Trauma Induced Coagulopathy

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Foreword

The absence of hemorrhage control identified as "trauma-induced coagulopathy" is a major contributor to mortality following potentially survivable trauma. This comprehensive and timely text integrates reviews on the biology of blood coagulation, including the plasmin–fibrin/fibrinolysis system, by describing human and animal studies of trauma-induced coagulopathy. These latter studies describe the role of platelets, the endothelium, the fibrinolytic system, the complement system, and inflammation, as well as recent discoveries associated with damage-associated molecular pattern molecules (DAMP) in both hemostatic and thrombotic pathology.

These reviews are linked to practical descriptions of those technologies presently available for assessing trauma-induced coagulopathy in clinical scenarios and also summarize their limitations. The most current clinical studies describing the therapeutic intervention trials with red blood cells, platelets, cryoprecipitate, whole plasma, and plasma derivatives, as well as current concepts regarding antifibrinolytic agents are summarized. The hypercoagulable state seen following successful attenuation of bleeding after injury, which is either associated with the primary injury or a consequence of those therapies used to treat the original pathology, is also discussed in detail.

While no surrogate for human pathology in the study of trauma is completely adequate, both the animal models and numerical modeling procedures described in this monograph have been advanced as mechanisms for the transition of laboratory-based hypotheses to the evaluation and therapeutic intervention associated with trauma.

This book provides a catalyst to link laboratory research-based assets with the clinical expertise of surgeons and physicians. This interaction should yield the comprehensive studies required to develop therapeutic and diagnostic techniques to thoroughly understand and effectively treat trauma-induced coagulopathy.

Burlington, VT — Kenneth G. Mann

Preface

Like many good ideas in clinical medicine, trauma-induced coagulopathy was the product of a routine multidisciplinary research meeting. Two research fellows, Eduardo and Hunter, were presenting their experimental plans and remarked that their study backgrounds were based on literature from a variety of disciplines in diverse journals. They proposed compiling a collection of seminal papers from the experts in the field to assist those interested in coagulation research. The idea was further stimulated by frequent questions from our colleagues on the surgical intensive care unit rounds who wanted to understand the basis for our new concepts in coagulation management that were not available in surgical texts. As the process unfolded, it became clear that multiple classic papers were required for each concept. The collection soon became too large for practical distribution, and the next evolutionary step was to extract information from each contribution to generate a reference handbook. We ultimately recognized that the individual components of the coagulation system were simply too complex to relegate to a summary in a handbook. Thus, we agreed the most useful reference would be a text of chapters written by those conducting research in various fields related to coagulation.

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Historical Perspective of Trauma Induced Coagulopathy

Keywords

History
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Plasmin
Platelets
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Thrombelastography
Thromboelastometry

Injury is the second leading cause of death worldwide [1], and the third leading cause of mortality in the United States [2]. Despite advances in emergency medical systems and trauma care, deaths from injury have increased in the United States over the last decade [3]. In both the civilian [4] and military [5] settings, uncontrolled hemorrhage is the leading cause of preventable death after injury. In civilian studies, 80 % of deaths from hemorrhage occur within the first 24 h, at a median time of 2 h [4]. Consequently, there is intense interest worldwide in the pathogenesis of coagulopathic bleeding after injury and its early management. While there have been substantial insights, the words of Mario Stefanini in his address to the New York Academy of Medicine in 1954 [6] remain applicable today: "The ponderous literature on the subject of hemostasis could perhaps be considered a classical example of the infinite ability of the human mind for abstract speculation. For several years, the number of working theories of the hemostatic mechanisms greatly exceeded and not always respected the confirmed experimental facts. In recent years, however, the revived interest in this field has led to an accumulation of new findings, which has been almost too rapid for their orderly incorporation into a logical working pattern. As a result, we have rapidly gone from a state of orderly ignorance to one of confused enlightenment, from which we have not emerged as yet."

The evolution of our understanding of the complexities of coagulopathy associated with trauma has been, in large part, the result of collaboration between civilian and military researchers and clinicians. The earliest reports

of coagulopathy in injured patients were generated from military research teams, often including civilian consultants, during major wars. These novel observations would then intensify hemostasis research in civilian centers. Ultimately, the resulting findings improved coagulopathy management in subsequent conflicts, and primed the environment for making new observations. The specific contributions to our understanding of coagulopathy, however, are somewhat difficult to ascertain from World War I through Vietnam because the primary focus was on optimizing shock resuscitation at a time when plasma or whole blood was employed to replace acute blood loss [7]. Nonetheless, several landmark contributions are well recognized.

In 1916 the US National Research Council formed a Subcommittee on Traumatic Shock that collaborated with the British Medical Research Committee to study wounded soldiers in the front lines of France. Among them was Walton B. Cannon from Harvard, who was perplexed by the inconsistencies of the prevailing toxin theory of shock. Based on observations made on the battlefield in France during 1918 [8], Cannon wrote "…the heart, nervous system and other organs are suffering from an insufficient blood supply" and later admonished "if the pressure is raised before the surgeon is ready…blood that is sorely needed may be lost." Cannon documented experimentally that stress, i.e., epinephrine infusion into animals, provoked hypercoagulability followed by hypocoagulability [9]. Cannon also stated prophetically "…shock is a loss of homeostasis, and without homeostasis the patient does not survive." In 1936, based on Cannon's observations and his own research at Vanderbilt and Johns Hopkins, Alfred Blalock [10] concluded "the work of recent years has shown that shock is dependent on an inadequate supply of blood to the tissues, which may be brought about by the most diverse causes," i.e., hematogenic, neurogenic, vasogenic, and cardiogenic.

In the spring of 1940, with major victories established by Germany and Japan, the US involvement in the war appeared inevitable. Military experts recognized that bottled whole blood would be logistically impractical and enlisted the expertise of Edwin Cohen, a Harvard biochemist, to deconstruct blood in order to deliver its components to the battlefield [11]. Cohen was successful in purifying albumin as well as preparing plasma. At the onset of World War II, the National Research Council's Committee on Transfusion recommended that dried plasma—not blood—would be used if combat occurred outside the continental United States because it was easy to prepare and transport, whereas whole blood had to be typed, cross-matched, and refrigerated. Based on the legendary work of consultant Edward D. Churchill [12] in North Africa, who concluded, "wound shock is blood volume loss," the policy was changed to whole blood administration and implemented in Italy in 1943.

In 1952, the Board for the Study of the Severely Wounded systematically reviewed the cause of death in 186 war casualties. The report was dominated by the discovery of a new syndrome "post-traumatic renal failure" that was attributed to prolonged hypoperfusion. This observation ultimately led to a paradigm shift in resuscitation, incorporating crystalloid as a fundamental component of initial fluid administration [13]. Contemporary studies in civilian hospitals, based on observations in trauma and burn patients, reported a "severe bleeding tendency" implicating fibrinolysis [14, 15]. The plasmin–antiplasmin system had been well characterized at this point [16]. Alternatively,

others postulated the loss of a labile clotting factor in whole blood and recognized the key role of platelets in hemostasis [17, 18]. In 1954, Stefanini [6] noted that postinjury hemorrhage persisting despite surgical control of bleeding was variously referred to as medical bleeding, diffuse bleeding diathesis, post-transfusion bleeding disorder, and disseminated intravascular coagulation (DIC), reflecting a general lack of consensus in the pathophysiology.

During the Korean War, William Stone is credited with promoting Surgical Research Teams in the combat zone in Korea [19]. Scott and Crosby [20], representing one such team, reported that the prothrombin time (PT) was doubled in combat casualties while platelet count and fibrinogen were increased. They also speculated that the cause was due to a labile clotting factor during blood storage. Artz and Fitts [21] observed that severely injured soldiers in the Korean Conflict required both return of shed blood and crystalloid for optimal survival, inspiring the later seminal work of Tom Shires [22] defining the scientific basis for crystalloids.

After the Korean War, civilian studies implicated a number of causative factors responsible for bleeding associated with major surgery requiring transfusion, including DIC [23], fibrinolysis [24], compromised viability of platelets in stored blood [25], and the loss of the labile factors V and VIII during storage [26]. The initial response to experimental hemorrhagic shock was hypercoagulability, followed by a progressive state of hypocoagulability with decreases in factors V, VIII, IX, X, and XI along with reduced fibrinogen and platelets [27]. The early clinical studies in Baltimore further identified a third phase of hypercoagulability in those who survived the intermediate period of hypocoagulability [28]. The authors concluded that in surviving patients, the oscillatory pattern converges into a "dynamic homeostatic state," whereas, in non-survivors, "fluctuations exceeded safe limits and behaved like a runaway system."

Based on the compelling experimental work by Shires et al. [22], the major change in resuscitation strategy in Vietnam was the administration of large volumes of crystalloid. This policy virtually eliminated acute kidney dysfunction, but led to a new entity coined "Da Nang Lung" [29], later termed the acute respiratory distress syndrome (ARDS) as the civilian counterpart [30]. The first large study on coagulation disorders in combat casualties from Vietnam was reported by Simmons et al. [31]. In their comprehensive analysis of 244 injured soldiers, the authors concluded that there is "an initial phase of hypercoagulability followed by hypocoagulability and this seemed best explained by DIC. "Massive transfusion was accompanied by a dilutional coagulopathy compatible with factor levels in stored blood. Platelet levels fell, but PT, partial thromboplastin time (PTT), and fibrinogen levels were less affected. "Fresh whole blood partially counteracts this dilutional state, but is rarely necessary," concluded Simmons. Miller et al. [32] studied 21 patients requiring a massive transfusion in Vietnam. Significant coagulation defects were not evident until 20 units of stored blood were administered. A dilutional defect in platelets appeared to be the primary cause for bleeding, and this was reversed with fresh whole blood administration. Interestingly, they reported no evidence of DIC or fibrinolysis. In 1974, John A. Collins [33] systematically reviewed the problems associated with massive transfusion and offered these observations: [1] "Early complete replacement of

blood volume in the massively bleeding patient lessens the impact of exchange transfusion with stored blood," [2] "An intact circulation is a very good defense against the metabolic problems of massive transfusion," and [3] "Historically as new problems associated with massive transfusion have been defined, they have almost always been grossly overstated."

Coagulation research in civilian institutions in the early 1970s began to elucidate the molecular events resulting in thrombin generation as the common end product of the extrinsic and intrinsic clotting pathways [34, 35]. In the clinical arena, trauma surgeons recognized that controlling bleeding from the liver was a priority to improve survival following trauma, but much of the work concentrated on techniques to achieve mechanical hemostasis with some mention of packing when bleeding continued [36–38]. It was also noted that tissue disruption from blunt trauma appeared to be associated with more problematic bleeding than penetrating wounds, stimulating resurgent interest in DIC and subsequent pulmonary microemboli [39, 40]. In the later 1970s, trauma surgeons began to recognize that bleeding following massive transfusion with stored blood required supplemental clotting factors. This literature is confounded by the fact that blood banks began to implement blood component therapy [41], a policy change that unmasked the prevalence of a trauma-related coagulopathy. In 1979, our group [42] and others [43–45] observed that the majority of patients succumbing to liver injuries died of a coagulopathy, after surgical control of bleeding. We recommended pre-emptive fresh frozen plasma (FFP): "If the patients remain hypotensive after the second unit of blood, FFP should be administered then and with every fourth unit thereafter." Furthermore, we advocated fresh whole blood "…if bleeding persists despite normal PT, PTT, and bleeding times" [42]. Stimulated by these findings, we analyzed a group of patients who developed life-threatening coagulopathy with major vascular injuries and noted the compelling association of metabolic acidosis and hypothermia. Confirming the independent effects of acidosis and hypothermia on coagulation experimentally [46], we proposed the "bloody vicious cycle" in 1982 [47], which subsequently became known as the "lethal triad" and now is often referred to as "resuscitation-associated coagulopathy." The concept of truncating definitive repair of all injuries in coagulopathic patients in the operating room, to allow for correction of hypothermia, acidosis, and coagulopathy in the intensive care unit, was the fundamental basis of "damage control surgery" introduced by Harlan Stone et al. in 1983 [48]. In studying our coagulopathic injured patients in 1982 [47], we noted that higher ratios of FFP to stored blood were associated with improved survival and advocated presumptive FFP:blood administration of 1:4 in the emergency department. Charles Lucas and Anna Ledgerwood also conducted animal work that supported the concept of pre-emptive FFP during massive transfusion [49]. In the later 1980s [50], the Detroit General Group systematically studied coagulation abnormalities in patients requiring a massive transfusion of stored red blood cells (RBC) and postulated them to be secondary to consumption of factors, reflected in standard measures of coagulopathy, i.e., PT, PTT, and thrombin time (TT). Collectively, the coagulopathy associated with severe trauma was postulated to be secondary to both consumption and dilution of clotting factors. There was also considerable inter-

est in the early administration of platelets due to the long-term observation of deteriorating platelet numbers in stored blood, although clinical trials failed to confirm a benefit of pre-emptive platelet administration [51].

In the ensuing decade much of the clinical investigation centered on optimizing the use of damage control surgery for refractory coagulopathy [52–54]. Coagulation research during this period was further complicated by the practice of aggressive crystalloid resuscitation targeting supra-physiologic oxygen delivery, promulgated by William Shoemaker et al. [55]. This resulted in an epidemic of compartment syndromes, with much attention diverted to the urgent need to decompress the abdomen following protracted shock that required high volume crystalloid resuscitation [56]. In retrospect, most of the compartment syndromes and, to a significant extent, coagulopathies were generated by overzealous infusion of crystalloid driven by the subsequently disproven concept of supra-physiologic oxygen delivery [57]. There is no question that chasing oxygen delivery with Swan-Ganz catheters and attempting to correct metabolic acidosis with large volume crystalloid loading added a substantial component of dilutional coagulopathy [58].

The first decade of the twenty-first century perhaps represents the most significant insights gained into trauma-associated coagulopathy in modern history, and many of these investigators responsible are authors in this monograph. Progress was unquestionably inspired by the revolutionary concept of the cell-based model of coagulation proposed by Hoffman and Monroe [59] who emphasized the fundamental role of platelets as a platform for clotting factor assembly and thrombin generation on damaged endothelium. Paradoxically, these new insights led to the unbridled use of activated factor VII, which was ultimately proven unjustified [60, 61]. In 2003, MacLeod et al. [62] from the University of Miami made the observation that 28 % of severely injured patients had an elevated PT on arrival to the hospital, and this was associated with an increased risk of mortality. At the same time, Karim Brohi [63] from the Royal London Hospital reported that 24 % of severely injured patients had prolonged clotting times, and extended their analysis to demonstrate this abnormality was independent of fluid administration and, consequently, termed the syndrome the “acute coagulopathy of trauma” (ACOT). Stimulated by his observations on the ACOT in London, Brohi pursued a trauma research fellowship with Mitch Cohen and colleagues in San Francisco. Together, in 2007, this civilian research team provided compelling evidence that activation of protein C is an integral component of ACOT [64]. Shortly thereafter, Par Johansson [65] from Copenhagen added evidence of endothelial glycocalyx degradation, stimulating interest in the endotheliopathy of ACOT. Additional evidence has implicated the innate immune response in general [66], and neutrophils specifically [67] in the pathogenesis of ACOT.

Simultaneous with these provocative studies in civilian trauma centers, the military recognized coagulopathy as the most common source of preventable death in soldiers in the war in Iraq [68]. When confronted with this challenge, Hess and colleagues from the US Army [69] suggested the best solution was to replace the acute blood loss with a blood component formula that would replicate the whole blood lost, thus the genesis of the 1:1:1 concept. In 2007, Borgman et al. [70] reported the US military experience in Iraq suggesting a

survival benefit for soldiers resuscitated with an FFP:RBC ratio approaching 1:1 when they required a massive transfusion (10 units of red blood cells (RBC) in 24 h). This report was extrapolated to support the proposed "damage control resuscitation" concept with 1:1:1 as the centerpiece. Although the relative simplicity of this recommendation is appealing, this concept is not intuitively scientific and has prompted vigorous debate that continues today [71–74]. Ultimately these debates stimulated the National Institutes of Health (NIH) to conduct a Trans-Agency Coagulopathy in Trauma Workshop, held in Bethesda in April 2010. Out of this meeting came the consensus that the term "trauma-induced coagulopathy" (TIC) would be employed to describe what was previously referred to as ACOT.

Conspicuous among the many questions is whether platelets should be given empirically with the initial administration of FFP and RBC units in patients at risk for TIC. In contrast to platelets and plasma for first-line therapy in the United States, the European approach has been to load fibrinogen [75]. The current limitation in assessing platelet function for hemostasis has hampered resolution of this topic [76, 77]. Further is the debate of the optimal ratio of FFP:RBC units in the patient at risk for TIC. The only randomized trial to date failed to demonstrate a survival advantage of a 1:1 versus 1:2 FFP:RBC ratio when delivered with platelets [78, 79].

The role of systemic fibrinolysis in TIC has added another layer of controversy, which was largely overlooked until the widespread implementation of global viscoelastic assays of hemostasis in trauma care, such as thrombelastography (TEG) and thromboelastometry (ROTEM) [80–83]. Unfortunately, the CRASH-2 trial reported in 2010 [84] prompted indiscriminate use of tranexamic acid (TXA), until the limitations of this study were recognized [85, 86]. Subsequently, it was generally acknowledged in the United States that TXA should be reserved for selected populations until randomized trials clarify the indications, including its role in traumatic brain injury patients. Recently the elucidation of early fibrinolysis shutdown [87] has added to the concern of routine TXA administration. Finally, the issue of whether goal-directed therapy via viscoelastic assays such as TEG or ROTEM is superior to a fixed ratio approach is ongoing. A large retrospective study indicated that TEG-driven resuscitation was more effective than 1:1:1 approach [88], and our recent single-institution randomized study [89] indicated that TEG was more effective in guiding a massive transfusion protocol than conventional laboratory tests (PT, PTT, platelet count, and D-dimers). In 2013, driven by these ongoing controversies, the NIH funded a Trans-Agency Research Consortium for Trauma-Induced Coagulopathy (TACTIC) in collaboration with the Department of Defense (DOD) with the aim of elucidating the underlying mechanisms of TIC from "road to rehabilitation."

In sum, the need to define the scientific basis for blood component administration and regulation of fibrinolysis in the critically injured patient is as clear today as it was 60 years ago and, as optimistically articulated by Mario Stefanini [6], we are making substantive progress. "While the multiplicity of hypotheses and the conflict of experimental findings still deny us a firm theoretical basis for the interpretation of the mechanisms of hemostasis, the impact of the advances of the last 10 years on the diagnosis and management

of the bleeding patient has been staggering. New diagnostic tests have greatly increased the accuracy of the diagnosis; broader interest in the isolation of coagulation factors and of platelets points to more specific methods of treatment in the near future. One feels that, with the unending ferment of ideas and fervor of investigation in this field, great progress lies ahead."

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Part I

Physiology of Hemostasis

Cell-Mediated Hemostasis

1

Maureane Hoffman

Introduction

In the 1960s two groups proposed a "waterfall" or "cascade" model of coagulation composed of a series of proteolytic steps in which activation of one clotting factor led to the activation of the next, finally leading to a burst of thrombin generation [1, 2]. While those schemes specifically addressed that we now refer to as the "intrinsic" pathway, the "coagulation cascade" is now depicted as a Y-shaped scheme, with distinct "intrinsic" and "extrinsic" pathways initiated by factor XII (FXII) and FVIIa/tissue factor (TF), respectively (Fig. 1.1). The pathways converge on a "common" pathway at the level of the FXa/FVa (prothrombinase) complex. The coagulation complexes are generally noted to require phospholipid and calcium for their activity. This scheme was primarily proposed as a model for how the multiple coagulation factors interacted to produce a fibrin clot. Of course, hemostasis researchers at the time knew that platelets were important for hemostasis. Because platelets are difficult to reproducibly prepare as a reagents, they were replaced with phospholipid vesicles in biochemical experiments and subsequently in our common clinical coagulation assays. The cascade model is a good depiction of the sequence of events in the prothrombin time (PT) and activated partial thromboplastic time (aPTT). The concept of a series of proteases acting as a biological amplifier was a breakthrough in understanding hemostasis and a number of other cellular processes, such as apoptosis. However, this protein-centered model does not account for many facets of hemostasis in vivo.

Many people recognized that the intrinsic and extrinsic systems could not operate in vivo as depicted by this model. For example, it appears in the cascade model as though the "intrinsic" and "extrinsic" pathways are redundant—each leads to the production of activated factor X (FXa). However, patients with a deficiency of FVIII or FIX (hemophilia A or B) have a severe bleeding tendency, even though they have an intact "extrinsic" pathway. Thus, the two pathways are not functionally redundant. In addition, even though deficiencies of each of the factors in the intrinsic pathway can produce long aPTT values, they have dramatically different risks of hemorrhage. Deficiencies of FXII are not associated with significant hemorrhage [3], deficiencies of FXI might or might not be associated with hemorrhage [4], but deficiencies of factors VIII and IX are consistently associated with hemorrhage [5].

Few, if any, medical professionals would assert that contributions from cells do not play a

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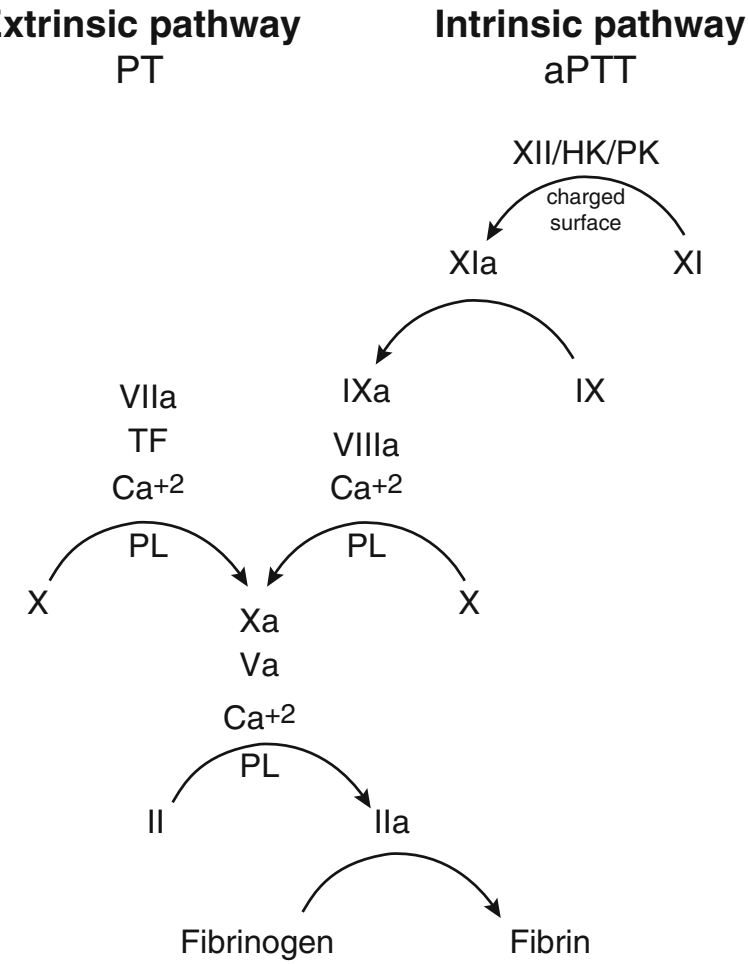

Fig. 1.1 The "extrinsic" and "intrinsic" pathways in the cascade model of coagulation. These two pathways are conceived as each leading to formation of the factor Xa/Va complex, which generates thrombin. *PL* indicates that the reaction requires a phospholipid surface. These pathways are assayed clinically using the prothrombin time (PT) and activated partial thromboplastin time (aPTT), respectively. *HK* high-molecular-weight kininogen; *PK* prekallikrein

role in hemostasis. However, we sometimes still behave as though we think that the results of coagulation screening tests are predictive of the risk of clinical bleeding. The purpose of this chapter is to highlight the roles of cells in localized hemostasis and introduce concepts of how hemostasis can become impaired in vivo.

Cell-Mediated Hemostasis

The key concept underlying the paradigm of "cell-mediated hemostasis" is that cells play active roles in regulating and localizing the coagulation reactions. The complement of receptors and other features of the surfaces of cells are critical to defining the roles of specific cell types in hemostasis. Many cells can participate in hemostasis and thrombosis, but the two critical players are platelets and endothelial cells. Localization of thrombin generation is critical in preventing the spread of clot formation to sites where it is damaging rather than protective. Platelets not only provide the surface on which procoagulant reactions take place, but they also control the rate and localization of thrombin production by adhering specifically to the site of injury. Endothelial cells, on the other hand, have several mechanisms by which they are actively antithrombotic, thus preventing the propagation

of clotting from a site of injury throughout the vascular system. A failure of cell-mediated regulation or localization can lead to failures of normal hemostasis, even when the protein components are within normal ranges. This concept seems to be particularly relevant to understanding the mechanisms of bleeding and thrombosis that are induced by trauma. The cell-based experimental model of coagulation [6–8] in combination with existing literature led to the hypothesis [9] that hemostasis occurs as overlapping steps, regulated by cellular components in vivo, as outlined below.

