even more, endotoxin is released into the circulation after the disruption of the intact bacteria due to cell lysis, acting as DAMPs [3]. LPS is recognized by Toll Like Receptor 4 (TLR-4), a receptor expressed both on immune cells and non-immune cells [4]. TLR-4 through the recognition of the Lipid A, represents PRRs expressed on the cell surface involved in the innate immune response [5]. Two key molecules are vital for the interaction between LPS and TLR-4: LPS binding protein (LBP), cluster of differentiation 14 (CD14), and the "Toll gatekeeper" called myeloid differentiation 2 (MD-2). CD14 is a LBP and exists both as a soluble and membrane-anchored form. CD14 acts as an endotoxin receptor, and it also supports LPS internalization and detoxification. LBP binds LPS and allows the transferring of LPS to MD2/TRL-4 complexes on cell surface (as shown in Fig. 2.1) [6, 7]. As a consequence, MD2/TLR-4 complex enters through the cell in the endosomal compartment and activates two intracellular pathways: Myeloid differentiation primary response 88 (MyD88)-dependent and MyD88-independent pathway [8] (as shown in Fig. 2.1):

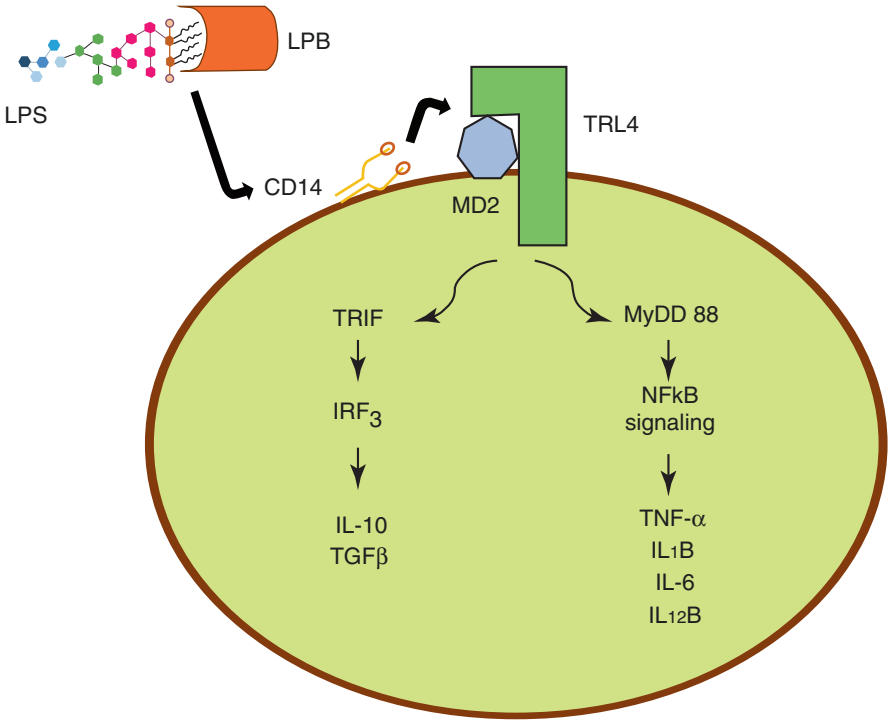

Fig. 2.1 LPS linkage to TLR-4 and intracellular pathways

- MyD88 pathway is a signaling cascade which involves interleukin-receptor-associated kinase (IRAKs) and ends up in the activation of a transcriptional program (i.e., mitogen-activated protein kinases, MAPK, and nuclear factor kappa-light-chain-enhancer of activated B cells, NF-κB). NF-κB induces the transcription of proinflammatory genes with subsequent production of cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL1B), interleukin-6 (IL-6), and interleukin 12B (IL12B) [9, 10].
- MyD88-independent pathway recruits TIR-domain-containing adaptor protein inducing interferon-β (TRIF) which activates interferon regulatory factor 3 (IRF3) [11]. IRF3 is involved in the activation of interferon-β (IFNβ) and induction of transcription of related genes. This cellular pathway regulates the late phase of transcriptional activation, inducing the production of interleukin 10 (IL10), leading to the development of endotoxin tolerance [12].

The consequent release of proinflammatory cytokines and chemokines triggers the classical manifestations of the antimicrobial and anti-inflammatory response (e.g., leukocyte activation and transmigration, increased capillary permeability and endothelial damage).

Sepsis is defined as "a life-threatening organ dysfunction caused by a dysregulated host response to infection" [13]. Consequently, sepsis is characterized by an uncontrolled, maladaptive activation of the inflammatory response and by the inability of the host to confine the inflammation response [14]. The uncontrolled systemic release of proinflammatory mediators was termed as "cytokine storm" [15]. Several bacterial factors as well as host factors are responsible for this excessive inflammatory response and the loss of the homeostasis between pro- and anti-inflammatory response, potentially leading to septic shock and multiple organ failure.

The dysregulated activation of the immune system involves not only the immune cells but also the coagulation and complement system. The cross-talk between inflammatory response, coagulation, and endothelial cells represents a critical aspect to take into account during sepsis. Neutrophils contribute to hyperinflammation through the release of proteases and reactive oxygen species (ROS), and through the production of neutrophil extracellular traps (NETs) [16]. NETs consist of a network of chromatin fibers containing antimicrobial peptides and proteases. The role of NETs is to contribute to the defense by trapping bacteria; however, its excessive production leads to intravascular thrombosis with secondary damage to tissues and organs [17]. NETs can also adhere and activate the endothelium, causing vascular injury by the disruption of endothelial and epithelial cells. Even more, the activation of the complement system, with subsequent release of C3a and C5a, exerts potent proinflammatory activities, such as recruitment of leukocytes, platelets, and activation of endothelial cells, representing a vital component of the innate immune response. The term "immunothrombosis" was introduced to support the concept that also the activation of the coagulation system can be considered as a part of the innate immune response to the host [18]. During sepsis the activation of coagulation becomes unbalanced to the procoagulant status, especially in the microvascular

compartment. An overactivation of coagulation, and consequent consumption of factors, may lead to disseminated intravascular coagulation [DIC] [19].

Endotoxin tolerance and compensatory anti-inflammatory response syndrome (CARS) describe an interest phenomenon characterized by a decreased responsiveness and sensitivity of LPS by the immune system [20]. This mechanism is still poorly understood and may be due to the sepsis-induced epigenetic reprogramming which leads to the immunoparalysis typical of sepsis. It has been seen that chronic exposure to lower concentration of endotoxin causes the generation of endotoxin-tolerant macrophages which produce lower levels of proinflammatory cytokines and higher levels of anti-inflammatory molecules (e.g., reduction of IL-1 and IL-6 release, decreased LPS-stimulated TNF production, and impaired NF- κB translocation) [21]. Even more, endotoxin-tolerant monocytes present an increased phagocytic ability [22]. Not only monocytes and macrophages, but all the other immune cells play a role in the development of tolerance secondary to TLR stimuli [23, 24]. This shift from a proinflammatory to an anti-inflammatory profile may represent a protective mechanism against the over-exuberant inflammation typical of septic shock. However, this pathophysiological adaptation is associated with high risks of secondary infections [25].

2.3 Pathophysiology of Endotoxin Organ Dysfunction and Shock

Endotoxin plays a pivotal role in the genesis of septic shock in Gram-negative bacterial infections (Fig. 2.2). The power of endotoxin to activate and deregulate the immune system may lead to a wide range of clinical manifestations, from singular organ damage to multiorgan failure.

In sepsis, the endothelium is considered to be a full-fledged organ. Endothelial cells (ECs) act as "unconventional" immune cells, and as such they undergo multiple changes which facilitate recruitment of leukocytes and should ultimately favor the elimination of pathogens. The interaction of the endotoxin with the endothelium leads the latter to carry out a reprogramming of its phenotype in a proinflammatory sense, through the production of cytokines, chemokines, procoagulant factors, and the expression of pro-adhesive molecules [26]. These modifications taking place in the endothelium should be aimed at limiting bacterial spread; however, severe or persistent endothelial phenotypic changes can contribute to impaired microcirculatory blood flow and tissue hypoperfusion.

Endothelial permeability is regulated by adherent and tight junctions, which are composed of cytoplasmic and transmembrane proteins, among which cadherins, occludins, and claudins. The cytokine storm that occurs during sepsis leads to the dysfunction of these proteins, thus damaging the glycocalyx and inducing apoptosis of the ECs, ultimately generating vascular hyperpermeability and interstitial edema. It has been demonstrated that LPS induces vascular endothelial (VE)-cadherin disruption through multiple mechanisms such as tyrosine phosphorylation, internalization, endocytosis, and lysosomal degradation; in parallel, there is evidence that

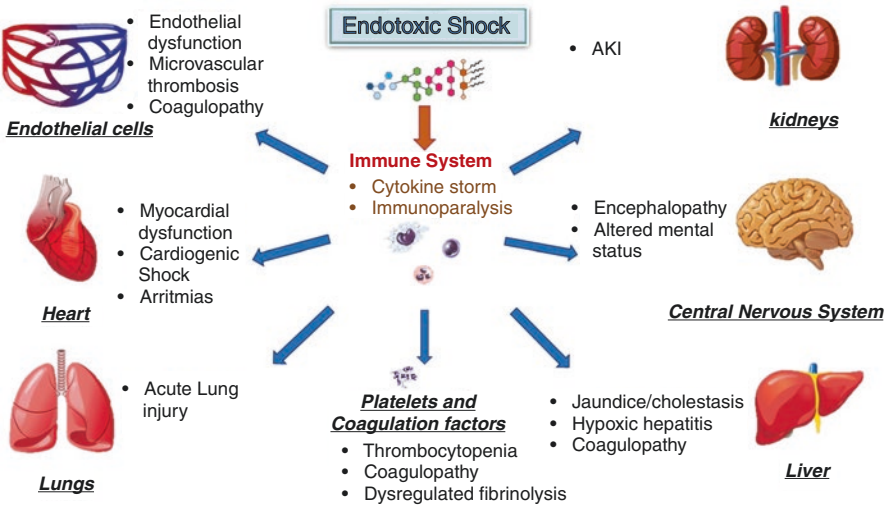

Fig. 2.2 Pathophysiology of endotoxic shock

inhibition of cadherin destruction prevents excessive endothelial permeability [27]. Endothelial integrity also depends on the conditions of its inner coating, the glycocalyx, which also plays a primary role in leukocyte trafficking. LPS and inflammatory mediators, such as TNF-α, reduce glycocalyx thickness, encouraging increasing macromolecules permeability and leukocyte adhesion [28]. Vascular tone is also impaired during endotoxemia [29]. The regulation of vascular tone depends on the balance between vasoconstrictor and vasodilator signals. During sepsis, an important aspect is represented by the vasodilation of the resistance vessels; however, increased stiffness and the compliance of arterial walls are also observed [30]. The impairment of vascular tone in sepsis depends at least in part on the release into the circulation of nitric oxide (NO), endothelin, and prostacyclin. During endotoxemia, inducible NO synthetase (iNOS) is expressed with subsequent production of large amounts of NO. In addition to its vasodilator effect, NO can interact with the superoxide anion forming peroxynitrite, which produces oxidative stress on ECs [31].

Even more, endothelium is also implicated in the homeostasis of coagulation and fibrinolytic pathways [32]. During sepsis, apoptotic ECs express overabundant amounts of tissue factor and, at the same time, TFPI, AT, thrombomodulin, and Activated Protein C (APC) are downregulated, leading overall to the accumulation of thrombin, which amplifies local and disseminated coagulation. The fibrinolytic pathway is also involved, as some of its important counter-regulatory mechanisms are missing. Low levels of APC are responsible for an antifibrinolytic stimulus, since in physiological conditions APC acts as an inhibitor of the plasminogen activator inhibitor Plasminogen activator inhibitor type-1 (PAI-1), activating fibrinolysis which limits an excessive amplification of the coagulation cascade. High plasma levels of PAI-1 in humans correlate with higher mortality [33].

Platelets also contribute to endotoxin-mediated coagulopathy and associated impaired microvascular blood flow. Although thrombocytopenia due to the consumption of thrombocytes and their entrapment in NETs often occurs in the septic patient, platelets contribute at the same time to the amplification of thrombosis and to the worsening of tissue perfusion, since platelets form aggregate with leukocytes and further activate the endothelium that, in turn, is already solicited through multiple pathways [34]. Furthermore, activated platelets promote the secretion of inflammatory mediators by ECs and amplify systemic procoagulant, pro-adherent, and proinflammatory activities. Even more, red blood cells in the septic patient with microvascular damage show a lower negative charge, which results in a greater tendency to form aggregates and with decreased deformability property [35].

In addition, endotoxemia exerts direct cellular effects in several organs (i.e., myocardiocytes, Kupffer cells, renal cells, and pneumocytes). Depression of cardiac activity is a common clinical manifestation during septic shock, and often begins as a reversible cardiogenic shock [36]. Endotoxin acts directly and indirectly on myocardiocytes inducing cellular dysfunction. The presence in the heart of both infiltrating and resident cells expressing TLR-4 makes the tissues of this organ vulnerable to damage induced by endotoxin, given the primary role that this receptor plays in the response to LPS. The damage occurs through the induction of apoptosis or through the activation of immune cells capable of releasing cytokines (IL-6 and TNF-α) which in turn generate and amplify cardiac injury [37]. In fact, several circulating factors during endotoxic shock, such as TNF-α, IL-1β, lysozyme c, endothelin-1, and reactive oxygen species (ROS), seem to have direct inhibitory effect on myocardiocytes. In addition to myocyte dysfunction, myocardial wall edema occurs in the setting of endotoxin shock, causing altered myocardial compliance and elastance.

Acute kidney injury is another common clinical manifestation during endotoxin shock [38]. There is certainly a reduction in blood flow to the kidney leading to organ hypotension, and alterations in the microcirculation; however, the main source of kidney damage appears to be a direct action of endotoxin, which induces the production of proinflammatory cytokines by renal cells. Since renal tubular cells constitutively express TLR-4, LPS stimulates proinflammatory cascades with impaired tubular transport. The dysfunction implies enhanced NaCl delivery to the macula densa and increased tubuloglomerular feedback with a reduction of glomerular filtration rate. Apoptosis of tubular cells also occurs [39].

Lungs are also affected by endotoxin-induced damage. Interaction between LPS and Alveolar Epithelial Cell type I and II (ATI and ATII) enhances apoptosis and activates inflammatory pathways [40]. Endotoxin inactivates the production of surfactant by ATII, causing fluidization of the film, thus impairing its function. Furthermore, the alveolo-capillary membrane is disrupted both by the proinflammatory response and by the generation of microthrombi in lung capillaries: the final result is barrier breakdown with increased endothelial permeability and edema [41]. All these pathological modifications lead to acute lung injury (ALI). LPS is also involved with the generation of fibrosis secondary to ALI; its interaction with

macrophages and fibroblasts increases cytokine storm and fibroblast proliferation and accelerates the development of pulmonary fibrosis [42].

The cytokine storm and endotoxin itself act on cerebral cells as well; however, the complex interactions that exist between the various types of cells in the central nervous system make it difficult to shed light on the exact mechanism by which brain injury in sepsis occurs. Taken together, the brain damage mechanisms that intersect in the septic patient are clinically manifested by a syndrome that takes the name of "sepsis-induced encephalopathy" [43]. Endotoxin can cross the blood–brain barrier (BBB), especially during the systemic inflammation secondary to sepsis, and it can even damage BBB up to its breakdown integrity [44]. Then, endotoxin exerts direct damage on microglial and astroglia cells through TRL-mediated cellular pathways. Apoptosis, autophagy, and oxidative stress also occur during endotoxemia. Moreover, LPS stimulates the production of neuropeptides, proopiomelanocortin, cocaine and amphetamine-related transcript and neuropeptides Y [45].

Finally, endotoxin is able to induce pathological changes even in the main types of cells present in the liver (i.e., hepatocytes—HCs, kupffer cells—KCs, and liver sinusoidal endothelial cells—LSECs) [46]. The increase in IL-6 levels, the main cytokine of liver inflammation, increases the production of acute phase proteins. LPS also causes increased production of other cytokines, such as TNF-α, IL-1β, IL-12, IL-18, ROS, and NO by KCs [47]. IL18 is the major responsible of liver damage, secondary to the secretion of IFN-γ, which results in apoptosis of HCs. Furthermore, one of the main effects of TNF-α is to recruit neutrophils in the liver, which are liable for further damage of hepatocytes. NO production is also involved in injury to HCs and LSECs [48]. LSECs are the main hepatic source of endothelin-1 (ET-1), a strong vasoconstrictor whose production is increased after endotoxin's stimulus. In turn, ET-1 involves the expression of TNF-α, IL-1, and IL-6 and the activation of NF-κB, and induces the synthesis of TNF-α in monocytes and macrophages [49]. ET-1 seems to be an early predictor of poor prognosis in patients with endotoxic shock [50].

2.4 Conclusions

Endotoxic shock is a clinical manifestation caused by the spread in the organism of LPS, a constitutive component of Gram-negative bacterial wall, during bacterial infections. The pathophysiology of endotoxic shock is complex and involves a dysregulated activation of the immune system, which induces a strong systemic release of proinflammatory mediators termed "cytokine storm."

The cellular mechanism implies the linkage between LPS and TRL-4, a receptor expressed by several kinds of cells, which triggers cellular pathways of transcription of genes for the production of inflammation mediators. The systemic inflammation generated after the stimulus of endotoxin can be deleterious both locally and systemically, leading to shock and multiorgan failure.

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Host Resistance to Endotoxic Shock

3

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3.1 Introduction

Endotoxic shock [1] develops from a dysregulated host response to endotoxin, which causes multiorgan dysfunction and may require several organ support therapies [2]. Endotoxin triggers inflammatory activation via the innate immune system that is primarily involved to protect the host from this threat. However, excessive endotoxin exposure may induce dysregulated hyperinflammation, which leads to sepsis, endotoxic shock, and consequent complications. In this context, scarce evidence has investigated the burden of endotoxin-induced immunosuppression, namely "endotoxin tolerance," as a mechanism of host resistance to endotoxic shock.

In this chapter, we will describe the concept of "endotoxin tolerance" and shed light on its pathophysiology, clinical manifestations, and relevance on patient-related outcomes.

3.2 The Concept of Endotoxin Tolerance

Small amount of endotoxin may be retrieved into the bloodstream at a concentration that approximates 3 pg/mL in healthy subjects, and 1–100 pg/mL in patients with chronic diseases, persistent inflammation, and microbiota dysfunction [3]. Endotoxin release into the bloodstream occasionally occurs from organs where

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_3.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_3

Gram-negative bacteria physiologically reside (e.g., the gut) and is uneventful [4]. However, when endotoxin load (>10 ng/mL) exceeds the host capacity of clearance, it triggers immune activation and may induce life-threatening, dysregulated, hyper-inflammatory responses [5]. On the contrary, repeated exposure to this molecule may induce immunosuppression, which is named "endotoxin tolerance." This condition was first reported by *Beason* in 1946 [6], and subsequently confirmed by further experimental models and studies on patients with infection [7]. Specifically, endotoxin tolerance may be considered a specific subgroup of the Compensatory Anti-Inflammatory Response Syndrome (CARS), a clinical condition that develops with the Systemic Inflammatory Response Syndrome (SIRS) after the exposure of the immune system to a specific threat [8]. For this reason, endotoxin tolerance may be considered as an epigenetic modification that occurs in response to endotoxin and leads to changes of gene expression [9]. As long as most of the studies on this topic have been experimental and carried out on monocytes, it remains unknown whether endotoxin tolerance may exert a protective role to prevent uncontrolled inflammatory bursts [10] or favor the development of infectious complications and worse clinical outcomes [8].

3.3 Molecular Pathways of Endotoxin Tolerance

Endotoxin is carried into the bloodstream by the lipopolysaccharide binding protein and activates immune cell response through the interaction with the toll-like receptor 4 (TLR-4), CD14, and MD-2 at the membrane level [11]. The TLR-4 activation by endotoxin induces a specific inflammatory response via (Fig. 3.1):

- the transcription factor NF-kB, via the MyD88 adaptor, that induces the transcription of pro-inflammatory genes like TNFα, IL1β, IL6, and IL12β. This pathway mediates short-term response to endotoxin exposure.
- the transcription factors IRF3 and STAT1, via the TRIF adaptor, that induce the transcription of IFNβ and interferon-inducible genes like CCL5 and CXCL10. This pathway mediates long response to endotoxin exposure.

Although most of the studies described an association between endotoxin tolerance and defects of the MyD88-dependent signaling cascade [12], further evidence identifies the most recently discovered TRIF pathway [13, 14] as the main determinant of this condition. Specifically, endotoxin tolerance appears as a model of gene reprogramming that leads to the inhibition of pro-inflammatory genes and upregulation of antimicrobial genes [15, 16], via histone deacetylation or methylation (silencing) of the former, and acetylation or demethylation (activation) of the latter [17]. As a consequence, this process ends up in inflammatory burst reduction coupled with significant antimicrobial defense increase, which may involve negative regulators of the TLR pathway like IRAK-M, MKP1, FLN29, ST2 [18–21], as well as microRNA [22]. Moreover, some evidence supports the existence of "heterotolerance" or "cross-tolerance to endotoxin," which means that such a condition may

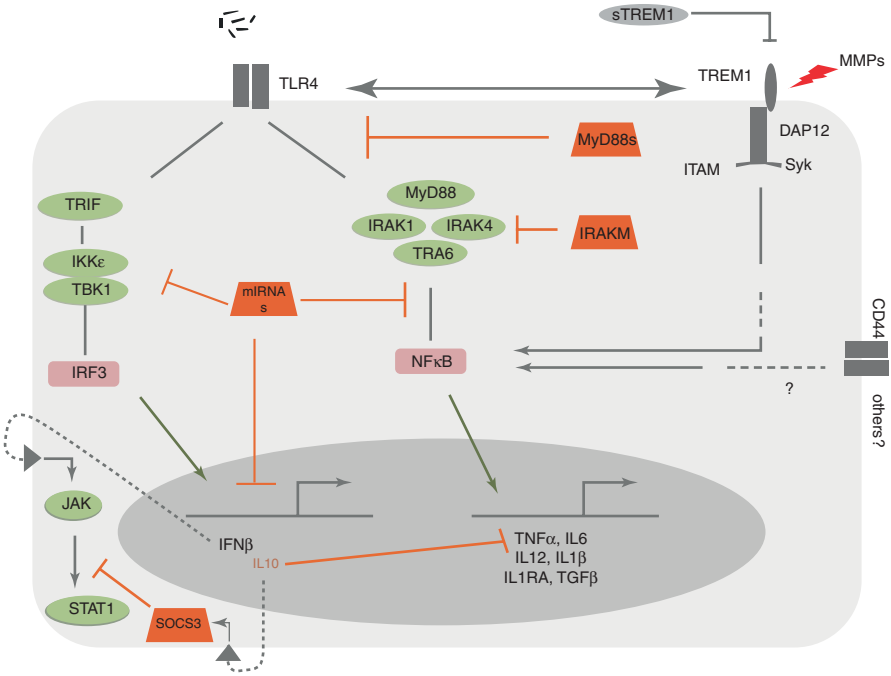

Fig. 3.1 Molecular pathways of endotoxin tolerance. From López-Collazo et al., Pathophysiology of endotoxin tolerance: mechanisms and clinical consequences, Critical Care 2013 with permission

be triggered by the exposure to TLR ligands independent from endotoxin (e.g., Gram-positive bacteria [23], damage-associated molecular pathways (DAMPs) [24], or chronic inflammation [25]). Finally, further research has shown that endotoxin tolerance may be reversed by exposing the endotoxin-tolerant monocytes to molecules (e.g., β-glucan [26]), that induce "trained immunity" in these cells.

3.4 Immune Cell Phenotype Modifications Associated with Endotoxin Tolerance

As compared to monocytes from healthy individuals, endotoxin-tolerant monocytes are characterized by downregulation of genes that codify for inflammatory cytokines (like TNFα, IL-6, IL-1a, IL-1b, and IL-12), chemokines [27–30], and antigen presentation pathways [16, 31], while showing upregulation of anti-inflammatory cytokines (like IL-10, TGFb, and IL-1RA) [28, 31, 32], scavenger receptors and antimicrobial genes [15, 16, 33] (Table 3.1). Moreover, endotoxin tolerance may involve other myeloid cells, like neutrophils and dendritic cells (DC). Specifically, endotoxin-tolerant neutrophils were characterized by reduced TLR4 expression and impaired respiratory burst [34], while endotoxin-tolerant DC showed low production of IL-12, TNFα, and IL-6, but enhanced synthesis of IL-10 and endocytosis

Table 3.1 Phenotypic characteristics of cells associated with endotoxin tolerance

Cells/tissues	Phenotypic characteristics
Monocytes	↓ Immune activation ↑ Immune suppression ↓ Antigen presentation ↑ Scavenger receptors ↑ Antimicrobial activity
Dendritic cells	↓ Immune activation ↑ Immune suppression ↓ Pathogen recognition ↓ Antigen presentation
Endothelium	↓ Low leukocytes adhesion

[35, 36]. On top of that, endotoxin tolerance has been retrieved in non-immune cells, like endothelial cells, that showed low activation and adhesion to leucocytes [37]. Finally, endotoxin tolerance may influence cellular metabolism and is associated with a transition from high-energy glycolysis to low-energy lipolysis [9].