Step 1: Initiation of Coagulation on TF-bearing Cells

The goal of hemostasis is to produce a platelet and fibrin plug to seal a site of injury or rupture in the blood vessel wall. This process is initiated when TF-bearing cells are exposed to blood at a site of injury. Tissue factor (TF) is a cofactor that binds to and enhances the activity of its partner protease, FVIIa. TF is structurally related to cytokine receptors, and unrelated to any coagulation factor [10]. This lineage emphasizes the close evolutionary and physiologic links between the coagulation system and components of the host response to injury. TF is a transmembrane protein [11] that is expressed by cells around the outside of blood vessels, as well as epithelia, organ capsules, brain and heart [12]. TF is normally not expressed by cells in contact with the blood. This distribution of TF has been described as a "hemostatic envelope" that can rapidly activate coagulation upon injury to vessels or critical organs.

The TF around vessels is bound to FVII even in the absence of injury [13]. Once bound to TF, zymogen FVII is rapidly activated by autoactivation or by low concentrations of FXa, FIXa or FVIIa [14]. The FVIIa/TF complexes catalyze activation of small amounts FX and FIX, even in the baseline state. The ongoing generation of small amounts of activated factors means that the coagulation system can be rapidly activated when injury allows blood to enter the extravascular space [15]. Most of the coagulation factors are small enough to leave the vasculature and reside at low levels in the extravascular space, and small amounts of their activation peptides are found in the lymph [16]. However, this baseline activation of coagulation factors is kept separated from other key components of hemostasis by an intact vessel wall. The very large components of the coagulation process are platelets and FVIII bound to multimeric von Willebrand factor (vWF). These components enter the extravascular compartment when an injury disrupts the vessel wall. Escape from the vascular space allows platelets and FVIII-vWF adhere to collagen and other matrix components at the site of injury.

The FXa formed on the TF-bearing cell interacts with its cofactor FVa to form prothrombinase complexes and generate a small "priming" amount of thrombin on the TF cells (Fig. 1.2). Experiments using a cell-based model have shown that minute amounts of thrombin are formed in the vicinity of TF-bearing cells exposed to plasma concentrations of procoagulants, even in the absence of platelets. The small amounts of FVa required for prothrombinase assembly on TF-bearing cells can be activated by FXa [17] or by non-coagulation proteases produced by the cells [18]. However, thrombin generation during initiation of coagulation in vivo is predominantly supported by the release of FV(a) from platelets that adhere to the site of injury [19]. Platelets release a distinct form of FV that has been taken up from plasma and processed to make it active as a procoagulant and resistant to inactivation by Protein C [20]. Thus, a collaboration between the TF-bearing cells and "first responder" platelets leads to the production of the small amount of thrombin that is critical to the Amplification Phase.

The factors Xa and IXa formed on TF-bearing cells have very distinct and separate functions in blood coagulation [7]. The activity of FXa formed by the FVIIa/TF complex is restricted to the TF-bearing cell because FXa that dissociates from the cell surface is rapidly inhibited by tissue factor pathway inhibitor (TFPI) or antithrombin

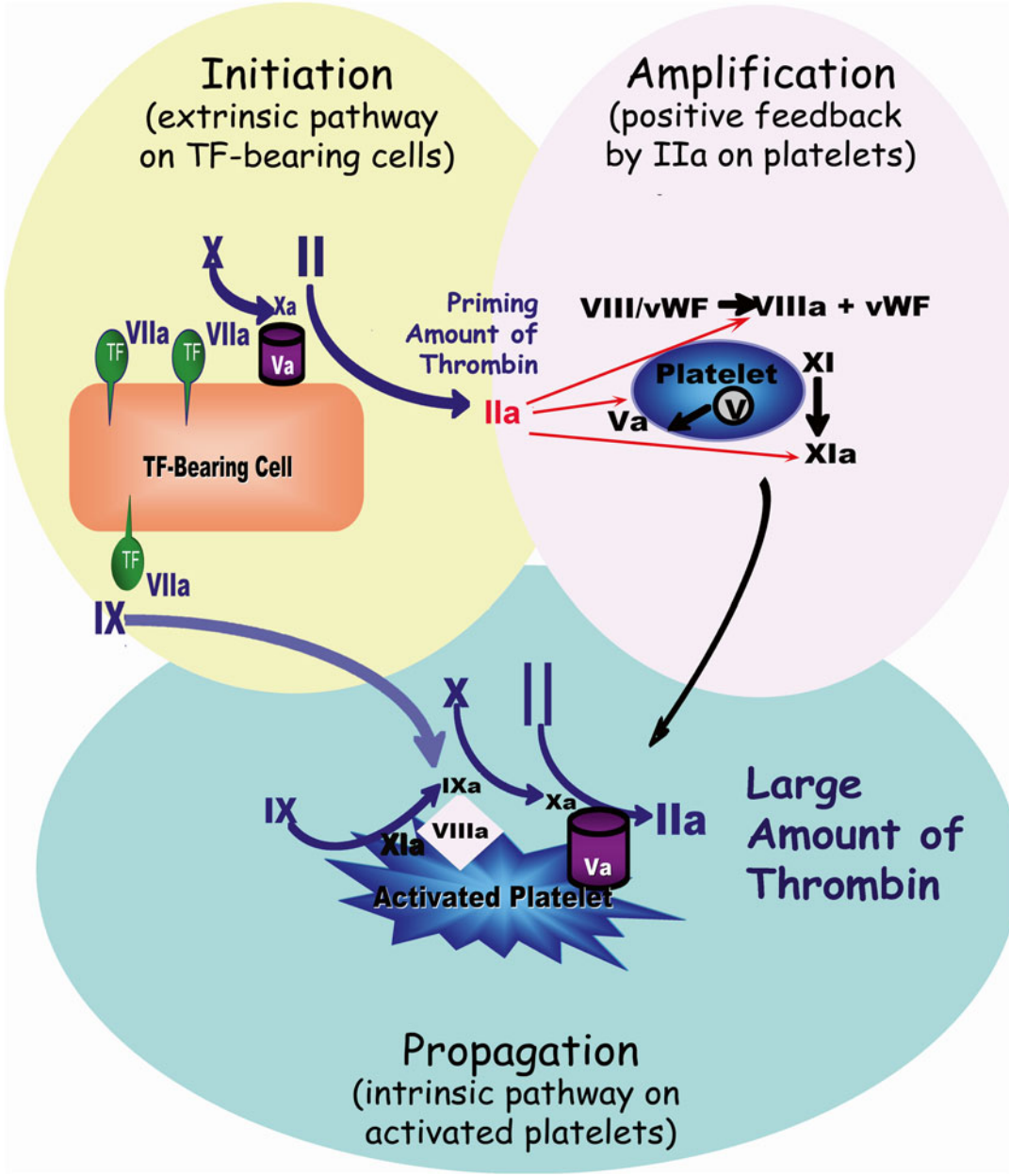

Fig. 1.2 Steps in a cell-based model of coagulation. Initiation occurs on the TF-bearing cell as activated FX combines with its cofactor, FVa, to activate small amounts of thrombin. The priming amount of thrombin generated on the TF-bearing cell amplifies the procoagulant response by activating cofactors, factor XI and platelets. The large burst of thrombin required for effective hemostasis is formed on the platelet surface during the propagation phase. Modified from [46]

(AT) in the fluid phase (Fig. 1.3). In contrast, FIXa can diffuse through the fluid phase because it is not inhibited by TFPI and is inhibited much more slowly by AT than is FXa. FIXa that reaches the surface of nearby activated platelets can bind to a specific receptor [21], and participate in platelet surface thrombin generation during the Propagation Phase.

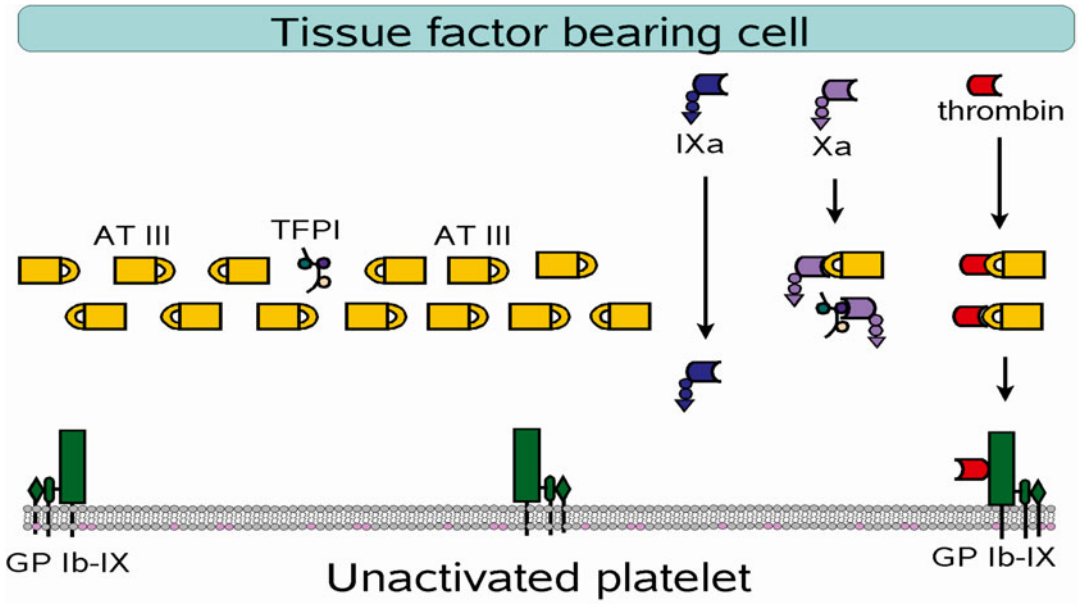

Fig. 1.3 The coagulation inhibitors, antithrombin (AT) and tissue factor pathway inhibitor (TFPI) provide a major barrier to the movement of activated coagulation proteases between cell surfaces. Thus, they tend to localize procoagulant activity to the surface on which it was formed. However, because FIXa has a greater ability to diffuse between cell surfaces, since it is not inhibited by TFPI and is much more slowly inhibited by AT than are thrombin (IIa) or FXa

Step 2: Amplification of the Procoagulant Signal by Thrombin Generated on the TF-Bearing Cell

Binding of platelets to the site of injury leads to partial platelet activation. This is probably initiated by binding to collagen in the extracellular matrix. As platelets are activated they release adenosine diphosphate (ADP) from their dense granules, which potentiates activation of additional platelets. Thrombin is a potent platelet activator both through glycoprotein (GP) Ib and the protease activated receptor (PAR)-1 [22–24]. PAR-4 also contributes to platelet activation by thrombin [23]. Simultaneous engagement of platelet receptors by both collagen and thrombin leads to the activation of platelets, yielding a highly procoagulant state [25]. These platelets have been referred to as COAT (COllagen And Thrombin stimulated) platelets [26]. The greatest procoagulant activity is likely generated on the first platelets to arrive at the site of injury; those that have bound to collagen matrix and also been exposed to thrombin. Once the exposed collagen matrix is covered by a platelet/fibrin layer, additional platelets that accumulate would not be activated to the "COAT" state—thus damping down the coagulation process as the area of the wound is covered by a hemostatic clot.

The activated platelets initially provide a surface for the feedback activation of coagulation factors V, VIII and XI by thrombin and amplification of the initial procoagulant stimulus. GPIb not only acts as a receptor mediating thrombin activation of platelets, but it also serves as a scaffold that binds FVIII-vWF and FXI [27] and facilitates their activation by thrombin. The activation of FXI by thrombin explains why FXII is not essential for hemostasis [28]. Although the small amount of thrombin generated during the Initiation Phase may not be sufficient to clot fibrinogen, it is sufficient to "prime" the clotting system for a subsequent large burst of platelet surface thrombin generation by: (1) fully activating platelets; (2) activating FV; (3) activating FVIII and dissociating it from vWF; and (4) activating FXI.

Step 3: Propagation of Thrombin Generation on the Platelet Surface

Platelets play a major role in localizing clotting reactions, since they adhere and aggregate at the sites of injury where TF is also exposed. They provide the primary surface for generation of the burst of thrombin needed for effective hemostasis during the Propagation Phase of coagulation. Once platelets are activated, the cofactors FVa and FVIIIa are rapidly localized on the platelet surface [6]. As noted above, the FIXa formed by the FVIIa/TF complex can diffuse through the fluid phase and also bind to the surface of activated platelets. FXI that was activated by the "priming" amount of thrombin can activate more FIX to IXa; supplementing the initial activation of FIX provided by FVIIa/TF. Once the platelet "tenase" (FIXa/FVIIIa) complex is assembled, FX from the plasma is activated to FXa on the platelet surface. FXa then associates with FVa to support a burst of thrombin generation of sufficient magnitude to produce a stable fibrin clot. The fibrin clot enmeshes and stabilizes the initial platelet plug for the time required for tissue repair to take place.

The large amount of thrombin generated on platelet surfaces is responsible for stabilizing the hemostatic clot in more ways than just initiating fibrin polymerization. In fact, most of the thrombin generated during the hemostatic process is produced after the initial fibrin clot is formed [29]. The burst of thrombin also stabilizes the clot by: (1) activating FXIII [30], which then crosslinks fibrin monomers; (2) activating thrombin-activatable fibrinolysis inhibitor (TAFI) [31], which reduces plasmin activation on the fibrin clot; (3) cleaving the platelet protease activated receptor (PAR)-4 receptor [32], which requires a higher concentration of thrombin than does PAR-1 [33]; and (4) being incorporated into the structure of the clot, where it can rapidly reactivate the coagulant response if the initial clot is disrupted.

Even though each phase of the cell-based model has been described as a discrete event, they should be viewed as an overlapping continuum. For example, thrombin produced on the platelet surface early in the propagation phase may initially cleave substrates on the platelet surface and continue to amplify the procoagulant response, in addition to leaving the platelet and promoting fibrin assembly. The cell-based model of coagulation shows us that the "extrinsic" and "intrinsic" pathways are not redundant. Let us consider the "extrinsic" or pathway to consist of the FVIIa/TF complex working with the FXa/Va complex; and the "intrinsic" pathway to consist of FXIa working with the complexes of factors VIIIa/IXa and factors Xa/Va. The "extrinsic" pathway operates on the TF-bearing cell to initiate and amplify coagulation. By contrast, the "intrinsic" pathway operates on the activated platelet surface to produce the burst of thrombin that leads to formation and stabilization of the fibrin clot.

What Controls Coagulation and Stops it Once it Gets Started?

Localizing production of thrombin to specific cell surfaces is critical to preventing uncontrolled coagulation leading to thrombosis or disseminated intravascular coagulation (DIC). Receptors on cells play an important role in localization. In addition, several plasma proteins play critical roles in limiting the coagulation process and restricting large-scale thrombin generation to the surface of activated platelets. These include antithrombin (AT, previously called antithrombin III), tissue factor pathway inhibitor (TFPI) and the Protein C/S system. AT and TFPI are important in localizing the activity of coagulation complexes to specific cell surfaces and preventing coagulation activation in the circulation and on endothelial surfaces. Protein C and Protein S are important in inactivating procoagulant complexes on endothelial surfaces.

AT is an inhibitor, not only of thrombin, but also of all of the coagulation proteases. Its effectiveness as an inhibitor is enhanced in the presence of heparin-like molecules. Proteases on cell surfaces are less susceptible to inhibition by

AT. Thus, the presence of AT in the fluid phase tends to limit the activity of the coagulation factors to the surface on which they were activated. However, AT can exert its effects on specific cell surfaces. The presence of heparan sulfates is thought to localize AT and enhance its activity on endothelial cells, thus contributing to the actively antithrombotic nature of healthy endothelium.

TFPI is a protease inhibitor that is proving to have a quite complex biology [34]. It is a direct inhibitor of FXa, and when in complex with FXa, can also inhibit the FVIIa/TF complex. TFPI is a very effective inhibitor of FXa in solution, and thus tends to limit FXa activity to the surface on which it was formed. Once FVIIa/TF has activated some FXa, TFPI/Xa is thought to act as a negative feedback regulator, and inhibit further FVIIa/TF activity. This would tend to limit the duration of the procoagulant stimulus after TF is exposed to blood. TFPI is also localized to endothelial cells, in addition to circulating in the plasma. One form of TFPI (TFPI-alpha) is localized to endothelial cells by binding to heparan sulfates. TFPI-alpha can directly inhibit prothrombinase activity. A second form (TFPI-beta) is covalently attached to the endothelial surface by glycosylphosphatidylinositol (GPI) linkage. Thus, TFPI isoforms make important contributions to the actively anticoagulant nature of the endothelium, as well as limiting the procoagulant effects of FVIIa/TF.

The Protein C/S system is also very important in preventing activation of coagulation on healthy endothelium. Thrombin is appropriately produced at a site of vascular injury. However, the endothelium has mechanisms to prevent generation of thrombin on its surfaces. Most endothelial cells express the endothelial Protein C receptor (EPCR) and thrombomodulin (TM)—both transmembrane proteins. As illustrated in Fig. 1.4, thrombin which diffuses away from a site of injury, and onto healthy endothelium, binds to TM. Protein C from the plasma binds to EPCR where it is activated by the thrombin/TM complex. Activated Protein C (APC), in concert with its cofactor Protein S, cleaves and inactivates FVa and FVIIIa (not shown in Fig. 1.4), thus preventing additional generation of activated factors on normal endothelial surfaces. Components of the Protein C system can also play a role in derangements of the coagulation system. For

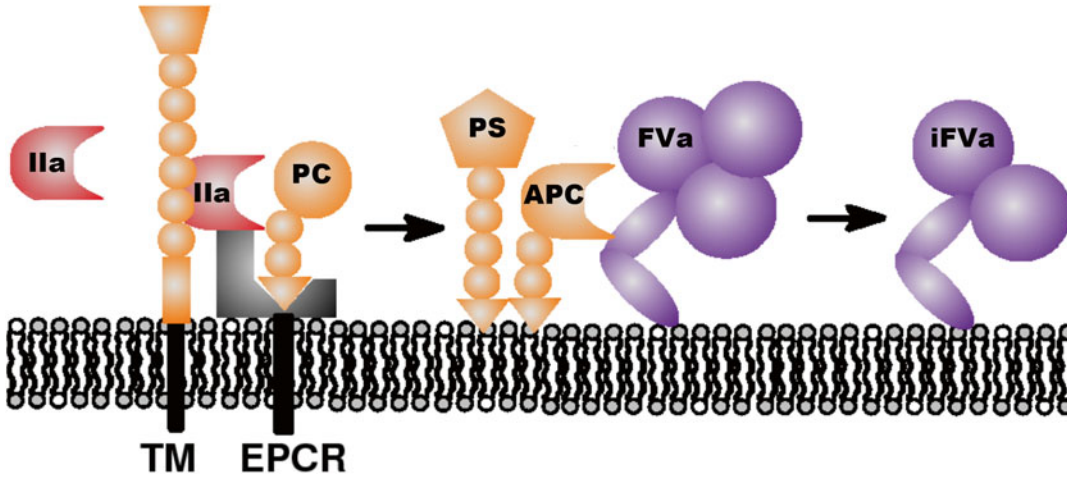

Fig. 1.4 The Protein C system has an antithrombotic effect by preventing activation of thrombin on endothelial surfaces. Thrombin (IIa) that reaches the surface of healthy endothelium binds to thrombomodulin (TM) which allows it to activate Protein C (PC) that has bound to the endothelial protein C receptor (EPCR). Activated Protein C (APC) in concert with its cofactor, Protein S (PS), cleaves and inactivates FVa and FVIIIa (not shown). This effectively prevents assembly of procoagulant complexes on normal endothelial surfaces

example, levels of both EPCR and TM on the endothelial surface are reduced as a consequence of inflammation, thus predisposing to thrombosis [35, 36]. By contrast, systemic activation of Protein C appears to play a role in the early coagulopathy of trauma which will be discussed in detail in Chapter 6 [37].

Many have considered the Protein C system to be the mechanism by which hemostatic coagulation reactions are terminated. Certainly extension of coagulation through the vascular tree is limited by the action of APC, as well as by AT and TFPI. However, it seems more likely to us that cleavage of cofactors by APC is not the mechanism for ending thrombin generation on platelet surfaces. This is because there is not a good mechanism to localize APC to platelets as there is to endothelial cells, and because FVa on platelet surfaces is resistant to inactivation by APC [20, 38]. It seems more likely that hemostatic coagulation is terminated, at least in part, by "paving over" the highly procoagulant "COATed" platelets that are bound to collagen, with layers of less highly procoagulant platelets. Therefore, we think of the Protein C system under normal conditions as being a relatively endothelial-specific antithrombotic mechanism, rather than an anticoagulant.

Fibrinolysis

Fibrinolysis is essential for removal of clots during the process of wound healing as well as for removing intravascular clots that might otherwise be manifest as thrombosis. Intravascular deposition of fibrin is also associated with the development of atherosclerosis. Therefore, an effective fibrinolytic system tends to protect against the chronic process of atherosclerotic vascular disease as well as the acute process of thrombosis. Conversely, defects of fibrinolysis increase the risk of atherothrombotic disease. For example, elevated levels of plasminogen activator inhibitor-1, an inhibitor of fibrinolysis, are associated with an increased risk of atherosclerosis and thrombosis [39] as are decreased levels of plasminogen [40]. Thus, effectiveness of hemostasis in vivo depends not only on the procoagulant reactions, but also on the fibrinolytic process. In the case of trauma, the coagulation reactions are in a race with the fibrinolytic process to form a clot stable enough to persist until healing can occur. In some cases this situation can be detected as a systemic hyperfibrinolytic state on whole blood testing, such as with the thromboelastogram. However, this situation is more often local, with plasmin activation occurring primarily at the site of injury and not necessarily being detectible in the blood. For further reading on the mechanism of Plasmin/Anti-plasmin system please refer to Chapter 3 and pathologic fibrinolysis from trauma in Chapter 9.

What Does All this Mean for Clinical Laboratory Testing?

It should be clear from the preceding discussion that commonly used clinical coagulation tests do not really reflect the complexity of hemostasis in vivo. That does not mean that the PT and aPTT are useless. We just need to understand their limitations. These "screening" coagulation tests are abnormal when there is a deficiency of one or more of the soluble coagulation factors. They do not tell us what the risk of clinical bleeding will be. As noted before, two patients with identical aPTT values can have drastically different risks of hemorrhage (XII vs XI vs IX vs VIII). All of our common coagulation tests including the PT, aPTT, thrombin clotting time, fibrinogen levels, and coagulation factor levels tell us something about the plasma level of soluble factors required for hemostasis. Their clinical implications must be evaluated by the physician ordering these tests. Thus, just because the PT and aPTT are within the normal range it does not follow that the patient is at no risk for bleeding. Conversely, a mild elevation in these clotting times does not mean that the patient is at risk for bleeding after an invasive procedure.

Several whole blood ("global") coagulation tests are jockeying for position as a means of evaluating overall hemostatic status in selected

clinical settings. While whole blood tests have the advantage that they may reflect the contributions of platelets and other blood cells to the hemostatic process, they still do not reflect the contributions of the tissue cells and local tissue conditions. Furthermore, the reagents used to initiate the coagulation reactions in these may not be very physiologically relevant. Thus any laboratory test requires skilled interpretation and clinical correlation in evaluating the true risk of bleeding. For further reading on these tests please refer to Chapters 17 and 18.

What Causes Bleeding in Previously Normal Patients?

Many patients that experience significant hemorrhage do not have an underlying bleeding tendency that can be identified prior to a bleeding episode. Bleeding following surgical or accidental trauma is often associated with the development of an acquired coagulopathy. It is important to recognize that mechanical control of bleeding is the first priority in trauma. With continued uncontrolled vessel bleeding the risk of developing coagulopathy continues as resuscitation and replacement with blood products does not fix the underlying cause of the problem, hemorrhage. The hallmark of coagulopathy is microvascular bleeding. In spite of being characterized by bleeding from very small vessels, microvascular bleeding can lead to massive blood loss, which cannot be mechanically controlled. Drivers of coagulopathic bleeding include consumption of coagulation factors and platelets, excessive fibrinolysis, hypothermia, and acidosis.

Consumption of Coagulation Components

We normally think of disseminated intravascular coagulation (DIC) when we talk of consumption. In DIC the normal localization of coagulation fails, and coagulation factors are activated throughout the circulation. This leads to production of microthrombi and also consumption of platelets, coagulation factors and coagulation inhibitors. However, clotting factors and platelets can also be consumed during appropriate physiological attempts at hemostasis. In this case it is appropriate to replace the depleted factors with transfusion therapy.

DIC can be much more complicated to manage [41]. The mainstay of treatment is to treat the underlying disorder, such as sepsis. DIC can also be triggered by tissue damage due to trauma. In early or mild/compensated DIC administration of low dose heparin may be considered to control the procoagulant response to inflammation, infection or malignancy. However, in more severe or advanced DIC replacement therapy may be necessary to treat the bleeding tendency associated with depletion of coagulation factors and platelets. Please refer to Chapter 13 for further reading on DIC management from trauma.

Dilution of Coagulation Components

In the face of significant hemorrhage, replacement of blood loss with non-blood solutions can result in dilution of the coagulation factors, platelets, and inhibitors. Thrombin generating ability is well preserved even when the coagulation factors and inhibitors are diluted by as much as 50 %. For continued reading on techniques to reduce dilution coagulopathy refer to the massive transfusion Chapter 23.

Hypothermia

Many patients become hypothermic during medical illness or following surgical or accidental trauma [42]. Hypothermia can directly interfere with the hemostatic process by slowing the activity of coagulation enzymes. Platelet adhesion and aggregation is also impaired, even in mild hypothermia [43]. Thus, hypothermic patients will have some degree if impairment in platelet function and coagulation enzyme activity even without other causes of coagulopathy.

Acidosis

Acidosis can have an even more profound effect on procoagulant function than hypothermia, though the two metabolic abnormalities often coexist. A drop in the pH from 7.4 to 7.2 reduces the activity of each of the coagulation proteases by more than half [44]. Thus, acidosis should be considered as a possible contributor to coagulopathic bleeding in medical and surgical patients.

What Happens After the Bleeding Stops?

Once hemostasis is completed the process of wound healing begins. The hemostatic plug must be stable enough to maintain hemostasis until healing is well under way, yet be removed so as not to interfere with tissue repair. At this point fibrinolysis is essential, through the action of plasmin in concert with leukocyte proteases. The neutrophils that initially accumulate at a site of injury are replaced over the course of a few days with macrophages that engulf and degrade cellular debris and components of the fibrin clot. The macrophages secrete cytokines and growth factors that facilitate the migration of fibroblasts and endothelial cells into the wound site. In the case of a skin wound, the dermis is replaced by highly cellular and vascular granulation tissue, while the surface epithelium proliferates and migrates from the margins to cover the surface of the wound. Many of the activities involved in wound healing are influenced by thrombin. Thrombin plays a major role in platelet activation and degranulation. Several key cytokines modulating wound healing are released from activated platelets, including transforming growth factor beta (TGFβ1), and platelet-derived growth factor (PDGF). Of course, the amount and rate of thrombin generated during hemostasis influences the initial structure of the fibrin clot—the framework on which cell migration takes place. In addition, thrombin has chemotactic and mitogenic activities for macrophages, fibroblasts, smooth muscle cells, and endothelial cells. Impaired hemostasis leads to a delay in wound healing [45]. Thus, generation of the "right" amount of thrombin during the coagulation process is not only essential for effective hemostasis, but sets the stage for effective wound healing.

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Thrombin-Antithrombin System

2

Susan C. Bock

Introduction to Antithrombin

Antithrombin (AT) is a plasma proteinase inhibitor that inactivates not only thrombin, but also factor Xa and coagulation enzymes (fVIIa-TF, fXIIa, fXIa, fIXa) that collectively mediate the generation of thrombin. Inherited and acquired antithrombin deficiencies cause thrombosis, and the homozygous deletion of AT is lethal. Rates of AT target enzyme inhibition and the associated anticoagulant activities can be accelerated as much 500- to 39,000-fold by cofactor HSPGs (heparin sulfate proteoglycans) and heparins, which are respectively present naturally in the vessel wall, and clinically via the administration of pharmaceutical heparins. The broad coagulation enzyme target specificity of antithrombin and its potentiation by HSPG/heparin enable AT to play a key role in maintaining circulatory system patency and providing protection against thrombosis.

The first section of this chapter reviews current understanding of the mechanisms underlying AT inhibition of coagulation enzymes, heparin/HSPG cofactor activation of AT, and inflammatory inactivation of antithrombin. Then, building on this foundation, AT dysregulation in the context of trauma, tissue injury, blood loss, and resuscitation is addressed. Finally, the outcomes of several AT supplementation studies in trauma patients are reviewed.

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AT is a Serpin and Uses the Serpin Proteinase Inhibitor Mechanism to Inactivate Its Coagulation Enzyme Targets

AT is a serpin (*ser*ine *p*roteinase *in*hibitor) and exhibits sequence, structural, and functional homology to other members of this large gene family [1–3]. AT uses the canonical serpin suicide-substrate inhibition mechanism illustrated on the left side of Fig. 2.1 to form stable, covalent inhibitory complexes with its coagulation enzyme targets. Native AT (Fig. 2.1a) is a globular molecule with a protruding reactive center loop (RCL) (*purple*) containing a substrate-like sequence that serves as "bait" for its target enzymes. Inhibitory complex formation begins when a target enzyme cleaves the scissile P1–P1′ bond (nomenclature of Schechter and Berger [4]) of the RCL to generate an acyl–enzyme complex in which the P1 residue (*purple sphere*) is covalently linked to the target enzyme active site serine, and in which the polypeptides generated by reactive loop cleavage become mobile and are rearranged [5, 6].

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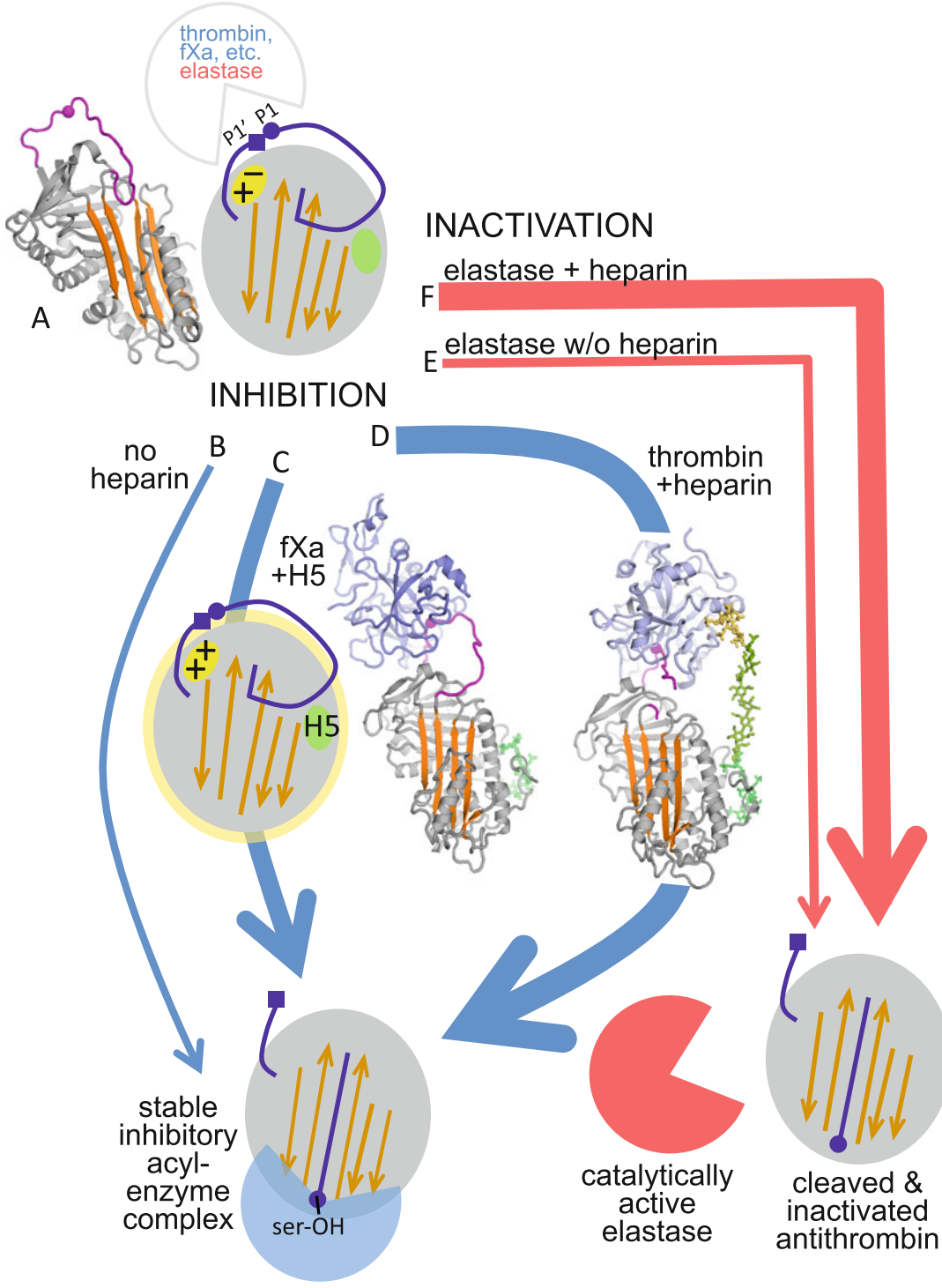
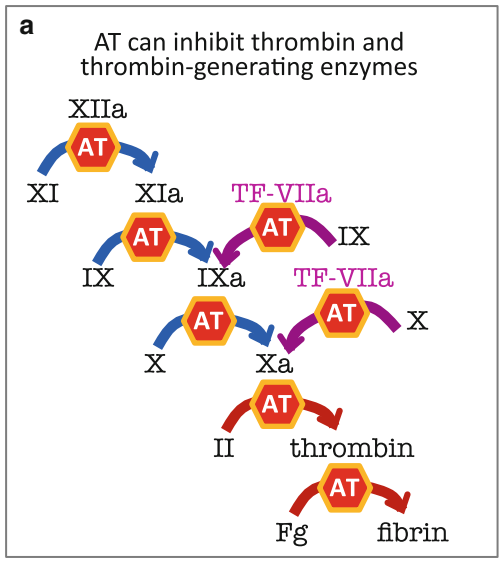


Fig. 2.1 Structural basis of AT inhibition and inactivation mechanisms (A) *Native AT. Left* shows the 2.6 Å 1E04i structure of the native conformation human antithrombin which circulates in blood. Cartoon on the *right* illustrates functionally important structural features. Reactive center loop (*purple*) is bound by the active sites of target enzymes which cleave the RCL at its P1 residue (*purple sphere*). AT central β-sheet A is *orange*. Note that in contrast to other serpins, the N terminus of the AT RCL is partially incorporated into β-sheet A of the native conformation. The *yellow* and *green* patches mark locations of AT fXa exosite and heparin pentasaccharide binding sites, respectively. (**B**) *Cofactor-independent, progressive AT inhibition of its target enzymes.* In the absence of heparin and HSPG cofactors, AT inhibits its targets via the formation of stable, covalent AT-target enzyme complexes like the

As illustrated in the cartoon representation of the stable inhibitor acyl-enzyme complex on the bottom left of Fig. 2.1, the P1-containing polypeptide incorporates into central β-sheet A (*orange*) of the inhibitor, translocating the covalently bound target enzyme approximately 70 Å to the opposite pole of the serpin, and initiating critical protein conformational changes in both molecules. The translocated target enzyme molecule is crushed against the hyperstable serpin molecule in a way that distorts its catalytic triad geometry and prevents deacylation and regeneration of active enzyme.

Although AT uses the canonical serpin inhibition mechanism, it is distinguished from other serpins in several notable ways. A first unusual characteristic at the structural level is the partial insertion of the AT native conformation reactive center loop as a sixth strand at end of the central A β-sheet that is nearest to the RCL (see Fig. 2.1a; most other serpins have entirely external RCLs and the A sheet is 5-stranded at the reactive loop end). Additional distinguishing features of AT relate to functional characteristics: its native conformation inhibits target enzymes at rates that are at least 1000-fold slower than typical serpin inhibition rates, and AT requires cofactor heparin or HSPG (heparan sulfate proteoglycan) binding to increase its target enzyme inhibition rates into the range that is typical for other serpins.

Fig. 2.1 (continued) one depicted in the cartoon at the *arrow head* end of pathway *B*. Inhibitory complexes form upon target enzyme cleavage of P1–P1′ peptide bond of native AT (**A**), which initiates a large protein conformation change wherein the N-terminal polypeptide of the RCL rapidly inserts into AT central β-sheet A as a sixth strand. This translocates the acylated target enzyme to "south pole" of AT, and produces inhibitor and target proteinase conformational changes which distort the enzyme's catalytic triad and prevent deacylation of the covalent linkage between the AT P1 residue and the target enzyme active site serine. Heparin cofactor-independent, progressive rates for AT inhibition of its clotting factor target enzymes are modest compared to rates obtained in the presence of heparin/HSPG cofactors. These differences are symbolized by the widths of blue arrows in pathways *B*, *C*, and *D*, and illustrated quantitatively in Fig. 2.2b. (**C**) *Conformational activation of fXa inhibition by heparin pentasaccharide.* A specific heparin pentasaccharide (H5) component of endogenous HSPGs and pharmaceutical heparins accelerates the rate of AT inhibition of fXa by ~300-fold using the mechanism illustrated in pathway **C.** In the native conformation of AT (**A**), a fXa recognition exosite *(yellow)* underlying the RCL contains elements mediating favorable and unfavorable (**+ −**) interactions with fXa. Binding of H5 pentasaccharide to the *green* heparin binding site induces AT conformational changes that are transmitted across the serpin, and convert the fXa exosite *(yellow)* to a form (**+ +**) that is highly favorable for interaction with fXa. The 3.3 Å 2GD4 structure to the right of the **C** pathway H5-activated AT cartoon image shows the enzyme active site of fXa (*blue*) interacting with the P1 residue (*purple sphere*) of the AT RCL loop, and recognition of the H5-exposed fXa exosite on AT by the enzyme. P1–P1′ cleavage, acylation of fXa, and translocation to form a stable inhibitory complex then proceed as described for progressive AT inhibition in pathway **B**. In summary, heparin pentasaccharide activates AT for inhibition of fXa by inducing a conformational change that leads to favorable interactions between the AT fXa exosite and factor Xa. (**D**) *Heparin approximation-mediated acceleration of AT thrombin inhibition.* H5 pentasaccharide binding to AT increases its rate of thrombin inhibition by less than two-fold. In contrast to the conformational activation mechanism described in pathway **C** for fXa, heparin activation of thrombin inhibition requires pentasaccharide-containing long chain heparins, which accelerate the AT thrombin inhibition rates by ~6000-fold via the bridging mechanism illustrated in pathway *D*. The 2.5 Å 1TB6 AT-thrombin-heparin ternary complex is shown. One end of the long heparin molecule (*light green*) binds to the pentasaccharide binding site of antithrombin, while its other end (*yellow*) binds to a heparin-binding exosite on thrombin (*blue*). Thus, cofactor bridging of AT and thrombin promotes interaction of thrombin's active site and the AT RCL, cleavage of the P1–P1′ bond, initiation of the serpin inhibitory conformational change and formation of a stable inhibitory complex as previously described. **(E, F)** *Neutrophil elastase-mediated inflammatory inactivation of AT in the absence and presence of heparin.* Neutrophil elastase cleaves at the P5–P4 peptide bond of the AT RCL with out forming a stable E–I complex. Instead, AT is converted to a thermodynamically stable 6-stranded cleaved conformation that is devoid of inhibitory activity because the RCL has been cleaved. Relative arrow widths of the *E* and *F* pathways indicate that elastase inactivation of antithrombin occurs more rapidly in the presence of heparin. Neutrophil elastase has a heparin binding site which bridges elastase and AT according to an approximation mechanism that is similar to the one illustrated in pathway *D* for long chain heparin activation of AT thrombin inhibition.



b Second-order association rate constants ($M^{-1}S^{-1}$) for inhibition of clotting enzymes by AT in the absence (uncat) and presence of unfractionated heparin (UFH)

target enzyme	k_{uncat}	k_{UFH}	fold increase
thrombin	7,700	47,000,000	6,100
fXa	2,600	6,600,000	2,500
fIXa	10	390,000	39,000
fXIa	360	180,000	500
fXIIa	39	66,000	1,700
fXIIa/TF/Ca^{++}	33	15,000	500

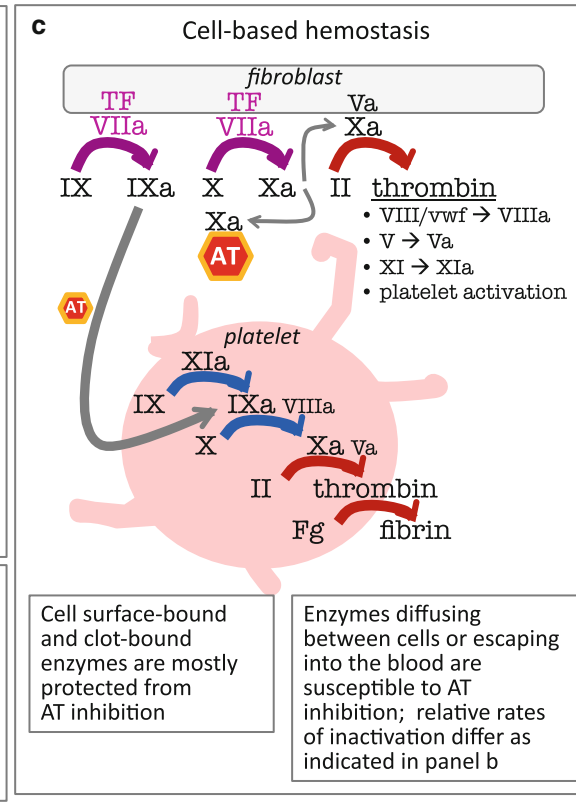


Fig. 2.2 AT inhibition of clotting enzymes in vitro and in a cell-based model of hemostasis (**a**) In vitro inhibition of coagulation enzymes by AT. Studies using plasma-based coagulation assays and purified proteins have established the in vitro inhibition of thrombin—and the extrinsic, intrinsic, and common pathway coagulation enzymes that generate thrombin—by AT. (**b**) Uncatalyzed and heparin cofactor-dependent rates for AT inhibition of thrombin, factors Xa, IXa, XIa, XIIa, and TF-VIIa. Note inactivation rates for different enzymes vary over ~3 orders of magnitude, both in absence and in the presence of heparin, and that there are also large differences in the magnitude of acceleration by heparin. Data from Olson et al. [19]. (**c**) AT in the regulation of cell-based hemostasis. The drawing highlights features of the Hoffman cell-based model of hemostasis exhibiting various degrees of sensitivity to AT inhibition. *Left side,* Factor IXa generated on TF-bearing cells during the initiation phase is inefficiently inhibited by AT during diffusion to platelets and accordingly available to contribute to the formation of additional tenase (fIXa-VIIIa) complexes on the platelet surface. *Center,* In contrast, AT (and TFPI) effectively inactivate fXa produced by and released from fibroblasts. *Right side,* Factor Xa remaining on fibroblasts associates with Va to form prothrombinase (fXa-Va). The prothrombinase generates thrombin for the amplification phase, wherein platelets are activated and tenase and prothrombinase complexes assemble on their surfaces. Robust generation of thrombin and fibrin follows during the propagation phase

AT Inhibits a Broad Spectrum of Coagulation Enzymes

The name "antithrombin" emphasizes that AT inhibits thrombin but is somewhat misleading in implying that AT is primarily an inhibitor of thrombin. In fact, AT has broad inhibitory activity against a broad range of coagulation enzymes including thrombin, fXa, TF-VIIa, fIXa, fXIa, and fXIIa (see Fig. 2.2a). Therefore AT regulation of hemostasis may occur at several levels, including thrombin-mediated fibrin clot formation, common pathway fXa-mediated thrombin generation, and effects on coagulation factors that are higher up in the intrinsic and extrinsic pathways. AT inhibition of thrombin may also

serve to regulate thrombin's non-coagulant functions, including platelet activation and vascular cell signaling and proliferation.

AT Inhibition of Its Target Enzymes is Accelerated by HSPGs and Heparin

As discussed earlier, the native conformation of AT is a considerably less efficient proteinase inhibitor than are most other members of serpin family. Rate constants for target proteinase inhibition by most serpins are in the 10^6–10^7 M^{-1} s^{-1} range. However, AT inhibits its target enzymes at rates that are about three orders of magnitude slower than typical serpins. Unlike other serpins, which have fully exposed reactive center loops, native ATIII circulates with the amino-terminal end of its reactive center loop partially inserted into its central A β-sheet [7] (see Fig. 2.1a). RCL insertion was originally thought to reduce AT inhibition rates by limiting target enzyme access to the arginine-393 P1–P1′ scissile bond [8]. However, it is now appreciated that the slow, cofactor-independent, "progressive" inhibition rates of native antithrombin in the absence of heparin (pathway **B** in Fig. 2.1) reflect the unfavorable presentation of the fXa-binding exosite (*yellow* in Fig. 2.1a). In native AT, the fXa exosite is comprised of elements that engage positively *and negatively* with fXa, (**+ −**) and therefore the rate for inhibition of fXa is low [9, 10].

Conformational Activation of fXa Inhibition by Heparin

Pathway **C** of Fig. 2.1 illustrates the mechanism for heparin activation of AT fXa inhibition. The native conformation of AT (**A**) bears a fXa recognition exosite (*yellow*) which is located under the RCL. This native conformation exosite contains elements mediating favorable and unfavorable (**+ −**) interactions with fXa. Binding of heparin pentasaccharide (H5) to the AT heparin binding site (*green*) [11] induces long range allosteric conformational changes that are transmitted across the serpin [12, 13] and expose favorable exosite features (**+ +**) which promote interaction with fXa by minimizing the repulsive interactions which were present in the native conformation exosite [10, 14–16]. Therefore, heparin pentasaccharide activates AT inhibition of fXa by inducing a conformational change that leads to favorable interactions between the AT fXa exosite and factor Xa, and accelerates the rate of inhibition by ~300 fold.

Heparin Approximation-Mediated Acceleration of AT Thrombin Inhibition

Pathway **D** of Fig. 2.1 illustrates the bridging mechanism responsible for heparin activation of AT thrombin inhibition. H5 pentasaccharide binding to AT increases its rate of thrombin inhibition insignificantly (<twofold). In contrast to the conformational activation mechanism described in pathway **C** for fXa, heparin activation of thrombin inhibition requires pentasaccharide-containing heparins of greater than 18 sugar units in length, which accelerate the rate of inhibitory complex formation by several thousand fold using an approximation mechanism wherein different regions of the same extended heparin chain bind to the pentasaccharide binding site on ATIII and to anion binding exosite II on thrombin [17, 18] as is illustrated in pathway D of Fig. 2.1, using the 2.5 Å 1TB6 structure of the AT-thrombin-heparin ternary complex. The bottom end of the long heparin molecule (*light green*) binds to the pentasaccharide binding site of antithrombin, while its top end (*yellow*) binds to anion -binding exosite II on thrombin (*blue*).

Progressive and Heparin Cofactor Activated Inhibition of Coagulation Enzymes by AT

Figure 2.2b presents a comparison of the rates at which AT inhibits six different coagulation enzymes under cofactor-independent, progressive (uncat) conditions, and heparin cofactor-activated conditions (UFH) [19]. As previously noted, AT uncatalyzed progressive inhibition rates for all of its coagulation enzyme targets are slow in comparison to those measured for other members of the serpin inhibitor family. However, the AT inhibition rates are significantly accelerated by heparin cofactor binding, and approach

the theoretical limits for serpins in the cases of thrombin and fXa.

AT Activation by Endogenous HSPGs

Heparin pentasaccharide-bearing HPSGs are the naturally occurring endogenous cofactor for AT activation. Small amounts of AT-binding HSPGs are present in the glycocalyx on the luminal side of the endothelium. On the abluminal side, the subendothelial basement membrane and extracellular matrix contain much greater concentrations of these cofactor molecules [20]. The observed asymmetrical distribution suggests that AT molecules bound and activated on luminal HSPGs may provide a basal level of scavenger inhibition activity against activated coagulation enzymes exposed to the endothelium, and that the magnitude of anticoagulant activity will be dramatically increased in the proximity of injured regions where endothelial damage liberates larger underlying pool of abluminal HSPGs and their associated bound and activated ATs.

The generation of increased anticoagulant activity via HSPG release and AT activation at sites of endothelium injury seems initially surprising in view of the need for hemostasis in this context. However, this mechanism for release of anticoagulant activity into the fluid phase of the blood may contribute to keeping injured vessels patent and capable of sustained systemic circulation, while platelet-based hemostatic mechanisms proceed in the vessel wall with little anticoagulant interference from AT as discussed in the section on cell-based hemostasis and Fig. 2.2, panel c. The physiologic importance of AT interactions with endogenous HSPGs is supported by the occurrence of lethal thrombosis in mice homozygous for an AT mutation that reduces binding to heparin/HSPG [21].

AT Role in Cell-Based Hemostasis

It is now recognized that in vivo hemostasis is more complex than the simple convergence of extrinsic and intrinsic enzymatic cascades into the common pathway (Fig. 2.2a). In a living organism, the individual enzymatic steps take place on distinct cell surfaces, which allows for regulation of hemostasis via spatial compartmentalization. Hoffman et al. have proposed a cell-based model of in vivo hemostasis that is organized into three phases: an early *initiation* phase that takes place on the surface of tissue factor (TF) bearing cells, an *amplification* phase that takes place on unactivated platelet surfaces, and a final *propagation* phase that takes place on the surface of activated platelets (for further details please refer to Chap. 1). The essentials of cell-based hemostasis [22–24] are reviewed below with special focus on AT contributions to regulation of the process.

As depicted at the top of Fig. 2.2c, injury exposes circulating fVII to tissue factor (TF) on fibroblasts, which results in bound and activated TF-VIIa that converts fX to fXa (central position on fibroblast). The activity of the generated fXa is restricted to the surface of the TF-bearing fibroblast; fXa molecules that dissociate and diffuse into the blood are rapidly inhibited by AT. As shown on the right side of the fibroblast, fXa molecules remaining on the fibroblast surface combine with fVa to produce prothrombinase and small amounts of enzymatically active thrombin, which plays several important roles during the ensuing amplification phase. Before proceeding to discuss amplification, it is additionally noted that fibroblast surface TF-VIIa also converts fIX to fIXa (left side of fibroblast), and that this source of fIXa will provide an important boost to thrombin and fibrin generation during the propagation phase.