3.5 Clinical Relevance of Endotoxin Tolerance in Sepsis and Non-infectious Diseases

Considering the in vivo relevance of cellular phenotype modifications associated with endotoxin tolerance, poor inflammatory capacity coupled with upregulation of anti-inflammatory cytokines would contribute to protection against endotoxic shock, and increased phagocytosis would allow efficient bacterial clearance. In contrast, impaired antigen presentation would possibly alter the development of an adaptive response and expose the host to the development of infectious complications [17]. Although the clinical impact of endotoxin tolerance as a mechanism of the host to resist endotoxin shock has been strongly advocated and confirmed by experimental research, its impact on patients' clinical outcome remains unknown.

Moreover, endotoxin tolerance may play a role in the pathophysiology of non-infectious disease like cystic fibrosis or acute coronary syndrome. Specifically, experimental research on circulating monocytes from patients with cystic fibrosis demonstrated that these cells are characterized by high phagocytosis ability and poor antigen presentation [16, 38], both resembling a state of endotoxin tolerance. Moreover, similar characteristics were described in patients with myocardial infarction that may represent a state of heterotolerance to endotoxin induced by DAMPs released by myocardial injury [39, 40]. However, the clinical impact of these findings remains unknown and warrants to be clarified in the future.

3.6 Conclusions

Endotoxin tolerance represents a mechanism of paramount importance for host resistance to endotoxic shock. However, the pathophysiology and clinical impact on long-term clinical outcomes of endotoxin tolerance remain unknown. Moreover,

this condition may be triggered by various stimuli and may influence the pathophysiology of infectious and non-infectious diseases. For these reasons, further investigations are warranted on this topic, in order to provide better understanding of this condition and potential therapeutic tools that may improve patient-related clinical outcomes.

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Endotoxin and Organ Cross-Talk

4

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4.1 Introduction

Despite outstanding efforts and advances in knowledge and technological evolution in sepsis and septic shock, these clinical conditions remain major causes of mortality and morbidity in intensive care units (ICUs) worldwide [1, 2]. Understanding the pivotal role played by endotoxins in triggering the complex and downward effects across the organ systems could be the key to solving the puzzle, as they are interconnected with each other. Clinically, we see the net result of multiple life-threatening organ dysfunctions. Sepsis management is intended to revert, support, stabilize, and maintain systemic alterations by administering effective antimicrobials and controlling active infection sources. Today, our response against sepsis has primarily targeted the elimination of likely pathogens and managing the resulting organ damage.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_4.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_4

The emergence of progressively multidrug-resistant pathogens and the gradual drying up of the antibiotic pipeline have led the fight against pathogens to reach critical proportions. Also, organ support interventions and the seeking to mitigate organ damage have limitations and pitfalls. It recalls the Indian proverb "closing the barn door when the horse has already bolted." The pathophysiological pathways of sepsis and the role of endotoxins lie somewhere between these two events. A simple homogeneous algorithm cannot explain the sepsis conundrum. Instead, it is intricate with complex interconnected pathways involving the host's biochemical and physiological processes. The current knowledge of all these mediators and their role in pathogenesis are still evolving.

4.2 Endotoxemia

The sepsis-3 definition defines it as a life-threatening organ dysfunction caused by a dysregulated host response to infection [3]. It points out that infection or simple invasion by an unusual pathogen is not sepsis. Host response, particularly the so-called innate immunity, is triggered whenever there is an infection. On repeated exposure to the same pathogen, adaptive or acquired immunity occurs. However, all these defense mechanisms need to be controlled and regulated. Sepsis occurs when the natural immunologic processes reach disproportionate and dysregulated dimensions. The precise moment at which dysregulation begins is still unknown. The sepsis-related framework incorporates normal physiological processes and feedback loops to fight against infection. The uncontrolled triggering of these processes leads to life-threatening organ dysfunction (Fig. 4.1). The major sepsis triggers are endotoxins or lipopolysaccharides (LPS).

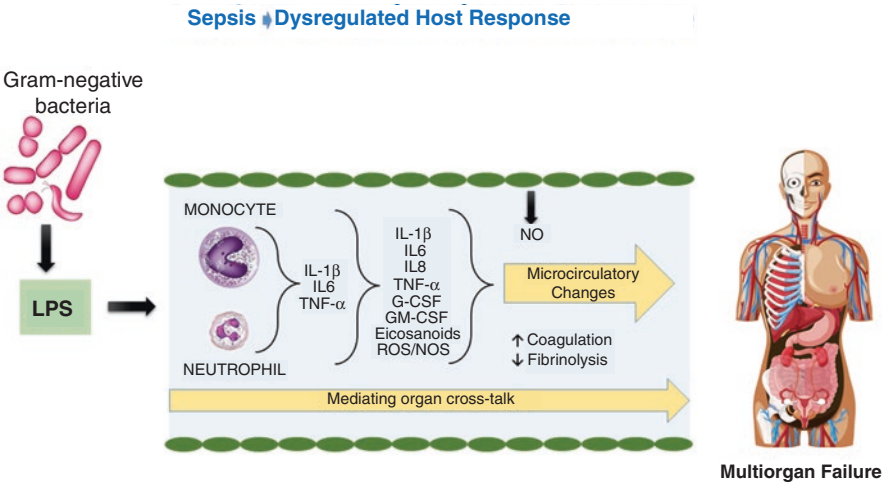

Fig. 4.1 Dysregulation of immune response leads to life-threatening organ dysfunction. *IL-1β* interleukin 1-beta, *IL-6* interleukin 6, *TNF-α* tumor necrosis factor alpha, *IL-8* interleukin 8, *GM-CSF* Granulocyte Macrophage Colony-Stimulating Factor, *ROS* reactive oxygen species, *NOS* Nitric-oxide synthases, *NO* nitric oxide

LPS are a group of glycolipids present in the outer membrane of gram-negative bacteria and have two components, a polar lipid head group (lipid A) and a chain of repeating disaccharides [4]. Most of the biological effects of LPS are reproduced by lipid A [5], although the presence or absence of the repeating oligosaccharide O antigen influences the magnitude of the response [6, 7]. LPS binds to a specific lipopolysaccharide-binding protein (LBP) [8, 9]. The LPS:LBP complex activates the CD14/toll-like receptor (TLR)-4 receptor complex on monocytes, macrophages, and other cells, triggering the production of inflammatory mediators [10–12]. LPS is a crucial mediator of sepsis in response to gram-negative bacteria. Systemic administration of LPS was one of the earliest approaches used to model the consequences of bacterial sepsis. A significant breakthrough in sepsis literature has been recognizing the interaction between LPS and gram-negative bacteria with cellular receptors like the CD14/TLR4/MD2 complex. Both immune activation [13] and immune suppression [14] play a central role in sepsis. Interestingly, mechanisms other than the immunologic also contribute to sepsis pathophysiology, such as endothelial activation, coagulopathy, and altered glucose and protein metabolism [15].

Endotoxins or LPS are an integral component of the outer membrane of gram-negative bacteria [16]. In the host, they act as pattern recognition molecules (pathogen-associated molecular patterns, PAMPs) by activating the innate immune system response at the initial stages [17]. The dual effect of endotoxin enables it to act both as an "alarm molecule," warning the host of bacterial invasion within the internal milieu, and a "trigger molecule" of the pro- and anti-inflammatory cascades to mount an effective counterbalance for antimicrobial elimination. For unknown reasons, a dysregulated host response develops in some cases that may culminate in multiple organ dysfunction and death [18]. The overall direct or indirect impact of endotoxemia manifests in organ systems like the lungs, heart, liver, kidney, gut, brain, and immune system (Fig. 4.2, Table 4.1).

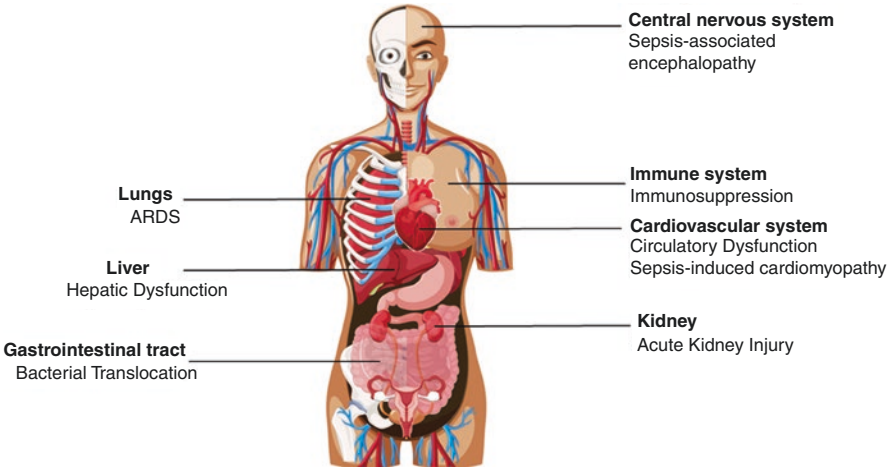

Fig. 4.2 Clinical consequences of endotoxin-induced organ damage. *ARDS* Acute respiratory distress syndrome

Table 4.1 Specific mechanisms and characteristics of endotoxin-induced organ dysfunction. *IL-1* interleukin 1, *IL-10* interleukin 10, *TNF-α* Tumor necrosis factor alpha, *DAMPs* damage-associated molecular patterns, *PRRs* pattern recognition molecules, *PAMPs* pathogen-associated molecular patterns, *TLR-4* Toll-like receptor 4, *IRAK-M* Interleukin-1 receptor-associated kinase, *TREM-1* Triggering receptor expressed on myeloid cells 1, *AKI* acute kidney injury; *ARDS* Acute respiratory distress syndrome

Endotoxin-induced organ damage	Mechanisms and characteristics
Sepsis-induced cardiomyopathy Circulatory dysfunction	• Biventricular dysfunction – Myocardial depressant factors (e.g., IL-1, TNF-α) • Vasodilation • Increased vascular permeability • Microcirculatory dysfunction
Sepsis-induced AKI	• Tubular injury and vacuolization – DAMPSs/PAMPS induced TLR-4-dependent pathways – Abnormal peritubular and glomerular microvascular flow • Oxidative stress of epithelial cells in tubular segments S1 and S3 • Renal perfusion low, normal or high
Sepsis-associated ARDS	• Endothelial barrier dysfunction – Increased vascular permeability – Damage of the extracellular matrix structure • Stimulation of alveolar type II cells and macrophages • Increased dead space and shunt in patchy areas
Sepsis-associated encephalopathy	• Acute or long-term cognitive impairment • Regional-specific lesions – Neurotransmitter dysfunction – Inflammation and ischemia of the neural tissue: Neurotoxicity – Blood–brain barrier dysfunction – Metabolic failure of brain tissue – Vascular abnormalities – Alterations in cerebral perfusion • Microglial dysfunction – Microglial activation – Deramification – Release of inflammatory and anti-inflammatory cytokines • Possible role of brain microbiota
Sepsis-induced immunosuppression	• Innate immunity dysfunction – Cellular apoptosis – Phagocytosis • Endotoxin tolerance – Gene reprogramming of monocytes and macrophages – Increased IL-10, IL-1, and IRAK-M activity – Decreased expression of TREM-1 – microRNAs

4.3 Sepsis-Induced Cardiomyopathy

Cardiac function abnormalities can occur in sepsis due to various reasons. Although the term "sepsis-induced cardiomyopathy (SIC)" usually refers to sepsis-induced left ventricular (LV) systolic dysfunction, impairment of both ventricles may be

present since the ventricles show considerable interdependence. The depressed LV contractility is independent of afterload variability [19].

Cardiac function undergoes different transitions throughout each sepsis stage. In the first stage, a low flow state develops due to relative hypovolemia (maldistribution of blood volume), absolute hypovolemia, and diminished filling pressures. The resulting volume loading increases cardiac output during resuscitation and augments organ perfusion. In the second phase, a hyperdynamic state appears, characterized by high cardiac output and low systemic vascular resistance. In most cases, minimal myocardial depression develops during these two phases. In the last stage, cardiac dysfunction becomes evident. Consequently, many patients cannot maintain adequate cardiac output, leading to progressive metabolic acidosis, multiorgan failure, and death [20].

The abovementioned model could be too simplistic. Parker et al. described that these phases overlap [21]. In their study, the authors found that more than half of patients had LV systolic dysfunction within 24 h of the onset of septic shock. In a previous study, Suffredini et al. found a significant decrease in the pulmonary capillary wedge pressure and LV end-diastolic volume index ratio after endotoxin injection in healthy volunteers. Also, they observed a moderate increase in LV end-diastolic volume after volume loading and a lower pulmonary capillary wedge pressure than the control group [22]. In a large series of 262 patients with severe sepsis or septic shock, Landesberg et al. showed the velocity of the mitral annulus in diastole (e' wave) was lower than 8 cm/s, suggestive of LV diastolic dysfunction [23]. In another study, Bouhemad et al. found that LV diastolic dysfunction incidence was around 40%, irrespective of LV systolic dysfunction [24].

Thermodilution and transesophageal echocardiography have allowed the early recognition of sepsis-induced right ventricle (RV) systolic dysfunction, alone or in association with LV dysfunction [25, 26].

In sepsis and septic shock, the release of multiple mediators is present. Myocardial depressant factors increase in the circulation, such as IL-1, TNF-α, and endotoxins, resulting in vasodilation, decreased LV ejection fraction, LV dilatation, vasoconstriction, and abnormal ventricular compliance. In addition, leukocyte aggregation increases, causing microembolization and vascular endothelial cell dysfunction, depending upon the balance of counteractive actions [27]. The net result is heterogeneous among patients. Danner et al. described that endotoxin plays a crucial role in affecting myocardial function [28]. Hobai et al. showed that prolonged exposure of adult rat ventricular myocytes to a mixture of LPS and inflammatory cytokines inhibits cell contractility. The effect is mediated by the inhibition of Ca2+ influx via L-type calcium channels and partially opposed by inhibiting Na+/Ca2+ exchange [29].

Sepsis-induced cardiomyopathy remains a serious condition related to sepsis, and its nature and pathophysiology are incompletely understood. Bacterial LPS and its interplay with endotoxin recognition molecules, such as TLR and MDs, have a central role in the complex mechanisms involved.

4.4 Sepsis and AKI

Sepsis is one of the leading causes of acute kidney injury (AKI) [30]. The classic hypothesis linking sepsis with AKI is the presence of diminished renal perfusion and subsequent renal parenchymal ischemia. However, a tiny proportion of post-cardiac arrest patients, a natural model of "warm ischemia," develop AKI [31]. Recently, it has been elucidated that sepsis-induced AKI can develop in the absence of signs of global renal hypoperfusion or "warm ischemia." Sepsis-induced AKI can occur in the presence of normal or even augmented renal blood flow [32]. AKI can occur in sepsis patients who are not considered high-risk and present without shock or hemodynamic instability. In vitro studies using cell cultures have shown that the cardinal features of sepsis-induced AKI can be reproduced in human epithelial tubular cells by exposing them to plasma from septic patients [33].

Sepsis-induced AKI manifests as a dramatic decline in glomerular filtration rate (GFR) and variable tubular dysfunction. Histologically, it is characterized by the presence of non-specific, patchy areas of tubular cell vacuolization and a remarkable absence of apoptosis or necrosis [34]. The cause is likely multifactorial rather than the result of an individual insult. Several concurrent mechanisms may be involved. These mechanisms include inflammation, a profound, heterogeneous distortion of microvascular flow at the peritubular and glomerular levels, stimulation of mitochondrial quality control processes, and cell cycle arrest [35].

During sepsis, circulating damage-associated molecular patterns (DAMPs) and PAMPs act as signal molecules for the immune system, alerting and activating a host response to infection. Epithelial and parenchymal cells, through their Pattern Recognition Receptors (PRR), like TLR, NOD-like receptors, and Retinoic acid-inducible gene I (RIG-I)-like receptors, can also recognize DAMPs and PAMPs [36]. About 21% of the cardiac output passes through the kidneys, and approximately 120–150 ml of plasma is filtered into renal tubules per minute. This process places the kidneys on the front line to be exposed to such mediators. DAMPs and PAMPs emerge elsewhere in extrarenal tissues and access the renal tubules by glomerular filtration or by proximity to the peritubular capillaries [35, 37]. Sepsis induces renal-wide expression of otherwise constitutively expressed TLR-4 [38], and DAMPs/PAMPs are actively recognized by tubular epithelial cells through TLR-4- and TLR-2-dependent pathways [39]. Although all nephrons in the kidney could be potentially exposed to these mediators, only patches of tubular cells show signs of distress due to these "danger signals" [40]. The patchy appearance could be attributed partially to the heterogeneous flow distribution due to regional microvascular dysfunction. Kalackeche et al. have shown that TLR-4-dependent LPS recognition in the tubular epithelial cells occurs in the S1 segment of the proximal tubule. The assembly of LPS with TLR-4 in the tubular epithelial cell produces internalization of LPS through fluid-filled endocytosis and triggers an organized oxidative outbreak in epithelial cells of the adjacent tubular segments (S2 and S3) though not in the S1 segment [39]. Endotoxin can be filtered through nephrons and internalized

by S1 proximal tubules through a TLR-4-dependent mechanism. The interaction between endotoxin and S1 can result in oxidative stress and injury in downstream tubular segments [41].

Sepsis-induced AKI pathophysiology remains not fully understood. In conjunction with microvascular dysfunction and regional or global renal hypoperfusion, endotoxin-mediated tubular injury can explain some of the observed pathophysiological changes. Tubulo-glomerular feedback and other unknown mechanisms may also have a role in sepsis-induced AKI.

4.5 Acute Respiratory Distress Syndrome (ARDS)

Pulmonary endothelial barrier dysfunction in patients with sepsis is an early outstanding event, resulting in acute respiratory distress syndrome (ARDS). LPS activity leads to cellular deformation and endothelial or epithelial cell gap formation resulting in endothelial and epithelial increased permeability. Activation of TLR on alveolar type II cells and resident macrophages induces the secretion of chemokines. Fluid leaks through and accumulates in the interstitium and inside the damaged alveolus. Consequently, the hallmark of sepsis-induced ARDS is increased dead space and shunt in patchy areas of the lungs in a heterogeneous manner [42]. The clinical effects are hypoxemia and hypercapnia. Inflammatory exudates occupy alveolar spaces with loss of lung volume. Pro-inflammatory cytokines are released, which causes increased neutrophilic inflammation and favors endothelial barrier dysfunction and increased vascular permeability. Therefore, endotoxin damage-mediated pathways foster other pro-inflammatory pathways and cause more inflammation in a vicious cycle [43]. LPS induces changes in the extracellular matrix structure in the lung tissue, increasing vascular permeability and causing pulmonary edema [44]. Bowler et al. showed that LPS given intrapulmonary to 10 healthy volunteers caused profound changes in protein expression, indicating the LPS-induced lung injury is multipronged and the loss of integrity of the epithelial barrier represents one of the main contributors [45].

The current understanding of LPS-induced lung damage remains limited. The whole process involves a nonhomogeneous pattern of events and a complex interaction of different molecules and interdependent pathways.

4.6 Sepsis-Associated Encephalopathy (SAE)

Sepsis and critical illness can lead to acute or long-term neurocognitive impairment. Sepsis-associated encephalopathy (SAE) [46] has a clinical spectrum ranging from delirium to coma. Delirium, an acute form of SAE, is an independent predictor of mortality, duration of mechanical ventilation, ICU length of stay, and short- and long-term cognitive impairment in ICU survivors [47]. SAE is a multifactorial process leading to diffuse cerebral dysfunction caused by a systemic inflammatory response without evidence of central nervous system infection. Though often

underdiagnosed, SAE is a significant manifestation of sepsis that can occur in up to 70% of patients admitted to the ICU. Alteration of mental status is a characteristic feature of sepsis [48]. Most patients with SAE have prolonged ICU stays and poor cognitive and functional outcomes [49, 50].

The pathophysiology of SAE is complex, and it may involve neurotransmitter dysfunction, inflammatory and ischemic lesions to the brain, microglial activation, and blood–brain barrier dysfunction [51]. Animal and human models have demonstrated that neuroinflammation, vascular changes, and metabolic failure can cause neural tissue damage [46]. These mechanisms are nonhomogeneous throughout the brain and may lead to regional-specific lesions. Brain mechanisms regulating autonomic, arousal, awareness, and behavioral functions are severely affected. The spectrum of SAE ranges from delirium to coma [52].

Microglial cells are the primary innate immune phagocytic cells residing in the brain. They exhibit various surface receptors interacting with the peripheral immune system through cytokine binding and DAMPs and PAMPs sensing [53]. Once these brain scavenger cells are stimulated through circulating endotoxins or TLR pathways, they undergo morphological, immunological, and metabolic changes. Morphological changes are characterized by a retracting of microglial processes, or deramification, by which microglial cells transform into amoeboid phagocytes. Immunological changes during microglial activation include the release of pro-inflammatory cytokines (e.g., interferon γ, TNF-α), and anti-inflammatory or immunomodulatory cytokines (e.g., IL-4, IL-10). Pro-inflammatory phenotypes are associated with neurotoxicity, whereas anti-inflammatory phenotypes could be neuroprotective [53]. The neuronal activation and dysfunction result from microglial activation through different pathways triggered by endotoxins.

Animal [54, 55] and human [56, 57] studies have shown non-infectious microglial activation. However, the recent finding of bacterial genomic material and living bacteria in non-encephalitic animals and humans has challenged this concept of non-infectious activation of microglia [58]. While brain microbiota is absent in healthy subjects, a different situation could occur in critically ill patients [59]. SAE is a major complication of sepsis, and its pathophysiology is complex and multifactorial. Some mechanisms involved include ischemia, autoregulation disturbance, inadequate cerebral perfusion, inflammatory mediators and immunomodulators, blood–brain barrier breakdown, and the release of neurotransmitters or neurotoxic substances. Endotoxin plays an important role in the whole process.

4.7 Immunosuppression

Sepsis involves a complex interplay of different pro- and anti-inflammatory events. The counterbalance of both mechanisms often determines the outcome and clinical manifestations. Initially, the host response is hyperinflammatory, which gradually evolves over several days into a more prolonged immunosuppressive phase [34, 60, 61]. However, recent studies have shown that pro- and anti-inflammatory responses occur early in sepsis, though the initial clinical manifestation reflects an early

dominant hyperinflammatory stage characterized by shock, fever, and hyper-metabolism [62–64].

In 1991, alterations in cytokine response (e.g., low production of tumor necrosis factor (TNF), IL-1, and IL-6) upon ex vivo challenge with LPS were observed in the blood of sepsis patients, suggesting alterations in the innate immunity [65]. In 1997, the concept of compensatory anti-inflammatory response syndrome (CARS) was first introduced, which hypothesized an anti-inflammatory or compensatory immune response in sepsis. Subsequently, the beneficial immune-stimulatory effects of IFNγ treatment in sepsis were reported [66]. In the early 2000s, the physiological response to injury, which initially encompasses a pro-inflammatory phase, was associated with a compensatory anti-inflammatory response leading to an immunosuppressive state [62]. The mechanisms involved are variable. Cellular apoptosis is one of the most well-known contributing factors to sepsis-induced immunosuppression [67, 68]. Long-term exposure to LPS or injection of sublethal doses of LPS in animals can induce a state of tolerance that reprograms the inflammatory response. This state is characterized by a reduced inflammatory cytokine production in vitro and in vivo after endotoxin challenge or other inflammatory stimuli [69]. In vitro, most myeloid cells lose their ability to respond to LPS following an initial challenge. This phenomenon has been called LPS or endotoxin tolerance (ET), an important biological phenomenon and a primary mechanism for immunosuppression in sepsis [70]. ET is a protective mechanism in which reprogramming of the inflammatory response due to high exposure to endotoxin leads to a transient state in which cells cannot respond to an endotoxin challenge. This process promotes healing and recovery of homeostasis while promoting susceptibility to secondary infections, increasing mortality. Central mechanisms of endotoxin tolerance are anti-inflammatory, such as IL-10 expression, increased interleukin-1 receptor-associated kinase M (IRAK-M) activity, low expression of TREM-1, and microRNAs which regulate gene expressions [71]. Based on the evidence discussed herein, ET represents an adaptive response of the innate immune system that protects against exaggerated inflammation. Accordingly, ET involves extensive gene reprogramming that supports the functional polarization of monocytes and macrophages, modulating the inflammatory response, promoting phagocytosis, tissue repair, and immunoregulatory functions. Besides, ET is also involved in different pathological conditions as a mechanism for immunosuppression. Such conditions often contribute to immune evasion, increased susceptibility to secondary infections, and even mortality.

4.8 Conclusions

Sepsis results from a heterogeneous interaction of molecules and pathways that negatively affect the organ systems. The role of endotoxin in triggering these events is well-known. However, further understanding of the genuinely interconnected pathways and organ-specific mechanisms will help elucidate novel interventions in earlier phases to regulate those complex networks of events leading to organ dysfunction. Basic research on sepsis pathophysiology should focus on elucidating

other endotoxin-driven mechanisms that lead to irreversible events causing organ dysfunction and whether organ-specific mechanisms promote organ damage elsewhere. Endotoxin tolerance comprises an exciting field that could be the key to solving the puzzle of late complications in sepsis.

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Endotoxin Measurement in Septic Shock

5

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5.1 Introduction

Systemic Gram-negative sepsis remains one of the most severe complications of hospitalized patients, principally in critical ill subjects. Despite important developments in critical care research and in molecular biology, the morbidity and mortality related sepsis is high [1]. Lipopolysaccharide (LPS), mostly used synonymously with endotoxin, plays an important role in the pathogenesis of sepsis, since 1800s, when it was first revealed as a Gram-negative cell wall toxin implicating in lethal shock [2]. Small quantities of LPS may stimulate the immune system inducing the strong activation against infection. On the contrary, the high amount of LPS into the blood may cause lethal septic shock syndrome. Particularly, LPS may trigger cellular biosynthesis, activate intracellular mechanisms of apoptosis, induce activation of inflammatory pathways with the consequent release of pro-inflammatory cytokine, such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-18 (IL-18), and other bioactive metabolites producing organ damage and septic shock. Different forms of LPS are produced by distinctive and specific species of bacteria: every type of LPS is characterized by a different toxicity.

Outer membrane of Gram-negative bacteria is characterized by an asymmetric structure: the inner cytoplasmic membrane wall consists of phospholipids, on the contrary, the outer leaflet contains a big amount of LPS. For the 75%, the outer

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_5.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_5

surface of Gram-negative bacteria consists of specific LPS. The space that separates the outer membrane from the inner membrane is defined as periplasmic space. LPS is a macromolecular glycolipid (10–20 kDa) and consists of three distinct domains that differ genetically, structurally, and antigenically: a hydrophobic membrane anchor called lipid A; a short chain of sugar residues with multiple phosphoryl substituents defined the core oligosaccharide; a serospecific polymer composed of oligosaccharide repeat-units called the O-antigen [3, 4].

The lipid A, highly conserved among the different species, consists of a phosphorylated *N*-acetyl glucosamine dimer with six to seven fatty acids attached and saturated. In general, some fatty acids are attached to *N*-acetyl glucosamine dimer while others are esterified to the three fatty acids. Lipid A chain has enormous architectural diversity when seen in different bacterial species. Variations can be in terms of the number and length of acyl chains, in addition, there may be other groups substituting at the positions of phosphate moieties. Lipid A is the bioactive domain of LPS: it provokes toxic effects and it is responsible for the myriad of in vivo and in vitro actions. In fact, lipid A has an important role in the stimulation of the innate immune system induced by Gram-negative bacteria and endotoxin [3, 5].

The oligosaccharide component is very important in the LPS structure. This is composed by two different portions: a hydrophilic polysaccharide chain associated with its immunogenicity, and the O-antigenic a periodic repeating hydrophilic oligosaccharide unit (linear or branched). The O-repeating component is extremely variable immunochemically determining an enormous quantity of O-specific serotypes. The O-antigen increases bacteria intracellular survival in some bacteria, protects from oxidative stress in others, prevents the internalization in host epithelial cells and contributes to bacteria motility in other species. In all cases, the immunogenicity of the O-antigen polysaccharide evokes a strong immunity response intermediating by specific antibody [3].

LPS could be present in two different forms: (1) “rough” including only lipid A and core subunits; (2) “smooth” including all units (LPS capped with O-antigen). For its chemical and structure features, LPS resulted to have a very good heat stability and a worthy resistance to oxidative stress and oxidative molecules. LPS has to be moved from the internal of the bacterial cell where it is produced to the outer membrane and the bacterial surface. This mechanism involves a specific transport pathway including the presence of a protein complex involving seven different proteins. This complex of protein is a bridge to help LPS to cross the periplasmic space and to arrive in the outer membrane. Specifically, a beta-barrel membrane protein allows the transport of LPS to the leaflet of the outer membrane. Finally, bacterial wall shedding and bacterial lysis permit the release of LPS in the host blood circle. Endotoxin binds to the host receptor Toll-like receptor 4 (TLR4) which is featured by a large, leucine-rich extracellular domain, a single transmembrane segment, and a short cytoplasmic tail. TLR4 is present on the surface of various cells, including neutrophils, monocytes, and macrophages. TLR4 establishes a heterodimer with co-receptor MD-2 and together participates in a common pattern for LPS recognition. A large amount of LPS induces inappropriately activation of the immune

system triggering an inflammatory response and extensive organ injury (for example sepsis) [4, 6].

In this context, identification, determination, quantification, and monitoring of LPS from various bacteria are very important and they are performed by LPS receptors as well as accessory proteins.

In the category of accessory protein, CD14 (cluster of differentiation 14) has a prominent role (CD14 binds LPS in the presence of soluble lipopolysaccharide-binding protein—LBP) and it is often used for the indirect detection of LPS.

5.2 Endotoxin Detection

Given the importance of determining endotoxins, in recent years a lot of research has been carried out to identify methods and devices for analyzing them. Currently, there are simple, rapid, highly sensitive and specific tests for the detection of endotoxins commercially available.

Rabbit Pyrogen Test The first method approved by US Food and Drug Administration was based on injecting the test solution into a rabbit and then measuring the rabbit's temperature [7]. Obviously, it was expensive and time period.

LAL (Limulus Amebocyte Lysate) Test One of the most used test due to its easiness. When exposed to LPS, the Amoebocytes from horseshoe crabs blood can form a clot after a protease cascade. Specifically, the protease cascade activating the proteolytic conversion of coagulogen to coagulin is induced by LPS [8, 9]. Practically, after incubation with the sample to analyze: in the presence of LPS, gelation occurs; in the absence of LPS, gelation does not occur.

Various commercial LPS detection kits are based on LAL gel clot assay, in which a piece of LAL gel will form a clot after the exposure to a certain amount of LPS.

During the years and through innovations, some novel and innovative methods, such as chromogenic [10], turbidimetric [11], or viscometric [12], have been introduced to enhance the LAL test. LAL is easy to use and cheap test, unfortunately, several factors may have an effect on the sensitivity of the assay: for example, some factors such as β-(1,3)-D-glucan, typical of fungi, algae, and yeast, may affect the results of the LAL test interfering with coagulation cascade [13].

Biosensors In recent years, many efforts have been made to find reliable methods to detect the level of endotoxins based on endotoxin-affinity components. In particular, some sensors have been developed with good results for detecting LPS.

A biosensor consists of two main components: a biological recognition element and a signal element. The former is used to identify a target molecule and the latter is used to translate the biological recognition into physically measurable signs.

Biosensors are described as devices that detect the presence of a target analyte [7]. Biosensors are user-friendly, rapid, and highly sensitive, and highly selective for specific molecules. Biosensors produce a measurable signal proportional to the

concentration of the target element and its variations. Usually, biosensors are based on the biological interactions between the sensing element and the target. The target element must possess some characteristics that influence the selectivity of the biosensor. There are many types of biosensor and are categorized in electrochemical sensors [7], magnetoelastic sensors [14], and quartz crystal microbalance-based sensors [3]. All these types have been utilized for the detection of biological marks.

Biosensors contribute significantly to advances in next-generation medicines. In particular, in recent years, different types of protein-based biosensors, peptide-based biosensors, and synthetic substrates with affinity to LPS have been developed to detect LPS by electrochemical or optical biosensors.

These biosensors, although useful in identifying LPS and its concentration, may also cross-bind to other molecules causing signal loss. Furthermore, these types of biosensors have high cost and are characterized by detection methods [7].

Currently, antibody-based biosensors are revolutionized diagnostics tools in this context. In fact, antibody-based biosensors offer sensitive and rapid analytical methods for the recognition of a vast array of pathogens and their associated toxins. Antibody-based biosensors are characterized by high specificity and affinity between antibody and target element [15]. For LPS detection, antibody-based biosensors are better than protein-based biosensors. In particular, LPS levels can be measured by chemiluminescence technology. Unfortunately, these types of biosensors are still expensive and time consuming [7].

Aptamers Aptamers are an alternative to antibodies in their role as biorecognition elements in analytical devices. Aptamers are ligands that form three-dimensional structures and bind to a target by molecular complementarity, electrostatic interactions, or hydrogen bonds [7, 16]. Aptamers are very good recognition molecules of biosensors owing to their exclusive features such as small size, high stability, high binding affinity and specificity, and simplicity of modification. In contrast to antibodies-based or proteins-based or enzymes-based biosensors, aptamer-based biosensors are more chemically stable with their target and can be modified and synthesized easily [17, 18]. In particular, aptamers with electrochemical sensors have been created to recognize LPS sensitively and selectively [3]. Several commercial techniques have been developed for monitoring LPS rapidly and easily, but they are often expensive methods. A combination of several techniques has been employed to analyze and detect LPS in biological samples. For example, LPS concentration was determined by reversed-phase High Performance Liquid Chromatography (HPLC) and quantified by mass spectrometry (MS)/MS combined with the LAL Test [19]. This method is not an easy and cheap technology to analyze LPS but it allows obtaining excellent results.

Endotoxin Activity Assay An excellent technique to measure LPS in a few minutes is Endotoxin Activity Assay (EAA), a rapid test for the detection of endotoxemia in whole blood. EAA is a quick and easy diagnostic test based on monoclonal antibodies that recognize endotoxin. In this method, LPS activity is measured as the corresponding oxidative burst of primed neutrophils by complexes of an anti-

endotoxin antibody and endotoxin as detected by chemiluminescence [20]. This method permits good results for the correct amount of endotoxin present in the patient's whole blood.

Certainly, emerging data and the search for new innovative methods are expected to open up new diagnostic options for LPS evaluation. This aim will be the focus of the next few years. In the meantime, the use of these techniques alone or combined together can help clinicians in the analysis and determination of LPS levels in patients and to identify appropriate therapies to lower these levels.

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Clinical Management of Endotoxemia: Antibiotics

6

Salvatore Lucio Cutuli, Veronica Gennenzi, Joel Vargas, and Gennaro De Pascale

6.1 Introduction

Endotoxemia is commonly caused by infections sustained by Gram-negative bacteria [1] that represent the most common pathogens isolated from critically ill patients with suspected infection [2]. In this setting, adequate antimicrobial therapy is pivotal to reduce pathogen load, in order to mitigate inflammatory dysfunction and tissue damage, with significant benefit on patient outcomes [3]. However, this intervention is challenged by concurrent patient and pathogen characteristics that may limit its efficacy. In this chapter, we will discuss the importance of adequate antibiotic therapy in patients with sepsis and provide an overview of the most recent evidence on antimicrobial therapy in patients with Gram-negative infection.

6.2 Timing and Adequacy of Antibiotic Therapy in Septic Shock

The early administration of adequate antimicrobial therapy was demonstrated to effectively improve the outcome of patients with sepsis [4–6] and several studies reported a direct association between timing of adequate antimicrobial administration and mortality [6–8]. For these reasons, the Surviving Sepsis Guideline for

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_6.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_6

management of sepsis and septic shock 2021 [3] issued a strong recommendation to administer antimicrobials within 1 h of septic shock recognition, and within 3 h in patients with high likelihood of sepsis. On the contrary, antimicrobials administration should be deferred in patients with a low likelihood of sepsis or septic shock, in order to prevent potential harms like allergic or hypersensitivity reactions, kidney injury, thrombocytopenia, Clostridium difficile infection, and antimicrobial resistance [3]. Accordingly, a stewardship program for antimicrobial administration [9, 10] has been strongly advocated and should account for the epidemiology of pathogens, the suspected source of infection, and the characteristics of the patients. Moreover, the pharmacokinetic (PK) characteristics of the drug and the spectrum of sensitivity to antimicrobials of the pathogen (pharmacodynamic, PD) should be considered, in order to optimize this therapy and allow prompt de-escalation [11]. However, positive microbiological cultures may be retrieved only in 65% of critically ill patients with suspected infection, for whom Gram-negative bacteria are prevalent [2]. In this setting, rapid molecular tests have raised interest to provide pathogen identification, in order to shorten adequate antimicrobial administration [12].

6.3 PK-PD Principles to Optimize Antimicrobic Treatment

The optimization of antimicrobial therapy is a key treatment intervention in the management of sepsis, both to maximize therapeutic success and limit the emergence of resistant pathogens [13, 14]. Specifically, critically ill patients with severe infections are at high risk of suboptimal antimicrobial dosing, mostly due to homeostatic changes associated with sepsis [organ dysfunction, increased volume of distribution due to endothelial permeability, fluid overload, and hypoalbuminemia], therapeutic interventions (e.g., extracorporeal organ support therapies), and comorbidities [15–18]. These conditions may influence PK characteristics of antimicrobials and challenge conventional drug dosing [13] (Fig. 6.1). Moreover, antibiotic dose should be targeted to PD, in order to overcome the in vitro minimum concentration of antimicrobic to inhibit (MIC) pathogen growth. Accordingly, antimicrobic dose optimization in critically ill patients is difficult to achieve and requires a personalized approach. In this setting, several strategies have been suggested like unit-level interventions (e.g., prolonged infusions), nomograms based on renal function or body weight and therapeutic drug monitoring (TDM) [15, 19, 20]. The latter involves the measurement of drug concentration at the tissue level (usually, the bloodstream or bronchial secretions) and may help to adjust antimicrobial dosing to overcome the MIC of the pathogen, mitigate the emergence of resistance and limit toxicity [19]. TDM is recommended for many antimicrobials used to treat Gram-negative infections, such as beta-lactams and aminoglycosides, whereas there are not specific recommendations for other classes of antimicrobial like polymyxins and fluoroquinolones [21, 22].

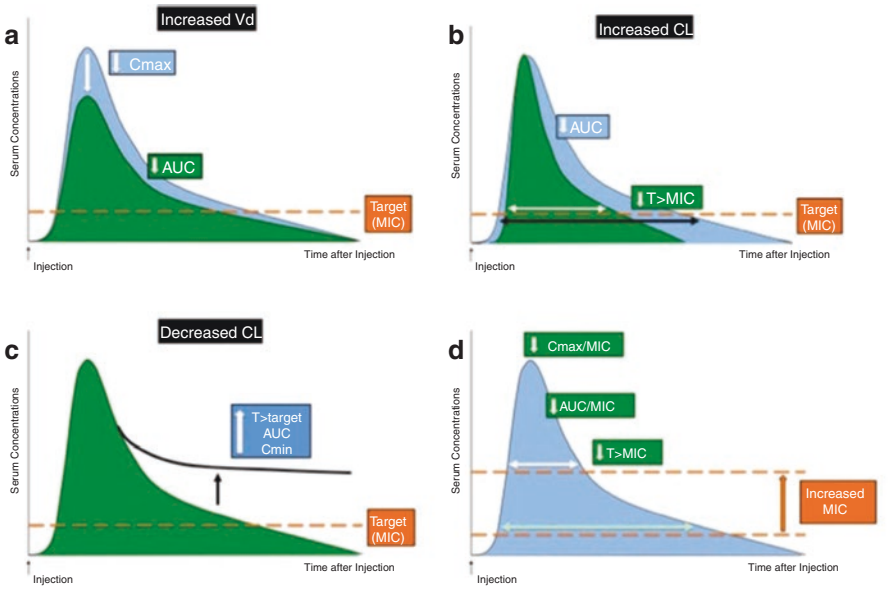

Fig. 6.1 PK/PD changes for antibiotics in critically ill patients. (**a**) Increased volume of distribution (*Vd*) will decrease the peak concentration (*C*max; relevant for drugs like aminoglycosides) and the area under the curve of drug concentrations over time (AUC; relevant for drugs like quinolones) of the drug in the first dosing interval. (**b**) Increased drug clearance (CL) will reduce the AUC and the time above the minimum inhibitory concentration (T>MIC, relevant for drugs like beta-lactams). (**c**) Decreased CL will increase the AUC, the T>MIC and the minimum drug concentration before the next administration (*C*min). (**d**) Increased MIC of the pathogen will result in decreased PD targets (*C*max/MIC, AUC/MIC and T>MIC). From Roberts et al., Examples of PK/PD changes for antibiotics in critically ill patients, Intensive Care Med. 2016 with permission [18]

6.4 The Placement of New Molecules Against Gram-Negative Bacteria

The emergence of multi-drug resistant (MDR) pathogens, characterized by high MIC for the majority of commonly used wide spectrum antimicrobials, challenges the adequate administration of this therapy. Recent evidence [2] showed that critically ill patients are at risk of Gram-negative infections sustained by MDR strains like carbapenem resistant (CR) or extended-spectrum beta-lactamase (ESBL) *Enterobacterales*, *Pseudomonas aeruginosa* (PA), and *Acinetobacter baumannii* (AB). For these reasons, many efforts have been invested to test the effectiveness of new drug with marked antimicrobial properties (Table 6.1) or to improve the use of "old" molecules with narrow therapeutic windows (e.g., polymyxins) [24]. Among the former, Ceftolozane/Tazobactam (TOL/TAZ), a combination of a fourth-generation cephalosporin with a b-lactamase inhibitor, was demonstrated effective to treat infections

Table 6.1 New drugs with marked antimicrobial properties against Gram-negative bacteria [23]

Drug	FDA/EMA infection approval	Dosage
Ceftolozane/ Tazobactam	Complicated intra-abdominal infections Complicated urinary tract infections Ventilator associated pneumonia	1.5 g q 8 iv in 1 h infusion 3 g q 8 iv (ventilator associated pneumonia)
Ceftazidime-Avibactam	Complicated intra-abdominal infections Complicated urinary tract infections Ventilator associated pneumonia	2.5 g q 8 iv in 2 h infusion
Meropenem-Vaborbactam	Complicated intra-abdominal infections Complicated urinary tract infections Hospital associated pneumonia Ventilator associated pneumonia Bacteremia	4 g q 8 iv in 3 h infusion
Imipenem/ Relebactam	Complicated urinary tract infections	1.25 g q 6 in 30 min infusion
Cefiderocol	Complicated urinary tract infections	2 g q 8 iv in 3 h infusion

Abbreviations: *EMA* European Medicine Agencies, *FDA* Food and Drug Administration

caused by Enterobacterales and MDR Pseudomonas Aeruginosa. For these strains, the time above the MIC (T > MIC) needed to produce bactericidal activity was much lower (approximately 30%) compared with other drugs of the same class [25, 26]. In this setting, a recent trial demonstrated that the efficacy of TOL/TAZ was not inferior to meropenem in patients with ventilator associated pneumonia (VAP) caused by Gram-negative bacteria [27]. On top of that, Ceftazidime-Avibactam (CAZ/AVI), a combination of a third-generation cephalosporin with a b-lactamase inhibitor, was demonstrated effective to treat infections caused by MDR bacteria with ESBL and Class A, C and some D (OXA 48) carbapenemases activities [28]. Moreover, Meropenem-Vaborbactam (MER/VAB) and Imipenem/Relebactam (IMI/REL) were demonstrated effective against Enterobacteriaceae KPC as well as MDR bacteria with class A carbapenemases (MER/VAB) [29, 30] and class A and C b-lactamase (IMI/REL) activities. Furthermore, other antibiotic combinations like aztreonam-avibactam were demonstrated to be effective against Enterobacteriaceae producing β-lactamases, ESBL and AmpC enzymes [31].

Finally, a new generation of cephalosporins, Cefiderocol, was demonstrated effective against KPC, NDM carbapenemases, MDR PA, AB and Stenotrophomonas maltophilia. This molecule has been approved to treat urinary infections, although it may play a role in the management of patients with pneumonia caused by these strains [32, 33].

6.5 Polymyxins in the Clinical Practice

Polymyxins are "old" antibiotics that were discovered in Japan in 1947 [34]. This group consists of cationic polypeptides (A–E), among which only Polymyxin B and E (colistin) have been used in clinical practice to treat Gram-negative infections.

Polymyxin B and Colistin are produced by *Bacillus* spp. [35] and consist of cyclic decapeptide molecule, positively charged and linked to a fatty acid chain. They cause lipopolysaccharide disruption and exert concentration-dependent bactericidal activity against many Gram-negative bacteria like *Acinetobacter* spp., *Klebsiella Pneumoniae*, *Escherichia Coli*, *Pseudomonas Aeruginosa*, and *Enterobacter* spp. The systemic use of polymyxin was abandoned after the 1970s, when some reports warned about their neurologic (only Polymyxin B) and renal toxicity. However, the emergence of MDR Gram-negative bacteria has raised interest towards these molecules and systemic administration of colistin is now considered a cornerstone of therapy in this setting, despite its narrow therapeutic window. In the same period, the use of Polymyxin B has been recovered as well and this molecule has been manufactured into cartridges of polystyrene fibers for endotoxin removal via extracorporeal blood purification therapy [36], in order to prevent its toxicity.

6.6 Conclusions

Timely and appropriate antibiotic therapy is of paramount importance in the management of patients with endotoxemia and sepsis. In order to optimize this intervention and prevent potential undesirable adverse events, antibiotic therapy should be driven by evidence-based stewardship programs that take into account the severity of organ dysfunction, pharmacokinetic/pharmacodynamic characteristics of the drug, and the emergence of multi-drug resistant pathogens. In this setting, several diagnostic tools and new drugs may help the clinician to overcome these issues and improve patient-related clinical outcomes.

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Clinical Management of Endotoxemia: Volume Support

7

Marzia Savi, Andrea Montisci, and Massimiliano Greco

7.1 Introduction

The goal of therapy in endotoxic and septic shock is the correction of tissue dysoxia by providing adequate end-organ perfusion. Therefore, it is pivotal to assess how to optimize patient hemodynamics, including cardiac output (CO) and mean arterial pressure (MAP). Cardiac output (CO) equals the amount of blood the heart receives in the unit of time, known as venous return (VR).

$$\mathrm{CO} = \mathrm{SV} \times \mathrm{HR} \tag{7.1}$$

where SV is stroke volume and HR is heart rate.

The venous return (VR) depends on the pressure gradient between the mean systemic pressure in the peripheral venous system (mean systemic filling pressure or Pmsf) and the mean right atrial pressure (RAP) divided by the venous vascular resistance (VVR).

$$VR = \frac{Pmsf - \mathrm{RAP}}{VVR} \tag{7.2}$$

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_7.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_7

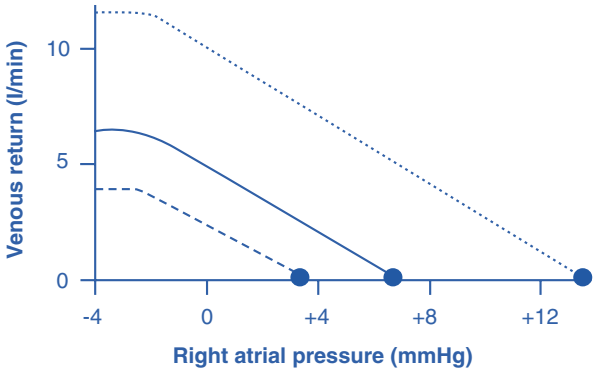

Fig. 7.1 Guyton curve: fluid infusion is performed to increase the stressed volume, mean circulatory filling pressure (Pmsf) increases and the venous return (VR) curve is shifted upward in an almost parallel manner. It is important to apply this concept during fluid resuscitation and optimize the gradient between Pmsf and right atrial pressure (RAP) to increase VR and improve oxygen delivery

Pmsf is the pressure in the whole vascular system when there is no fluid motion as if the heart ceases to beat.