During the amplification phase of cell-based hemostasis, platelets and factors V, VIII, and XI are activated by a small number of thrombin molecules, which were produced on TF-bearing fibroblasts and were able to escape AT inhibition before encountering their substrates. Active tenase (fIXa/VIIIa) and prothombinase (fXa/Va) complexes assemble on the activated platelets (Fig. 2.2c, center).

Upon assembly of the tenase and prothrombinase complexes, activated platelets bound to the exposed fibroblasts and collagen are now primed for the propagation phase of hemostasis, during which efficient and robust generation of throm-

bin occurs and catalyzes the formation of a fibrin network that physically stabilizes the clot. Factor Xa generated by the fIXa-VIIIa tenase on the activated platelet is protected from inhibitors, and can move directly into prothrombinase complexes with fVa. Prothrombinase production on the surface of activated platelets is further boosted by additional tenase complexes formed from fIXa produced by fibroblast TF-VIIa [25]. Due to relatively inefficient AT inhibition of fIXa (Fig. 2.2b), active fIXa molecules generated during the initiation phase are able to escape inhibition during diffusion from the fibroblast to the platelet. The additional fIXa assembles into "extra" tenase complexes on the activated platelets, boosts downstream prothrombinase production, and contributes to robust thrombin and fibrin network generation and to growth as well as stabilization of the clot.

Propagation continues in the proximity of the clot whilst thrombin and procoagulant factor concentrations remain sufficient to activate additional platelets and to support tenase and prothrombinase assembly and thrombin and fibrin generation at sufficient levels.

Thrombin and fIXa and fXa molecules not trapped within the clot or binding to platelet surfaces may escape into the blood circulation where they will be inhibited by plasma AT at progressive (heparin-independent) rates. Progressive inhibition by plasma AT (~2.4 μM (140 μg/mL) [26]) is usually sufficient to prevent the nonproductive, systemic activation and consumption of coagulation factors and thrombosis. However, as discussed below, the AT and other endogenous anticoagulant systems may be overwhelmed in the contexts of severe trauma or inflammation.

Inflammatory Inactivation of AT

As discussed previously and illustrated on the *left* side of Fig. 2.1, the serpin inhibition mechanism used by AT begins with target enzyme recognition and cleavage of the P1–P1′ linkage in the exposed reactive center loop. However, instead of being released from the enzyme upon cleavage of the RCL polypeptide, large protein conformational changes in both the inhibitor and its target proteinase occur, and lead to formation of a stable covalent AT-target proteinase inhibitor complex. Accordingly, an *intact reactive center loop* is absolutely necessary for the serpin protease inhibitor mechanism to work, and non-target proteinase cleavages of the RCL, which cause the conformational change to occur prematurely, destroy the protease inhibitor functionality of serpin [27].

In the contexts of injury and inflammation, neutrophil elastase is released into the tissues systemically and cleaves the P5–P4 peptide bond of the AT RCL [28, 29]. For further details on neutrophils and inflammation in trauma, please refer to Chap. 10. Elastase cleavage converts AT to the inactivated conformation illustrated at the bottom of pathways **E** and **F** in Fig. 2.1. The inactivation arrow of pathway **F** is larger than that for pathway **E** because heparin accelerates the inactivation of AT using a heparin bridging mechanism. Neutrophil elastase has a heparin binding site, therefore as in the case of thrombin (pathway D), heparin approximation increases the enzyme and inhibitor association rate, but in this case results in AT cleavage and inactivation, instead of inhibition [30].

AT and Thrombosis

AT inhibits thrombin and the enzymes that generate thrombin. The complete absence of antithrombin is lethal [31], while partial reductions of AT permit over-activity of the coagulation system, and the increase the risk of thrombosis. Heterozygous AT deficiencies in mice [32] and in humans [33, 34] increase risk for renal and venous thrombosis, respectively. For further description of the clinical presentation, diagnosis and management of AT deficiencies, please refer to Chap. 27.

Acquired AT reductions may also occur as a consequence of trauma, sepsis, burns, malignancies, extracorporeal circulation, and surgery. In these contexts, which are frequently accompanied by inflammation, AT deficiency is associated with consumptive coagulopathies.

Functional AT activity declines because of stoichiometric consumption during neutralization of activated coagulation enzymes and inflammatory cleavage and inactivation of AT. The remainder of this chapter focuses on AT dysregulation in the settings of trauma and trauma induced coagulopathy.

Dysregulation of Antithrombin in Trauma

Trauma disrupts the physiological equilibrium between endogenous procoagulant and anticoagulant systems, including antithrombin. AT reduction in trauma is due to its stoichiometric consumption during the inhibition of activated clotting enzymes, its cleavage and inactivation by neutrophil elastase, and auto-heparinization secondary to endothelial glycocalyx shedding. For further description of endothelial glycocalyx shedding seen in trauma, please refer to Chap. 7. The immediate post-trauma drop in plasma AT activity may be followed by a gradual restoration, which is associated with better outcomes, or by persisting low levels of AT, which are associated with poor prognosis.

Reduction of Plasma AT Activity During Trauma

The first studies of trauma induced acquired AT deficiencies were conducted in the 1980s and early 1990s and used chromogenic substrate assays to measure plasma antithrombin activity [35–38]. These studies noted greater reductions in plasma AT activity (into the range of 50–60 % of normal human plasma values) for more severe cases of trauma. The gradual restoration of AT activity was also observed to associate with better outcomes and survival. In contrast, persisting low AT was associated with unfavorable outcomes, including acute respiratory distress syndrome (ARDS), sepsis and death, [35, 36] as well as limb loss after reconstructive vascular surgery due to thrombosis [38].

Kinetics of Plasma AT Activity Loss and Recovery Following Trauma

A variety of different blood sampling schedules have been used for studies of AT in traumatic injury. The first sample may be obtained at the accident scene, or not collected until up to 12 h after hospital admission. During the first 24 h of hospitalization, blood may be drawn multiple times, or only once, and after that, at daily or several-day intervals during follow up periods ranging from 1 to 3 weeks. In addition to sampling-time protocol differences, critical care interventions, including resuscitation, hemodilution, and heparin administration, may also affect measurements of plasma AT activity. Nevertheless, despite the above caveats, a general overall pattern of AT depletion and recovery emerges.

A study of 30 patients in which blood samples were drawn before primary resuscitation at the scene of the accident [39] showed that plasma AT activity begins to drop immediately upon injury. In patients with minor injuries [injury severity score (ISS) 9–17], AT activity did not fall below 70 % for the first sample, however, for severe (ISS >18) and very severe (ISS >32) injuries, the reduction in AT activity was more pronounced, and fell into the 60 % or lower range.

At 6 h after hospital admission, AT activity increased in all groups and reached into the normal range (80–120 % of NHP) for patients with ISS <32. AT activity for the severely injured group remained in the 50–70 % range. Other studies with extensive serial sampling of trauma patients are in general agreement with the pattern described above, [35, 40–43] although the inflection point when plasma AT activity begins to increase again may occur on day 1 or day 2.

Temporal Correlation of Plasma Thrombin-Antithrombin and Thrombelastography (TEG) Hypercoagulability with Plasma AT Activity Reductions are Evidence for Trauma Induced Consumption of AT by Activated Coagulation Factors

Theoretically, the reduction of plasma AT inhibitory activity that is observed early after traumatic

injury could be due to its stoichiometric consumption as inhibitory complexes with activated coagulation enzymes, or to cleavage and inactivation of AT by neutrophil elastase, or to both (see Fig. 2.1). Three studies have reported serial measurements of thrombin-antithrombin (T-AT) complexes in plasma from trauma patients [40, 44, 45]. All found T-AT complex levels to be markedly elevated on day 1, corresponding to the period when plasma AT activity is most reduced and there has been recent massive tissue factor liberation by the trauma. T-AT levels then decreased on days 2–4, during the period when plasma AT activity rebounds. One of the studies also measured hypercoagulability by TEG and determined that the prevalence of a hypercoagulable state was 62 % on day 1 and then decreased to 26 % on day 4 [45].

In summary, T-AT and hypercoagulability data provide direct evidence that AT participates in the regulation of activated clotting factors released after trauma, and that consumptive coagulation plays a role in the trauma induced depletion of plasma AT activity.

Plasma AT Activity as a Predictor of Deep Vein Thrombosis (DVT) and Disseminated Intravascular Coagulation (DIC) in Trauma Patients

A large study on the effect of critical injury on plasma antithrombin activity enrolled 157 trauma patients, of which 77 % had blunt and 23 % penetrating injuries [41]. The mean ISS was 23 (SD +/− 11, range 4–66). Blood samples were drawn upon emergency room arrival, at hours 8, 16, 24, and 48, and on days 3, 4, 5, and 6. Plasma antithrombin activity was measured by chromogenic anti-fXa assay.

Low AT levels were predictive of DVT (diagnosed by ultrasound based on clinical suspicion) and DIC thromboembolic complications by logistic regression analysis. Both nadir and average plasma AT activity predicted DVT (diagnosed using color flow duplex ultrasound) with p values of 0.030 and 0.042, respectively. The nadir levels usually occurred within the first 24 h after admission. Logistic regression analysis also demonstrated that nadir, maximum and average AT levels were also predictive of DIC (all $p < 0.0001$). AT was lower in the DIC group overall and at each time point by repeated measures ANOVA.

Inflammatory Inactivation of Antithrombin

As discussed in introductory Section "Inflammatory Inactivation of AT" on mechanisms of AT inhibition and inactivation, in vitro cleavage of the AT reactive center loop by neutrophil elastase converts the serpin to a stable inactive conformation (Fig. 2.1e) that is devoid of protease inhibitor and anticoagulant function [28, 29]. Moreover, elastase inactivation of AT is accelerated by the AT cofactor heparin (Fig. 2.1f), to which neutrophil elastase can also bind with high affinity [30].

Collectively the above in vitro solution phase experimental results suggest that in vivo, inflammatory responses in tissues where injury has exposed blood to HSPGs may rapidly switch off AT anticoagulant function, and promote intravascular coagulation and thrombus formation. Accordingly, AT activity reductions that are typically attributed to its massive stoichiometric consumption during coagulation may—at least partially—result from neutrophil elastase cleavage and inactivation of the inhibitor, which occurs independently of inhibitory complex formation type consumption with target enzymes. Several studies with serial measurements of plasma elastase and AT levels in trauma patients with DIC or organ failure support this concept.

A study examined elastase and AT with respect to DIC [46]; plasma elastase was assayed in complexes with its inhibitor α1-antrypsin by ELISA, and plasma antithrombin activity determined using standard methods. At the first blood sampling (within 12 h after admission, day 1), but not on days 3 or 5, significant increases in plasma elastase and significant decreases in plasma antithrombin activity were observed for DIC patients in comparison with non-DIC patients. These data indicate that higher levels of elastase are present in patients who have lower antithrombin levels

and develop DIC, and are supportive of an elastase contribution to reduced AT levels in some trauma patients.

Also of relevance to the in vivo question of elastase inactivation of AT is a study of multiple organ failure (MOF) in severe trauma patients. This study [43, 47] divided patients into MOF non-survivors, MOF survivors, and survivors without MOF groups. Plasma elastase and antithrombin levels were determined over a 2-week course. Plasma elastase was significantly higher ($p < .01$) for MOF patients (survivors and non-survivors) vs patients without MOF over the entire observation period. Beginning on day 3, non-surviving MOF patients had significantly higher plasma elastase than did surviving MOF patients ($p < .05$). Plasma antithrombin levels were significantly lower for non-surviving and surviving MOF patients vs. patients without MOF ($p < .01$) through out the study, and the values for non-surviving MOF patients were significantly decreased compared to survivor MOF patients ($p < .05$) after the first week. Thus, there was a significant (and temporally logical) inverse relationship between antithrombin activity and elastase levels, and antithrombin levels also demonstrated a negative relationship with the severity of organ failure. A later study from the same group reported similar results [48].

In summary, the above clinical studies suggest that inflammatory, elastase-mediated cleavage and inactivation of AT contributes to the pathogenesis of coagulopathies in trauma patients with DIC and organ failure.

Glycocalyx Shedding and Auto-heparinization

As illustrated in the introductory section, heparins dramatically increase rates of AT target enzyme inhibition (Fig. 2.1b, c, d and Fig. 2.2b), and also the rate of its cleavage and inactivation by neutrophil elastase (Fig. 2.1e, f). Therefore, the cofactor interaction with heparins can cause anticoagulant—as well as procoagulant—effects.

In the body endogenous heparin cofactor activity is provided by heparan sulfate glycosaminoglycan molecules. Some of them are attached to syndecan-1 proteins and form the HSPGs (heparan sulfate proteoglycans) of the endothelial glycocalyx [49]. For further description of the endothelial glycocalyx, please refer to Chap. 7. The glycocalyx is shed into the circulation during hemorrhagic shock [50], and ensuing "auto-heparinization" has been proposed to exert an anticoagulating effect by way of heparan sulfate potentiation of AT clotting factor inhibition [51]. In a study of 77 trauma patients, differences in kaolin-TEG vs heparinase-TEG were used to identify the subset ($n = 4$, 5 %) with the highest degree of endogenous heparinization. These patients had fourfold increased syndecan-1 levels, higher ISS, INR, thrombomodulin, and IL-6, and lower protein C. Thus, they had profound endothelial damage, coagulopathy, and inflammation. A trend towards plasma AT activity deficiency, which did not reach statistical significance ($p = 0.055$), was also observed [51].

The AT that was present may have been activated by auto-heparinization to be more efficient in procoagulant enzyme neutralization. Alternatively, given the inflammatory status of these patients, auto-heparinization may have contributed to reduction of the potential anticoagulant effect of AT by increasing the rate at which it is cleaved and inactivated by neutrophil elastase [30]. In either case, it is reasonable to believe that the effects of auto-heparinization on AT inhibition and/or inactivation contribute to the overall coagulopathic state in these trauma patients.

AT Supplementation in Trauma

Early Trials Focusing on DIC and Organ Failure Prevention in Trauma Patients

As discussed in Section "Reduction of Plasma AT Activity During Trauma", multiple studies have demonstrated that plasma AT activity begins to decrease immediately after trauma. Patients who have less severe reductions—and whose plasma AT activity deficits improves during the first week—experience better outcomes and survival than do patients who develop severe AT activity

deficiencies that do not resolve, and predict non-survival.

Although it has been recognized that antithrombin is just one of the several endogenous anticoagulants that becomes dysregulated in trauma and critically ill patients, early trials of antithrombin supplementation [52–54] were conducted to explore whether correction of AT deficiency might nonetheless reduce mortality. The straightforward premise of these early trials was that normalization of AT activity by concentrate administration would be helpful for reducing post-traumatic intravascular coagulation and the subsequent development of DIC and organ failure. Furthermore, additional favorable advantages might accrue from AT interruption of thrombin non-coagulant functions that contribute to trauma pathogenesis (e.g., platelet activation, signaling, leukocyte recruitment). For further details on the pathophysiology, diagnosis, and management of DIC, please refer to Chap. 13.

None of the early trials of AT supplementation demonstrated improvements in survival of the treated patients. However, based these studies it was noted that AT supplementation permitted better control of alterations of the clotting system that are responsible for DIC [52], that AT replacement may reduce the incidence of renal impairment [54], and that infusion of high doses of AT might benefit certain severe DIC patients in whom rapid and strong thrombin generation was the main problem [53].

A final common conclusion from all of these early studies was that a large, randomized, double-blinded study would be required to properly assess the value of AT substitution therapy in trauma patients.

High-Dose AT for Attenuation of Inflammation and Prevention of Organ Failure in Trauma Patients

A prospective, placebo-controlled, double-blinded study of antithrombin supplementation in severely injured patients was reported in 1998 [42]. It is relevant to note that high-dose antithrombin was utilized in this trial, and that there was a new focus and efficacy objective, which was distinct from those of previous AT replacement studies in trauma and critical care patients. Specifically, "the primary intention of the [high-dose antithrombin III treatment of severely injured patients] study was not the treatment of DIC but the attenuation of the inflammatory response and the decrease in organ dysfunction" [42].

During the period when this AT supplementation in trauma study, as well as a large clinical trial on AT supplementation for sepsis [55], were being designed and conducted there was great excitement about anti-inflammatory properties of antithrombin. AT anti-inflammatory properties were attributed to endothelial prostacyclin release and attenuated cytokine liberation and leukocyte activation secondary to antithrombin binding to vascular HSPGs. A series of papers published between 1995 and 2007 reported that AT induction of prostacyclin attenuated endotoxin-induced pulmonary vascular injury and hypotension, ischemia-reperfusion (I/R)-induced renal and hepatic injury, crush and I/R-induced spinal cord injury, and hepatic metastasis of colon cancer cells in experimental rat models. Thus, the rationale for expecting AT to exert protective anti-inflammatory effects against organ failure in humans seemed solid, and was a motivating factor for clinical studies of AT supplementation in trauma and sepsis.

Recently, however, the animal work forming the basis for anti-inflammatory properties of AT has come into question, and some papers have been officially retracted [56]. Nevertheless, at the time the trauma and sepsis AT supplementation clinical trials were conducted, the anti-inflammatory potential of AT constituted a large part of the justification for these studies.

Therefore, the primary objective of the High-Dose Antithrombin III Treatment of Severely Injured Patients trial was to evaluate the effects of early and high-dose administration of AT on clinical measures of the inflammatory response, and its secondary objective was to evaluate the effects of AT on plasma indicators of DIC and the systemic inflammatory response. Forty consecutive patients with severe blunt trauma (ISS $\geq$29) were enrolled into the double-blind study, and randomized to receive plasma-derived high dose

AT (Pharmacia & Upjohn) (20,000 IU per patient during a 4 day period) or placebo (human serum albumin) within 6 h after trauma. Additional AT or an equivalent protein weight of placebo was infused at 6 hourly intervals over the first 4 days to obtain plasma AT activity at 140 % of normal concentrations. During this period, AT activity averaged ~70 % in placebo-treated patients.

The primary efficacy response variables for the study were the incidence and severity of multiple organ dysfunction. Durations of liver and multi organ failure were significantly shorter for the AT supplemented group, but exhibited no significant differences with respect to respiratory failure and ARDS. Mortality, respiratory failure incidence, mechanical ventilation duration, and length of ICU stay were not significantly different for the control and AT groups.

The secondary efficacy objective of the study was to evaluate AT supplementation effects on DIC and the systemic inflammatory response. In the AT group plasma prothrombin tended to be elevated, and prothrombin fragment F1+2 and thrombin-AT complex tended to be lower on the first day. No differences between groups were observed for PTT, PT, platelets, PAI-1, sTNF receptor II, neutrophil elastase, IL-1 receptor antagonist, IL-6, and IL-8. DIC occurred in six patients (30 %) of each group.

In summary, the overall conclusions of the trial were that early and high-dose administration of antithrombin to patients with severe blunt trauma does not attenuate the posttraumatic inflammatory response or disseminated intravascular coagulation, or significantly improve outcome.

It is noted that a larger trial of high-dose antithrombin for sepsis was also conducted at about the same time as the trauma trial, and obtained similar negative results [55]. As in the trauma trial, the sepsis trial did not exclude concomitant heparin, which a large subset of the patients received. In retrospect heparin was speculated to have interfered with potential therapeutic benefits of AT. Reanalysis of the sepsis trial data showed that for patients without concomitant heparin, 28-day mortality was lower for the AT group compared to the placebo group, and that this trend continued through 90 days, whereas it was not observed with groups receiving heparin [57].

Accordingly, it is pertinent to address the question of concomitant heparin administration in the trial of high-dose AT in trauma patients [42]. It is reported that “many” of the trauma study patients received heparin as prophylaxis for thromboembolic complications. Therefore, similar caveats about the negative results from the trauma trial can be raised, and lead the authors to conclude that “further study of high-dose antithrombin III therapy without the use of heparin in patients with sepsis and trauma is warranted.”

This view was based on the idea that heparin interfered with anti-inflammatory functions of AT by competitively inhibiting AT binding to the endothelium and subsequent prostacyclin production. However, the recent retraction of work which formed the foundation for proposal of the AT-prostacyclin anti-inflammatory pathway has cast some doubt on antithrombin anti-inflammatory properties [56].

Trauma induced coagulopathy is a complex pathology in which multiple components of endogenous procoagulant and anticoagulant systems are dysregulated and also undergo consumption. Unlike hereditary factor and inhibitor disorders which are mostly due to deficiency of a single isolated component, the multifactorial nature of TIC may cause it to be inherently more difficult to correct using simple replacement therapies.

Conclusion

The causes of trauma induced coagulopathy are multifactorial and the course for each patient is complex and distinct in its own way. A better understanding of the basic physiological functions of antithrombin and its dysregulation will support efforts to develop strategies to mitigate TIC.

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Plasmin-Antiplasmin System

3

Nicola J. Mutch and Nuala A. Booth

Fibrin as a Substrate

Plasmin is a potent trypsin-like serine protease that cleaves any substrate after lysyl or arginyl bonds. It activates growth factors and prohormones, actions that are outside the scope of this review, but its main substrate in vivo is fibrin. Many of the cleavage sites in fibrin have been revealed by study of fibrinogen, which, as a soluble protein, is easier to analyse (reviewed by [1]). The ordered degradation pattern (Fig. 3.1) is detailed here as it is essential to the understanding of what is measured in assays of D-dimer and other fibrin degradation products (FDP). The first cut is to the α-chain of fibrinogen, releasing the αC fragments; the remainder of the molecule is called fragment X (~260 kDa). Fragment X is then cut in the α-, β- and γ- chains across the coiled coil that connects the central E and terminal D domains of fragment X. The cleavage occurs in two steps, first splitting the molecule asymmetrically to generate fragment Y (~160 kDa) and fragment D (~100 kDa), and then cleaving fragment Y into a second fragment D and fragment E (~60 kDa), which contains the amino-terminal portion of all six polypeptide chains.

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Newly formed fibrin is degraded by plasmin with the same cleavage pattern as fibrinogen, showing that no major structural reorganization occurs during fibrin polymerization [2, 3]. In contrast, when fibrin is cross-linked by the transglutaminase factor XIIIa (Fig. 3.2, right) its degradation is slower and different products arise. D-dimer, which consists of two fragments D from adjacent fibrin monomers, cross-linked via their γ-chain remnants, is generated. This covalent dimer, bound non-covalently to fragment E, is DD/E complex. This fragment also occurs in long arrays held together by uncleaved coiled coils [4]. Larger FDP reassociate with one other and with fibrin [5], so the substrate for fibrinolysis is not a single entity but a complex and dynamic one, in which both formation and degradation occur simultaneously. The clearance of FDP from the circulation is via the kidney and also liver, depending on the actual fragment [6, 7].

Fibrin as an Active Surface for Plasmin Generation and Activity

Fibrin is at the heart of the lytic cascade and plays a key role in "orchestrating its own destruction" [1]. This behaviour will be explained by considering the proteases and inhibitors that regulate the system, stressing throughout the governing role of fibrin.

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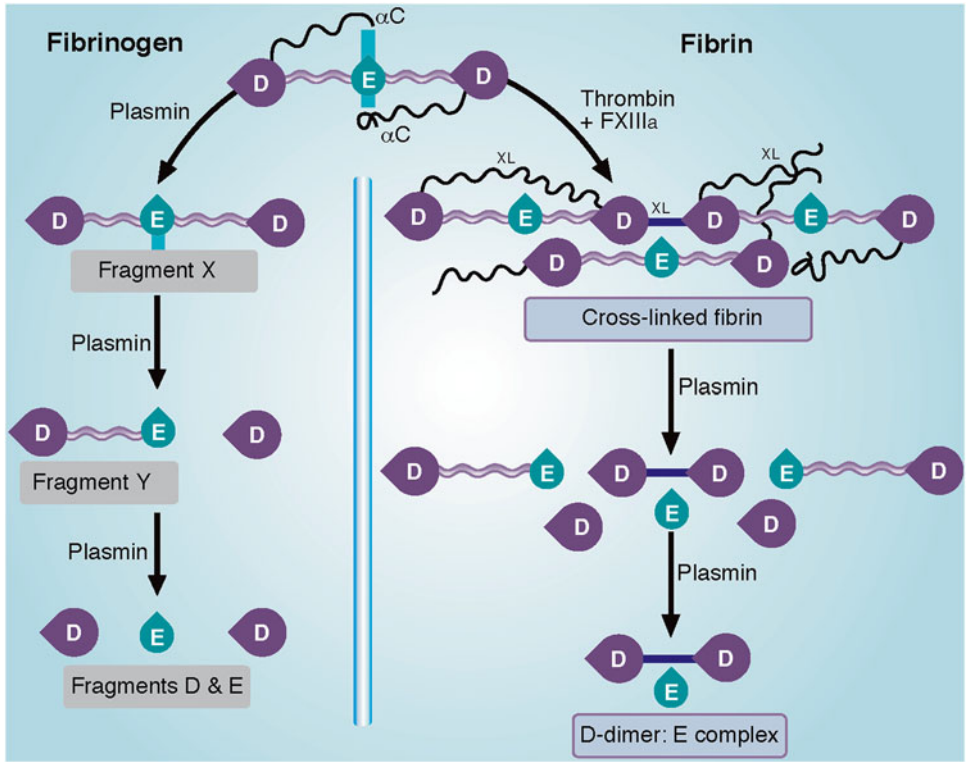

Fig. 3.1 Plasmin degradation of fibrin(ogen). Fibrinogen (*top*) is a three-domain globular protein with extending αC domains. Fibrinogen is degraded asymmetrically (*left panel*). Plasmin initially cleaves the αC domains from fibrinogen generating fragment X, which consists of all three domains connected by coiled coils, but lacks the Aα chains and the Bβ1-42 sequence. The second cleavage occurs across the coiled coil that connects the central E and terminal D domains, generating fragment Y, which is composed of the central E-domain connected by a coiled coil to the D-domain. Fibrin is formed by cleavage of fibrinopeptide A and B from fibrinogen by thrombin (*right panel*). Thrombin also activates the transglutaminase factor XIII (FXIIIa) which cross-links (XL) fibrin longitudinally between the D-domains and within the α-chain extensions. Cleavage of the two-stranded protofibrils by plasmin initially removes the cross-linked α-chains, followed by the coiled coils to liberate a series of fibrin degradation products (FDP), the smallest being DD/E. Larger complexes, such as DY/YD, are also released from cross-linked fibrin and are subsequently degraded to the DD/E moiety

Plasminogen

Plasminogen is a 92 kDa glycoprotein, abundant in plasma (Table 3.1). It is a classic zymogen, a single-chain molecule, activated by cleavage of one peptide bond to produce plasmin, in which the two chains are held together by two disulphide bonds. It is composed of several discretely folded domains. From the N-terminus, these are the activation peptide, a pan apple domain, kringles 1–5 and the protease domain (Fig. 3.2). The kringles give plasminogen the capacity to bind to cells and other proteins; the most relevant to this chapter are fibrin, α_2AP and TAFI. Such binding has profound effects on plasminogen activation. Plasminogen is primarily produced by the liver and is classified as an acute-phase protein [8]. Cells other than hepatocytes can produce plasminogen, for example eosinophils, kidney, cornea, brain and adrenal medulla; such plasminogen is more likely to have local effects acting on substrates other than fibrin [9–12]. Human deficiency of plasminogen is uncommon but, when it occurs, it is often association with fibrin deposition, for instance in ligneous conjunctivitis [13].