The venous system can be approximated as a system of vascular pipes which brings blood back to the heart pump. Those pipes are kept open by the presence of an amount of blood determining the *unstressed volume*, but their wall-compliance allows them to receive a certain amount of extra volume, the so-called *stressed volume*.

In order to increase VR, it is viable to increase the stressed volume which is the component that determines flow. Pmsf is determined, eventually, by the total stressed volume in the circulation and the sum of the compliances of all the regions, including the pulmonary and cardiac compartments.

Whereas RAP and CO are easily measured, many efforts have been made to estimate Pmsf [1]. An increase in CO is obtained when the administration of fluids causes a greater increase in Pmsf than in RAP, implementing the VR gradient (Fig. 7.1).

7.2 Vasoplegia and Endotoxemia

Septic shock is characterized by vasoplegia, a pathological condition with low systemic vascular resistance that causes a drop in MAP in the presence of a normal or raised CO (hyperdynamic state) [2].

Endotoxins, a class of phospholipids called lipopolysaccharides (LPS) present in the outer membrane of gram-negative bacteria, are directly involved in the genesis of vasoplegic shock.

Under predisposing conditions, such as trauma, burns, hepatopathy, and ischemic shock, endotoxins can be released after the lysis of the cell, or endotoxins can translocate from the gastro-enteric (GE) tract to the bloodstream.

Endotoxins play a direct role in hypotension, favouring capillary leak, altering nitric oxide production, and inducing hyposensitivity to vasopressors; it also plays an indirect role caused by the damage of the glycocalyx leading to further fluid loss.

The degree of hypotension resistance to standard therapy impacts on outcomes in terms of mortality, in a vicious circle that seems difficult to be broken. One of the mechanisms involved in vasoplegia is nitric oxide (NO) synthesis. NO is a major driver of acute vascular dysfunction in shock [3]. During sepsis, especially in the case of endotoxemia, proinflammatory cytokines (TNF, IL-1, INF gamma) and LPS may induce the hyperproduction of NO by the inducible form of NO synthase (iNOS), thus worsening hypotension, cardiodepressive and vascular hyporeactivity in septic shock [4]. Treatment with inhibitors of iNOs has been shown to improve hemodynamic variables and survival in several animal models of septic shock [5], whilst there is still uncertainty on the long-term effects of this treatment in human septic shock.

Fluid resuscitation therapy improves survival and attenuates capillary perfusion deficits and inflammatory responses by a mechanism related to the limitation of levels of NO in animal models [6, 7].

7.3 Fluid Therapy: Rationale and Modalities

Why? In case of septic shock, the restoration of end-organ perfusion is mandatory. The inflammatory storm as well as the direct effect of endotoxins on endothelium induce hemodynamic instability. First-line agents are fluids due to their ability to increase the stressed volume and vasopressors, that *shrink* the vascular pipes, converting part of the unstressed volume into stressed volume.

What? Many types of solutions are available for fluid administration (Table 7.1). Currently, some study groups [8] promote the use of intravenous *balanced* solutions, including crystalloids with a minimal effect on the homeostasis of the extracellular compartment, the acid-base equilibrium, and the electrolyte concentration, such as Ringer's and rehydrating solutions. Furthermore, the term balanced has been recently applied also to fluids with a lower chloride content (Cl^-). Since chloride administration may impact on renal function even at low doses by causing both tubular dysfunction and arteriolar vasoconstriction [9], 0.9% saline should be restricted to cases of hypovolemic hyponatremia or hypochloremic metabolic acidosis.

The usage of synthetic colloids is contraindicated [10], even if their administration has not been completely abandoned as shown in the FENICE study [11]. Estrada et al. showed that patients with severe sepsis assigned to fluid resuscitation with HES 130/0.42 had an increased risk of death at day 90 and were more likely to require renal replacement therapy than those receiving Ringer's acetate [12]. There is still a controversial role of albumin; SSC 2021 suggests administering albumin to avoid an exaggerated volume of crystalloids to restore hemodynamic stability (weak recommendation, low quality of evidence). Albumin has antioxidant and

Table 7.1 Choosing the fluid: characteristics of the principal crystalloid (blue rows) and colloid solutions (yellow rows)

Fluid	Osmolarity (mOSM/L)	pH	Na+ (mEq/L)	Cl (mEq/L)	K+ (mEq/L)	Mg++ (mEq/L)	Ca++ (mEq/L)	Organic anion (mEq/L)	Dextrose (g/L)	Colloid oncotic pressure (mmHg)	SID
Hypertonic Saline 7.2%	2396	/	1197	1197	0	0	0	0	0	0	0
Saline 0.9%	308	5	154	154	0	0	0	0	0	0	0
Ringer's lactate	275	5.5-7	130	109	4	0	3	Lactate (28)	0	0	27
Ringer's acetate	273	6-7	132	110	4	0	3	Acetate (29)	0	0	29
Rehydrating III	312	5.5-7	140	103	10	3	5	Acetate (47)	0	0	55
Dextrose in water 0.5%	252	4	0	0	0	0	0	0	50	0	0
Frozen fresh plasma	300	variable	140	110	4	0	0	0	0-4	20	12
Human serum albumin 25%	/	/	0	0	0	0	0	0	0	200	/
Hetastarch 6% 450/0.7 Hespan	310	5.5	154	154	0	0	0	0	0	26	0

anti-inflammatory effects, reduces vascular permeability, and leads to the restoration of microcirculatory hemodynamics, as shown in animal models [13].

How? The best strategy to test the patient's fluid responsiveness is to administer an adequate amount of fluid in a predefined time interval. Cecconi et al. suggest performing a fluid challenge by administering 4 ml/kg of crystalloids and watching out for dynamic parameters variation in the following 15 min [14]. The choice of a small amount of fluid to assess the volume responsiveness aims at reducing the risk of fluid overload. Continuous CO monitors are among the best options to evaluate the response to a fluid challenge. An increase of at least 10–15% of SV and CO is considered as a positive response.

Static preload markers, including central venous pressure, have been used to predict preload dependency and fluid responsiveness for many years. Still, they are not recommended since they have been repeatedly shown to be unreliable [15]. As alternatives, *dynamic* indices have been introduced. For adults with sepsis or septic shock, the recently reported SSC [10] suggests using dynamic measures over physical examination and static parameters alone (weak recommendation, low quality of evidence).

Dynamic indices are based upon the changes in cardiac output (CO) or stroke volume (SV) resulting from various changes in preload conditions, induced by heart-lung interactions, postural manoeuvres, or by the infusion of small amounts of fluids.

Examples of dynamic indices to guide fluid resuscitation are passive leg raising (PLR) combined with cardiac output measurement (CO), fluid challenges against

stroke volume variation (SVV) or pulse pressure variation (PPV), and increase of SV in response to changes in the intrathoracic pressure.

The hemodynamic effects and the reliability of these *dynamic* indices of fluid responsiveness are well described. From their respective advantages and limitations, it is also possible to describe their clinical interest and the clinical setting in which they are applicable.

7.3.1 Passive Leg Raising

PLR is a readily available method that can be performed to assess fluid responsiveness in spontaneously breathing patients, without the administration of exogenous fluids. It corresponds to an autologous transfusion of about 300 ml of blood from the venous compartment to the heart. It remains valid even in cases when PPV or SVV indices are not reliable (i.e. arrhythmias, spontaneous breathing effort). PLR requires continuous CO monitoring; the patient is considered a responder if an increase of 10% of CO is observed. It is contraindicated in case of traumatic brain injury, suspected raised intracranial pressure, and deep vein thrombosis in the inferior limbs.

7.3.2 Central Venous Pressure

Central venous pressure is a method for estimating RAP. It is a poor predictor of fluid responsiveness and may reflect preload only in extreme conditions. Furthermore, an elevated CVP neither indicates an adequate preload nor should prevent a fluid challenge if indicated. Indeed, it offers an outlook on RV performance; for instance, a marked rise in CVP during a fluid challenge can reveal ventricular failure [16].

Moreover, central venous oxygenation saturation (ScvO2) is a global indicator of tissue oxygenation, helpful in guiding resuscitation in the early stages of septic shock.

7.3.3 Pulse Pressure Variations (PPV)

During positive-pressure inspiration, the increase of intrathoracic pressure initially causes an increase in LV preload, reduction in LV afterload, and increase in CO and systolic blood pressure (SAP), with a complete reversal during positive-pressure expiration. Pulse pressure (PP) is the difference between systolic blood pressure and diastolic blood pressure [17]. Beat-to-beat variation in PP > 13% seems to be a specific and sensitive indicator of preload reserve. Changes in SVV also predict fluid responsiveness and can be measured by arterial waveform analysis. The following conditions reduce the predictive performance of PPV and SVV test:

arrhythmias—including extreme bradycardia or tachycardia, RV failure, spontaneous breathing, tidal volume < 8 ml/kg, reduced lung compliance, pneumoperitoneum, open thorax.

7.3.4 Inferior Vena Cava (IVC) Collapsibility Index

The inferior vena cava collapsibility index (in spontaneously breathing patients) and the inferior vena cava distensibility index (in mechanically ventilated patients) are calculated by using IVC maximum and minimum diameters [18]. The IVC diameter is principally determined by its transmural pressure that, in turn, depends more on the level of backward pressure (i.e. RA pressure) and on intraabdominal pressure than on the level of pleural pressure [19]. During mechanical ventilation, the cyclic rise in pleural pressure induced by tidal ventilation increases the intramural pressure of both RA and IVC, therefore IVC tends to dilate. Variations of the IVC diameter are amplified in preload-dependent patients, but they may be reduced, or even abolished, in the presence of elevated RA pressure. A fully distended IVC could result from hypervolemia but also from RV dysfunction or severe pulmonary hypertension.

$$\textit{IVC distensibility index} = 100 \times \frac{\text{maximum inspiratory diameter} - \text{minimum expiratory diameter}}{\text{minimum expiratory diameter}}$$

7.3.5 End-Expiratory Occlusion Test (EEOT)

In patients on positive-pressure mechanical ventilation, each insufflation decreases RV preload and tends to obstruct venous return. The interruption of mechanical ventilation (MV) stops this cyclic impairment in VR, leading to a transient increase in cardiac preload. EEOT is performed by imposing an end-expiratory pause lasting at least 15 s. If CO increases at least of 5% in response to this manoeuvre, the patient is considered a fluid responder. EEOT is also reliable in patients under protective MV (i.e. ARDS), differently from SVV and PPV.

7.3.6 Velocity Time Integral (VTI)

Other techniques for monitoring fluid responsiveness include variation of the VTI, measured by bedside echocardiography by obtaining an apical-5-chamber view. As the diameter of the Left Ventricular Outlet Tract is assumed not to change during respiratory and cardiac cycles, variations in VTI reflect changes in SV. Also peak velocity variation (Vpeak) predicts fluid responsiveness using a 12% threshold [20].

7.3.7 Hemodynamic Monitoring Devices

Several monitoring devices are currently available. Among them, calibrated techniques combine transpulmonary thermodilution and pulse contour analysis to measure cardiac output and assess fluid responsiveness. They normally require a central internal jugular line and an arterial line (the femoral artery is the preferred cannulation site).

Volumetric techniques offer an overview of static and dynamic parameters, and provide useful insights into other derived parameters such as extravascular lung water (*EVLW*) and pulmonary vascular permeability index (*PVPI*) in case of PiCCO monitoring. Specifically, EVLW corresponds to the amount of fluid that is accumulated in the interstitial and alveolar spaces, whilst PVPI reflects the integrity of the alveolar-capillary membrane. Both these parameters are useful to guide fluid management of patients at risk of fluid overload, such as during septic shock and ARDS, warning against excessive fluid administration [21].

7.4 Conclusions

Targeted fluid replacement plays a key role in endotoxic shock, with the aim of balancing resuscitation and avoiding additional harm.

Contemporary studies suggest that an excessive amount of fluid may be harmful, as demonstrated by improved outcomes through the restriction of intravenous fluids in acute lung injury and septic shock. Microscopically, the already compromised function of endothelial glycocalyx can be additionally damaged by an inappropriately high volume administration [22]. We suggest titrating fluid resuscitation by targeting specific hemodynamic and metabolic parameters, using balanced solutions and avoiding normal saline and colloid solutions and allocating albumin to a later stage of resuscitation when a significant amount of fluids has already been administered. Further research on this topic is warranted, to avoid crossing the threshold between active management and noxious actions, a daily challenge in critical care medicine.

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Clinical Management of Endotoxemia: Corticosteroids

8

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8.1 Introduction

In the last decades, corticosteroids have been widely used in septic patients despite hundreds of observational studies and randomized clinical trials (RCTs) showing unclear results [1]. In fact, the role of corticosteroids in the treatment of life-threatening infections is still under debate [2, 3]. The reasons for such controversy are numerous and potentially due to different therapeutic regimens, patient heterogeneity, type of infection, and great "inter-patient" variability of immune responses to infection (i.e., hyper- versus hypo-inflammatory states).

Specifically, sepsis is a highly lethal syndrome resulting from dysregulated immune and metabolic responses to infection, strongly compromising host homeostasis. Activation of the hypothalamic–pituitary–adrenal axis and subsequently adrenocortical-glucocorticoid production are important regulatory processes to maintain homeostasis during sepsis [4]. However, in some patients, this balance, between endogenous corticosteroids and infections, is frail and could lead to life-threatening conditions such as excessive inflammation (well documented by rapid assays of mediators of acute phase response and cytokines such as C-reactive protein, interleukins 6 and 8, procalcitonin), vascular defects, hypoglycemia, and severe dysfunction of the hypothalamic–pituitary–adrenal axis, named critical-illness-related corticosteroid insufficiency or CIRCI [1–4].

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_8.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_8

Thus, despite a potential clinical benefit to counterbalancing the excessive initial pro-inflammatory response due to an acute infection, the real benefits of the use of corticosteroids are still under investigation.

According to the latest Cochrane systematic review, published in 2019, there is moderate evidence that corticosteroids may reduce 28-day hospital mortality among patients with sepsis, while they reduce Intensive Care Units (ICU) and hospital lengths of stay (high-certainty evidence). However, corticosteroids clearly increase the risk of hypernatremia and muscle weakness, and likely increase the risk of hyperglycemia, relevant adverse effects potentially able to worsen patient outcome [2].

Later on, the Surviving Sepsis Campaign, in 2021, did not confirm the concepts mentioned above and suggested the use of iv hydrocortisone at a dose of 200 mg/day to treat septic shock only when adequate fluid resuscitation and vasopressor therapy are unable to restore hemodynamic stability (weak, low/moderate-quality evidence) [5].

In conclusion, more studies are still needed to assess the real benefit of corticosteroids during severe endotoxemia and their influence on long- and short-term outcomes.

8.2 Corticosteroids and Immunomodulation in Sepsis

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to an infection [4]. According to recent studies, the annual incidence of sepsis is 48.9 million cases with 11 million deaths worldwide, representing 19.7% of all global deaths [6, 7].

The infection causing sepsis may originate from different sites, such as the respiratory tract (64%), the abdomen (20%), and the urogenital tract (14%); and may be due to gram-positive or gram-negative bacteria, fungi, viruses, and parasites.

Based on experimental animal models, the mechanism of sepsis can be classified into three categories [8]:

1. administration of a cytokine or toxin (i.e., tumor necrosis factor [TNF] or lipopolysaccharide [LPS])
2. administration of a pathogen (i.e., gram-positive or negative bacteria) or
3. disruption in the animal's protective barrier, causing bacterial invasion

Moreover, during sepsis, an excessive inflammatory response and a blunted adrenal response to the infection, extensively documented in last decades, are common and proportionally associated with disease severity and adverse short- and long-term survival [9–11].

Sir William Osler was the first author to describe this phenomenon in the beginning of the twentieth century. He observed that individuals with severe infection tend to die from the exaggerated inflammatory response rather than from infection itself [12]. Hence, he hypothesized that modulating this disproportionate response

may reduce mortality from severe infections. This represents the concept of "immunomodulation" [12–14].

Moreover, in animal models of sepsis, most deaths occur within the first 5 days after infection initiation, due to an initial dysregulated hyper-inflammatory state. However, some patients can survive after the initial hyper-inflammatory state and die later because of subsequent nosocomial infections, usually pneumonia [4]. Specifically, as the septic condition persists, the host immunologic response shifts from a hyper-inflammatory state to anti-inflammatory one, in which the patient is immunocompromised.

Taking inspiration to previous experimental models, when LPS is administered to healthy subjects, the concomitant administration of hydrocortisone reduces plasma levels of TNF-α [15] and decreases the number of eosinophils [16], circulating levels of phospholipase A [17], serum levels of nitrite/nitrate, interleukin [IL]-6, IL-8, and markers of neutrophil activation.

Moreover, corticosteroids lower *ex vivo* whole blood production of IL-1 and IL-6 in response to LPS and this "attenuate" mechanism contributes to fight septic cascade [12, 17–20].

Based on this sepsis-induced immunological impairment, corticosteroids have been widely employed as adjuvant therapies for different types of infections, such as severe sepsis and septic shock, severe community-acquired pneumonia, and bacterial meningitis, with the aim to modulate the initial hyper-inflammatory response [1, 12–14].

Despite these assumptions, the role of corticosteroids in the treatment of life-threatening infections remains controversial because of the following:

1. the ideal pro-inflammatory response for one particular patient in a particular time on the course of an infectious episode is unknown.
2. the adequate inflammatory status for each distinct episode of infection in a given individual is unclear.
3. the potential clinical biases (genetic factors, comorbidities, severity of the disease, type and site of infection).
4. finally, as early diagnosis, resuscitation with fluids and vasopressors, and prescription of broad-spectrum antimicrobials have reduced the rates of shock and early deaths, one of the most prevalent and potentially lethal consequences of severe sepsis remains the onset of an early immunosuppressive state ("immunoparalysis") [1]. In this scenario, exposing the patient to corticosteroids would potentially aggravate the immunosuppression.

8.3 Hypothalamic–Pituitary–Adrenal Axis in Sepsis

In septic patients, the excessive inflammatory response is often associated with an inappropriate response by the adrenal cortex [1, 4]. This condition can range from actual absence of adrenal cortical response (as in case of Waterhouse–Friderichsen syndrome, i.e., the occurrence of bilateral adrenal hemorrhage in the setting of a

severe infection as meningococcemia) to a blunted adrenal response where, despite producing high levels of cortisol, these concentrations are relatively low as related to the increased needs at tissue level during sepsis.

The activation of the hypothalamic–pituitary–adrenal (HPA) axis by immune cell-derived cytokines is an important regulatory process to guarantee homeostasis and survive the life-threatening impact of excessive inflammation on the host [21]. The HPA axis is based on a circadian and ultradian rhythm characterized by peak levels during the active phase, which usually occurs in the morning in humans.

Specifically, during infections the activity of the HPA axis increases as a consequence of inflammation, abnormal release of cytokines, and emotional stress [22, 23]. So, the hypothalamus secretes corticotropin-releasing hormone (CRH), which subsequently induces secretion of adrenocorticotropic hormone (ACTH) by the anterior pituitary and subsequently glucocorticoids (GC) from the adrenal cortex, and cholesterol is used as a substrate. Adrenal GC production controls total GC levels in the circulation, but extracellular binding proteins and intracellular enzymes regulate GC activity locally. Moreover, GC level negatively impacts the activity of the HPA axis via the paraventricular nucleus of the hypothalamus and the anterior pituitary, or indirectly by decreasing the expression of inflammatory cytokines [24].

Critical illness is often associated to an impairment of the HPA axis with inadequate cellular corticosteroid activity, usually proportionate to the severity of the patient's disease, a condition named "critical illness related corticosteroid insufficiency" (CIRCI), which was demonstrated to increase mortality rates [1, 25].

Interestingly, a French study found that CIRCI could be easily diagnosed in patients with septic shock using conventional blood analysis [26], providing the basis for a clinical stratification of patients at higher risk of death or for prompt identification of individuals who could benefit from corticosteroid replacement in "stress" doses, defined as the lowest dose needed to treat or supply the inadequate adrenal response to stress. The aim of the "stress" dose is to reverse shock and mitigate inflammation without exposing the patient to immunosuppressive doses that had already proven harmful in many clinical trials [27, 28].

8.4 Desirable and Undesirable Effects

Considering the strength of the evidence in the early 2000s, in the first version of the Surviving Sepsis Campaign guidelines corticosteroids were proposed as adjuvant therapies for severe sepsis and septic shock, being employed in up to 80% of the patients [29].

A study from Leuven et al. has shown that hypercortisolemia was probably due to reduced cortisol breakdown and suppressed expression of cortisol-metabolizing enzymes, leading to corticotropin suppression [30]. This not only challenged the notion of inadequate adrenal response in sepsis, but also showed that the "stress" doses were potentially excessive, increasing the risk of side effects [31].

Currently, the lack of demonstration of improved survival or cardiovascular function in more recent RCTs has decreased the enthusiasm for a routine use of corticosteroids in septic patients [5, 17].

According to the meta-analysis conducted for the development of the 2016 Surviving Sepsis Campaign guidelines, patients treated with corticosteroids only showed a weak increase of vasopressor-free days [mean difference (MD) 1.5 days; 95% confidence interval (CI) 0.8–3.11 days]. On the contrary, corticosteroids increased many adverse effects, above all neuromuscular weakness (risk ratio [RR] 1.21; 95% CI 1.01–1.45), without a clear effect on short- or long-term mortality. The overall quality of evidence was moderate. In conclusion, the panel judged the desirable effects (shock resolution, vasopressor-free days) not to outweigh the undesirable effects of low dose corticosteroid, thus did not support a recommendation for the corticosteroids use, especially if adequate fluid resuscitation and vasopressor therapy were able to restore hemodynamic stability [32].

The last Cochrane systematic review on the use of corticosteroids for treating septic shock, including 61 RCTs and a total of 12,192 patients [2], found that, compared to placebo or usual care, corticosteroids probably resulted in a slight reduction of 28-day mortality (RR 0.91, 95% CI 0.84–0.99; 11,233 participants; 50 studies; moderate-certainty evidence) and hospital mortality (RR 0.90, 95% CI 0.82–0.99; 8183 participants; 26 trials; moderate-certainty evidence), while not affecting long-term mortality (RR 0.97, 95% CI 0.91–1.03; 6236 participants; seven studies; low-certainty evidence). Corticosteroids reduced length of hospital stay for all participants (MD −1.63 days, 95% CI −2.93 to −0.33; 8795 participants; 22 studies; high-certainty evidence) and, to a lesser extent, the length of intensive care unit (ICU) stay for all participants (MD −1.07 days, 95% CI −1.95 to −0.19; 7612 participants; 21 studies; high-certainty evidence). Corticosteroids did not seem to augment the risk of superinfection (RR 1.06, 95% CI 0.95–1.19; 5356 participants; 25 studies; moderate-certainty evidence), while they increased the risk of muscle weakness (high-certainty evidence), hypernatremia (high-certainty evidence) and probably also hyperglycemia (moderate-certainty evidence). Noteworthy, there was moderate-certainty evidence that steroids do not affect gastroduodenal bleeding, stroke, or cardiac events, while low-certainty evidence indicated that corticosteroids may cause tiny, if any, differences in neuropsychiatric events.

Finally, in 2021, the Surviving Sepsis Campaign guidelines discouraged the use of corticosteroids when adequate fluid resuscitation and vasopressor therapy are able to restore hemodynamic stability (weak, low/moderate-quality evidence) [5].

8.5 Corticosteroids Dose in Sepsis

Focusing on the optimal dose, timing of initiation, and duration of corticosteroid therapy, all these elements remain still uncertain.

Considering the most recent RCTs, the “typical” dose is 200 mg per day of iv hydrocortisone in divided doses. However, inclusion criteria and duration of hydrocortisone therapy were very different between studies, as described below:

1. In the ADRENAL trial the "eligible" patients were those on any dose of vasopressor or inotrope for more than 4 h to maintain a mean arterial pressure (MAP) >60 mmHg; hydrocortisone was administered for a maximum of 7 days or until ICU discharge or death [33].
2. In the APROCCHSS trial, the included patients were dosed on ≥0.25 μg/kg/min or ≥1 mg/h of norepinephrine or epinephrine, or any other vasopressor for ≥6 h to maintain a MAP ≥65 mmHg; hydrocortisone was administered for 7 days [33].
3. In the VANISH trial enrolled adult patients who had septic shock requiring vasopressors despite fluid resuscitation within a maximum of 6 h after the onset of shock. Patients were randomly allocated to vasopressin (titrated up to 0.06 U/min) and hydrocortisone, vasopressin and placebo, norepinephrine and hydrocortisone, or norepinephrine and placebo. Specifically, 200 mg of hydrocortisone was administered daily for 5 days and then tapered over further 6 days [34].

Additionally, Zhang et al. recently published a Bayesian network meta-analysis for a head-to-head comparison of the therapeutic efficacy and safety of currently used corticosteroids in sepsis [35]. A total of 35 RCTs and 8859 patients were included. Methylprednisolone and dexamethasone were more effective in reducing short-term mortality than placebo (RR 0.65, 95% credible interval 0.40–0.93 for methylprednisolone versus placebo; RR 0.42, 95% credible interval, 0.24–0.84 for dexamethasone versus placebo). Hydrocortisone and hydrocortisone plus fludrocortisone were superior to placebo in days to shock resolution. Methylprednisolone was better than placebo in improving ventilation-free days (MD 7.71, 95% credible interval 1.15–14.42). In addition, the optimal therapeutic dosage was 200–400 mg per day of hydrocortisone or equivalents (RR 0.83, 95% credible interval, 0.64–0.98), and duration was 4–7 days (RR 0.78; 95% credible interval, 0.57–0.96). In conclusion, this study provided moderate evidence that the dosage of 200–400 mg per day of hydrocortisone or equivalent for 4–7 days was most likely to benefit septic patients.

With regard to the best modality of administration of hydrocortisone during septic shock, Tilouche et al. recently randomized 50 adult patients with septic shock requiring more than 2 mg/h (approximately 33.3 mg/min) of norepinephrine after adequate fluid administration to receive hydrocortisone 200 mg/day by continuous infusion (50%) or by boluses of 50 mg every 6 h (50%) throughout the prescription of vasopressors with a maximum of 7 days [36]. Although the occurrence of side effects and mortality were similar between the study groups, hydrocortisone administered by intermittent bolus was associated with higher shock reversal at day 7 compared with a continuous infusion [36].

In the above mentioned Cochrane systematic review on the use of corticosteroids for treating septic shock [2], only three studies considered the effect of continuous infusion of corticosteroids vs. intermittent bolus administration remains unclear, resulting in a very low certainty of evidence [2].

Finally, the last Surviving Sepsis Campaign international guidelines suggest the use of iv hydrocortisone at a dose of 200 mg/day given as 50 mg intravenously every 6 h or as a continuous infusion only when adequate fluid resuscitation and

vasopressor therapy [norepinephrine or epinephrine ≥0.25 mcg/kg/min at least 4 h after initiation] are not effective in restoring hemodynamic stability (weak, low/moderate-quality evidence) [5].

8.6 Conclusions

In conclusion, efficacy and potential risks of corticosteroids in septic patients have not been well assessed. According to the last RCTs, meta-analyses, and updated international guidelines, corticosteroids do not affect short-term and long-term mortality, while they seem to achieve a small reduction in the length of hospital and ICU stay. Corticosteroids are associated with a higher risk of hypernatremia and hyperglycemia, while the effects on superinfection and gastroduodenal bleeding are unclear.

Adherence to the current international guidelines for the use of corticosteroids in clinical practice is advisable.

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Clinical Management of Endotoxemia: Vasoactive and Cardiostimulant Drugs

9

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9.1 Introduction

Endotoxin has well-known vasoplegic and cardiodepressant effects. All of the information on vasopressors and inotropes provided in this chapter apply to all patients with distributive or cardiogenic shock, including endotoxemic patients.

Vasopressors induce vasoconstriction, thereby limiting vasoplegia and elevating mean arterial pressure (MAP), while inotropes increase cardiac contractility. These drugs are routinely used in clinical practice to control tissue perfusion in patients with shock.

Endotoxemic patients may develop septic shock, which is a complex condition characterized by circulatory, cellular, and metabolic abnormalities usually associated with adverse patient outcomes. Septic shock is defined by persistent hypotension requiring vasoactives to maintain a mean arterial pressure of 65 mmHg or higher and a serum lactate level above 2 mmol/L (18 mg/dL) despite adequate volume resuscitation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_9.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_9

Under these circumstances, besides antibiotics administration and source control (i.e., etiological treatments of infection and sepsis), fluid resuscitation, vasopressors, and inotropes are the cornerstone of hemodynamic support in patients with septic shock.

9.2 Vasoactive Agents

According to the Surviving Sepsis Guidelines [1], norepinephrine is recommended as the first-line vasopressor for treating hypotension in patients with endotoxic shock. It acts on both alpha-1 and beta-1 adrenergic receptors, thus producing vasoconstriction and an increase in cardiac output (see after). Being an alpha-1 vasopressor, norepinephrine increases MAP during endotoxic shock without any concomitant increase in heart rate. Furthermore, its beta-1 adrenergic effect could increase myocardial contractility and improve cardiac function during septic shock. Norepinephrine may improve coronary artery perfusion in patients who were previously hypotensive by increasing diastolic arterial pressure. Finally, the increase in arterial pressure may increase left ventricular afterload, thus inducing the Anrep response (i.e., a physiological response of the ventricle resulting in increased intrinsic contractility) [2]. In addition, patients with hypotensive septic shock admitted to the intensive care unit (ICU) commonly have reduced ventriculo-arterial coupling with a marked decrease in arterial elastance. In fact, left ventricular systolic performance, while influenced by arterial pressure, is also determined by ventriculo-arterial coupling, which reflects the relationship between the left ventricular contractility (end-systolic elastance) and the arterial vascular stiffness (arterial elastance) [3–5]. Thus, if ventriculo-arterial coupling is either too large or too small, poor left ventricular performance or left ventricular failure may occur, and this ratio is independently influenced by both arterial elastance and end-systolic elastance (Fig. 9.1). Norepinephrine has been shown to increase arterial elastance in septic

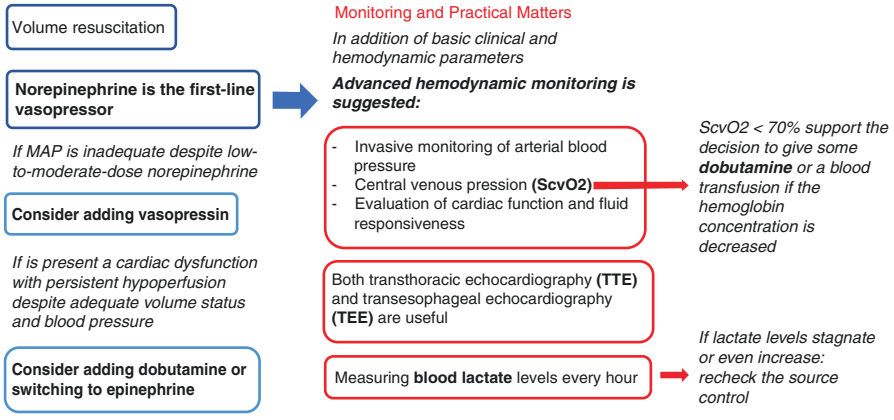

Fig. 9.1 Vasoactive agents management in endotoxic shock

patients with increased cardiac output when the ratio of arterial to end-systolic elastance is normalized. In fact, in the setting of reduced baseline arterial elastance, norepinephrine-increased arterial elastance improves left ventricular ejection by restoring normal coupling quantified as an increased stroke volume despite a small increase in arterial pressure [6–8].

In patients with endotoxic-associated vasoplegic distributive shock, norepinephrine increases stressed volume by decreasing unstressed circulatory volume; this effect would increase mean systemic pressure for the same total blood volume. In preload responsive patients, this mechanism will increase the pressure gradient for venous return, improve blood flow back to the heart, and increase cardiac output. Finally, through its alpha- and beta-adrenergic effects, norepinephrine may induce immunoparalysis. While alpha adrenergic receptors result into both pro- and anti-inflammatory actions, beta-adrenergic stimulation exerts anti-inflammatory effects [9–11].

Norepinephrine is more effective than dopamine and is nowadays suggested as the first-line vasoconstrictor for septic shock. A systematic review and meta-analysis [12] including 32 trials (total of 3544 patients) is cited in the SSC [1]. Compared to dopamine, norepinephrine was associated with a decrease in all-cause mortality and a lower risk of major adverse events and cardiac arrhythmias. No other mortality benefit was demonstrated for the comparisons between norepinephrine and epinephrine, phenylephrine and vasopressin/terlipressin. Hemodynamic data were similar between the different vasopressors, with some advantage for norepinephrine in central venous pressure, urinary output, and blood lactate levels. Evidence suggests that norepinephrine, as compared with dopamine, is associated with survival benefit, improved hemodynamic profile, and reduced adverse event rate. Although the beta-1 activity of dopamine may be useful in patients with myocardial dysfunction, the increased risk of arrhythmias limits its use.

Targeted continuous intravenous infusion is suggested for norepinephrine to maintain hemodynamic targets during septic shock. However, considering the numerous side effects associated with the pharmacological stimulation of adrenergic receptors (including increased oxidative stress, interaction with cellular energy metabolism, and/or modulation of the inflammatory response), a new concept called "decatecholaminization" has recently emerged, which involves use of non-catecholamine vasopressors to decrease catecholamine exposure [13]. Many studies reveal that high doses of administered catecholamines and high levels of circulating catecholamines are associated with poor outcomes and serious side effects, including myocardial injury and peripheral ischemia. Although necessary and life-saving in the early fight or flight reaction to any insult, prolonged adrenergic stress is harmful and contributes to organ dysfunction in septic shock. While high catecholamine levels could be a marker of disease severity, they may also be a perpetrator of other organ dysfunctions. To minimize catecholamine dosing, in addition to volemic adjustment and optimization of sedatives and other hypotensive/myocardial depressant agents, a combination of vasopressor drugs is recommended [14].

Studies as VANISH [15] and VASST [16] have demonstrated the catecholamine-sparing effect of vasopressin in sepsis and septic shock. Early use of vasopressin in

combination with norepinephrine may help reduce the adrenergic burden associated with traditional vasoactive agents. Vasopressin (antidiuretic hormone) binds V1 receptors on vascular smooth muscle, resulting in vasoconstrictive activity and increased arterial blood pressure. These studies show that vasopressin concentration is elevated in the early stages of septic shock but decreases to normal range in most patients between 24 and 48 h as shock continues. This finding has been called "relative vasopressin deficiency" as vasopressin should be elevated in the presence of hypotension. The significance of this finding is unknown. If MAP is inadequate despite low-to-moderate dose norepinephrine, addition of vasopressin is suggested. The VANCS study [17] suggests that vasopressin can be used as a first-line vasopressor agent in postcardiac surgery vasoplegic shock and improves clinical outcomes.

For adults with endotoxin-induced cardiac dysfunction and signs of persistent hypoperfusion despite adequate fluid resuscitation and arterial blood pressure, dobutamine may be administered with norepinephrine or epinephrine may be used as an alternative to norepinephrine. In patients with septic shock and persistent hypotension despite treatment with norepinephrine and vasopressin, addition of epinephrine is suggested. Furthermore, epinephrine has been suggested as a second or third-line vasopressor for patients with septic shock.

No randomized controlled trial compared dobutamine with placebo in patients with severe sepsis and septic shock. In an indirect comparison, a network meta-analysis showed that dobutamine with norepinephrine had no clear impact on mortality compared to no inotropic agents [18]. No evidence supported the superiority of dobutamine over epinephrine. Therefore, the SSC [1] considered the desirable and undesirable consequences to be comparable for both drugs and issued a weak recommendation to add dobutamine or switch to epinephrine in patients with septic shock and cardiac dysfunction with persistent hypoperfusion despite adequate fluid status and MAP [19, 20].

Selepressin is a highly selective V1 agonist that has been studied for administration in septic shock in two randomized trials [21, 22]. Selepressin has been shown to effectively maintain MAP > 60 mmHg without co-administration of norepinephrine. Unfortunately, the follow-on phase of the study was stopped for futility, with no significant differences between any of the key endpoints (ventilator- and vasopressor-free days, 90-day all-cause mortality, 30-day RRT-free days, 30-day ICU-free days); adverse event rates were also similar between groups [22]. A meta-analysis of the two studies showed no significant differences in mortality [1]. Since selepressin failed to demonstrate clinical superiority over norepinephrine, the SSC [1] considered the desirable and undesirable consequences to be in favor of norepinephrine and issued a weak recommendation against the use of selepressin as first-line therapy. Selepressin does not induce release of the procoagulant Willebrand factor; unlike the mixed vasopressin type 1a receptor/vasopressin type 2 receptor agonist arginine vasopressin, the selective vasopressin type 1a receptor agonist FE202158 does not release von Willebrand factor. Also, it is not currently commercially available.

In the SSC [1], weak recommendations are available for other drugs to be used in combination with vasoactive and inotropic drugs, such as angiotensin II, terlipressin, and levosimendan.

Angiotensin II is a physiologic substance with marked vasoconstrictor effects, triggered through stimulation of the renin-angiotensin system. The endotoxin associated with Gram-negative sepsis has the potential to inactivate the angiotensin-converting enzyme. In diseases affecting the pulmonary capillary endothelium, such as acute respiratory distress syndrome (ARDS) due to endotoxinemia and pneumonia sustained by gram negative bacteria, angiotensin-converting enzyme activity is altered at an early stage, resulting in a reduced ability to convert Angiotensin I to Angiotensin II. Angiotensin II is antagonized by the endogenous vasodilator, nitric oxide (NO), and each has a role in influencing the production and function of the other. A meta-analysis found no difference in mortality rates between angiotensin II and norepinephrine [1]. There was no clear increase in adverse events associated with use of angiotensin II. In the ATHOS-3 study [23], angiotensin II effectively increased blood pressure in patients with vasoplegic shock who did not respond to high doses of conventional vasopressors. Since the available evidence is of very low quality and clinical experience in sepsis and, therefore, demonstration of safety remains limited, the panel considered that angiotensin should not be used as a first-line agent. However, having demonstrated physiological efficacy, it could have a role as an adjunctive drug to provide a "balanced" approach to vasopressor therapy [24].

Terlipressin is a prodrug that is converted to vasopressin lysine by endothelial peptidases, producing a "slow-release" effect and giving an effective half-life of about 6 h. Terlipressin is more specific for the V1 receptors and has been studied in nine clinical trials of patients with sepsis, with or without cirrhosis. The SSC meta-analysis [1] showed no difference in mortality, but an increase in adverse events such as digital ischemia was observed in patients receiving terlipressin; diarrhea was also more common in the terlipressin group. There were three cases of mesenteric ischemia in the terlipressin group compared with one in the norepinephrine group. Therefore, the panel considered the undesirable consequences to be higher with terlipressin and made a weak recommendation against its use in patients with septic shock [25].

Levosimendan acts on the cardiovascular system through various mechanisms. The main indication for its use is acute heart failure. In septic shock, it is a second-line drug. Its use is currently encouraged in cases of acute heart failure where β-blockers are suspected of contributing to the state of hypoperfusion. A certain degree of septic heart disease is common in advanced stages of septic shock and contributes to the persistence of hypotension, in which cases the use of levosimendan may be indicated [26]. To date, trials comparing levosimendan with dobutamine are scarce, and do not show a clear mortality advantage. Patients with severe septic shock often require very high doses of norepinephrine to reach the target MAP, thus potentially leading to adverse side effects. In this kind of patients, levosimendan may provide a "catecholamine-sparing effect" [27]. The half-life of levosimendan is approximately 1 h; its active metabolite can reach 80 h, leading to persistence of cardiovascular effects for approximately 7–9 days after discontinuation of a 24-h infusion.

9.3 Use in Clinical Practice

The dose-response curves of vasopressors and inotropes depend on the hematic concentration of the drug. However, their hemodynamic effects depend on multiple factors, including the high interpersonal variability of receptors, pharmacodynamic interaction, and strong reliance on the patient's clinical, hemodynamic, and pharmacological status. Furthermore, these drugs act on different receptors involved in different hemodynamic responses and may have both direct and indirect effects through activation of the autonomous system. Close multiparametric hemodynamic monitoring should be carried out when administering vasopressor and cardiostimulant infusions in patients with endotoxic shock [28, 29].

Assessment of volemic status is crucial, and adequate resuscitation of intravascular volume should be obtained before vasopressor prescription. Early goal-directed therapy has been used for severe sepsis and septic shock in the intensive care unit. This approach involves adjustments of cardiac preload, afterload, and contractility to balance oxygen delivery with oxygen demand [30, 31].

The resuscitation phase should be followed by an optimization phase in which the objective of treatment is to ensure adequate transport of O_2 to the peripheral organs to prevent organ damage related to hypoperfusion and/or edema. In the optimization phase, advanced hemodynamic monitoring is suggested which may include, in addition to basic clinical and hemodynamic parameters (diuresis and water balance), central venous pressure, evaluation of cardiac function and fluid responsiveness.

Despite concerns about the studies on early targeted therapy, monitoring of central venous oxygen saturation ($ScvO_2$) is suggested, because a low value (<70%) may assist in the decision to give some dobutamine or a blood transfusion if the hemoglobin concentration has decreased.

Measuring blood lactate levels every hour after shock development is useful to determine the decrease due to clearance. If lactate levels stagnate or even increase, it would be necessary to reassess source control.

Basic (Rapid) Assessment by Cardiac Echo (RACE) plays a particularly pivotal role in the hemodynamic evaluation of septic shock. An analytical study of sepsis in the MIMIC-III database showed that CCUS can effectively reduce the 28-day mortality rate of critically ill patients with sepsis. Both transthoracic echocardiography (TTE) and transesophageal echocardiography (TEE) should be available, the latter being considered part of today's technologically advanced physician's armamentarium. Besides guiding the de-escalation phase, which follows the optimization phase, hemodynamic monitoring should minimize flow and assess the need for negative water balance in case of fluid overload [32, 33].

Because of considerable variability in cardiovascular effects (arrhythmias, ischemia, hypertension or hypotension), use of these drugs should be guided by the results of continuous hemodynamic monitoring.

The dose should be titrated up to achieve effective blood pressure or end-organ perfusion, as evidenced by criteria such as urine output or mental status. If the maximal dose of a first agent is inadequate, then a second drug should be added to the

first (Fig. 9.2). In situations where this is ineffective, such as refractory septic shock, anecdotal reports describe the addition of a third agent, although no controlled study has demonstrated the utility of this approach [1].

These drugs can be administered intravenously either as a bolus dose or by continuous infusion. A central venous catheter must be used to avoid extravasation and subsequent tissue necrosis. Low-dose administration through a peripheral venous catheter over a limited period has been shown to be safe.

Responsiveness to these drugs may decrease over time due to tachyphylaxis. Doses should be constantly titrated to adapt to this phenomenon and changes in the patient's clinical condition.

Dosage increase should not be attempted simply because of persistent or worsening hypotension, without reconsidering the patient's clinical situation and the appropriateness of the current strategy.

Finally, few clinical studies have been conducted to compare the efficacy and safety of one drug versus another and determine whether their use improves patient outcomes [34]. Decision to use these drugs is therefore based on expert opinion, considering their molecular mechanism of action and according to evidence derived from the few currently available clinical studies [35, 36].

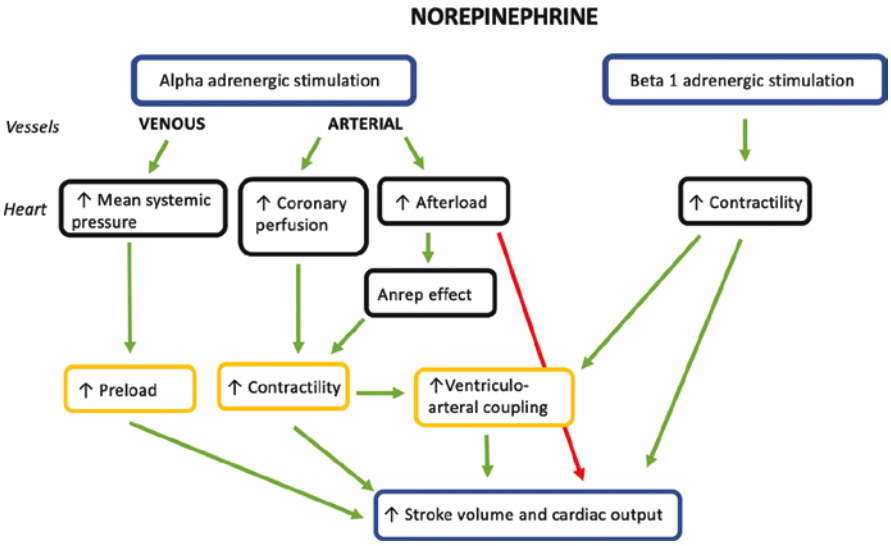

Fig. 9.2 Schematic representation of the potential mechanisms by which norepinephrine might increase cardiac output and stroke volume in patients with sepsis and septic shock. Blue boxes represent the primary receptor stimulation, black boxes their immediate effect in the heart, and yellow boxes the functional impact of those effects. The green arrows represent the positive consequences while the red ones represent the negative consequences compared to the effects present in the boxes

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Clinical Management of Endotoxemia: Source Control

10

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10.1 Introduction

In recent years, the issue of source control in septic patients has been debated and discussed both in guidelines and randomized trials. The term "source control" encompasses all those physical measures used to control a focus of invasive infection and to restore the optimal function of the affected area [1]. Appropriate source control is a key principle in the management of sepsis and septic shock [2]. Intra-abdominal infections and soft tissues infections are the sites where a source control is more feasible and more impactful. Source control may include drainage of an abscess, debriding infected necrotic tissue, removal of a potentially infected device, or definitive control of a source of ongoing microbial contamination [3]. Foci of infection readily amenable to source control include intra-abdominal abscesses, gastrointestinal perforation, ischemic bowel or volvulus, cholangitis, cholecystitis, pyelonephritis associated with obstruction or abscess, necrotizing soft tissue infection, other deep space infection (e.g., empyema or septic arthritis), and implanted device infections [3].

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_10.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_10

10.2 Timing

Source control of infectious foci was associated with improved survival in recent observational and cluster randomized studies [4]. Source control should be achieved as soon as possible following initial resuscitation in septic shock [5, 6]. While there are limited data to conclusively issue a recommendation regarding the timeframe in which source control should be obtained, smaller studies suggest that source control within 6–12 h is advantageous [5–8]. Studies generally show reduced survival beyond that point.

Kim et al. [8, 9] found lower 28-day mortality in septic shock patients who underwent source control, but no association between the time to source control and 28-day mortality. Surviving Sepsis Campaign (SSC) in 2021 recommended that the target time (no more than 6–12 h after the establishment of the diagnosis) of performance of source control was sufficient for most cases [2]. However, studies considered by SSC guidelines included only single disease entities and the definition of rapid source control was different in each study considered [7, 9, 10].

A prospective, observational study including 1011 critically ill patients with severe sepsis or septic shock found that performance of source control within the first 6 h was associated with 16% lower 28-day mortality [6]. Another prospective observational study [11] found significantly lower mortality, even after adjustment for confounding factors (patients undergoing source control were older, and a higher proportion had shock). However, the authors could not demonstrate that source control was time dependent. Patients who received early source control also received better early resuscitation, suggesting that these patients might have been sicker; however, they found no significant differences in baseline characteristics between patients who received early source control and those who received late source control. Yet, despite better early management, the mortality for patients receiving early source control was similar to those receiving late source control. The most likely explanation is that the clinical team considered source control more urgent in patients who underwent earlier source control and that the multivariate analysis failed to measure this confounder.

There are at least three reasons for delaying source control in severely septic patients:

1. Small foci of infection might not be clinically evident at first.
2. Physicians aware of the need for source control might delay intervention in apparently stable patients to enable nonemergency source control.
3. Surgical intervention might be deferred to allow necrosis to define itself anatomically to optimize intervention (e.g., in necrotizing pancreatitis) [12].

Determining the impact of early versus late source control would require formal randomization and prospective trials in more homogenous populations of patients and specific sources of infection [13, 14]. Clinical experience suggests that without adequate source control, many severe presentations will not stabilize or improve despite rapid resuscitation and provision of appropriate antimicrobials. Tellor et al.

[10] showed that inadequate source control and administration of inappropriate antibiotics were independent predictors of mortality. Lack of adequate source control was the strongest predictor of mortality, which is consistent with other analyses of complicated intra-abdominal infections [14].

However, what actually represents "adequate" source control is controversial. In general, the authors considered the goal of source control to be drainage of infected fluid collections, debridement of infected tissue, and definitive measures to avoid further contamination, as outlined by Marshall [15]. However, it has been increasingly recognized in recent years that less invasive techniques can constitute adequate source control. For instance, percutaneous drainage of an infected fluid collection is well accepted as a means of source control, as long as the goal of elimination of a substantial amount of the microbial inoculum and prevention of ongoing contamination can be achieved [14].