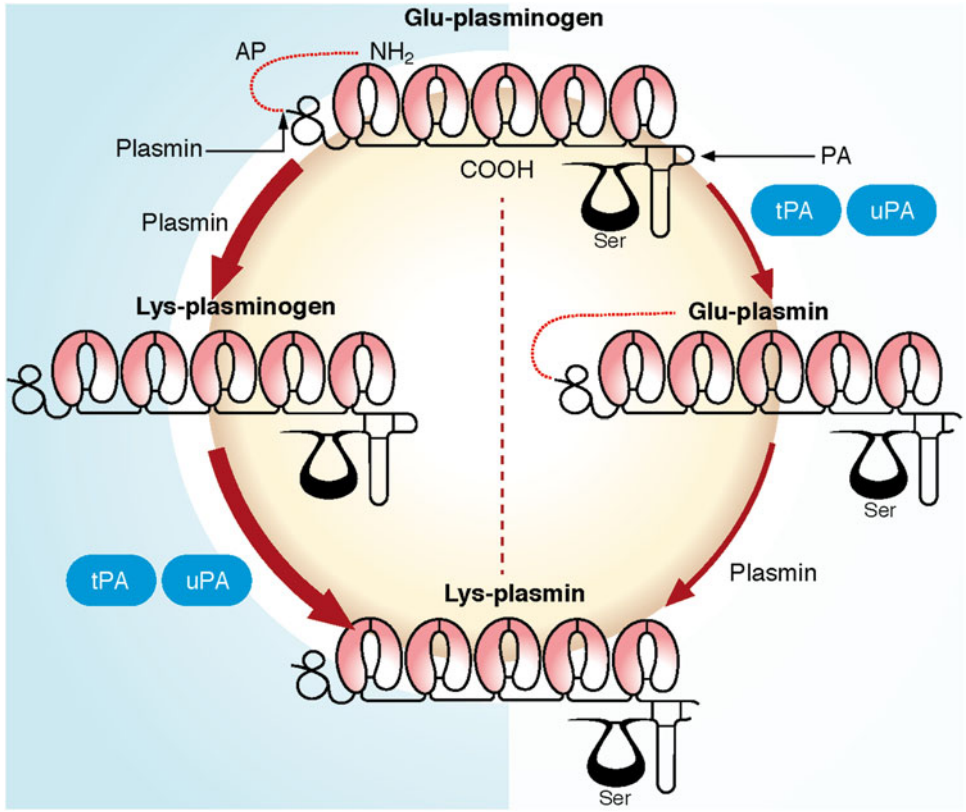

Fig. 3.2 Plasminogen activation. Plasminogen activator (PA) cleaves at Arg 561-Val562, separating the B (light, protease or catalytic) and the A (heavy, kringle) chains. Glu-plasminogen and Glu-plasmin forms both contain the amino-terminal activation peptide from Gln1 to Lys 76 (shown in *red*). Plasmin can cleave this activation peptide (*left side*), generating Lys-plasminogen, an intermediate form that interacts with fibrin more efficiently and is more readily cleaved by tPA and uPA. It is this pathway of plasmin generation that occurs more readily, as indicated by the shading and heavy set arrows. Plasmin can also cleave the activation peptide from Glu-plasmin, generating Lys-plasmin (*right side*). The five kringle structures of the A-chain modulate binding of plasminogen to both fibrin and cell receptors. The catalytic centre contains the typical Ser-His-Asp residues and is the major site of interaction with its principal inhibitor, α_2-antiplasmin

Native plasminogen has several variants, in terms of limited proteolysis, degree of glycosylation and genetic polymorphism. For the purposes of this review we will consider only the two main variants, Glu-plasminogen, the full-length form, and Lys-plasminogen, which has been processed to a variable extent at the N-terminus by trace plasmin. These two forms differ markedly in how efficiently they are activated (Fig. 3.2). Glu-plasminogen is a relatively closed structure [14], whereas Lys-plasminogen is more flexible and open; it binds plasminogen activator about tenfold more effectively [15–17]. Lys-plasminogen also binds to fibrin with higher affinity than Glu-plasminogen. The same is true of binding to plasminogen receptors, a group of proteins that are exposed on cell surfaces and bind plasminogen via lysine residues [18]. Thus, through several mechanisms, Lys-plasminogen is activated more readily, especially on the fibrin or cell surface [19].

Plasminogen Activators

The principal plasminogen activators are tPA and uPA, while the contact pathway plays a role in

Table 3.1 Plasma balance of the principal proteins of the fibrinolytic system

Protein	Mr (kDa)	Molar concentration	Plasma $t_{1/2}$	Function
Plasminogen	92	2 μM	2.2 days	Zymogen
tPA	68	70 pM	4 min	Protease
scuPA	54	40 pM	7 min	Zymogen
PAI-1	52	200 pM	8 min	Inhibitor
α_2-Antiplasmin	70	1 μM	3 days	Inhibitor
TAFI (pro-CpU)	60	75 nM	10 min[a]	Inhibitor
C1-inhibitor	105	1.7 μM	3 days	Inhibitor
α_2-Macroglobulin	725	3 μM	–	Inhibitor
PAI-2	46/70	<70 pM	–	Inhibitor
Factor XII	80	375 nM	2–3 days	Zymogen
Prekallikrein	88	450 nM	7–10 days	Zymogen
HMW kininogen	110	600 nM	9 h	Cofactor

[a]Activated form

some contexts. Activation of plasminogen is always by cleavage of Arg561–Val562 bond, yielding plasmin. It may be helpful to consider the life cycle of a plasminogen activator in terms of synthesis and release into the circulation, neutralization by inhibitors and clearance from the circulation by receptor-mediated mechanisms.

tPA is produced by endothelial and other cells as a single chain but is exceptional in that it is an active serine protease and not a true zymogen [20]. It circulates at low concentrations, mostly in complex with its primary inhibitor, PAI-1 [21, 22]. The plasma half-life is very short (Table 3.1) and shows a circadian rhythm, with lowest levels at night. Plasma tPA can be increased approximately 4-fold under experimental conditions by venous occlusion or by drugs that induce acute endothelial release, such as bradykinin, histamine and β-adrenergic agents [23, 24]. Exercise also augments adrenalin-mediated tPA release, but also decreases clearance from the circulation [25]. Both tPA and tPA-PAI-1 complex are cleared by the low-density lipoprotein-related protein receptor (LRP) system [26].

tPA contains a finger domain and two kringle domains; the finger domain is the basis for its affinity for fibrin [27, 28]. This characteristic is crucial because tPA is a poor plasminogen activator in solution and requires fibrin as a cofactor. Fibrinogen is not able to accelerate plasminogen activation by tPA, as the sites are only exposed in fibrin [29]. Single-chain and two-chain tPA bind fibrin in a comparable way [30] with plasminogen increasing the affinity of tPA for fibrin some 20-fold [31], due to formation of a ternary complex. In the absence of fibrin, the K_M values range from 9 to 100 μM plasminogen [32–34]. In most studies, this K_M value is 3–4 fold lower with two-chain tPA than with the single-chain form, a difference that essentially disappears in the presence of fibrin, when both forms of tPA yield K_M values ranging from 0.16 μM to 1.1 μM plasminogen [32, 34]. These concentrations are readily achieved in blood (Table 3.1). One clear reason for the experimental range in these data is that the kinetics are non-linear [33, 35, 36], with a dual phase activation. Starting with Glu-plasminogen and tPA in the presence of fibrin, the initial K_M of 1.05 μM plasminogen was observed. Following plasmin formation and generation of partially digested fibrin, binding of both plasminogen and tPA increased [37–41], so that the K_M was decreased to 0.07 μM plasminogen, with no change in k_{cat} [36].

uPA is synthesized by several cell types, particularly those with a fibroblast-like morphology, but also by epithelial cells [42], monocytes and macrophages [43, 44]. uPA can activate solution-phase plasminogen; it does not require fibrin as a cofactor. This behaviour, which is in marked contrast with tPA, is sometimes interpreted to suggest that uPA is unimportant in fibrinolysis and cer-

tainly it has roles in other processes, such as extracellular matrix degradation, cell migration, wound healing, inflammation, embryogenesis, and invasion of tumor cells and metastasis [45, 46].

uPA has three domains: an epidermal growth factor (EGF) domain, a kringle, and a protease domain. The uPA kringle has no affinity for fibrin. Its main binding, via the EGF domain located in the amino-terminal fragment, is with a specific uPA receptor, uPAR, described later in this chapter. uPA is expressed in its single-chain (sc) form, which has trace proteolytic activity; full activity requires cleavage of Lys158–Ile159 [47]. This can be achieved by several enzymes, the most relevant being plasmin [48, 49], factor XIIa, and kallikrein [50]. Normal plasma contains scuPA at relatively stable concentrations of 2–4 ng per mL [51, 52] with little circadian fluctuation [53]. While endothelium is not a major source of uPA, there are reports of increased uPA following venous stasis [52], DDAVP infusion [54], and strenuous physical exercise [55], probably explained by decreased clearance from the circulation by receptor-mediated mechanisms. Under normal circumstances uPA activity is not detected in plasma, but both leukocyte-associated and free scuPA are elevated in leukaemia [56] and other disorders, including liver disease [57]. If generated, uPA is rapidly cleared from plasma, in a manner that depends on hepatic blood flow [58]. The LRP system binds and internalizes scuPA and uPA-PAI-1 complexes [26, 59, 60]. The asialoglycoprotein receptor, on parenchymal liver cells, also removes nonsialated uPA from the circulation [58].

Contact activation is a distinct process resulting from the interactions of four proteins, factor XII (FXII), prekallikrein (PK), factor XI (FXI) and high molecular weight kininogen (HK). Negatively charged surfaces such as polyphosphate [61, 62], RNA [63], misfolded proteins [64] and collagen [65] stimulate reciprocal activation of FXII to FXIIa and of PK to kallikrein in association with its non-enzymatic cofactor, HK. The process is accelerated by zinc ions which induce a conformational change in FXII [66–70] and HK [71–73], thereby augmenting surface interactions. The downstream targets of these proteases have been debated as this pathway is associated with coagulation via cleavage of FXI to yield FXIa, inflammation by generation of bradykinin from HK and fibrinolysis.

Of note, while FXII is classified as a coagulation factor it is structurally related to tPA, uPA and plasminogen [74, 75] and can function in plasminogen activation by different mechanisms. FXIIa directly activates plasminogen (Fig. 3.3) albeit relatively poorly compared to tPA and uPA [76–78]. However, the reaction is markedly enhanced by negatively charged surfaces such as dextran sulphate[44] and polyphosphate (unpublished observations *Mutch NJ*). Circulating plasma concentrations of FXII are four orders of magnitude higher than tPA and uPA, and combined with the increased plasma half-life suggest that in certain environments or conditions *in vivo* it could facilitate plasminogen activation [79].

Kallikrein generated by FXII dependent [50, 80] and independent [81] pathways is a kinetically favourable activator of scuPA (Fig. 3.3) which in turn activates plasminogen. Finally, the vasoactive peptide bradykinin, described above in the inflammatory arm of the contact pathway, also indirectly impacts fibrinolysis by stimulating tPA release from endothelial cells [82, 83]. These three functionally distinct mechanisms implicate the contact pathway as a modulator of plasminogen activation, but further studies are necessary to define its contribution in different milieu.

Inhibitors of Plasmin Generation and Activity

The proteases of the system are controlled by inhibitors, most of which act directly on the proteases and form inactive complexes with them. PAI-1 and α_2AP are members of the serpin family, which inhibit plasminogen activators and plasmin respectively via a reactive centre loop that mimics the protease substrate (reviewed by [84]). A second mode of action, exemplified by TAFIa, is modulation of the generation of fibrinolytic activity.

PAI-1 is the principal inhibitor of tPA and uPA and inhibits both with second-order rate

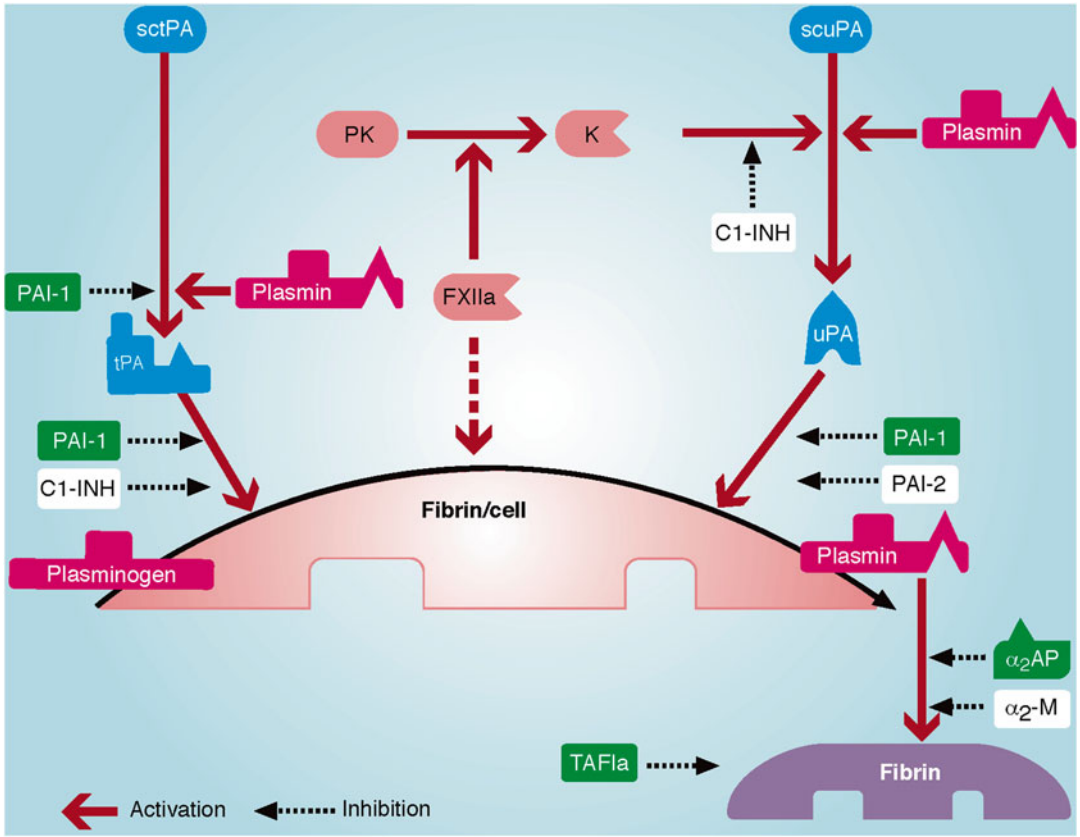

Fig. 3.3 Significant players in the fibrinolytic system. *scuPA* single-chain urokinase plasminogen activator, *sctPA* single-chain tissue plasminogen activator, *α_2AP* α_2-antiplasmin, *α_2M* α_2-macroglobulin, *C1-INH* C1 inhibitor, *PAI-1* plasminogen activator inhibitor 1, *PAI-2* plasminogen activator inhibitor 2, *TAFI* thrombin-activatable fibrinolysis inhibitor, *FXIIa* factor XIIa, *PK* prekallikrein, *K* kallikrein. Activation of plasminogen to plasmin usually occurs on a surface, either fibrin or a cell membrane. Once formed plasmin degrades fibrin as described in Fig. 3.1

constants greater than 10^7 M^{-1} S^{-1}, close to the diffusion limit [85]. It does not inhibit scuPA, which is largely inactive, but it does associate with scuPA non-covalently [86]. It is an unusual serpin in that it spontaneously loses activity by insertion of its reactive centre loop into the core of the molecule [87]. This inactive form was originally termed "latent", which unfortunately gives an impression that the latent material is physiologically activated. Reactivation is indeed possible, but only by chemical denaturation and refolding [88]. It was characterized originally as a product of endothelial cells but it is synthesized by most cells in culture, including megakaryocytes [89], endothelial cells [90], hepatocytes [91], and adipocytes [92–94]. PAI-1 is synthesized in its active form and circulates in plasma in complex with vitronectin, which stabilizes the active form substantially lengthening its plasma half-life [95].

PAI-1 plasma concentrations are approximately 20 ng per mL [96–98] but reported values range, even in normal individuals, from barely detectable to 40 ng per mL. The variations may be circadian; PAI-1 plasma concentration peaks in the morning [99–101] and in addition, PAI-1 is an acute-phase protein [102]. Understanding its behaviour in response to stress is complicated by the fact that it is synthesized by a wider range of cells than the classic acute-phase proteins and that it is responsive to many stimuli. Some variations in PAI-1 measurements may be method-

ological. It is necessary to exclude platelets and their release products in analysis of plasma PAI-1, especially in measurements of antigen, since platelets are the major pool (more than 95 %) of circulating PAI-1 antigen [103]. Platelet contamination is less of an issue for measurements of PAI-1 activity in plasma, because of the lower activity of platelet PAI-1 [97, 98]. PAI-1 in plasma is in excess over tPA (Table 3.1); therefore, most of the tPA is in complex with PAI-1. Immunological assays of either protein generally measure both free and complexed forms, requiring care in interpretation. Gram-negative septicemic patients have dramatically elevated plasma PAI-1 concentrations, as much as 50-fold over normal, and are associated with high mortality [104] High circulating PAI-1 is associated with a range of disease, including cardiovascular disease [105, 106] and cancer [107]. The causal significance remains unclear, and it seems that high PAI-1 does not independently predict disease when factors like obesity, diabetes, and elevated triglycerides are taken into account [108]. There is a guanine insertion/deletion polymorphism at position 675 in the PAI-1 promoter [109], which is associated with differences in circulating PAI-l [110] but the predictive power of this polymorphism appears to be low [108, 111]. Deficiency of PAI-1 in humans is rare but it causes a lifelong bleeding disorder, characteristically after a delay, consistent with normal clotting but premature lysis of hemostatic plugs at sites of vascular trauma [112–115]. Fibrinolytic inhibitors such as tranexamic acid decrease plasminogen activation and therefore are effective in normalizing hemostatic function in such patients [114, 115].

α_2AP is the principal inhibitor of plasmin, the term fast-acting being used to stress the rapid inhibition, with a second-order rate constant of 4×10^7 per M per second [116]. Its plasma concentration is 1 μM, about half the molar concentration of plasminogen; it has to be remembered that plasma plasminogen is seldom, if ever, entirely converted to plasmin, so the inhibitor is usually in excess. It is synthesized in the liver and consequently decreased in patients with advanced impairment of hepatic function. The $t_{1/2}$ of the native inhibitor is approximately 3 days, whereas the covalent plasmin/α_2AP (PAP) complex is cleared with a $t_{1/2}$ of approximately 0.5 days [117].

α_2AP has several forms, all depending on limited proteolysis at N- and C-termini. The processing appears not to affect the inhibitory capacity of α_2AP which depends on the reactive centre loop. Newly produced α_2AP (Met form) has 12 residues at the N-terminus that can be cleaved to yield N-terminal Asn [118] by an antiplasmin cleaving enzyme (APCE) [119]. Both forms are equally represented in plasma [120]. The N-terminal processing is important because it reveals Gln2, in the processed, Asn form, the Gln2 being cross-linked to Lys 303 of the fibrin(ogen) Aα chain [121, 122]. In contrast, in the Met form, Gln2 is blocked [123]. Fibrin to which α_2AP is cross-linked resists lysis by plasmin and this observation was central to discovery of the first deficiency of α_2AP [121]. Consistent with this, antibodies that react specifically with cross-linked α_2AP stimulate lysis of fibrin [124].

Comparison of α_2AP with other serpins shows that it has a C-terminal extension of some 50 residues [118]. This full-length form and a shortened form are both detectable in normal human plasma [125]. The full-length form binds plasminogen but the processed form, which is still a potent inhibitor of plasmin, cannot bind plasminogen [126]. The enzyme responsible for this C-terminal cleavage has not yet been characterized. The ratio of two forms, plasminogen binding to non-binding, is approximately 2:1 in plasma. This was still true even in advanced liver cirrhosis [57], despite these patients having impaired synthesis of α_2AP.

Binding of α_2AP to plasminogen competes with the plasminogen-fibrin interaction, because via the same lysine binding site (Fig. 3.4). Plasmin formed on fibrin is therefore relatively protected from the action of α_2AP [127], a key finding in the control of fibrinolysis [127]. The experimental basis for this concept used lysine analogues, in the presence of which α_2AP was about 100 times less effective in inhibiting plasmin [116]. The exact Lys residues responsible for binding the C-terminal region of α_2AP to plasminogen are not conclusively defined. One study showed a major effect of Lys452, but that

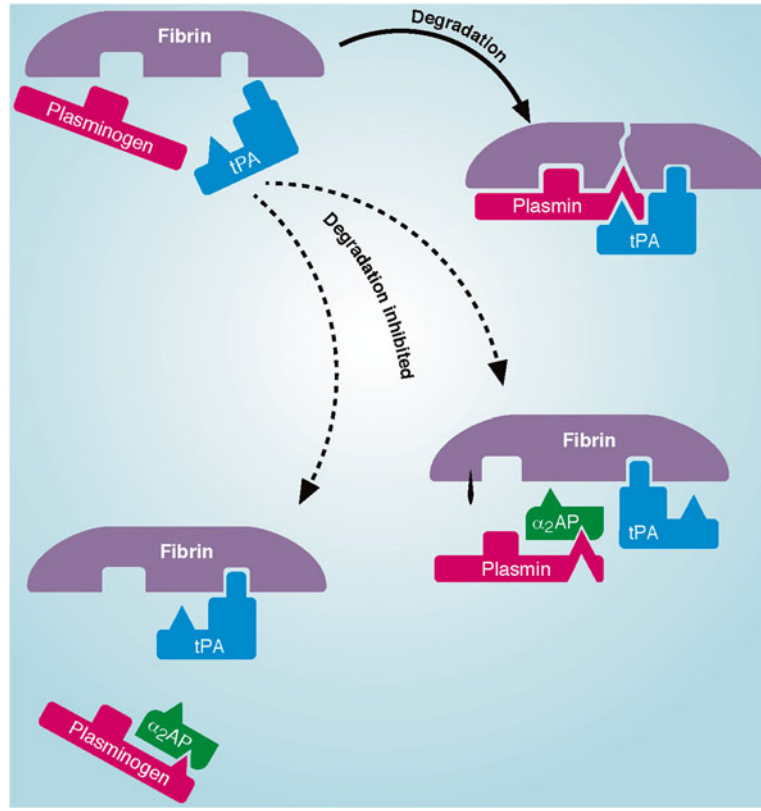

Fig. 3.4 Localization of plasmin(ogen) and tPA on fibrin; interference by α_2-antiplasmin. Fibrin binds plasmin(ogen) and tPA directly and acts as a cofactor in plasminogen activation, thereby augmenting its own destruction. α_2-Antiplasmin (α_2AP) can bind to plasmin(ogen) in solution obstructing binding to fibrin. α_2AP is also cross-linked to fibrin, via the action of factor XIIIa, which prevents plasmin from binding to fibrin and neutralizes the plasmin activity. These events hamper plasminogen activation on fibrin and/or inhibit plasmin activity and thereby limiting fibrin degradation

other internal Lys residues "tether" the kringles [128]. A different study, in which Lys residues were systematically mutated, suggested that Lys436 had the greatest effect [129].