10.3 Intra-abdominal Infections

The timing and adequacy of source control are important in the management of intra-abdominal infections (IAIs); late and/or incomplete procedures may have severely adverse consequences on outcome especially in critically ill patients.

IAIs include several different pathological conditions and are usually classified into uncomplicated and complicated [16]. In uncomplicated IAIs, the infectious process only involves a single organ and does not proceed to the peritoneum. Patients with such infections can be managed with either surgical source control or with antibiotics alone. In complicated IAIs (cIAIs), the infectious process extends beyond the organ and causes either localized peritonitis or diffuse peritonitis. The treatment of patients with complicated intra-abdominal infections involves both source control and antibiotic therapy. Peritonitis is classified into primary, secondary, or tertiary peritonitis [16]. Primary peritonitis is a diffuse bacterial infection without loss of integrity of the gastrointestinal tract in absence of an identifiable source of infection during surgical exploration; this is rare and mainly occurs in infancy and early childhood as well as in cirrhotic patients. Secondary peritonitis, the most common form of peritonitis, is an acute peritoneal infection resulting from loss of integrity of the gastrointestinal tract or from infected viscera. It is caused by perforation of the gastrointestinal tract by direct invasion from infected intra-abdominal viscera. Anastomotic dehiscences are common causes of secondary peritonitis in the postoperative period. Tertiary peritonitis is a recurrent infection of the peritoneal cavity that follows either primary or secondary peritonitis. It is a complication of a secondary peritonitis and may be termed also "ongoing peritonitis" or "persistent" peritonitis [17]. The primary objectives of intervention include (a) determining the cause of peritonitis, (b) draining fluid collections, and (c) controlling the origin of the abdominal sepsis.

Diagnosis of IAIs is primarily clinical. Patients with IAIs typically present with rapid-onset abdominal pain and signs of local and systemic inflammation. Hypotension and signs of hypoperfusion such as oliguria, acute alteration of mental

status, and lactic acidosis are indicative of ongoing organ failure. Physical evaluation may limit the differential diagnoses to better direct decisions regarding a proper management plan including the selection of appropriate diagnostic testing, the need for initiation of antibiotic therapy, and whether emergent intervention is required. Inflammatory markers such as C-reactive protein (CRP) and procalcitonin (PCT) have been evaluated in the diagnosis of bacterial infection. CRP is an acute phase protein promptly released during an inflammation. Since systemic bacterial infection is often associated with an inflammatory reaction, it represents an indirect marker of infection and inflammation [18]. Conversely, PCT rapidly increases in the presence of bacterial and fungal infections but not viral infections or noninfectious inflammation [19]. Ultrasound (US) and computed tomography (CT) have been used over the last two decades to complete the clinical assessment of patients with IAIs.

10.3.1 Appendicitis

Acute appendicitis is one of the most common general surgical emergencies worldwide and the most common cause of intra-abdominal sepsis. Although several infectious agents are known to trigger or be associated with appendicitis [20] the full range of specific causes remains unknown [6]. Recent theories focus on genetic factors, environmental influences, and infections. The rate of perforation varies from 16 to 40%, with a higher frequency occurring in younger age groups (40–57%) and in patients older than 50 years (55–70%) [21]. Appendiceal perforation is associated with increased morbidity and mortality compared with non-perforating AA. The mortality risk of acute but not gangrenous AA is less than 0.1%, but the risk rises to 0.6% in gangrenous AA. On the other hand, perforated AA carries a higher mortality rate of around 5%. In the nineteenth century, surgeons started performing appendicectomie do surgery became the most widely accepted treatment.

Current evidence shows laparoscopic appendectomy (LA) to be the most effective surgical treatment, being associated with a lower incidence of wound infection and post-intervention morbidity, shorter hospital stay, and better quality of life scores when compared to open appendectomy (OA) [22].

Recent systematic reviews and meta-analyses of RCTs have concluded that the majority of patients with uncomplicated AA can be treated with an antibiotic-first approach [23]. The success of the non-operative approach requires careful patient selection and exclusion of patients with gangrenous AA, abscesses, and diffuse peritonitis.

The antibiotic-first strategy can be considered safe and effective in selected patients with uncomplicated acute appendicitis. Patients who wish to avoid surgery must be aware of a risk of recurrence of up to 39% after 5 years.

In-hospital surgical delay up to 24 h is safe in uncomplicated acute appendicitis and does not increase complications and/or perforation rate in adults. Surgery for uncomplicated acute appendicitis can be planned for the next available list

minimizing delay wherever possible (better patient comfort, etc.). Several systematic reviews of RCTs comparing laparoscopic appendectomy (LA) versus open appendectomy (OA) have reported that the laparoscopic approach for AA is often associated with longer operative times and higher operative costs, but it leads to less postoperative pain, shorter length of stay, and earlier return to work and physical activity [24].

10.3.2 Cholecystitis

The estimated overall prevalence of gallstones is 10–15% in the general population. Between 20 and 40% of patients with gallstones will develop gallstone-related complications, with an incidence of 1–3% annually; acute calculus cholecystitis (ACC) is the first clinical presentation in 10–15% of the cases [25]. Cholecystectomy is the most common therapeutic approach for ACC and is considered the standard of care for gallstone disease for the majority of patients. Conservative management with fluids, analgesia, and antibiotics is an option for people with mildly symptomatic acute cholecystitis (i.e., people without peritonitis or those who have worsening clinical conditions). In patients with moderate or severely symptomatic cholecystitis or in those with mildly symptomatic acute cholecystitis who prefer surgery, laparoscopic cholecystectomy is preferred over open cholecystectomy [26]. The optimal timing of uncomplicated cholecystectomy is within 7 days from hospital admission and within 10 days from the onset of symptoms.

Acute cholangitis is associated with significant mortality [27]. The mortality rates in acute cholangitis have been declining (88 to <10%) with the advent of readily available biliary decompression via endoscopic retrograde cholangiopancreatography (ERCP). In cases where ERCP is unsuccessful, alternative therapies include percutaneous transhepatic biliary drainage and/or surgical decompression, although these modalities carry significant morbidity [28]. Lee et al. [29] demonstrated that acute bacteremic cholangitis with organ failure is associated with worse outcomes, specifically acute kidney injury and septic shock. Studies have suggested that early ERCP reduces mortality resulting from cholangitis, including inpatients with co-existent gallstone pancreatitis [30]. Khashab et al. [18] report that delaying source control with ERCP beyond 72 h in patients with acute cholangitis was significantly associated with a worsening composite endpoint of death, persistent organ failure, and length of ICU stay. Jang et al. [31] have shown that ERCP performed within 24 h in patients with mild to moderate cholangitis associated with choledocholithiasis have shorter lengths of hospital stays. Karvellas et al. [32] showed that, in patients with septic shock, endoscopic biliary decompression >12 h after the onset of shock and delayed receipt of appropriate antimicrobial therapy were both significantly associated with adverse hospital outcome. This might suggest that early initiation of antimicrobial therapy and urgent biliary decompression (within 12 h) could potentially improve outcomes in this high-risk patient population.

10.3.3 Perforation

Gastrointestinal perforation complicated by septic shock is associated with high mortality and morbidity. The best time to initiate surgery is difficult to determine. It is common to stabilize circulatory dynamics before surgery [3]; however taking a long time to initiate surgery may result in death from sepsis [14]. Perforated peptic ulcer (PPU) is a complication of peptic ulcer disease. The incidence has been estimated at six to seven per 100,000 inhabitants [33]. Mortality rates as high as 25–30% have been reported [34, 35]. Surgical delay in PPU is a well-established negative prognostic factor. However, the evidence derives from studies with a high risk of bias [36], and no study has assessed the association between hourly surgical delay and adverse outcome. Buck et al. [37] showed that every hour of delay from admission to surgery was associated with an adjusted 2.4% decreased probability of survival compared with the previous hour.

Duodenal perforation represents a rare but potentially life-threatening condition. The mortality rate ranges from 8 to 25% [38]. The incidence of peptic ulcer disease has decreased in recent years. This can partly be explained by the use of proton pump inhibitors (PPIs) and eradication treatment for Helicobacter pylori. Management of duodenal perforations includes conservative, endoscopic, and surgical strategies. The type of treatment should be individualized and depends on the mechanism of injury, the timing, location and extent of the injury and the clinical state of the patient.

Acute left-sided colonic diverticulosis (ALCD) is common in Western countries with its prevalence increasing throughout the world, which is likely due to changes in lifestyle [39]. ALCD ranges in severity from uncomplicated phlegmonous diverticulitis to complicated diverticulitis including abscess and/or perforation. In patients with suspected ALCD, diagnosis is based on clinical history and signs (acute pain or tenderness in the left lower quadrant), laboratorial inflammation markers (C-reactive protein (CRP) and white blood cell count (WBC)), and radiological findings (contrast-enhanced CT scan). Immunocompromised patients may fail standard, non-operative source control. As such, most of these patients require urgent surgical intervention, and this is associated with a significantly higher mortality rate [40].

In patients with CT findings of pericolic extraluminal gas, guidelines suggest a trial of non-operative source control with antibiotic therapy; however, high mortality associated with sepsis requires maintaining a high index of clinical suspicion for deterioration and more aggressive management. WSES expert panel recommends antibiotic therapy in patients with pericolic extraluminal gas [41]. Approximately 15–20% of patients admitted with acute diverticulitis have an abscess on CT scan. The treatment of abscess always requires antibiotic therapy. If the abscess is limited in size, systemic antibiotic therapy alone is considered safe and effective in removing the abscess and solving acute inflammation with a pooled failure rate of 20% and a mortality rate of 0.6% [42]. When abscess diameter is larger, antibiotics could fail to reach the adequate concentration inside the abscess leading to an increased failure rate. The size of 4–5 cm may be a reasonable limit between antibiotic

treatment alone, versus percutaneous drainage combined with antibiotic treatment in the management of diverticular abscesses [43]. When the patient's clinical conditions allow it and percutaneous drainage is not feasible, antibiotic therapy alone can be considered. However, careful clinical monitoring is mandatory. In patients with generalized peritonitis, the authors suggest performing laparoscopic peritoneal lavage and drainage only in very selected patients. It consists of the laparoscopic aspiration of pus followed by abdominal lavage and the placement of abdominal drains, which remain for many days after the procedure. Finally, they suggest Hartmann's procedure (HP) for managing diffuse peritonitis in critically ill patients and in selected patients with multiple comorbidities and damage control surgery (DCS) with staged laparotomies in selected unstable patients with diffuse peritonitis due to diverticular perforation.

Azuhata et al. [5] hypothesized that the outcomes of patients with GI perforation with associated septic shock could be improved by initiating surgery immediately after admission in order to control the infectious lesions entirely (early source control) with the support of early hemodynamic stabilization by initial resuscitation in accordance with EGDT. Therefore, they developed a protocol including early source control and EGDT for GI perforation with septic shock. Among the patients in which surgery was started within the first 2 h, the 60-day survival rate was 98%. As the time to initiation of surgery increased, the survival rate decreased and was 0% for the group that waited more than 6 h.

10.3.4 Soft Tissues and Skin Infection

These kinds of infections represent the third most frequent cause of severe sepsis and septic shock following pneumonia and intra-abdominal infections in some series [44], but one of those that source control measures can be more evident.

Skin and soft tissue infections (SSTIs) encompass a variety of pathological conditions that involve the skin and underlying subcutaneous tissue, fascia, or muscle, ranging from simple superficial infections to severe necrotizing infections.

The spectrum of diseases that are included in this group can present differently, according to causative microorganism, or extension or clinical symptoms. A clinical categorization depending on the presence of septic shock and the urgency of requirement for surgical procedures in order to achieve source control has been described [45] with worst outcomes in those with inadequate therapy and sepsis. Source control in these infections comprises topical actions, incision and drainage, debridement, up to amputation (Fig. 10.1).

Necrotizing soft tissue infections can be caused by polymicrobial (Type I) or monomicrobial organisms (Type II). Monomicrobial infections account for 10% of NSTI and are most commonly caused by Group A β-hemolytic streptococci, especially the toxin producing strains of S. pyogenes. Other less common organisms include Vibrio vulnificus (Type III NSTI) which is found in marine environments, Aeromonas hydrophila, found in fresh or brackish water; and Clostridium perfringens. Polymicrobial infections account for the majority of infections and involve a

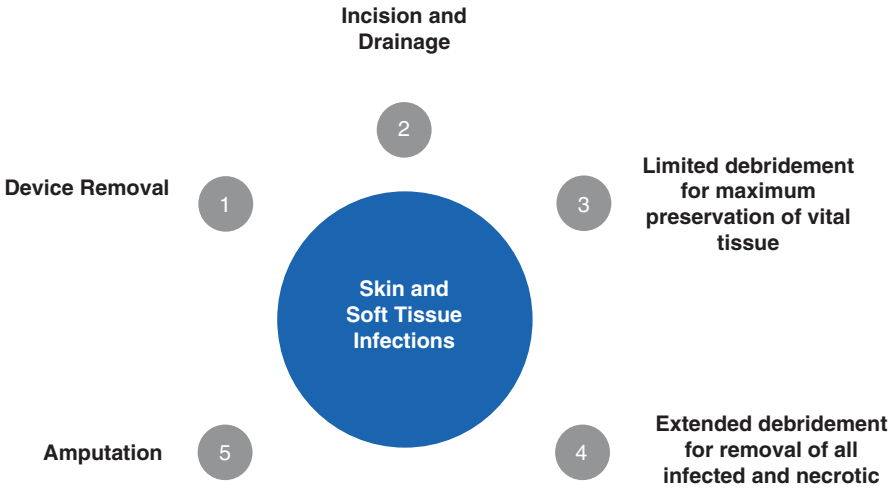

Fig. 10.1 Skin and soft tissue infections

combination of bacteria, including Staphylococcal, Streptococcal species, Escherichia coli, Bacteroides fragilis, or Clostridium species.

Broad-spectrum antibiotics that include gram-negative, gram-positive, and anaerobic coverage should also be initiated immediately after the diagnosis is suspected and continued until adequate source control is achieved.

Early surgical debridement with complete removal of necrotic tissue is essential to decrease mortality and other complications in patients with NSTIs. It is the most important determinant of outcome in necrotizing infections. This was well described in a study by Bilton et al. [46] in which patients with NSTIs, who had adequate surgical debridement (early and complete), were compared to those with either delayed or incomplete debridements. The mortality in the latter group was 38% compared to 4.2% in the group receiving early adequate surgical treatment. Delay in source control in patients with NSTIs has been repeatedly associated with a greater mortality.

In a retrospective study [47] of 121 patients with Vibrio vulnificus-related necrotizing infection, it was found that a substantial reduction in mortality risk was achieved by initiating surgical treatment within 12 h after admission compared with delaying either 12–24 h or more than 24 h after admission to initiate surgical treatment. Another review including both adults and pediatric patients supports [48] early (<12 h) initial debridement for NSTI to decrease mortality.

Guidelines suggest to plan the first re-exploration within 12–24 h and to repeat re-exploining outcomes in necrotizing infections when surgical re-debridements are performed in early versus delayed intervals. Scheduled re-explorations should be done at least every 12–24 h after the initial operation or sooner if clinical local or systemic signs of worsening infection become evident, as well as with worsening laboratory parameters.

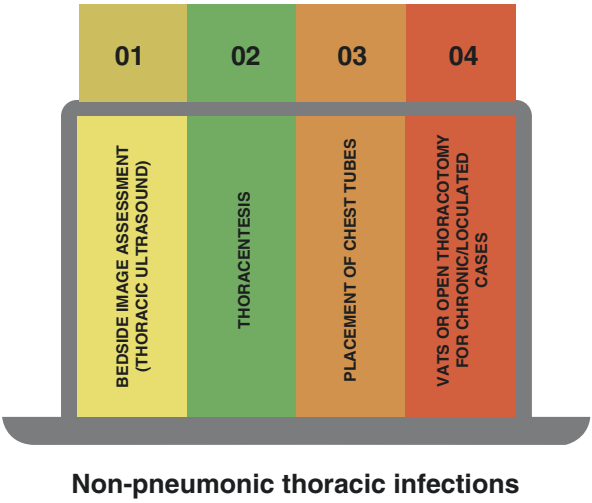

Fig. 10.2 Non-pneumonic thoracic infections

10.4 Non-pneumonic Thoracic Infections

Pleural infection is a non-rare complication for pneumonia with an approximate annual incidence of up to 80,000 cases in the UK and the USA combined. The associated mortality and morbidity is high; in the UK 20% of patients with empyema die; almost 20% of these empyema episodes require surgical intervention as source control measure [49]. In recent years, the use of thoracic ultrasound at the bedside to determine the presence of effusions especially in septic shock patients at the ICU has increased. Recent recommendations on this matter [50] suggested as first approach the use of thoracic ecography, following diagnostic sampling thoracocentesis, and if necessary the placement of a chest tube. The role of video assisted thoracoscopy and open thoracotomy can be reserved for those chronic or loculated cases (Fig. 10.2).

10.5 Conclusion

Septic shock is a time-dependent emergency that requires a multidisciplinary approach to improve outcome and reduce mortality and morbidity. All possible strategies should be implemented to control the source of infection in the first hours after diagnosis.

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Clinical Management of Endotoxemia: Treatment of DIC

11

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11.1 Introduction

Disseminated intravascular coagulation (DIC) is a severe clinical condition, which involves considerable activation of both coagulation and fibrinolysis in the circulating blood [1, 2]. It is characterized by organ failure and a tendency to bleed. The pathological feature of DIC is characterized by extensive thrombus formation in the microvasculature due to coagulopathy despite differences in underlying causes. Chan et al. revised this definition and proposed a unified theory, which consider DIC during sepsis primarily a result of microthrombogenesis, due to activation of Ultra Large Von Willebrand Factors (ULVWF) and proposed the new definition of an endotheliopathy-associated vascular microthrombotic disease (EA-VMTD) [3, 4].

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_11.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_11

11.2 Pathophysiology

11.2.1 The Coagulation Cascade

The induction of DIC is multifactorial, but the first critical event is the activation of tissue factor (TF) by different mechanisms. TF is expressed in many cells, as macrophage, monocyte neutrophils, and endothelial cells [5]. During sepsis pro-inflammatory cytokines, pathogen-associated molecular pattern (e.g., endotoxin) or damage-associated molecular pattern (e.g., cellular lysis products) act on monocyte/macrophage via Toll like receptor and activate TF [6]. TF is upregulated as sepsis worsens and induces coagulation through activation of factor VII and thrombin. Yang X et al. demonstrated that TF and VII factor activation depends also on gasdermin, a protein that induces release of calcium to the inner membrane of macrophage through a caspase-dependent reaction [7, 8].

The second important event is platelet activation through ULVWF, which is upregulated during sepsis and it is released from activated endothelium and not inhibited by anti-thrombotic proteins, as ADAMS T3 [8]. These two procoagulant events turn on microvascular thrombosis and the macro, which in conjunction with hemodynamic derangement contributes to multi-organ failure. [9].

These procoagulant events are associated with alteration of normal endothelial cell physiology and normal fibrinolytic processes. The glycocalyx, nitric oxide (NO), thrombomodulin, protein C, tissue factor pathway inhibitor, and antithrombin III maintain the normal vascular homeostasis: during sepsis all these factors are deranged and contribute to a prothrombotic environment [10, 11].

The fibrinolytic process exerts a protective effect through the activation of a prothrombotic and hyperfibrinogenemia state. The initial response to coagulation activation in bacteremia and endotoxemia is an increase of the fibrinolytic capacity, due to enhanced release of tissue plasminogen activator (tPA) from the endothelium and acceleration of tPA-induced plasminogen activation by fibrin [12]. However, levels of plasminogen activator inhibitor 1 (PAI-1), the main inhibitor of fibrinolysis, increase during the course of an inflammatory reaction and a prothrombotic status is inducted through the activation of TAFI [13–16].

11.2.2 Cytokines Endotoxin and Coagulopathy

This procoagulant activity is exacerbated by the dysfunction of the platelets, which induce the initial coagulant process and then amplify the inflammatory and prothrombotic cascade.

Platelet-derived MPs (microparticles) arise from activated platelets and induce the release of interleukin (IL)-1b, IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1) and monocytic (IL-1b, Tumor necrosis factor (TNF), IL-8) cytokines. The procoagulant activity of MPs is much stronger than activated platelets and correlates with a huge release of pro-inflammatory mediators [17]. IL-6 is one

of the major mediators involved in coagulation: it induces TF expression. At different cellular levels, it activates endothelial cells and impairs anticoagulant mechanisms [18, 19].

However, also endotoxin interferes with the coagulation. Many studies evaluated this relationship with discordant results. In vitro and in vivo animal models demonstrated the potential of LPS to initiate clotting [20, 21]. Clinical models patients with sepsis demonstrate shorter coagulation time and clot formation times. Neither platelet counts or function nor conventional clotting parameters were influenced by endotoxin concentration [22].

In contrast to these data, Zacharowski et al. observed hematologic parameters characterized by a consumption coagulopathy, with platelets decrease, fibrinogen consumption, and increase of vWF and PAI-1. These data were significantly related to increases of R and K times and decreases in MA evaluated by the thromboelastography [23].

These contradictory data could stem from different animal models, different times of study and not homogeneous laboratory parameters.

Recently, the pandemic Covid-19 storm complicated this issue, as the coagulation response during Covid infection has new aspects in relation to sepsis model, which interferes with a prompt diagnosis and therapy [24].

11.3 Clinical Features of DIC

Depending on the underlying disease, the intensity of coagulation activation, and the deficiency of the natural anticoagulant pathways, DIC may present as either a latent and compensated activation of coagulation with subtle hemostatic dysfunction and overt DIC with both bleeding and thrombotic manifestations. This may include both microvascular thrombosis and thrombosis of larger vessels, first of all in septic patients [25].

11.3.1 Differential Diagnosis in ICU

Whereas many clinical pathological conditions may result in thrombocytopenia, this requires a differential diagnosis and different therapeutic approach [26].

First, primary pseudothrombocytopenia must be excluded. Thereafter, drugs affecting platelet function and therefore blood deficiency should be excluded. In addition, some clinical events, such as increased blood loss and hemodilution, can also reduce the number of platelets. Many extracorporeal treatments can interfere with coagulation and simultaneously activate an inflammatory response. The immune-mediated disorder and post-transfusion purpura should be excluded. Renal and hepatic diseases can induce hypersplenism and hemolytic-uremic syndrome. Finally, myelodysplastic syndrome, cancer, and HTCP must be considered.

11.3.2 Diagnosis

11.3.2.1 Laboratory Findings

Laboratory findings have a prominent role for the diagnosis of DIC in ICU. Levi M et al. revised the most important criteria for the diagnosis of DIC [27].

Today, there are five different diagnostic scoring systems for DIC established by the ISTH, the Japanese Ministry Health and Welfare (JMHW), the Japanese Association for Acute Medicine (JAAM), the British Committee for Standards in Haematology (BCSH), and the Italian Society of thrombosis and Hemostasis. Recently a new score—sepsis induced coagulopathy (SIC)—dedicated to DIC in septic shock has been proposed, considering that delay for diagnosis is a major drawback of the above score system.

SIC is composed of three items: (1) presence of organ dysfunction; (2) decreased platelet count; (3) increase of PT-INR. Some studies reported a high predictive value for this score.

11.3.2.2 Thromboelastography

Thromboelastography (TEG) is a point-of-care test that quickly measures the rate (reaction time®, clot formation speed (K), and alpha angle, strength (maximum amplitude (MA)), and stability (lysis after 30 min (LY30)) of clot formation. It correlates with bleeding and thrombosis in cardiac surgery, neurosurgery, trauma and liver surgery, but is seldom used in septic patients with derangement of coagulation.

Nevertheless, it is useful for quickly identifying patients at increased risk of DIC at admission and also for guiding targeted therapy for coagulopathy. Moreover, during the management of extracorporeal treatment, TEG is essential to titrate the exact dosage of the anticoagulant end/or choose the right one, a light of new clinical scenario (e.g., platelets decrease during heparin). Recently, two studies identified MA decrease (<60 mm) and prolonged K time, decreased angle, and increased R value as predictors of early DIC in septic patients, with a correlation of laboratory findings [28].

The basis of DIC treatment is the removal of the underlying causative factor. However, DIC will most often progress even after appropriate treatment of the underlying disease. Ideally, an effective treatment for DIC would distinguish between hyperfibrinolytic and hypofibrinolytic degradation, in which prothrombotic vs. hypofibrinogenemia should be differentiated. However, laboratory diagnostic means for such distinctions are not universally available and the DIC treatment during sepsis remains controversial [29].

11.4 Extracorporeal Support During Septic DIC and the Coagulation Response

Extracorporeal blood purification is increasingly used in septic patients with organ failure, including coagulation dysfunction, which may result in DIC. Moreover, anticoagulation must be used to avoid clotting of the membrane: either heparin or regional citrate has many effects on platelets and coagulation factors, which may worsen the clinical condition. Only recently, Villa et al. addressed this important

point in a multicenter prospective study, aimed to describe the incidence and the associated factors of premature clotting of the oXiris membrane [30]. Interesting, either the hematological factors, the anticoagulant but also the pro-inflammatory molecules had a role in clotting the membrane, confirming the link between coagulation and inflammation. All these factors must be carefully evaluated when patients with organ failure need extracorporeal support to ensure an optimal treatment.

11.4.1 Blood Purification with oXiris Filter: Effect on Endotoxemia and Coagulation

11.4.1.1 Clinical Experience

From January 2012 to September 2020 143 patients with sepsis septic shock (Sepsis III definition) and AKI (AKIN classification) required ICU admission to Aurelia Hospital and European Hospital in Rome. One hundred one patients received CRRT with oXiris filters and completed the study. In these patients Endotoxemia [*EAA, Endotoxin Activity Assay; Spectral Diagnostics, Inc., Toronto, Ont., Canada*], PAI-1[*Human PAI-1 ELISA Kit*] SIC, *Sepsis–induced coagulopathy*, Thromboelastography—[*Haemonetic 5–6*] was evaluated at T0 (Basal time) and T1 (after 72 h) [31]. Endotoxin was detected in all the patients at T0. Its value at T0 was 0.73 ± 0.14 units.

At T0 8.5% of patients had low EAA activity (<0.39 units), 28% medium EAA activity (0.40–0.59 units), and 63% of patients high EAA activity (>0.60 units), confirming the massive release of endotoxin in septic patients with AKI (Fig. 11.1). At T1, EAA decreased to 0.52 ± 0.17 units ($p < 0.01$ vs. T0). At this time the percentage of patients with EAA high activity decreased with the changes of EAA (Fig. 11.1). IL-6 changes mirrored this improvement. The number of platelets, in part, correlated with the EAA activity (Fig. 11.1).

At T0 patients with laboratory signs of DIC were 19% at T0 and 22% at T1 (p = NS), but when DIC was assessed by TEG (MA < 60 mm) the percentage of patients with DIC was 10%. This is at variance with a recent study, in which all patients with septic shock had a tendency toward hypocoagulability with alteration of all TEG values.

This confirms the safety of RRT with oXiris filter, when anticoagulation with citrate is used and is in agreement with the course of TEG parameters (Table 11.1).

Patients with DIC assessed by TEG, in comparison to non-DIC patients, had less decrease of IL-6 and EAA, confirming that clearance of pro-inflammatory mediators could improve the coagulation (Fig. 11.2). Finally, we evaluated the course of PAI-1 during RRT with oXiris. PAI-1 is a key inducer of DIC in septic patients, is triggered by many pro-inflammatory mediators and increases the risk of microthrombosis in septic patients. Recent evidence suggest that PAI-1 overexpression is the hallmark of sepsis-associated DIC, as hypofibrinogenemia is less present in septic patients and decrease of platelets and PT prolongation is more present [32].

PAI-1 decreased during oXiris treatment, probably adsorbed as other pro-inflammatory mediators by the membrane: this may, in part, explain the stability of coagulation and the few episodes of bleeding in the patients (Fig. 11.2).

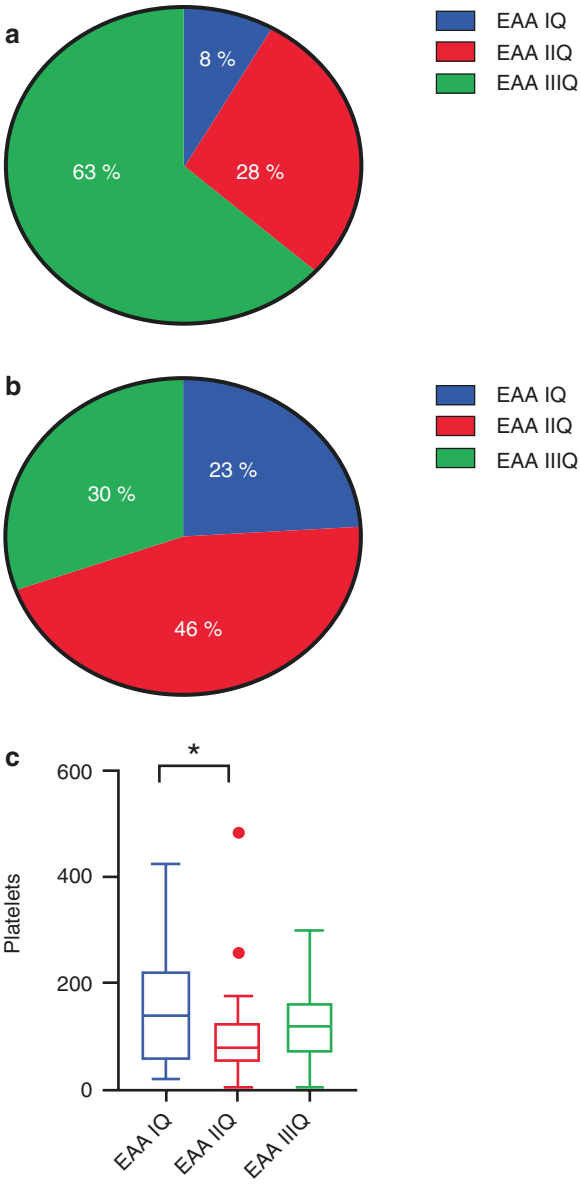

Fig. 11.1 (**a**, **b**) show the effect of the oXiris treatment on EAA activity: the patients with higher EAA activity at T0 decreased by 50% at T2. Patients with low EAA activity increased by 30% at T1. At (**c**) the effect on platelets number is shown

Table 11.1 TEG parameters during RRT with oXiris filter

Parameters	T0	T1
R [min]	9.65 ± 3	9.59 ± 4
K [min]	2.1 ± 0.5	3.7 ± 0.9
Ang [grade]	65 ± 7	62 ± 9
MA [mm]	65 ± 5	59 ± 8
K [min]	2.1 ± 0.1	3 ± 0.2

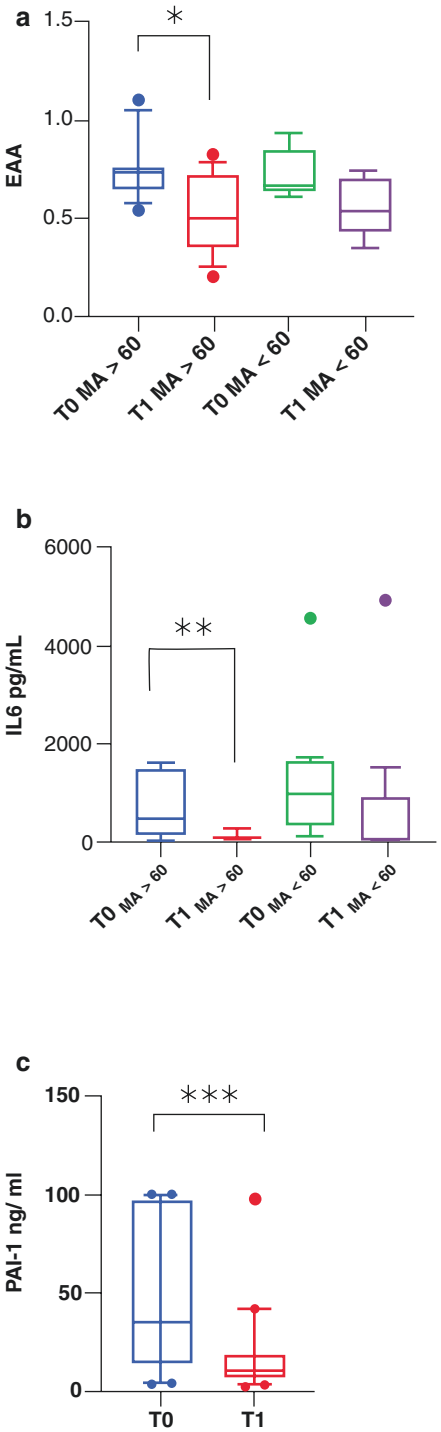

Fig. 11.2 (**a**, **b**) indicate the different effect of DIC, evaluated by TEG, on the changes of EAA and IL-6. (**c**) indicates the different effect of DIC, evaluated by TEG, on the changes of PAI-1. The effect was more prominent for the IL-6. In all the patients, not stratified on MA data, the changes of PAI are shown: the oXiris modulates significantly the prothrombotic effect of PAI-1 ($p < 0.01$)

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Clinical Management of Endotoxemia: Metabolic and Nutritional Support

12

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12.1 Introduction

Metabolic endotoxemia is a systemic condition in which the increase in plasma lipopolysaccharide (LPS) levels induced by infections (especially gram-negative bacteria) may lead to chronic inflammation-related diseases and hyperinflammation [1]. Metabolic endotoxemia is often exacerbated by dietary intake, and the gut epithelium represents an efficient barrier to preventing the absorption of LPS. However, in critically ill patients, the metabolic endotoxemia state and gut microbial dysbiosis are mainly exacerbated by other mechanisms, including invasive procedures (i.e., mechanical ventilation, endotracheal intubation, intravascular catheterization, surgical interventions), enteral or parenteral feedings, antibiotic use, vasopressors, proton pump inhibitors, and opioids that may alter the health of microbiome and facilitate the access of microbes and LPS [2]. Once entered the bloodstream, LPS binds to toll-like receptor-4 (TLR-4), leading to the activation and amplification of the inflammatory response [3], which in severe and critical patients might exacerbate multiorgan involvement and failure [2]. Hence, new therapeutic strategies are needed to overcome a possible chronic inflammatory condition. Therapeutic

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_12.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_12

advances have been obtained with dietary interventions, anti-endotoxins, antibodies, approaches to neutralize the toxicity of the Lipid A of LPS like polymyxins [1, 3], and novel but still confined therapeutics like fecal microbiota transplantation to restore an appropriate microbiota composition [2]. However, further investigations are needed to define the correct management of endotoxemic patients clearly. Therefore, this chapter aims to describe and highlight current advances in endotoxemia's clinical and therapeutic management.

12.2 Gut Microbiota and Metabolic Endotoxemia

In healthy conditions, the mucus layer represents the first-line protective barrier against microbial invasion, being a chemical barrier consisting of secretions, immune molecules, antimicrobial peptides, and cytokines. This layer is crucial to limiting contact between the microbiome and epithelial cells. Mainly, tight junctions' integrity is essential, acting as a barrier preventing the diffusion of solutes, molecules, and ions [1]. The gut homeostasis is also influenced by the bidirectional axis, which means that metabolites derived from the gut or lung bacteria and hormonal and inflammatory signals from the brain can affect each other (enteroendocrine system, adrenal axis, immune function) [2].

Critically ill patients, highly susceptible to metabolic and inflammatory dysfunction, may present increased tight junctions' permeability, altering the absorption of nutrients and allowing bacteria translocation. Indeed, in the acute phase of intensive care unit (ICU) admission, they can experience hyperinflammation, energy expenditure, and catabolic metabolism. In contrast, during ICU stay, other mechanisms like post-ventilation-acquired dysphagia, ICU-acquired weakness, metabolic dysregulation, and new infections may contribute to altered gut homeostasis and dysbiosis [1]. Within this context, the gut microbiota is considered the primary source of endotoxins and LPS. At the intestinal level, LPS alters the epithelial tight junction protein assembly (occludens and zonula occludens-1) of the gut epithelium and translocates from the lumen of the intestinal tract to the bloodstream, thus resulting in systemic endotoxemia and hyperinflammation [1] (Fig. 12.1). TLR-4 activation by LPS mediates this process. LPS is a pathogen-associated molecular pattern (PAMP), a component of the outer membrane of gram-negative bacteria, and a potent activator of the inflammatory response by interacting with TLRs (that belong to the pattern recognition receptors—PRRs, nod-like receptors—NLRs, and mannose) expressed by immune cells such as dendritic cells, macrophages, and non-immune cells. Each TLR recognizes specific microbial components (PAMPs) and activates a proinflammatory signaling pathway like nuclear factor-kB (NF-kB), and interferon regulatory factors (IRFs) [1]. TLR-4 recognizes bacterial LPS (lipid A moiety PAMP) with the potential for systemic inflammation, cytokines storm, and sepsis [4]. At molecular levels, LPS-binding protein (LBP) on the cell surface recognizes and binds to LPS, followed by the interaction of LPS with cluster differentiation (CD)-14 on the cell surface. Myeloid differentiation protein-2 (MD-2) forms a complex TLR-CD14-MD-2 that triggers two distinct signaling pathways:

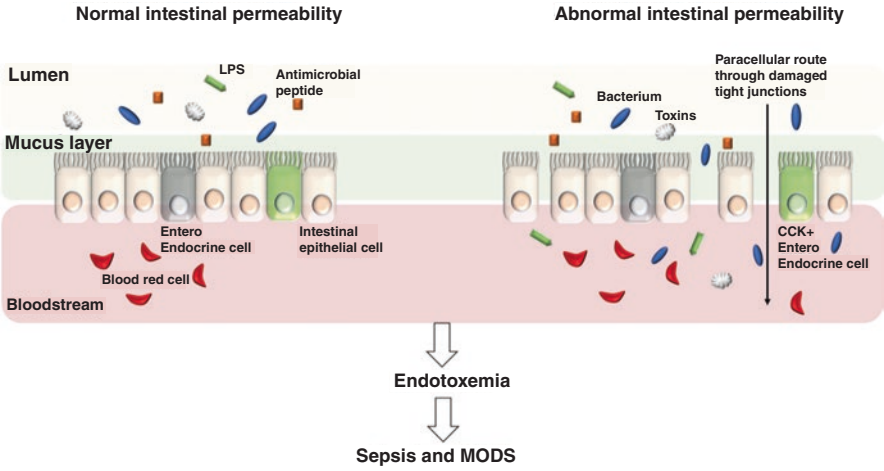

Fig. 12.1 Gut microbiota and LPS. Metabolic endotoxemia is a systemic condition in which the increase in plasma lipopolysaccharide (LPS) levels induced by infections may lead to chronic inflammation-related diseases and hyperinflammation

MyD88-dependent and independent pathways for early and late response respectively with final new gene expression [1].

Regarding clinical diagnostic and therapeutic approaches, endotoxins appear to be prevalent in several patients who meet standard clinical criteria for sepsis, particularly from gram-negative (suggested by the high levels of the endotoxin activity (EA) assay) [5]. Endotoxin has also been proposed as a prognostic marker in sepsis, multiorgan dysfunction, procalcitonin, and lactatemia [5, 6]. However, the latest update from the Surviving Sepsis Campaign (SSC) does not recommend using procalcitonin plus clinical evaluation to start antimicrobial therapy, but this approach is suggested only when de-escalating the antibiotic therapy. On the other hand, lactatemia appears to be a reasonable marker for predicting mortality [6]. Despite that: (1) a high level of EA is often identified in septic patients; (2) EUPHAS studies I and II and the EUPHRATES showed promising results, and (3) a recent meta-analysis confirmed decreased mortality using blood purification over not applying [7–10], the latest SSC guidelines suggest against the use of polymyxin B [6]. Therefore, other less invasive approaches should be mentioned and implemented, including nutritional support.

12.3 The Timing, Dose, and Titration of Enteral Nutrition in Septic/Endotoxic Shock

Early recognition followed by prompt initiation of antimicrobial provision, fluid resuscitation, and supportive care measures remains the cornerstone of septic shock management [6]. However, the overall role, timing, dose, and titration of enteral

nutrition (EN) in septic shock remain less clear. When luminal nutrients are introduced into the hypoperfused gut of septic patients, blood may be redistributed into the splanchnic circulation (splanchnic "steal"), causing an expense of systemic perfusion and increasing enterocyte workload that may clinically manifest as increased cellular hypoxia, lactate production, and increasing vasopressor dose. The result is cellular ischemia that can increase the risk of gut-related complications [11]. In addition, critically ill patients with septic shock present unbridled catabolism, proteolysis, and heightened inflammation, contributing to gut epithelial barrier dysfunction and gut dysbiosis. In the early acute phase of critical illness, EN has been shown to preserve gut epithelial barrier function reversing gut dysbiosis [12]. Since 2011, seven RCTs with at least one EN arm have enrolled patients with circulatory shock, and two of these trials (REDOX and NUTRIREA-2) [13–15] had a circulatory shock as an inclusion criterion. Particularly, the EN started in NUTRIREA-2 trial was at a full-target dose rate of 20–25 kcal/kg/day in critically ill adults with shock showing that early isocaloric enteral nutrition did not reduce mortality or the risk of secondary infections but was associated with a greater risk of digestive complications compared with early isocaloric parenteral nutrition. Initiating low-dose (trophic) EN may be reasonable in adequately resuscitated patients with septic shock. However, high-quality RCTs are needed to address the efficacy of this strategy and explore mechanisms for the postulated gut benefits of early EN in septic shock [16]. Although there is no consensus definition of high dose, a patient receiving an NE dose >0.5 mcg/kg/min is often considered a refractory shock. Clinical trials evaluating the impact of lower doses of EN are lacking. More data are needed to compare EN doses in hemodynamically unstable patients. Overall, NUTRIREA-2 and recent meta-analyses comparing early EN with parenteral nutrition (PN) demonstrate that early PN may be a safe option when early EN will not or cannot be provided. There is no direct evidence on how to adjust the EN rate in patients with septic shock.

12.4 Controversies of Parenteral Nutrition

In ICU, about 10–15% of patients cannot be fed with EN and need PN, that provides fluids, dextrose, amino acids, lipid emulsion, electrolytes, vitamins, and minerals. In the past years, patients were often provided with excessive calories to meet the elevated energy demands and to reverse the hypercatabolism of critical illness. Overfeeding contributed to hyperglycemia, hyperlipemia, increased infectious complications, and liver steatosis. Although the mechanism of PN side effects is multifactorial and not well understood, PN is associated with high morbidity and mortality [17], especially in critically ill patients and it can be associated with skeletal muscle weakness, increased rate of hospital acquired infection, impaired wound healing, and prolonged ICU stay [18]. The CALORIES trial showed no difference in mortality and infectious complications in ICU patients receiving EN or PN within 36 h from admission and up to 5 days [19]. In 2016, a systematic review and meta-analysis evaluated the effect of the route of nutrition administration on clinical

outcomes in critically ill patients [20]. There were no differences in mortality between enteral and parenteral administration, but patients receiving EN significantly reduced infectious complications and ICU length of stay [20]. No difference was found in hospital length of stay or mechanical ventilation days. Patients with moderate to severe protein energy malnutrition may benefit from PN if EN is not possible [21]. Unfortunately, available controlled clinical trials on the efficacy of PN are not well designed [22], and most of them are limited to a small number of patients, different critical illnesses, and inappropriate blinding strategies [23].

12.5 The Influence of Probiotics, Symbiotics, and Prebiotics on Endotoxemia

The intestinal epithelium barrier (i.e., a dense mucous layer containing secretory IgA and antimicrobial peptides) has essential functions in preventing systemic translocation of antigens and pathogens, allowing absorption of nutrients. Subchronic inflammation, secondary to traversing of fragments of gut-derived Gram-negative bacteria (lipopolysaccharides or endotoxin) into intestinal mucosa to enter the circulation, is one of the accepted theories that explain the contribution of gut microbes in the development of diseases [24]. Endotoxin can stimulate an innate immune response from the adipose, liver, and skeletal muscle tissues, leading to increased production of proinflammatory cytokines [25]. Gut microbiota dysbiosis, endotoxemia, and systemic inflammation contribute to disease pathophysiology in patients with critical illness (Fig. 12.2). Although emergent literature favors a probiotic supplementation for enhancement of barrier function, preventing endotoxin influx, we have conflicting results in the literature. Sabico et al. [26], in their 3-month RCT on the endotoxin-lowering effects of an 8-strain probiotics supplement among participants with type 2 diabetes mellitus, found that while circulating endotoxin levels in the probiotics group were no different than placebo after 3 months of intervention. Sharma et al. investigated the role of probiotics on gut permeability and endotoxemia in patients with acute pancreatitis, not finding any significant effect of probiotics on gut permeability or endotoxemia [27]. Prebiotics favor the proliferation of health-promoting bacteria such as Bifidobacteria and Lactobacilli, increasing the production of short-chain fatty acids (SCFAs), which can regulate the incretin axis and reduce inflammation [28, 29]. Particularly, oligosaccharides (oligofructose and galacto-oligosaccharide) were reported to support the growth of Lactobacillus as prebiotics [3, 30]. Few studies have evaluated the effects of dietary intervention on gut flora in critical care patients. Because probiotic strains feed off prebiotic substrates, the symbiotics are combined in a supplement to act synergistically to promote host gastrointestinal health. Seifi et al. [31] discovered that symbiotic supplementation could reduce serum endotoxin and inflammatory markers, but without any effects on the clinical outcomes. Further high-quality clinical trials are needed to conclusively prove the benefits of probiotics, symbiotics, and prebiotics and their effects on serum endotoxin and inflammation of adult patients with a critical illness.

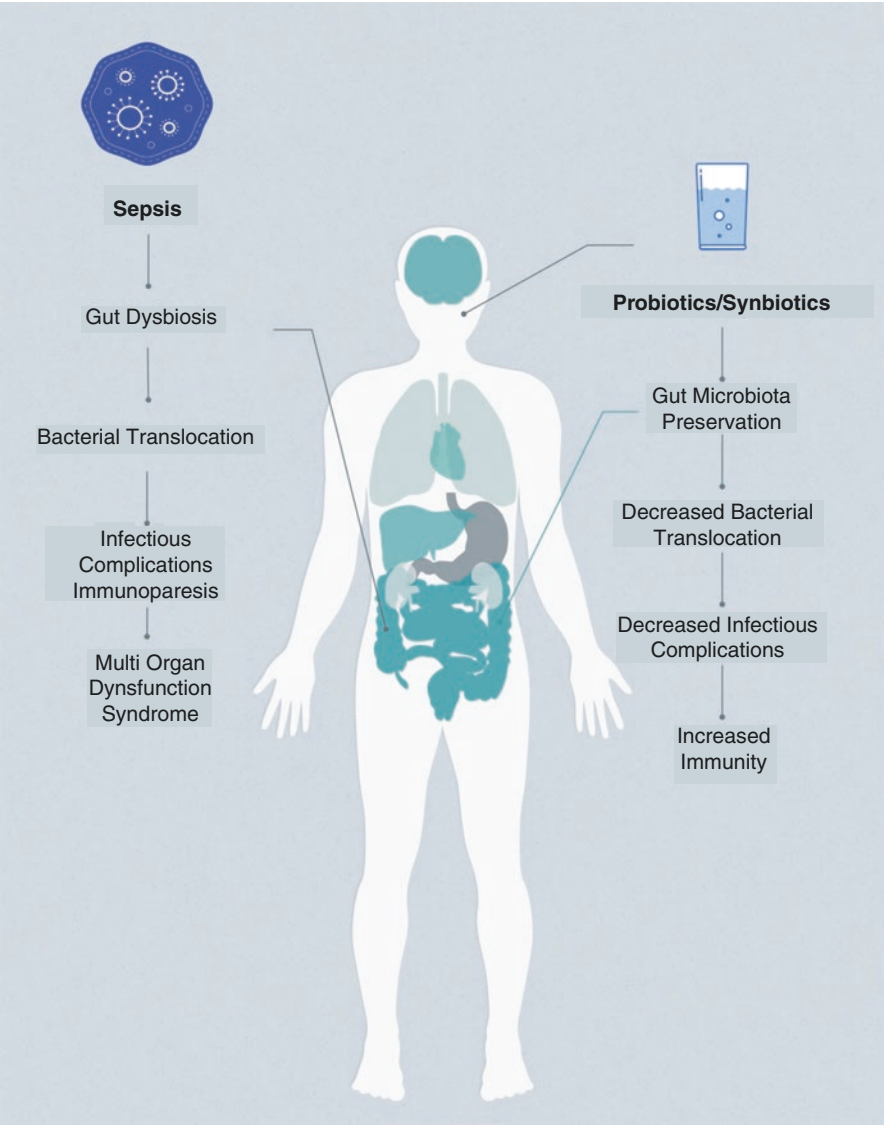

Fig. 12.2 Gut and probiotics/symbiotics. Deteriorated microbiota following sepsis cause systemic inflammation, infectious complications, immune-paresis, and multiple organ failures. Probiotics and synbiotics help to maintain gut microbiota and prevent infectious complications

12.6 Fecal Microbial Transplantation to Mitigate Multiple Organ Dysfunction in the ICU

Fecal microbial transplantation (FMT) could be an effective strategy to manipulate the microbiome. FMT is a procedure where stool from a healthy donor is collected, filtered, and given to the patients via an NG tube or the rectum. It has been successfully used in Clostridium difficile infections (CDI) [32] with a cure rate of about 90% [33] and it could be used in critically ill or immunocompromised patients, inflammatory bowel disease, septic shock, and antibiotic-associated diarrhea, and to eliminate colonization by multidrug-resistant organisms [34].