Thrombin-activatable fibrinolysis inhibitor (TAFIa; also known as carboxypeptidase B, U, or R [130]) removes C-terminal lysyl residues from fibrin, which as previously stressed are important in the binding of plasminogen [131]. TAFI is produced as a zymogen (or procarboxypeptidase) and is activated by the thrombin/thrombomodulin complex [132] or by plasmin in the presence of glycosaminoglycans [133]. Its activation by thrombin makes it an important molecular link between fibrinolysis and coagulation [134]. TAFI is produced in the liver but there is considerable variation in normal circulating concentrations [131, 135] and only a fraction need be activated for full physiological impact [132]. Its activity is controlled by its instability, with an effective plasma half-life of only about 10 min [136]. The function of TAFIa was shown in clot lysis assays; potato tuber carboxypeptidase inhibitor relieves the inhibition [134, 137]. This approach and more sensitive and specific assays for TAFIa have shown that the carboxypeptidase must be maintained at a threshold level to be effective in modulating fibrinolysis; this level fluctuates in relation to plasmin concentration [138]. Several polymorphisms in the TAFI gene have been reported, resulting in four isoforms [139, 140]. These isoforms explain the normal wide range in concentration, but do not correlate strongly with disease [140, 141]. Elevated TAFI appears to be a mild risk factor for venous thrombosis [142], and it also increases in inflammation, correlating with other acute-phase markers [143].

Increased fibrinolytic activity in haemophilia patients is explained by defective TAFI activation. Most thrombin is formed after clot formation, mainly by back activation of FXI by

thrombin, with deficiencies in FXI resulting in a mild to moderate tissue-specific bleeding disorder (Haemophilia C). In the absence of FXI clots lyse more readily [144] which is associated with feedback activation of FXI by thrombin [145]. The enhanced generation of thrombin augments TAFI activation stabilizing clots against premature lysis [146, 147]. Addition of TAFI, thrombomodulin, or factor VIII to haemophilia A plasma restored normal fibrinolysis [148]. Consistent with this, incorporation of anti–factor XI antibodies or inhibition of TAFI in a rabbit model resulted in an almost 2-fold increase of endogenous thrombolytic activity [149].

We described earlier the role of the contact pathway in facilitating plasminogen activation. The role of FXIa in sustaining thrombin generation and therefore TAFI activation implicates the contact pathway in antifibrinolytic as well as profibrinolytic mechanisms.

Other Inhibitors

In most situations, PAI-1, α_2AP and TAFI provide the main control for plasmin generation and activity, but there are other inhibitors that may have roles in specific circumstances, which will now be introduced briefly.

PAI-2 is an inhibitor of uPA purified from human placenta and the cell line U-937 [150, 151]. The real function of PAI-2 may not be as a PA inhibitor [152], as mice with a disrupted PAI-2 gene do not present any major phenotypic abnormalities [153]. The intracellular location of this serpin suggests roles other than inhibition of PA. It is about 10-fold less effective than PAI-1 in inhibition of uPA and is a much poorer inhibitor of tPA [151]. In the circulation, monocytes are the main reservoir of PAI-2 [154] and may increase fibrin stability on migration into thrombi, especially because PAI-2 is cross-linked to fibrin [155]. Normal plasma does not contain measurable PAI-2, except in pregnancy, where it rises steadily to reach approximately 250 ng/mL by the third trimester [156]. In placental dysfunction and intrauterine growth retardation [157–159], the rise in plasma PAI-2 is much smaller. PAI-2 also occurs in plasma of patients with acute myeloblastic leukaemia (M_4 and M_5) [160], and in patients with sepsis [161]. Local PAI-2 activity appears to be relevant to a number of cancers and this may provide clues to its physiological function [162, 163].

α_2-Macroglobulin (α_2M) is a non-serpin inhibitor of wide specificity. This breadth of targets and its relatively high plasma concentration (2.5 g per L; 3 μM) make it an effective backup inhibitor of many proteases, including plasmin, tPA and uPA [56]. α_2M is a tetramer made up of a pair of dimers containing two reactive sites. When proteases are inhibited by α_2M, they generally retain activity towards small peptide substrates, but are unable to cleave larger targets.

C1-inhibitor is a highly glycosylated serpin that inhibits the C1r and C1s proteases of complement C1. It also inhibits the contact proteases, FXIIa, FXIa and plasma kallikrein, as well as tPA, plasmin and uPA. It circulates in plasma at a relatively high concentration (1.7 μM). When tPA is in excess over PAI-1, complex formation with C1-inhibitor is observed [22, 57, 164]. Its diverse targets suggest that it will have a role in controlling contact phase–dependent fibrinolysis and the conversion of scuPA to uPA (Fig. 3.3).

Regulation of Plasmin Generation and Activity

So far, we have highlighted three important concepts: zymogen activation, protease inhibition and, crucially, the role of fibrin in promoting activation of plasminogen and protecting plasmin from inhibition. Further discussion requires consideration of particular situations, so we will now examine the balance of the various proteases and inhibitors in plasma, on platelets, cell surfaces, and thrombi.

Plasma Balance

Plasminogen, the central player of the fibrinolytic system, circulates at approximately five orders of magnitude higher than tPA and scuPA (Table 3.1).

Plasminogen is turned over relatively slowly, with a half-life of 2.2 days for Glu-plasminogen and 0.8 days for Lys-plasminogen, while tPA and scuPA have plasma half-lives of only minutes. From this we can infer that the rates of synthesis, release and clearance are low for plasminogen and much higher for the PA, illustrating the more dynamic part of the system. Similar considerations apply to the main inhibitors. PAI-1 is present in plasma at only 400 nM, while α_2AP circulates at 1 μM, and again the plasma half-lives are in marked contrast.

Fibrinolytic activity is not normally detectable in plasma because plasminogen is a true zymogen, and therefore inactive, while the one active PA in plasma, tPA, is normally controlled by an excess of PAI-1. Even if the PAI-1 were insufficient for full neutralization, then α_2AP, C1-inhibitor and α_2M would act as backup inhibitors. The other potential activator, scuPA, is not sufficiently active to begin the process of plasminogen activation, since it needs prior activation by plasmin or kallikrein. Any trace of plasmin generated in plasma would be quickly neutralized by α_2AP, again supported as necessary by other inhibitors, especially α_2M. So the quiescence of the system, in plasma, is maintained by tight control of protease activity, both at the level of existence of plasminogen as a zymogen and at the level of control by inhibitors, primarily PAI-1 and α_2AP.

Cellular and Platelet Contributions

A few years after appreciation of the central role of fibrin in controlling activation of plasminogen and protection of plasmin [127] it became clear that many of the same general characteristics applied to cell-based or platelet-based fibrinolysis, with more efficient activation of plasminogen and protection of cell-bound plasmin from inhibition by α_2AP [165]. Plasminogen binding to platelets and cells was first reported in 1985 [166]. The proteins responsible include $PlgR_{KT}$, α-enolase, S100A10 (functioning with annexin A2), actin, cytokeratin 8, and integrins $\alpha_{IIb}\beta_3$ and $\alpha_M\beta_2$ (reviewed by [167]). The binding of plasminogen to these receptors tends to be low affinity but high capacity, with some cell surface proteins only found on cells undergoing apoptosis. Not all these proteins express the C-terminal Lys that is expected of a plasminogen-binding protein. $PlgR_{KT}$ is a true membrane protein and is synthesized with a C-terminal Lys residue. tPA binding to cells occurs via annexin II and also directly to $PlgR_{KT}$ [167]. Other reports on tPA receptors, which have been characterized in less detail [168, 169], may be of the same or similar molecules.

uPA and scuPA bind to a well-characterized receptor, uPAR (CD87), with high affinity (K_D 10^{-9} to 10^{-11} M) depending on the cell type [170]. uPAR is not a transmembrane protein but is attached to membranes via a glycosylphosphatidyl inositol (GPI) anchor. Binding of uPA to uPAR elicits signaling [171], via other intracellular proteins. Other proteins also bind uPAR, including vitronectin and integrins in complex with caveolin [172]. The uPA/uPAR complex on some cells is associated with Endo 180, also known as uPAR-associated protein (uPARAP) [173], and has a role in collagen IV internalization [174]. uPA bound to uPAR is still inactivated by PAI-1 but not as fast as in solution [175]. The uPA/PAI-1 complex is then internalized, while uPAR is recycled to the surface, a process that also involves LRP [60, 176]. uPAR has clear roles in migration and metastasis. In terms of fibrin degradation, we must distinguish between activation by scuPA and uPA. In the case of uPA, which can freely activate plasminogen in solution, binding to uPAR seems not to affect plasminogen activation but the activity of scuPA is increased by two orders of magnitude when it is bound to uPAR on the surface of monocytes [177, 178]. An elegant experiment in which a uPA variant was directly anchored to the cell surface showed a stimulation of plasminogen activation similar to that achieved by binding to uPAR [179]. This is consistent with the principal function of uPAR being one of localization of uPA to the cell surface rather than enhancement of catalytic activity. The same co-localization and reciprocal activation of scuPA and plasminogen occurs on platelets [49], which

do not express uPAR, indicating there are additional receptors yet to be discovered. Other studies show that cellular binding of plasminogen and (sc)uPA do not have to be on the same cells or surface to facilitate fibrinolysis [180].

Platelets make several contributions to clot stability and lysis. On the pro-lysis side surface-bound plasmin is formed from local plasminogen and protection is afforded from α_2AP. On the anti-lysis side there is the physical barrier to lysis that results from clot retraction, added to which platelets have a pool of FXIII [181] that stabilizes fibrin. Further, platelets are a source of the three main inhibitors of fibrinolysis, PAI-1, α_2AP and TAFI (Fig. 3.5). These pools result from synthesis of the inhibitors in megakaryocytes. In addition it has been reported that platelets synthesize PAI-1 [182]; however, it is difficult to exclude contamination of the platelet preparations by monocytes [183]. Platelet-rich plasma has some 19-fold higher PAI-1 antigen than platelet-poor plasma [97], so that platelets account for 95 % of the circulating PAI-1 antigen. In terms of activity, this PAI-1 is less active than plasma PAI-1, but platelets still account for some 50 % of the total circulating active PAI-1. The platelet pools of α_2AP and TAFI are not as substantial, accounting for less than 1 % of the total blood pool [184, 185] but may have functional significance in particular niches.

Studies on human thrombi revealed that the inhibitors of fibrinolysis, especially PAI-1, accumulate in great excess over proteases [186, 187], providing an explanation as to why established thrombi are resistant to lysis. Observations made on human thrombi also show they retain substantial amounts of coagulant and fibrinolytic activity [188, 189]. Of course, such diverse material is hard to work on in a quantitative way and there are obviously differences in venous and arterial thrombi and between mural and luminal thrombi. Our studies in Chandler model thrombi showed that these thrombi lyse spontaneously, with fibrinolytic activity that could be ascribed primarily to uPA but to a lesser extent to tPA, elastase and cathepsin G [190]. This spontaneous generation of fibrinolytic activity [189] was dependent on polymorphonuclear cells, primarily neutrophils, generating local uPA activity on uPAR [190]. Plasma α_1-antitrypsin was crucial in protecting the activity from neutrophil elastase [191]. The integrin $\alpha_M\beta_2$ is important in the generation of such local activity [192]. The discovery of a role for local uPA in thrombus lysis ran counter to the usual proposition that tPA role is fibrin degradation and uPA mediates other cellular events. There is, however, compelling support for it from a number of other studies, including failure of thrombi from uPA gene knockout mice to resolve [193]. In that model, the uPA activity was associated primarily with monocytes, which migrate into thrombi [194] and express fibrinolytic activity [43]. Indeed, monocyte-bound uPA has been shown to reduce thrombus size in a model of venous thrombosis [195].

Questions That Remain

What Initiates Fibrinolysis?

The available evidence suggests that the Glu-plasminogen to Lys-plasminogen conversion is the initiating event. It has the required features of leading to large scale amplification as the plasminogen-binding sites on fibrin are revealed by partial lysis, and formed plasmin is protected from α_2AP. Part of the same question is which PA is responsible for the first molecules of plasmin that allow Glu-plasminogen to be converted to Lys-plasminogen? In the context of fibrin, with no cells or platelets, it may be tPA, a few molecules of which may be free of PAI-1, that provides initiation, especially since its single-chain form is active and not as readily inactivated by PAI-1. This has been suggested by Thorsen (1992) in his well-established bi-phasic lysis [196], where a small amount of plasminogen on fibrin fibres is activated, then degrades fibrin to generate C-terminal lysine residues that bind additional plasminogen and perhaps tPA, leading to the second faster phase of tPA-mediated fibrinolysis [197]. The molecular interactions and specific binding sites involved have been extensively reviewed [29]. Experiments using tPA

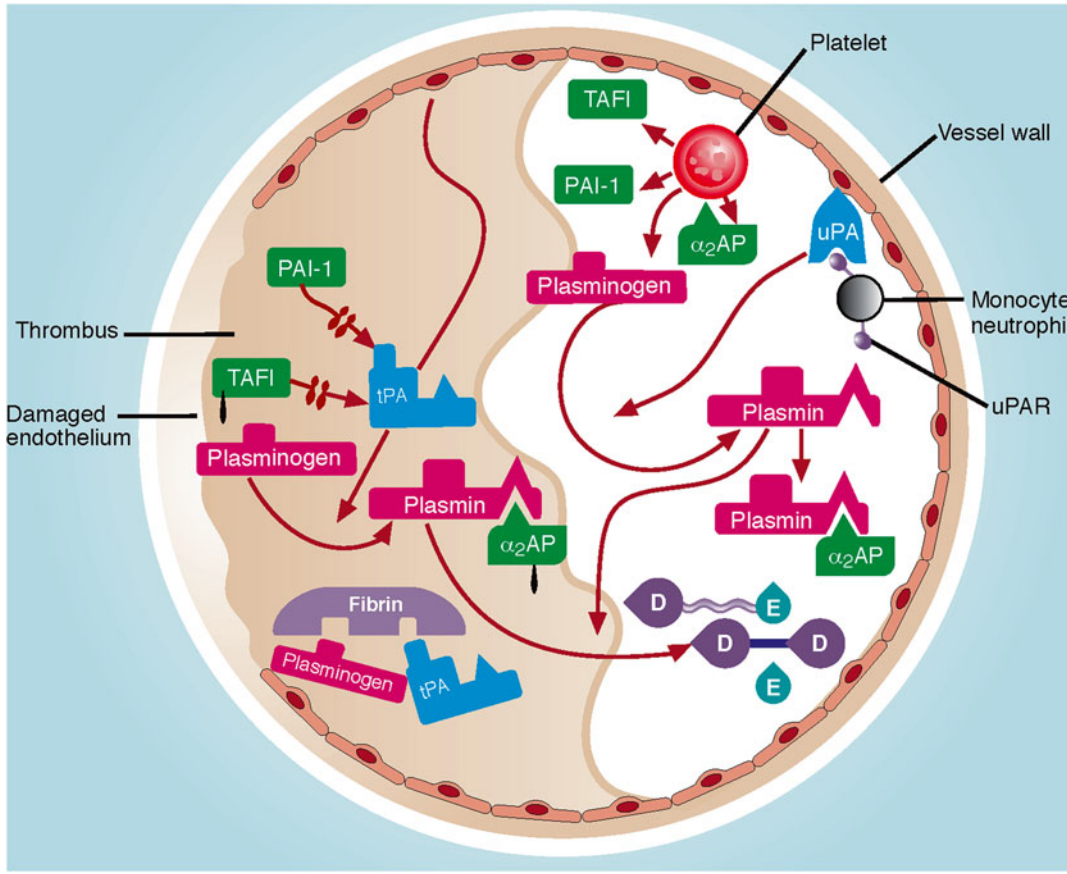

Fig. 3.5 The balance of fibrinolysis in the injured vessel wall. Schematic representation of the different modes of plasmin formation, inhibition and clearance within a damaged vessel wall, with a partially occluding thrombus. Solution, cell surface and fibrin phases of plasmin formation are represented. Plasminogen circulates at a relatively high concentration (2 μM) and is readily incorporated into a forming thrombus by virtue of its fibrin-binding capacity. tPA is largely derived from the endothelium and only circulates at low concentrations with high turnover. tPA-mediated plasminogen activation is slow in solution, but is enhanced several fold when bound to its cofactor, fibrin. uPA is found in the circulation derived from monocytes and neutrophils. uPA does not exhibit fibrin specificity and readily activates plasminogen in solution and while bound to its cell surface receptor, uPAR. Association of uPA with uPAR provides a focal point for plasmin generation. Plasmin degrades fibrin into fibrin degradation products, represented here as D-dimer and DD/E complexes. The system regulated by several inhibitors; only the principal ones are shown here for clarity. Complexes of active enzyme and inhibitor are rapidly cleared from the circulation via low-density lipoprotein receptor. PAI-1 circulates at low concentrations, but a large pool is released from platelets upon activation. PAI-1 can inhibit tPA and uPA in solution and in the presence of fibrin or cell surfaces, but this latter process is generally less efficient. α_2-antiplasmin (α_2AP) is abundant in plasma and a minor pool is also released from activated platelets. α_2AP inhibits plasmin generation in solution, but plasmin formed on cell or fibrin surfaces is relatively protected. α_2AP is cross-linked directly onto fibrin localizing it at the site of plasmin generation. TAFI is found in plasma and platelets and can be cross-linked to fibrin. TAFI is activated by thrombin/thrombomodulin complex or plasmin to generate TAFIa which down-regulates plasminogen activation on fibrin, by removing the C-terminal lysines residues that are important for binding of plasminogen to fibrin. This intricate sequence of events and interactions modulate fibrin accumulation in the body in a precise and coordinated manner. The many different feedback loops and surfaces involved localize reactions thereby preventing excessive plasmin generation in the circulation

variants show that the finger domain of tPA plays a more dominant role in the interaction with fibrin than the kringle 2 interaction with C-terminal lysine residues [198]. This suggests that it is the binding of plasminogen to partially degraded fibrin that is the crucial step in the rapid second phase of fibrinolysis. This central role of plasminogen may suggest that the PA responsible for activation is less crucial than previously assumed. Our experiments with TAFI demonstrated a similar delay in lysis regardless of the PA used [199] and we interpreted this as plasminogen primarily controlling fibrin-bound plasmin generation.

If a cell membrane is present then it may be scuPA, bound to cellular uPAR or on platelets, that yields the initial protease activity. This is suggested on the basis of several experimental systems, including data showing that the ordered addition of scuPA and then tPA [200] is potentially more effective than either agent alone. Our own work on Chandler model thrombi underlines the importance of the scuPA/uPA system in spontaneous lysis [190] but affirms the involvement of other proteases, especially tPA [189]. When in association with cellular uPAR scuPA binds PAI-1 and other serpins reversibly [86]. This has been interpreted in terms of receptor-bound scuPA initiating proteolytic activity, with conversion to uPA achieving inhibition thereby regulating the activity [201].

How Best to Measure Fibrinolysis?

Fibrinolysis, like other cascade systems, coagulation and complement, can be studied by various means. Individual components can be quantified, either as antigen or activity, and under defined situations can provide clear answers. However, the complexities of the system mean that a change in one factor can influence measurements of another and therefore it is important to interpret results with caution. As an example, tPA activity is challenging to measure in plasma, as it is at the limit of detection of most assays. Elevated PAI-1 may depress the activity that is measured. Frequently a manipulation of plasma is necessary to reveal tPA activity, including acidification of plasma or preparation of a euglobulin fraction, where tPA, plasminogen and fibrinogen are retained. Most inhibitors are removed but about 50 % of PAI-1 is retained [97] and these facts must be borne in mind for valid interpretation. As mentioned previously circulating tPA is variable, whether at the level of synthesis or release; therefore it is vital to consider the time-course as each sample represents a snapshot. Rapid hepatic clearance of tPA and of tPA-PAI-1 complex from the circulation rapidly restores the system to normality, allowing key events to be overlooked.

It is often essential to measure more than one analyte for a fuller appreciation of the system. Ideally the aim is to know how much enzyme is free and/or active and how much has been converted to a complex, such as tPA-PAI-1. A combination of ELISA and activity assays may provide a clear picture, but only if the specificity of the ELISA is known in some detail. Ideally, the measurement of PA would be complemented by examining a consequence of the elevation, for instance the fibrin degradation products produced, which of course reflect the presence of fibrin substrate, or generation of plasmin-α_2AP complex. The essential feature of ELISA for a complex is the use of antibody to one of the proteins, e.g. α_2AP, as a capture system and an antibody to the second moiety, e.g. plasmin, in the detection system. The capture antibody in this example will bind free α_2AP and α_2AP in complex, giving rise to potential competition and misrepresentation of the results. This element limits the use of these assays to situations where the free protein is decreased, for instance in liver disease, where α_2AP is lower than normal. Other approaches to measuring overall fibrinolytic activity in plasma include measurement of a zone of lysis on a fibrin plate, clot lysis assays and zymography. These can all be useful but there are limitations associated with most individual assays. For instance, in plasma clot lysis assays, the effects of FXIIIa cannot be reproducibly observed [202]. In addition, the overwhelming effects of α_2AP make it difficult to see inhibition by PAI-1. Failure to be alert to such considerations

gives rise, in the literature, to many inappropriate interpretations about the relative importance of particular proteases or inhibitors. In all assays the balance of enzyme to inhibitor ought to be as close to physiological as possible. When tPA is added it should be at a low concentration, always remembering that it is a catalyst, not a reagent that is consumed. The literature abounds with examples where PAs are added at high concentrations, simply to speed up the assay. This distorts a system that is designed to be delicately poised and generates artefacts of the experimental system rather than a true reflection of what goes on *in vivo*.

Detailed analysis of the fibrinolytic system is only practical for small numbers of samples but, for large clinical cohorts, the aim is to obtain an insight from a limited number of assays. Not surprisingly this has promoted the use of overall measures of activity, such as global assays of fibrinolysis, which have inherent advantages and some limitations. Thromboelastography is rapid, widely available and easily applicable to large sample sizes. However, most studies add tPA as a stimulus. In this situation added enzyme should be kept to a minimum, to avoid generating results that are far from physiological. Another global assay quantified fibrin degradation products after collection of blood samples onto thrombin [203]. Comparison of samples with and without aprotinin gives a measure of global fibrinolytic capacity, an approach that has proved useful clinically [204]. It should be noted that thrombin greatly enhances endogenous fibrinolytic activity, probably be inactivation of PAI-1 among other mechanisms. This consideration serves as a useful aide-mémoire that fibrinolysis is not an independent system. As Ratnoff reminds us "The coagulation, fibrinolysis, complement and kinin pathways are studied separately by scientists for their convenience. In life, they form a seamless web" [205]. Undoubtedly we choose our approaches and molecules of interest to us, and may well ignore other players, by virtue of the experimental system used. These choices may be convenient, but we must bear in mind the selection bias introduced into the system.