Donor microbiota can engraft in the recipient, increasing the microbiota diversity and restoring normal bowel function. In addition, bile acids, proteins, bacterial components, and bacteriophages influence the host homeostasis. However, the impact of FMT on an immunosuppressed patient with an altered microbiome is unknown and it has at least theoretical risks. In addition, treatments with antibiotics could alter the microbiome after FMT [35]. Even if a large amount of data from CDI allows us to make assumptions about safety and adverse effects, there is no international consensus on this procedure [36].

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Strategies to Reduce Endotoxin Activity

13

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13.1 Endotoxin as a Therapeutic Target

Endotoxin is a lipopolysaccharide that can be released in whole blood through Gram-negative bacteria membrane wall lysis or for direct translocation from the gut compartment.

Endotoxin is the most potent trigger of the septic cascade; a gut dysbiosis can lead endotoxin increase up to 1000-fold [1] driving a septic process and its presence has been shown to alter the expression of more than 3700 unique genes, many of which are involved in the inflammatory response [2]. The pathophysiology of sepsis involves a complex interplay between several molecular pathways, pro- and anti-inflammatory responses, release of cytokines, and activation of the coagulation cascade, the complement system and cellular components of inflammation [3]. The dysregulated host response, triggered by endotoxin, may lead to a life-threatening organ dysfunction. In this chapter we will discuss various approaches to neutralize endotoxin and its deleterious effects, including extracorporeal blood purification techniques and pharmacological immunomodulating strategies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_13.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_13

13.2 Extracorporeal Endotoxin Removal Strategies

Different medical devices for extracorporeal blood purification have been developed in the years in order to target the various stages of immune dysregulation [4, 5]. Table 13.1 shows the main devices currently available in clinics and described in literature for extracorporeal endotoxin removal specifically.

13.2.1 Polymyxin B Hemoperfusion Therapy

Among blood purification techniques for the treatment of septic shock endotoxin based (endotoxic shock), Polymyxin B hemoperfusion (PMX-HP) therapy (medical device Toraymyxin®, Toray Industries Inc. Tokyo, Japan) is the most well described in literature.

Starting from Japan and available in Europe since 2002, more than 200,000 patients has been treated with PMX-HP and more than 400 peer review articles are available in literature.

Toraymyxin® is a medical device containing polystyrene/polypropylene fibers to which polymyxin B has been covalently immobilized [6]. After different years, Toraymyxin® continues to be the only one device with Polymyxin B, the most potent endotoxin neutralizer. Although the Toraymyxin® medical device was designed to adsorb endotoxin, other mechanisms of immunomodulation have been demonstrated. Some of these mechanisms are caused by the neutralization of endotoxin, while others result from the direct adsorption of activated immune cells

Table 13.1 Current medical devices for extracorporeal removal of endotoxin

Medical device	Manufacturer	Active component	Specificity	Device Adsorption capacity (DAC) µg (Endotoxin Unit)	Other mechanisms
Toraymyxin®	Toray Industries Inc.	Polymyxin B	Specific	**64 µg—(640,000 EU)** Manufacturer Technical Data and Literature	Specific adsorption of activated immune cells
oXiris®	Baxter	Polyethylenimine (PEI), cationic	Non-specific	**1–8 µg (10,000–80,000 EU)** Literature	Non-specific removal of inflammatory mediators
Alteco® LPS Adsorber	Alteco Medical AB	Synthetic peptide	Specific	**1–8 µg—(10,000–80,000 EU)** Literature	N.D.

[7–9]. The EUPHAS RCT enrolled abdominal septic shock patients and demonstrated significant improvements in the primary endpoints of hemodynamics and organ function. Furthermore, 28-day mortality improved significantly from 53% in the control group, to 32% in the PMX-HP study group ($p = 0.03$) [10]. The most recent EUPHRATES trial showed improved hemodynamics [11] and a subsequent post hoc analysis [12] considering patient with endotoxic shock with Endotoxin Activity Levels (EA) between $0.6 < EA < 0.9$ and MODS score > 9, showed that PMX-HP was related with a significant improvement in mean arterial pressure, ventilator-free days, and survival. The TIGRIS trial is currently undergoing to clarify these results further. In addition to RCTs, the data registry EUPHAS-2 is ongoing collecting data from current clinical practice.

13.2.2 Other Blood Purification Techniques

The oXiris hemofilter (oXiris, Baxter, IL, USA) is an AN69-based membrane with a surface modification treated with a polyethyleneimine (PEI) and grafted with heparin. Broman et al. [13] enrolled 16 patients requiring CRRT for septic shock-associated acute kidney injury and endotoxin levels > 0.03 EU/ml. The patients were prospectively randomized to receive CRRT with an oXiris filter or with a standard filter. The median baseline plasma endotoxin level at T0 was 0.27 [0.15–0.63] EU/ml in the oXiris group ($n = 8$) and 0.10 [0.03–0.16] EU/ml in the standard filter group ($n = 8$, $p = 0.06$). Endotoxin levels decreased significantly using the oXiris filter compared to the standard filter. No further reduction in endotoxin levels occurred during the 2nd treatment.

Alteco LPS Adsorber® (Alteco Medical AB; Lund, Sweden) is a hemoperfusion adsorption column filled with porous polyethylene plates with an endotoxin-specific synthetic peptide. The peptide covers the surface of a porous polyethylene matrix designed to provide an optimal binding surface. The only available RCT on 32 patients showed no benefit compared with a sham device when using a LPS Adsorber in addition to standard care [14].

13.3 Immune-Modulating Strategies

The pivotal role of the immune system during sepsis provides a rationale for immune modulation treatments as adjuvant therapies [15].

13.3.1 Recombination Cytokines

Recombination cytokines are pharmaceutical analogues of endogenous cytokines and colony-stimulating factors [16]. Recombination cytokines such as IFN-γ and GM-CSF may be used to augment the immune response during sepsis-induced

immune paralysis, but their role in clinical practice remains controversial and warrants further research [17].

13.3.2 Therapies Targeting Specific Pro-inflammatory Mediators

The monoclonal antibody (mAb) against TNF-α and a recombinant human IL-1 receptor antagonist, Anakinra, represent two examples of immune modulators that have been tested for their suitability in patients with sepsis [18] but both failed to demonstrate benefit in terms of survival [19–22].

13.3.3 Immune Checkpoint Inhibitors

One of the features of sepsis-induced immunosuppression is upregulation of the T-cell exhaustion marker programmed cell death protein (PD1) and its corresponding ligand (PD-L1), which leads to the suppression of T-cell function and the consequent decreased production of key and increased apoptotic cell death [23]. Preclinical sepsis models and analyses of blood samples from patients with sepsis suggest that blockade of this pathway with anti-PD-1/PD-L1 antibodies might restore immune cell function [24, 25].

13.3.4 Therapies Targeting Epigenetic Modifications

Trained immunity is thought to be mediated by epigenetic reprogramming [26, 27]. For instance, b-glucan can stimulate changes in histone acetylation in multiple sites, thus enhancing memory of infection [28]. Some authors have suggested that trained immunity might be considered as the functional opposite of sepsis-induced immune tolerance [29]. Thus, future strategies for immune augmentation may explore the option of therapeutically inducing trained immunity and reversing immune paralysis through active manipulation of epigenetic enzymes.

13.3.5 Corticosteroids

Glucocorticoids are natural steroid hormones with pleiotropic effects, including the upregulation of anti-inflammatory protein expression and downregulation of proinflammatory protein expression through binding of the transcription factor glucocorticoid receptor (GR) [30]. In the 2021 Surviving Sepsis Campaign Guidelines, the use of intravenous corticosteroids is recommended for adults with septic shock and requirement for >0.25 mcg/Kg/min vasopressors for at least 4 h [31]. The standard corticosteroid of choice is represented by Hydrocortisone IV 200 mg/day. This recommendation, while weak and supported by moderate quality of evidence, is referred to septic shock in general, not specifically to endotoxic septic shock.

13.3.6 Intravenous Immunoglobulins (IVIG)

Intravenous immunoglobulin (IVIG) therapy has been suggested to be beneficial in sepsis and septic shock by modulating the immune response, neutralizing bacterial toxins and stimulating leucocytes and serum bactericidal activity [32]. Polyclonal standard IgG and IgM-enriched preparation are available, both obtained from plasma of healthy donors. Pathogen clearance is obtained using both preparations; however, IgM presents specific properties in the neutralization and clearance of toxins [33].

The protective effects of IVIG are attributed to their pleiotropic actions [34]. Although immunoglobulin research developed on the idea that their role was to protect the host from infection, results from experiments hint that they may play a dual antithetical role as proinflammatory or anti-inflammatory agents [35]. In fact, they may be also beneficial in the late phases of sepsis characterized by a profound depression of innate and adaptive immunity [35–37]. IVIG exerts a direct anti-apoptotic effect on lymphocytes and facilitates the clearance of apoptotic cells by an IgM-mediated mechanism that may counteract sepsis-induced immune dysfunction [38]. However, the 2021 Surviving Sepsis Campaign guidelines recommend against the use of intravenous immunoglobulins in patients with sepsis and septic shock due to lack of evidence [31, 39].

13.3.7 Stem Cells

Studies in animal models indicate that treatment with allogeneic mesenchymal stem cells (MSCs) reduces organ dysfunction and mortality [40]. Initial studies performed in mice demonstrated the potential for MSC therapy to decrease injury after pulmonary endotoxin instillation, elucidating the role of MSC-secreted mediators in reducing TNF-α and macrophage inflammatory protein-2 and increasing IL-10 concentrations, thus promoting injury resolution and tissue repair [41]. The MSC *secretome* and MSC-derived macrovesicles as well as embryonic stem cell-derived MSCs also proved effective in decreasing endotoxin-induced injury [42, 43].

13.3.8 Vitamin C

Endotoxemia and sepsis result in acute vitamin C deficiency, which is likely due to metabolic consumption of the molecule [44]. A meta-analysis concluded that in a mixed population of ICU patients, vitamin C administration had no significant effect on survival, length of intensive care or hospital stay [45]. Moreover, in the CITRIS-ALI RCT, vitamin C compared with placebo in patients with sepsis and ARDS did not significantly improve organ dysfunction scores or alter markers of inflammation and vascular injury [46]. Other studies investigated the potential benefits of administering vitamin C together with hydrocortisone and thiamine for

theoretical synergistic effects of these molecules [47] but these studies also did not report any positive effects [48, 49].

13.4 Conclusions

Considering the scientific and clinical evidence currently available in literature, among therapies for endotoxin neutralization and modulation of the host response, Polymyxin B hemoperfusion among blood purification therapies and immunoglobulins among the pharmacological therapies seem the most consistent options. Future studies evaluating a combined use of Polymyxin B hemoperfusion and enriched IVIG could be considered.

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Extracorporeal Removal of Endotoxin

14

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14.1 Adsorption Mechanism and Hemoperfusion for Endotoxin Removal

Adsorption is the mechanism under which a solute is restrained by a sorbent material through physical-chemical interaction. Among extracorporeal therapies, hemoperfusion relies on adsorbent cartridges in which the blood pass through leads the sorbent material to retain specific molecules. Adsorption is present also in CRRT when the dialyzer membrane applied in the extracorporeal circuit includes also the capability of binding specific molecules [1]. The performance of extracorporeal adsorption device for LPS should be based on Endotoxin Burden and Device

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/978-3-031-18591-5_14.

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S. De Rosa, G. Villa (eds.), *Endotoxin Induced-Shock: a Multidisciplinary Approach in Critical Care*, https://doi.org/10.1007/978-3-031-18591-5_14

Adsorption Capability (DAC). The endotoxin burden is the quantity of circulating LPS in a patient affected by (endotoxemic) septic shock (endotoxin activity assay (EAA) ≥0.6 based on MEDIC trials results) [2]. EAA levels higher than 0.9 are not found to have a beneficial effect on mortality, suggesting the insufficient capacity to control the endotoxin burden in more critically ill patients with sepsis through extracorporeal removal [3, 4]. The *Device adsorption capability (DAC)* is a specific property of a single device and it can be defined as the amount of endotoxin that a single device is able to remove from the whole blood. The following Table 14.1 shows the calculated values of the DAC for specific adsorption devices. Extracorporeal hemoperfusion treatments are based on cartridges containing polymyxin B-immobilized fibers (Toraymyxin PMX-F; Toray Industries, Tokyo, Japan): PMX-HP is covalently immobilized to an insoluble substrate as a ligand and is used as a selective absorber for endotoxin [5–7]. Polystyrene and polypropylene conjugated fibers, with island-sea type-conjugated fibers and polypropylene (island component) to provide reinforcement to the fibers, were utilized as substrate fibers. In addition, α-Chloroacetamide methyl groups were chemically introduced into the polystyrene molecule to provide a moiety to which the polymyxin B could be fixed [7]. PMX-HP, characterized by five primary amino groups derived from α,γ-diaminobutyric acid, was covalently immobilized on the surface of the fibers through the chemical reaction between the primary amino groups of polymyxin B and an active chlorine atom of the functional groups. The polymyxin B-immobilized fibers cartridge is characterized by a plastic case containing a knitted roll of polymyxin B-immobilized fiber fabric [8]. The low flow through the column is unidirectional and moves radially from the center to the outside of the roll, improving adsorption capacity through a homogeneous distribution of blood within the column (Fig. 14.1). The PMX-HP procedure is practiced through a whole blood extracorporeal circulation and a vascular access for hemoperfusion that usually is via a central vein.

Table 14.1 Calculated values of the DAC for specific adsorption device

Device	Toraymyxin TORAY	LPS Adsorber ALTECO	oXiris BAXTER
Main device component	Polymyxin B	Synthetic Peptide	Polyethylenimine (PEI), cationic
Device adsorption capacity, DAC (bovine blood)	(64 μg)	(1–8 μg)	(1–8 μg)
Reference	Technical data sheet + literature	Literature	Literature

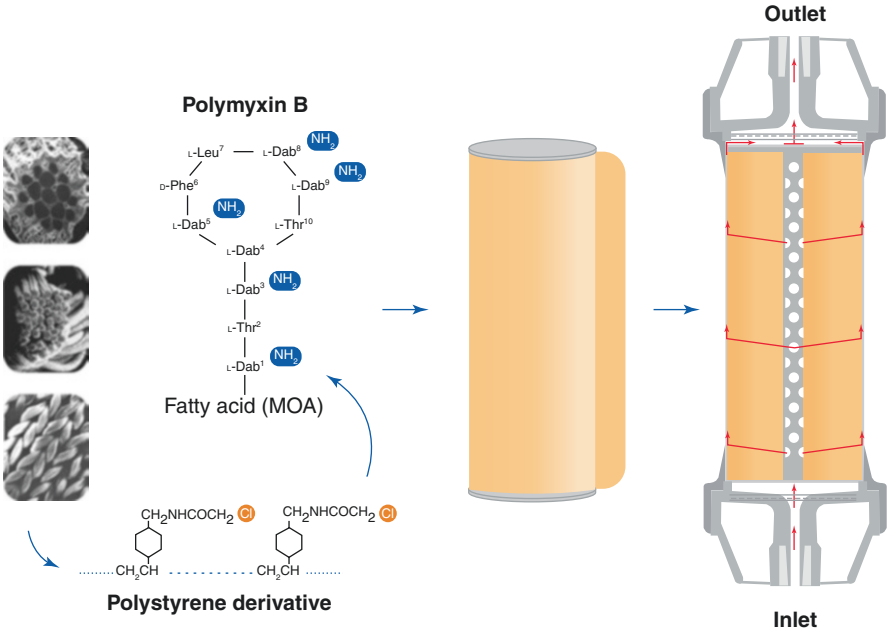

Fig. 14.1 Structure of cartridge

14.2 Clinical Use of PMX-HP Therapy

Several studies reported benefits through PMX-HP in septic patients, including improved hemodynamics, increased ratio of partial pressure arterial oxygen and fraction of inspired oxygen (PaO2/FiO2), decreased 28-day mortality, and decreased endotoxin levels [9–17]. Cruz et al. [9] showed that two sessions of PMX-HP added to conventional therapy significantly improved mean arterial pressure and vasopressor requirement and reduced 28-day mortality by 32% in the PMX-HP group and 53% in the conventional therapy group. Payen et al. [18] investigated whether PMX-HP reduces mortality and organ failure in peritonitis-induced septic shock from abdominal infections. The 28-day mortality was 27.7% in the PMX-HP group and 19.5% in the conventional group and was not significant. However, investigators did not enroll a critically sick patient and only for 68% of treated patients completed the two scheduled sessions of PMX-HP for the column clotting and hemodynamic instability. Dellinger et al. [19] investigated whether adding PMX-HP to conventional medical therapy improves survival compared with conventional therapy alone among patients with septic shock and high endotoxin activity value

with EAA. The survival rate of the PMX treated group was 37.7% and the control group was 34.5%. There was no significant difference in mortality at 28 days, and in the population with a MODS of more than 9, the PMX group was 44.5% and the control group was 43.9%. Secondary and exploratory end point analyses showed that the change of mean arterial pressure in day 3 was significantly higher than the control group both in all patients' population and in patients with MODS more than 9.0. Investigators supposed that for the patients who have overwhelming blood endotoxin burden, the dose and duration of PMX-HP as applied in this trial may have been insufficient to significantly reduce the endotoxin burden. Klein et al. [3], in a post-hoc analysis of the same study, evaluated the impact of PMX-HP on several endpoints in 194 septic shock patients with EAA level between 0.60 and 0.89. The 28-day mortality (26.1%) in the PMX-HP group was significantly lower than that (36.8%) in the sham group. Accordingly, TIGRIS trial is currently underway in septic shock patients with MODS > 9 and EAA level between 0.6 and 0.89 to elucidate the efficacy of PMX-HP in these patients. Systematic Reviews with Meta-Analysis for PMX-HP were performed with conflicting results. Terayama et al. [20] in their Systematic Review and Meta-Analysis found that PMX-HP was associated with lower mortality. Conversely, Fujii et al. [21] found that organ dysfunction scores over 24–72 h after PMX-HP treatment did not change significantly. They concluded that there is currently insufficient evidence to support the routine use of PMX-HP to treat patients with sepsis or septic shock. The results of systematic review with meta-analysis could not give a definitive answer. Further rigorous RCTs targeting the pre-defined adequate patients who are likely to benefit from PMX-HP are warranted to define the clinical role of PMX-HP. Beyond the pathophysiological rationale of the use of PMX-HP for endotoxin removal, the precise clinical indication for its initiation is widely debated in the literature.

14.3 Other Blood Purification Technique: The oXiris

The oXiris hemofilter (oXiris, Gambro Hospal, Sweden) is an AN69-based membrane, surface treated with a polyethyleneimine (PEI) and grafted with heparin, making itself capable of adsorbing both endotoxin and cytokine. Broman et al. [22] in a recent study enrolled 16 patients requiring Continuous Renal Replacement Therapy (CRRT) for septic shock-associated acute renal failure and who had endotoxin levels >0.03 EU/ml were prospectively randomized in a crossover double-blind design to receive CRRT with an oXiris filter or with a standard filter. Endotoxin levels decreased significantly using the oXiris filter compared to the standard filter. No further reduction in endotoxin levels occurred during the second treatment period in the crossover setting. Ongoing human randomized controlled trials are currently assessing the oXiris® membrane, compared to standard membranes (ECRO study, NCT03426943; oXiris study, NCT02600312), or to polymyxin B (ENDoX study, NCT01948778). The results of these studies will give more information on the oXiris® indications in the upcoming years.

14.4 The Golden Hour for Extracorporeal Removal of Endotoxin in Endotoxic Shock

PMX-HP can be life-saving when treating bacterial infections but are often used inappropriately. Although most clinicians are aware of the existence of a golden hour for septic shock, most underestimate this problem in their own hospital. Clinicians should always optimize antimicrobial management and source control to maximize the clinical outcome of the patients and apply this bridge therapy. The necessity of formalized systematic approaches to the optimization of extracorporeal blood purification therapy (EBPT) in the setting of septic shock has become increasingly urgent. De Rosa et al. [23] based on their clinical experience, strongly suggest starting extracorporeal endotoxin removal within 4 h after source control and starting antibiotic therapy. When organ failure develops, extracorporeal therapies may replace or support the function of several organs such as heart, kidney, liver, and lungs. If AKI KDIGO stage 2–3 is present, they start CRRT to support renal function after the first PMX-HP treatment. The further and evident severe unresponsive shock (VIS > 35) and sequential organ failure assessment score (SOFA > 15) and/or with a high level of EAA (higher than 0.9) should be carefully evaluated and should corroborate the extracorporeal endotoxin removal initiation. During the 2009 H1N1 [24] and 2020 COVID-19 [25] pandemic PMX-HP demonstrated the improvement of oxygenation index (PaO2/FiO2) for the patients with the severe respiratory failure.

14.5 Case Vignette: Endotoxic Shock

Miss M was a 57-year-old woman admitted to the hospital for a perforated ulcer on the anterior gastric wall. A partial gastrectomy and omental resection was performed. The patient's abdominal incision was not sutured because of high intra-abdominal pressure. After 48 h from surgery, her systolic blood pressure ranged from 60 to 80 mmHg with mean arterial pressures ranging from 50 to 70 mmHg while she was receiving multiple vasopressors (norepinephrine at 0.2 $\mu g/kg^{-1}$ min^{-1}). Her laboratory investigations were as follows: Hemoglobin 9.1 g/dl, White Blood Cells 1.52×10^9/l, Neutrophils 80.3%, Platelet Count 52×10^9/l, BUN 65 mg/dl, Creatinine 1.8 mg/dl, Albumin 3.1 g/dl, C-reactive protein 6.11 mg/dl, Procalcitonin (PCT) 100 ng/ml, Lactate 2.1. The patient had severe anasarca after requiring more than 5 l of fluid resuscitation, and was hypotensive despite large doses of noradrenaline (0.3 $\mu g/kg^{-1}$ min^{-1}). Within 3 h from surgery, Endotoxin Activity Assay (EAA) was 0.79 units, creatinine was 2.4 mg/dl with UO < 0.3 ml/kg/h over 12 h (KDIGO stage 2). Bacterial culture test of peritoneal exudate showed Enterobacter aerogenes and Pseudomonas aeruginosa. In view of EAA and unstable hemodynamics, decision was taken to initiate direct hemoperfusion using PMX-B. After priming the cartridge and blood lines, 2 h of direct hemoperfusion was performed using a blood flow rate of 100 ml/min and heparin anticoagulation. After 2 h of PMX-HP, sequential extracorporeal therapy in sepsis was commenced with

Hemofeel dialyzer on Intensa machine in order to remove inflammatory mediators and support kidney function. The prescription was set up as post-dilution, CVVHDF with the dose of 30 ml/kg/h, and no anticoagulation was applied. Hemofeel filters were changed every 24 h to insure the adsorption efficiency. After 24 h from first treatment: EAA was 0.63 and a second 2 h-cycle of PMX-HP was performed. SETS was continued with Hemofeel filter with the same prescription. After 80 h treatment with Hemofeel: the patient's vital signs have been stabilized and infection was well controlled. The dose of noradrenaline was progressively reduced and finally ceased; SOFA score decreased from 15 to 11. Her urine output was gradually increased from 125 to 3095 ml per day. Inflammation-related parameters such as PCT concentration decreased from 100 to 14.5 ng/ml over the 80 h treatment period. Surgical management of the patient's abdominal cavity was complicated. Surgeons closed all remaining open sections of his abdominal incision in the rehabilitation phase. After a total of 71 days ICU treatment, Multiorgan Dysfunction caused by septic shock was ameliorated. Then the patient was discharged from the ICU and transferred to the general ward.

14.6 Conclusions

This chapter discusses the effect of extracorporeal endotoxin removal strategies, and particularly, performed by using PMX-HP technique. Although the horizons of research are wide and constantly advancing, still several mechanisms are not clearly understood. In this respect, further research could allow the best treatment to the right patient and at the right time.

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