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Part II

Pathogenesis of Trauma Induced Coagulopathy

Thrombin Formation

4

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The Setting

Vascular damage exposes tissue factor to flowing blood and triggers a series of proteolytic activation events associated with the tightly regulated hemostatic response. The activation events convert inactive precursors to active proteinases and cofactors that function in concert with cells exposing phosphatidylserine on their outer membrane leaflet and localized at the site of injury [1–6]. Thrombin is the ultimate protease in the clotting cascade (Fig. 4.1) and is responsible for catalyzing blood cell activation and fibrin formation. It also plays a key regulatory role in determining flux through the cascade and its own formation. Low levels of thrombin formed following initiation of the cascade catalyze the proteolytic activation of factors XI, V, and VIII to greatly amplify flux through the cascade. Later in the clotting response, thrombin bound to thrombomodulin on the endothelium catalyzes protein C activation. Activated protein C downregulates flux through the cascade by catalyzing the inactivation of factors Va and VIIIa produced by thrombin early in the clotting response. The clotting process is shut down by the irreversible inhibition of thrombin and the other serine proteinases of coagulation by serine proteinase inhibitors (Serpins), such as antithrombin III, that circulate at high concentrations in plasma and act as suicide substrates. While it is convenient to discuss these details as discrete phases of the coagulation response, the different reactions occur contemporaneously with the net result at any time following vascular damage being determined by the relative contributions of the procoagulant, anticoagulant and inhibition reactions. Resultant clot formation is varyingly determined by the rate, amplitude, and total amount of thrombin produced in its transient production following vascular damage (Fig. 4.2). These features outline, in broad strokes, the key features of the response following vascular damage from a thrombin-centric perspective. The central regulatory role of thrombin in the clotting response emanates from its ability to act with pan-specificity on numerous protein substrates essential for the regulation of coagulation, anticoagulation, fibrinolysis, and cellular responses in blood and the vasculature. Because of its key and opposing roles in the regulation of coagulation, it logically follows that targeting the active site of thrombin for therapeutic

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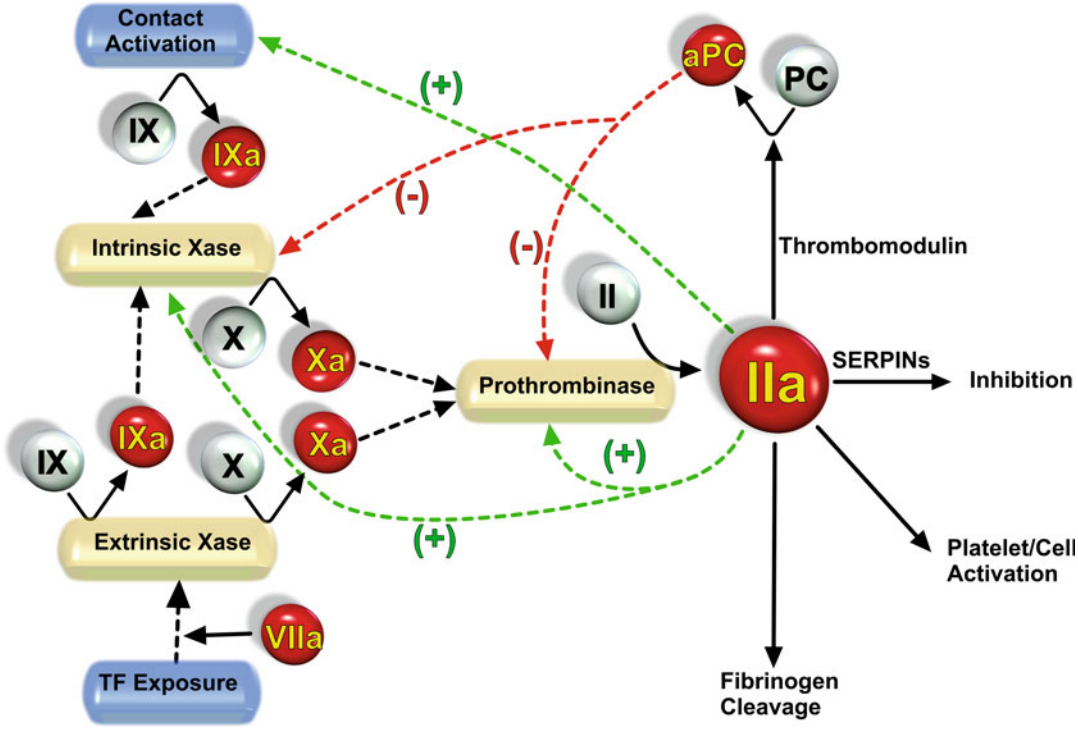

Fig. 4.1 The blood coagulation cascade. The zymogens are colored *grey* and proteinases are colored *red*. Negative feedback reactions are denoted by (–) and *red dashed lines* while positive feedback reactions are denoted by (+) and *green dashes*

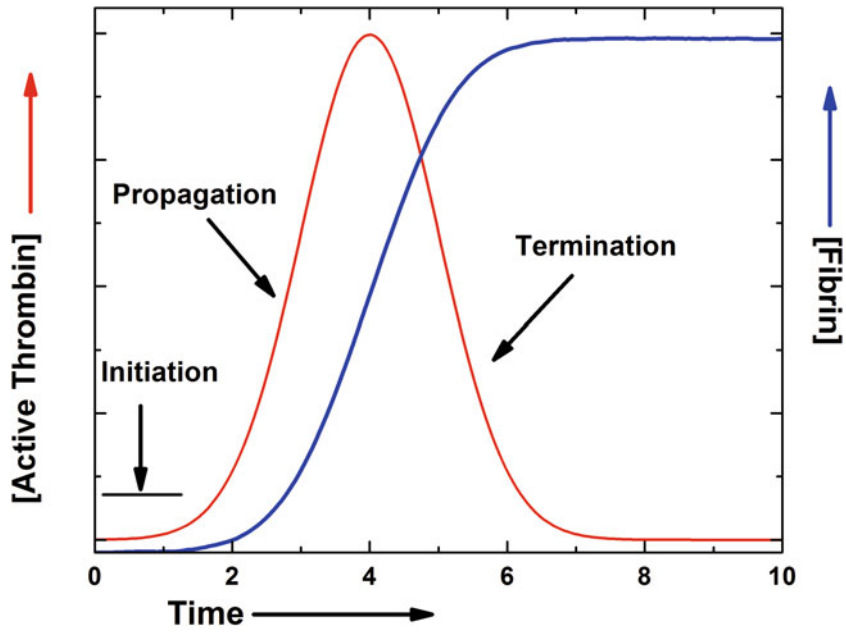

Fig. 4.2 Fate of thrombin following initiation of coagulation. In the initiation phase, positive feedback reactions enhance flux through the cascade to yield an explosive increase in thrombin formation in the propagation phase. Termination results following initiation of the anticoagulant reactions and elimination of proteinases by reaction with serpins. Fibrin is expected to evolve as the first derivative of the thrombin trace provided fibrinogen consumption is minimal

gain with inhibitors will carry significant risk for dysregulation of the hemostatic response. A second logical problem posed by therapeutic targeting of the active site of any of the coagulation serine proteinases relates to the potentially harmful and unpredictable consequences of shielding the proteinase in a reversibly inhibited complex that is protected from irreversible inhibition and removal by Serpins.

The Catalyst for Thrombin Formation

The conversion of prothrombin to thrombin represents the first committed step in coagulation as it is the first nonredundant activation reaction in the pathway between the initiation of coagulation and thrombin production (Fig. 4.1). This imbues the catalyst for thrombin formation with key functional and regulatory significance for coagulation, evident from severe bleeding in patients with defects in the catalyst [7]. Unlike thrombin, which shows relatively broad substrate specificity, the other serine proteinases of coagulation are far more selective for their substrates. Their specificity and selectivity arises from the membrane-dependent association of cofactors with their respective proteinases. This architecture is exemplified by the prothrombinase complex responsible for catalyzing the conversion of prothrombin to thrombin. Prothrombinase is composed of a complex of the serine proteinase factor Xa, and its cofactor, factor Va, that assembles through reversible protein–protein and protein–membrane interactions on membranes containing phosphatidylserine (Fig. 4.3). A wealth of biochemical and biophysical insights into the assembly and function of prothrombinase have been derived in the last 30 years from studies using purified proteins, synthetic phospholipid vesicles containing an optimal ratio of phosphatidylcholine and phosphatidylserine (PCPS). Although Xa is a competent serine proteinase with a fully formed active site, it acts as a very poor activator of prothrombin. Addition of saturating concentrations of membranes and Va dramatically improves the steady-state kinetic constants for prothrombin activation (Table 4.1). These improvements in substrate affinity and catalytic power attributed to the effects of membranes and the cofactor result in the proverbial 500,000-fold increase in the rate of thrombin formation at the physiological concentration of prothrombin. This is a generalizable phenomenon as comparable functional changes accompany the assembly of the other coagulation

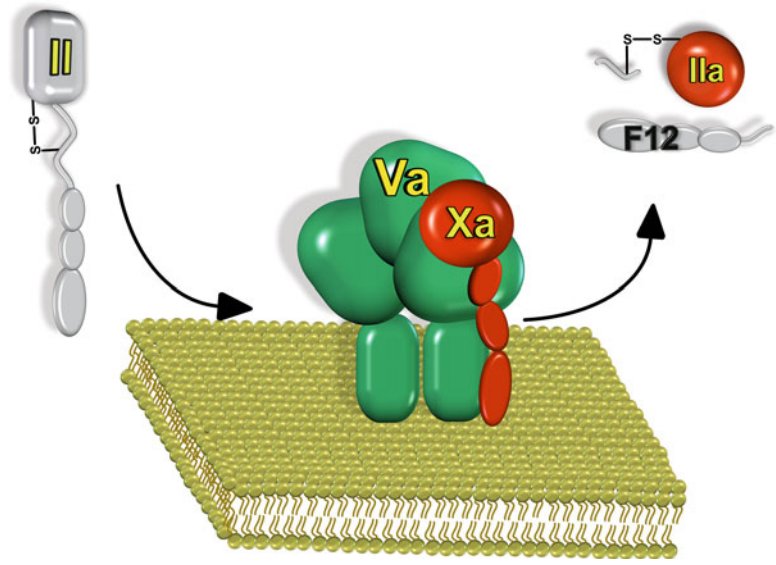

Fig. 4.3 Membrane assembled prothrombinase. Prothrombinase assembles through membrane-dependent interactions between the cofactor (factor Va) and the proteinase (factor Xa) on membranes containing phosphatidylserine. The complex cleaves the zymogen prothrombin (II) to thrombin (IIa) and the activation peptide fragment 1.2 (F12). Taken with permission from reference [65]

Table 4.1 Steady state kinetic constants for prothrombinase

Sustrate		Prothrombin[a]		Peptidyl substrate[b]	
Enzyme species[c]	Km (μM)	Vmax/E_T (s^{-1})	Relative rate[d]	Km (μM)	Vmax/E_T (s^{-1})
Xa	84	0.01	1	98	172
Xa/PCPS	0.7	0.05	203	98	181
Xa/Va/PCPS	0.4	108	512,000	200	179

[a]Kinetic constants for bovine prothrombin cleavage by bovine Xa alone [63], or for the activation of human prothrombin by human enzyme constituents in the presence of membranes [64]
[b]Kinetic constants for the cleavage of Spectrozyme Xa (methoxycarbonyl-D-cyclohexylglycyl-glycyl-L-arginine-p-nitroanilide) taken from reference [35]
[c]The enzyme species correspond to Xa alone, Xa saturably bound to membranes (Xa/PCPS) and Xa assembled into prothrombinase (Xa/Va/PCPS) using saturating concentrations of membranes and Va
[d]Relative rates at 1.4 μM prothrombin calculated from the steady state kinetic constants

enzyme complexes containing homologous constituents that interact in an analogous way [1]. On the one hand, these functional effects are considered essential for highly accelerated zymogen activation limited to the site of vascular damage leading to rapid clot formation. It follows from reason that the paradoxically poor activity of the proteinase alone in activating its biological substrate in the absence of membranes and cofactor is an equally important feature necessary for limiting thrombin formation in the absence or beyond the site at which damaged or activated cells expose phosphatidylserine.

Membranes and Function

All interacting species, proteinase, cofactor and substrate bind reversibly to membranes containing phosphatidylserine. Factor Xa and prothrombin are vitamin K-dependent proteins that contain γ-carboxyglutamic modifications at their N-terminal Gla domains. This domain mediates their binding to membranes in a Ca^{2+}-dependent manner [1]. Unlike the vitamin K dependent proteins, membrane binding by factor Va is not Ca^{2+}-dependent and likely occurs through the two discoidin-like C domains at its C-terminus [1]. Mechanistic details aside, all three interacting species bind to the membrane surface in an oriented fashion which lies at the heart of how membrane binding modulates function.

Factors Xa and Va interact with a physiological irrelevant affinity (Kd ~2 μM) in solution. Enzyme assembly requires the initial binding and independent binding of Xa and Va to the membrane surface followed by their surface-limited interaction to form prothrombinase with ~1000-fold enhanced affinity (Kd ~1 nM) [8, 9]. The efficient interaction on the membrane surface arises from their oriented binding to the membrane and the function of the membrane as a wave guide to limit dimensions and accelerate productive collisions. Linkage between protein–protein and protein–membrane interactions further contributes to enhanced affinity and permits prothrombinase to assemble efficiently at picomolar concentrations, far lower than would be predicted from knowledge of the individual binding constants [9]. These points outline the essential role played by membrane binding by reactants in facilitating prothrombinase assembly.

Because prothrombin can also bind membranes, it follows that similar phenomena must apply to its delivery and cleavage by the membrane assembled enzyme. An influential paper published 30 years ago sought to provide a comprehensive mechanistic interpretation of how membrane binding by prothrombin contributes in a fundamental way to the enhanced function of membrane-bound prothrombinase [10]. In this interpretation, preventing membrane binding by prothrombin was viewed as the functional equivalent of preventing the assembly of prothrombinase leading to catastrophic 3000-fold decrease in rate. It now seems likely to be a considerable over-estimate of the contribution of the substrate–membrane interaction to the rate of thrombin

formation as rate is only decreased by a modest fourfold for a prothrombin variant lacking γ-carboxyglutamic modifications necessary for membrane binding [11]. Conversely, membrane binding plays a major role in the presentation of the substrate to membrane-bound prothrombinase and the regulation of the pathway for substrate cleavage, covered in more detail below.

The perennial criticism of mechanistic inferences drawn from studies using purified proteins and synthetic membranes is the lack of relevance to the situation in vivo wherein the activated platelet adherent at the site of vascular damage has long been considered the most important surface for the assembly and function of prothrombinase [12]. There is little arguing that the platelet surface is complex and likely imposes fundamentally different physical and perhaps chemical constraints on prothrombinase assembly and function than pure phospholipid vesicles containing only phosphatidylcholine and phosphatidylserine. The long-standing suggestion for a specific platelet receptor(s) for the constituents of prothrombinase are yet to be borne out [13]. It now appears increasingly likely that the litany of functional differences ascribed to the platelet can adequately be attributed to the limiting exposure of phosphatidylserine [11]. Recent studies imaging prothrombinase following laser damage to the mouse microcirculation further question the preeminent role ascribed to platelets in supporting enzyme assembly and function. Instead, it appears that the damaged and activated endothelium is the major site supporting the binding of factors Xa and Va [14]. Relating concepts developed from biochemical and biophysical studies in purified systems to explain prothrombinase function on naturally relevant membranes represents a major outstanding challenge in the field.

The Cofactor and Function

The need for a cofactor for efficient thrombin formation has presented a long-standing puzzle in the field. This arises from the fact that the catalytic domain of factor Xa closely resembles trypsin, yet trypsin does not need a cofactor to act on its protein substrates with high efficiency. The early observation that factor Va seemingly increased the rate constant for catalysis for thrombin formation suggested a self-evident mechanism for cofactor function [1]. Factor Va must bind Xa and alter the active site to make it more complementary to the transition state and thereby lowers the free energy barrier for catalysis [15]. While this idea was supported by Va-dependent changes in spectroscopic probes incorporated into the active site of Xa, the unanswered question is whether these perturbations detected at the active site are relevant to function [16, 17]. Studies using a transition state irreversible peptidyl inhibitor and a variety of peptidyl substrates for Xa have failed to provide any evidence to suggest Va-dependent increases in active site function [15]. Indeed, the large increases seen in the kinetics of prothrombin activation upon the assembly of prothrombinase are not accompanied by significant changes in peptidyl substrate cleavage (Table 4.1). This implies that the questions of protein substrate specificity of prothrombinase and the mechanism by which factor Va functions within prothrombinase are closely related questions not properly assessed by peptidyl substrates that target the active site of Xa within prothrombinase. These ideas are further supported by the repeated failure of peptidyl substrate studies to adequately account for the cleavage site preference of prothrombinase (or Xa) within prothrombin [18–20].

Prothrombin Activation

Thrombin formation requires proteolysis at two sites within prothrombin. Consequently, there are two possible pathways for prothrombin activation (Fig. 4.4). Initial cleavage of prothrombin following Arg^{271} yields the N-terminal pro-piece fragment 1.2 (F12) and the zymogen prethrombin 2 (P2), which is then further cleaved following Arg^{320} to yield the disulfide-linked two chain form of thrombin. Cleavage of the bonds in the opposite order yields the proteinase meizothrombin (mIIa) as an intermediate. Its subsequent processing at Arg^{271} yields the final reaction products. The cleavage pathway that predominates is

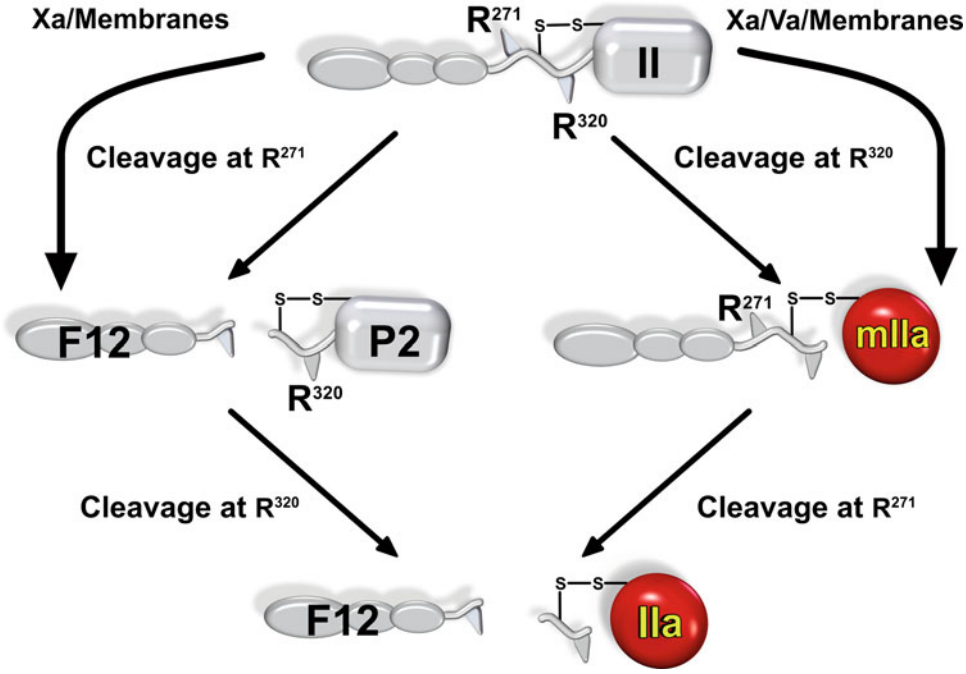

Fig. 4.4 Pathways for prothrombin activation. The conversion of prothrombin to thrombin results from cleavages following Arg^{271} and Arg^{320}. Initial cleavage following Arg^{271} yields the pathway on the *left* and produces the zymogen, prethrombin 2 (P2) and the propiece, fragment 1.2 (F12) as intermediates. P2 requires further processing at Arg^{320} to yield thrombin. The pathway on the *right* arises from initial cleavage following Arg^{320} which produces the proteinase meizothrombin (mIIa) as an intermediate. Further cleavage following Arg^{271} is required to yield IIa and the propiece, F12. The *left arm* of the pathway predominates in the absence of Va while activation almost exclusively proceeds through the *right arm* for prothrombinase. Taken with permission from reference [65]

dependent on the presence of factor Va and either adequate phosphatidylserine content of membranes or the ability of prothrombin to bind the membrane surface [21]. In the absence of factor Va, the reaction proceeds primarily via cleavage at Arg^{271} leading to the formation of P2 and F12 as intermediates. When prothrombinase is assembled with saturating concentrations of Va and membranes with high phosphatidylserine content (25 % w/w), prothrombin activation proceeds essentially exclusively via initial cleavage at Arg^{320} and the formation of meizothrombin as an intermediate [22]. Variable flux towards thrombin formation via the two arms of the pathway is evident when Xa is saturated with very high concentrations of Va in the absence of membranes, when membranes containing 5 % phosphatidylserine are used to assemble prothrombinase, when prothrombinase is assembled on activated platelets versus endothelial cells and when prothrombin with a deficit in γ-carboxyglutamate content is used as a substrate [11].

The biological significance of the pathway of prothrombin activation lies in the fact that P2 is a zymogen while mIIa is a proteinase that is defective in several of the procoagulant activities of thrombin but retains the ability to bind thrombomodulin and function as an anticoagulant by catalyzing protein C activation [23, 24]. The biochemical significance lies in the wealth of insights that this process reveals regarding the substrate specificity of prothrombinase, aspects of the role of cofactor and the substrate–membrane interaction in thrombin formation and the putative importance of the conversion of zymogen to proteinase in regulating the process of prothrombin activation. Because cleavage of precursors at multiple sites, frequently in ordered

fashion, is commonplace in coagulation, prothrombin activation provides a template for the consideration of the complicated enzymology associated with such cleavage reactions.

The interrelationship between substrate specificity and cofactor function is again revealed by the fact that the large increase in catalytic function that occurs with the addition of factor Va is associated with the ability of prothrombinase to distinguish between the two cleavage sites within prothrombin. Thus, while both cleavage sites within prothrombin are available to be cleaved by added proteinase, near-absolute discrimination between the two cleavage sites is evident from the fact that when prothrombin engages prothrombinase only the Arg^{320} site is available for cleavage by the enzyme complex. Cleavage at Arg^{271} only occurs following the formation of mIIa. Because mIIa is a serine proteinase and prothrombin is a zymogen, it follows that the zymogen to proteinase transition plays an important role in determining the sequential action of prothrombinase on prothrombin and its derivatives. Another complexity revealed here is that regardless of the pathway of prothrombin activation, thrombin formation results from the action of prothrombinase or Xa in two sequential enzyme-catalyzed reactions. Thus, steady state kinetic constants measured by adding prothrombin and measuring the rate of thrombin formation cannot be meaningfully interpreted. This suggests fundamental problems in deriving useful mechanistic interpretations using such kinetic measurements that have been used to frame the problem since the early 1980's.

Proteinase Formation

Thrombin and the other serine proteinases of coagulation are members of the S1 peptidase clade of which chymotrypsinogen is the archetypic member [25]. Consequently it is most useful to consider the areas of interest related to proteinase function within the catalytic domain using the homologous numbering system according to chymotrypsinogen (denoted by a c following the residue number, eg. Ile^{16c}) [26]. In the case of prothrombin and its derivatives, cleavage of the Arg^{320}-Ile^{321} peptide bond, which corresponds to Arg^{15c}-Ile^{16c}, is the key step in the conversion of the zymogen to proteinase. Cleavage reveals a new N-terminus in the catalytic domain which inserts in a sequence-specific way into the N-terminal binding cleft and forms a salt-bridge with Asp^{194c}. This process, aptly termed "Molecular Sexuality" by Wolfram Bode, is associated with ordering of four activation domains, 16c-21c, 142c-152c, 184c-194c, and 216c-233c, which are disordered in the zymogen. The net outcome includes correct formation of the primary specificity pocket (S1) which allows the binding of Arg-containing substrates at the active site. There is a flip in the amide bond of Gly^{193c} to form the oxyanion hole which is necessary to stabilize the transition state. These are the primary changes that allow the proteinase product to bind substrates with high affinity and catalyze their cleavage with a greatly increased catalytic rate constant, yielding 10^4–10^5-fold improvements in catalytic function in comparison to the zymogen. Thrombin also utilizes surfaces removed from the catalytic site, termed anion binding exosites (ABE1 and ABE2), to interact with its substrates and ligands (Fig. 4.5). Proteinase formation reorders the 70c loop to mature and enhance the binding function of ABE1. Na^+-binding to a specific site in the proteinase is also enhanced such that the cation is not bound in the zymogen but is part of the stabilized and folded structure of the proteinase. A final observation is that formation of the proteinase destabilizes ligand binding to ABE2 [27].

The most relevant ABE2 ligand for prothrombin activation is the F12 pro-piece that interacts with the proteinase domain through the fragment 2 (F2) region. Because of the destabilizing effect of proteinase formation on ABE2 ligation, the zymogen binds F12 with high affinity (~10^{-8} M), while thrombin binds F12 with ~100-fold weaker affinity [27]. The result of this discrepancy is that F12 greatly enhances the cleavage of P2 at Arg^{320} by binding the zymogen through F2 region and facilitating its binding to membranes through the F1 domain [27]. In contrast, the analogous but weaker interaction between thrombin and

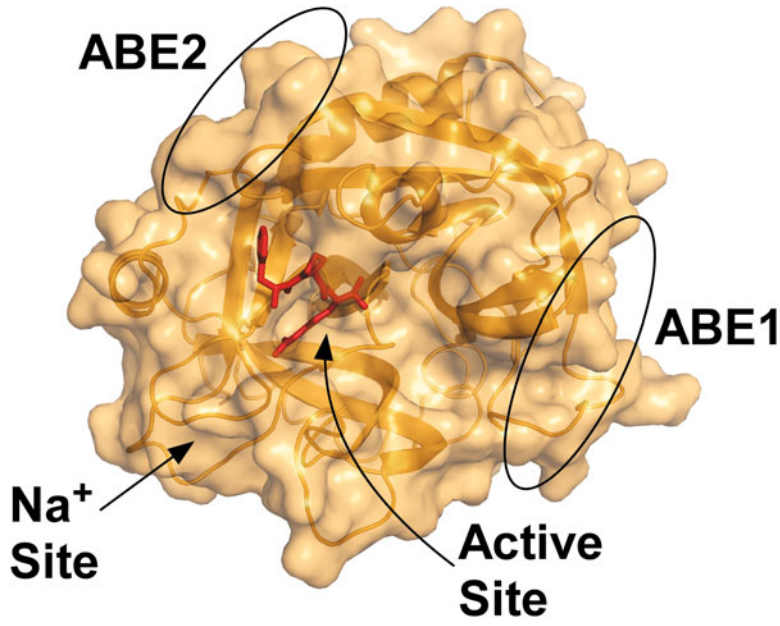

Fig. 4.5 X-ray structure of thrombin. The structure of human thrombin inhibited by D-FPR-chloromethyl ketone (1PPB) is shown in the standard orientation. The positions of the active site, ABE1, ABE2, and the Na^+ binding site are identified. The inhibitor is rendered in *red sticks*

F12 facilitates the release of the proteinase from the membrane surface at which it was produced. This is not the case for mIIa in which the proteinase domain is in covalent linkage with the F12 region. Consequently, despite being a proteinase variant of thrombin, mIIa can bind membranes with approximately the same affinity as does prothrombin allowing its accumulation on the membrane surface and its ability to cleave substrates in a membrane-dependent fashion [28]. Membrane binding by mIIa and the presence of the covalently bound F12 region are likely responsible for its apparently different sub-set of activities in comparison to thrombin.

Specificity of Prothrombinase for Prothrombin

Because of the interpretation problems posed by the sequential enzyme-catalyzed reactions in thrombin formation, mechanistic insights awaited the development of a series of recombinant substrate forms and isolated intermediates to allow studies of all possible four cleavage reactions. Early studies focused on using P2 in the absence of F12 as the simplest possible substrate for prothrombinase with a single cleavage site and lack of complexity arising from its ability to bind membranes [29]. Even these initial approaches provided evidence for a drastic difference in the mechanisms underlying protein substrate recognition in comparison to the specific binding and cleavage of peptidyl substrates that provided the formalisms for considering the mechanistic details of prothrombinase function until then. Short peptidyl substrates interact in a very limited way with the active site of Xa within prothrombinase to form a Michaelis complex prior to their cleavage and product release. A hallmark of this mechanism is the observation of competitive inhibition by reversible inhibitors that target the active site of factor Xa [29]. In contrast, the same inhibitors were found to be classical noncompetitive inhibitors of P2 cleavage by prothrombinase, implying that occluding the active site has no effect on the affinity of the protein substrate for prothrombinase. This can be accommodated by a multistep model for protein substrate binding in which the initial interaction between the substrate and prothrombinase occurs at extended surfaces on the enzyme (exosites) distant from the active site, followed by its active site docking and catalysis before product release (Fig. 4.6). Exosite binding is the main determinant for substrate affinity while the active site

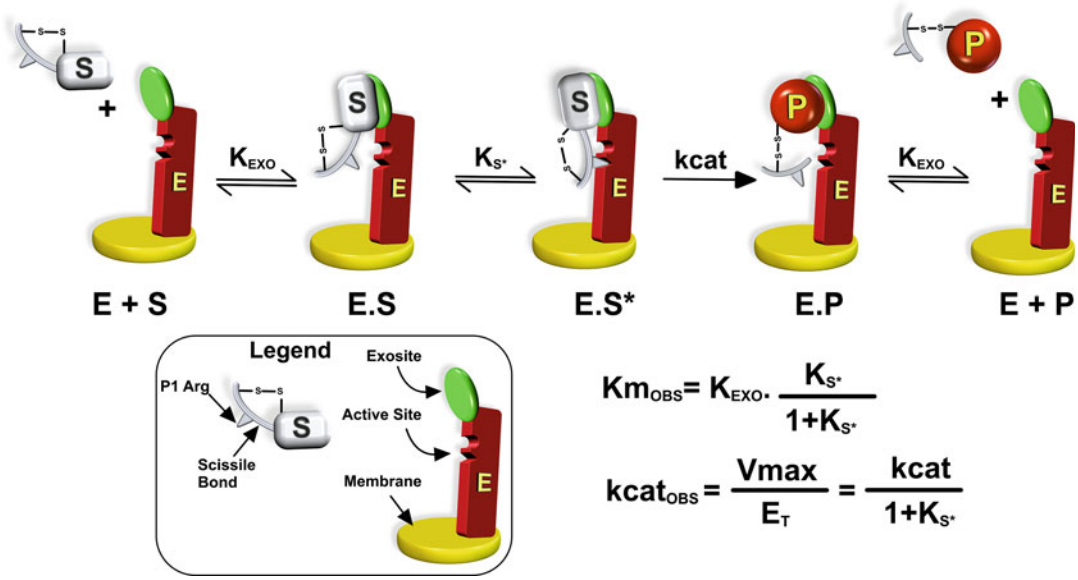

Fig. 4.6 The dock and lock pathway for protein substrate recognition by prothrombinase. The scheme illustrates the pathway for the recognition and cleavage of P2 by prothrombinase. The initial binding interaction between substrate (S) and prothrombinase (E) to form ES results from exosite-dependent interactions which leaves the active site unligated. Exosite binding is followed by a unimolecular binding step in which structures flanking the cleavage site engage the active site of the enzyme before catalysis can occur. The product (P) is also bound to E by exosite interactions before it is released. The graphic legend highlights the important features of S and E. The composite nature of the steady state kinetic constants is illustrated by derivation employing the rapid equilibrium assumption. Ks* is defined as [E.S]/[E.S*]. Taken with permission from reference [65]

docking step occurs in a unimolecular step before cleavage and contributes to the perceived rate constant for catalysis. The resultant composite nature of the steady state kinetic constants (Fig. 4.6), that derive from this mechanism illustrate how the active site docking step influences the observed rate constant for catalysis. This suggests that large improvements in $kcat_{obs}$ can result from simply modulating active site binding to prothrombinase and likely represents a component mechanism of how factor Va functions as a cofactor within prothrombinase.

An important implication of this observation is that the sequence-specific cleavage of prothrombin by prothrombinase is enforced by the exosite-mediated presentation of the scissile peptide bond to the active site of Xa within the complex rather than simply docking of the Glu/Asp-Gly-Arg sequence preceding the two scissile bonds with the active site. This idea has been tested with recombinant substrate variants in which residues before the scissile peptide bond were replaced with those found in other coagulation zymogens not ordinarily cleaved by prothrombinase [30]. Prothrombinase efficiently cleaved these prothrombin variants with a similar apparent affinity but with a modest change in the rate of catalysis. Thus, in contrast to prevailing ideas in the field, the active site of Xa within prothrombinase can accommodate and cleave a wide range of peptidyl sequences provided the protein substrate can engage the enzyme through exosite binding and facilitate their presentation for active site docking. In an unexpected twist, the geometric constraints enforced by exosite binding were also not found to be absolute as prothrombinase was surprisingly tolerant of an N-terminal shift of the cleavage site by one or two residues with cleavage being lost at greater shifts [31]. This suggests some flexibility in the polypeptide tether linking the exosite binding region and sequences flanking the cleavage site that engage the active site of the catalyst in the second step.

How Ordered Cleavage of Prothrombin is Achieved

A suite of recombinant mutants of full length prothrombin and the isolated intermediates allowed similar studies for each of the four possible reactions of prothrombin activation even in the presence of membrane binding (Fig. 4.7). Each possible half reaction exhibited the same dock and lock behavior initially noted with P2 and provided insights into why prothrombinase acts on prothrombin in an ordered fashion. The substrates for each of the four half reactions were cleaved with equal affinities [22]. Even though the II_{QQ} variant is uncleavable, it also bound prothrombinase with the same affinity as the other substrate forms. This is consistent with a primary role for exosite tethering rather than active site docking in determining binding affinity. Preferential cleavage of prothrombin at Arg^{320} first followed by cleavage at Arg^{271} was evident from equal values of $kcat_{obs}$ for three of the four half reactions while $kcat_{obs}$ was decreased 30–40 fold for cleavage at Arg^{271} in II_{Q320}. These findings explain why 97 % or more of the flux towards thrombin formation catalyzed by prothrombinase would occur via the formation of meizothrombin. When interpreted within the context of the composite nature of $kcat_{obs}$ for the dock and lock mechanism of substrate recognition (Fig. 4.6), the findings imply that when prothrombin binds prothrombinase, Arg^{320} readily engages the active site while Arg^{271} does not. Defective active site engagement by Arg^{271} is corrected by prior cleavage at Arg^{320} to form mIIa. Indeed, these ideas have been independently confirmed by binding studies of the individual cleavage sites in this suite of prothrombin variants to engage the active site of Xa within prothrombinase [32].

Ordered Prothrombin Cleavage and the Zymogen to Proteinase Transition

The ordered action of prothrombinase on prothrombin has its roots in the constraints arising from a single kind of exosite tethering interaction

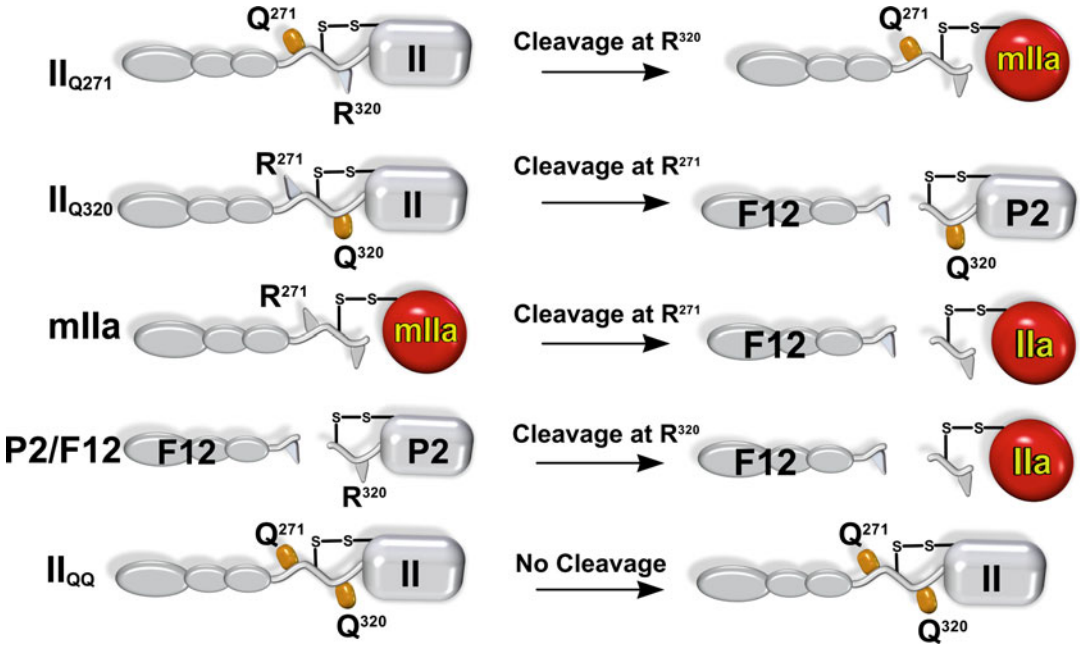

Fig. 4.7 Prothrombin variants necessary to resolve the kinetics of all possible steps of prothrombin cleavage. The cleavage reaction measured using the different substrate variants are indicated with the appropriate product either without prior cleavage (by substitution of one Arg with Gln) or following prior cleavage at one site. Taken with permission from reference [65]

between substrate and enzyme that is responsible for the presentation of two distant sites, expected to be separated by ~30Å, for active site docking and cleavage. The second major contributor is the well-established conformational transitions that accompany the conversion of zymogen to proteinase following cleavage at Arg^{320} to form mIIa [33]. Geometric constraints imposed by exosite tethering allow Arg^{320} to readily dock at the active site of Xa within prothrombinase while the distant Arg^{271} site cannot (Fig. 4.8a). When the proteinase is formed by prior cleavage at Arg^{320}, the comparably tethered mIIa substrate allows the previously distant Arg^{271} site into the vicinity of the active site for docking and cleavage (Fig. 4.8b). These concepts underlying the sequential presentation of the two sites to prothrombinase can be further illustrated by the manipulation of prothrombin between zymogen-like and proteinase-like forms. By exploiting the sequence-specific nature of the molecular sexuality process in proteinase formation, prothrombin variants were created that could be cleaved normally at Arg^{320} but failed to undergo maturation to proteinase. Despite prior cleavage at Arg^{320} the resultant mIIa was very poorly processed at Arg^{271} and persisted as an intermediate (Fig. 4.8c). Conversely, covalent stabilization of uncleaved prothrombin in a proteinase-like conformation enhanced its cleavage at Arg^{271} with a concomitant decrease in the rate of cleavage at Arg^{320} (Fig. 4.8d). This summarizes the essential role of exosite-dependent

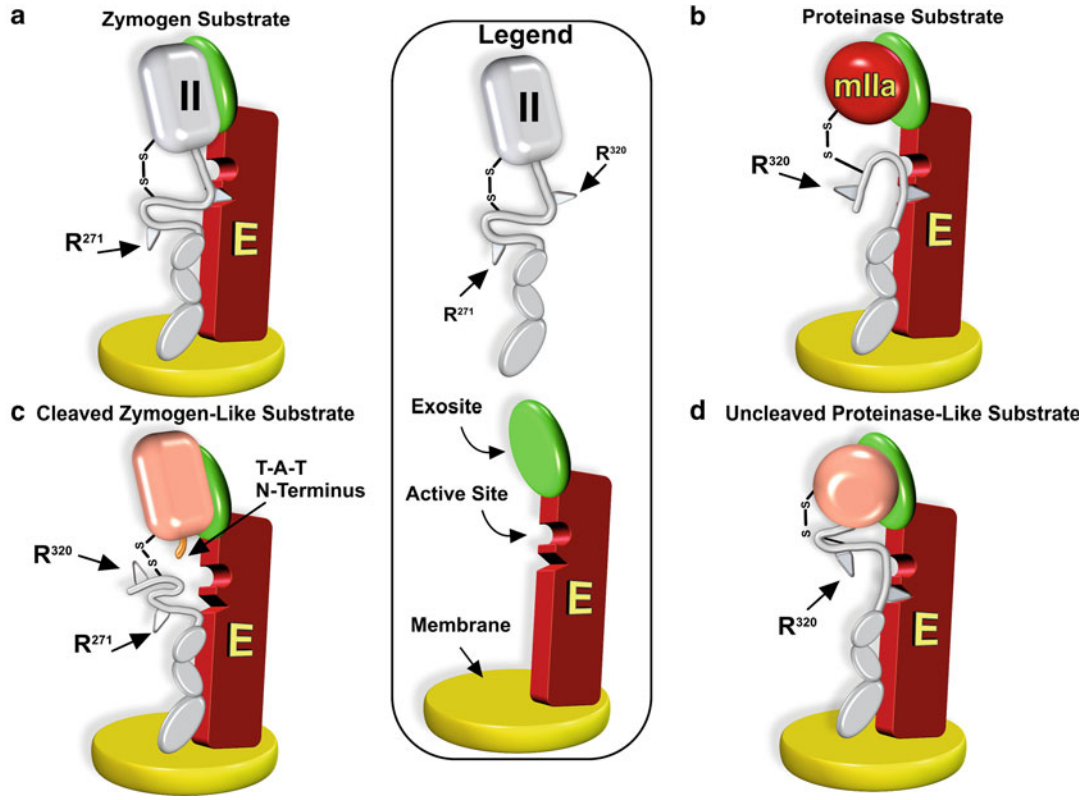

Fig. 4.8 Active site docking by the two substrate sites is driven by the zymogen or proteinase-like character of exosite-bound substrate. Exosite-binding constrains substrate presentation such that when the substrate is the zymogen, Arg^{320} preferentially engages the active site for cleavage (*Panel A*). Conversely, the Arg^{271} site readily engages the active site when the substrate is the proteinase (*Panel B*). Prior cleavage at Arg^{320} in a variant that remains zymogen-like yields impaired cleavage at Arg^{271} (*Panel C*). Conversely conformational activation and stabilization of uncleaved prothrombin in a proteinase-like state yields increased cleavage at Arg^{271} at the expense of cleavage at Arg^{320} (*Panel D*). Taken with permission from reference [65]

substrate tethering and the geometric constraints imposed by the zymogen to proteinase transition in regulating prothrombin cleavage.

Protein–Protein and Protein–Membrane Interactions in Exosite-Tethering

Evidence points to a significant role for surfaces in the catalytic domain of factor Xa, removed from the active site, in contributing to the substrate binding exosite [34, 35]. This can be deduced from studies with nematode anticoagulant peptide C2 and a monoclonal antibody that bind Xa within prothrombinase and act as competitive inhibitors of protein substrate cleavage without engaging the active site or affecting active site function of prothrombinase. It also follows that exosite-dependent substrate tethering must be mediated by substrate structures distinct from residues flanking the two scissile bonds. Indeed, a fragment from the C-terminus of the proteinase domain within prothrombin, physically separable from substrate structures bearing the cleavage sites, can act as a classical competitive inhibitor of protein substrate cleavage [36]. Such inhibition is also achieved without affecting active site function within prothrombinase. Finally, considerable evidence has accumulated over the years implicating an important functional role for interactions between prothrombin and its derivatives and factor Va within prothrombinase [37–40]. While some of these observed interactions may be most relevant for the activation of factor V, probes that inhibit the binding of prothrombin derivatives to factor Va also inhibit protein substrate cleavage. Thus, it is likely that exosite binding results from interactions between the substrate and extended surfaces in both factors Va and Xa within prothrombinase. Inhibition of substrate binding can be accomplished by occluding either component interaction. This scenario implies that the exosite mediated dock and lock strategy for protein substrate recognition by prothrombinase may not apply to the function of Xa in the absence of Va. Thus, a large fraction of the rate enhancing effects of the cofactor probably derive from a change in the mechanistic strategy by which an otherwise poor substrate for active engagement is now effectively recognized and cleaved by prothrombinase. This conclusion is fundamentally different from the ideas that have prevailed until recently and could probably never have been reached without the kind of detailed mechanistic understanding developed over the years.

Because membrane binding is mediated by N-terminal fragment 1 region of prothrombin distant from the cleavage sites, it falls under the definition of an exosite interaction that could play an important role in tethering the substrate to prothrombinase. If so, altered exosite-dependent tethering because of suboptimal or defective interactions between the substrate and membrane would be expected to alter substrate affinity and the geometric constraints on the bound substrate that affect active site docking and the pathway for substrate cleavage. Both criteria are met when the four half-reactions of prothrombin activation are studied with the appropriate recombinant single cleavage site substrate variants but without γ-carboxyglutamate modifications to eliminate their interaction with membranes (Fig. 4.9) [11]. In this case, loss in geometric constraints is evident as a decrease in $kcat_{obs}$ for cleavage at Arg^{320} with a concomitant increase in $kcat_{obs}$ for cleavage at Arg^{271} in previously uncleaved prothrombin [11]. These reciprocal changes lead to a switch in the pathway for prothrombin cleavage from one that is essentially entirely via the formation of mIIa in the case of the membrane binding variant to one in which the flux to thrombin formation is ~80 % via the formation of P2. The most surprising result from these compensating changes is a very modest three- to fourfold decrease in the rate of thrombin formation. This flies in the face of conventional wisdom in field for the past 40 years where large numbers of investigators have sought to correlate the progress of warfarin anticoagulation or bleeding with plasma levels of uncarboxylated prothrombin [41]. There is no arguing that warfarin is an anticoagulant; it is just the effects of warfarin on a reduced rate of thrombin formation are most likely exerted at the level of the other vitamin K dependent proteins and

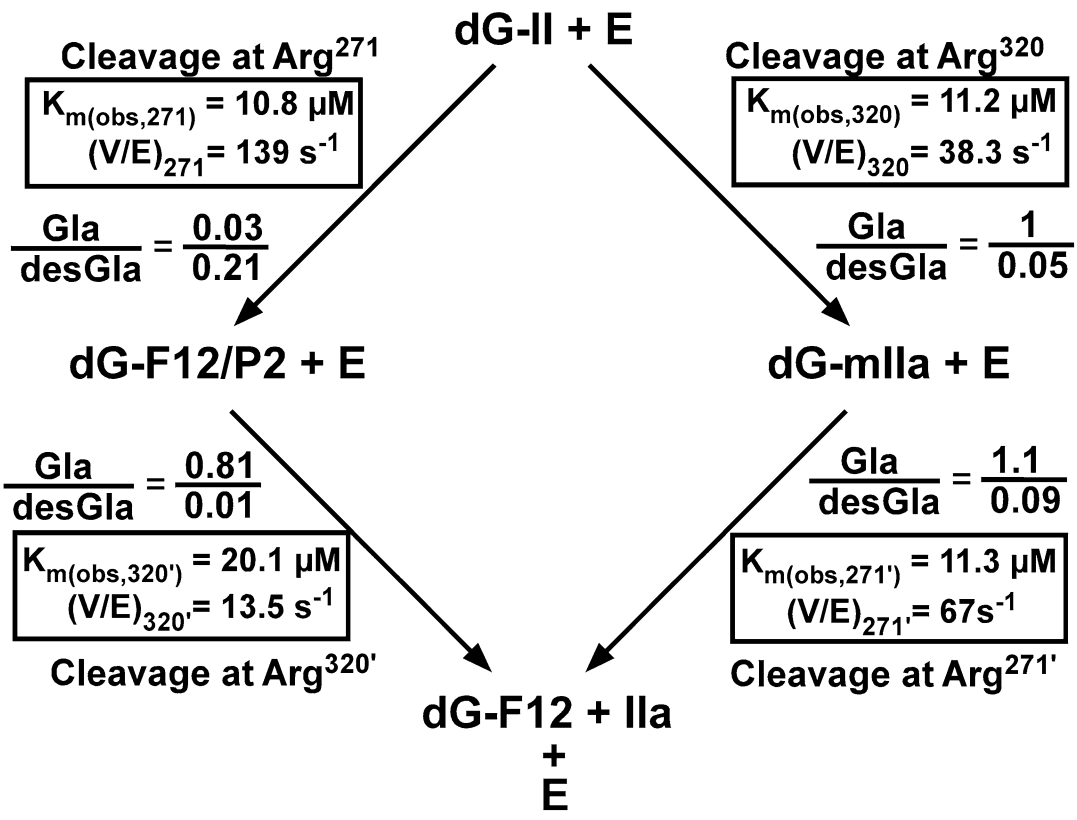

Fig. 4.9 Kinetics of cleavage of prothrombin with defective γ-carboxylation and membrane binding. The various des-gammacarboxy substrate forms are denoted as dG. Steady state kinetic constants for the individual steps are illustrated as are the rates for the individual steps at physiological concentrations of prothrombin for normal (Gla) and des-gammacarboxy (desGla) forms. E denoted prothrombinase

not on prothrombin. Thus, protein–protein and prothrombin–membrane interactions are component exosite binding interactions that work in concert to tether prothrombin to prothrombinase. Disrupting the membrane binding component is sufficient to relax the constrained presentation of the substrate to the enzyme and alter the pathway for thrombin formation (Fig. 4.10).

These concepts shed new light on the function of prothrombinase on natural membranes such as activated platelets or endothelial cells which are expected to expose limiting amounts of phosphatidylserine on their external leaflets [42]. Indeed, prothrombinase assembled on these cell surfaces forms thrombin by variable extents through the two possible activation pathways which can be replicated on synthetic membrane vesicles with low phosphatidylserine content [11]. The take home messages here are that prothrombinase function on physiologically relevant membrane surfaces can adequately described by biochemical mechanisms developed in purified and defined systems and that the amount of mIIa formed as an intermediate will be dependent on the cell type and its extent of activation in vivo.

Thrombin: The Multifunctional Proteinase

Interestingly, the exosite-dependent strategy employed by prothrombinase to achieve very narrow substrate specificity is also employed by thrombin to achieve pan-specificity in its myriad of roles as an effector and regulator of coagulation. This was initially evident from the ability to

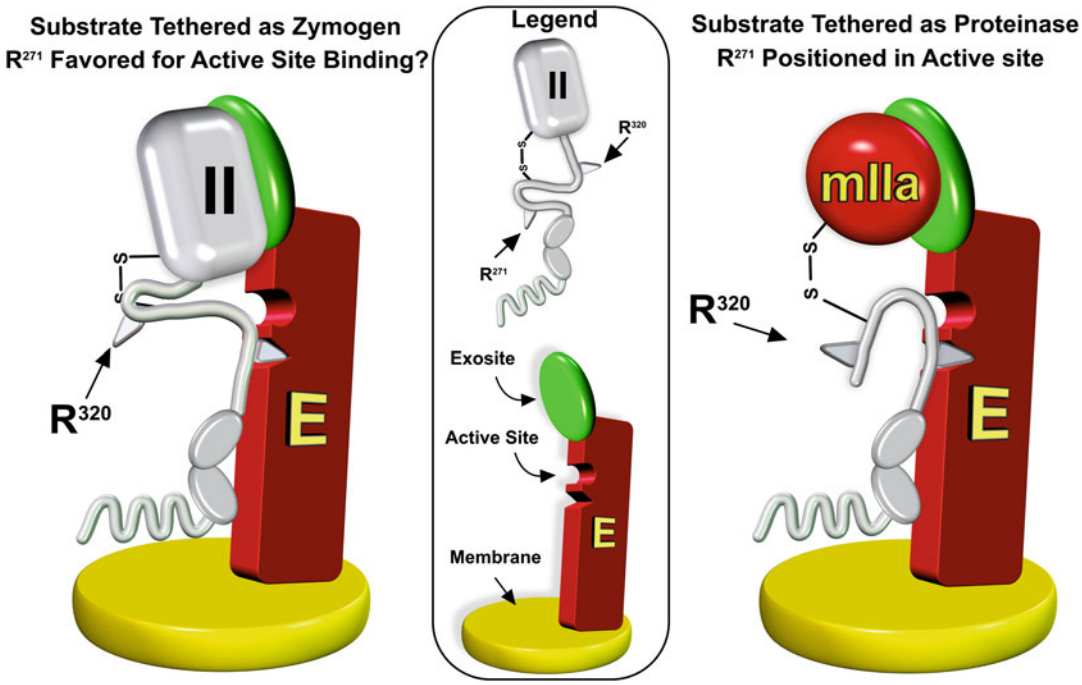

Fig. 4.10 Exosite-dependent tethering of substrate forms with defective γ-carboxylation to prothrombinase. The N-terminal Gla domain is shown as unfolded. In the enzyme-bound zymogen, loss of membrane binding now readily permits active site engagement by the Arg^{271} site. In the case of the previously cleaved proteinase intermediate (mIIa) membrane binding by the substrate has minimal impact on cleavage at the Arg^{271} site. The graphical legend highlights the salient features of the enzyme and substrate

greatly reduce its ability to clot fibrinogen by limited proteolytic cleavage but without impacting the ability of thrombin to cleave peptidyl substrates [43]. Subsequent and elegant studies of the inhibition of thrombin by the leech salivary inhibitor, hirudin, indicated that the femtomolar affinity for the interaction derived from bidentate binding with ABE1 and the catalytic site bridged by a polypeptide linker [44, 45]. The voluminous biochemical and structural studies that ensued have implicated an interaction motif with variations in detail on this theme, in explaining the binding and cleavage of a wide range of substrates for thrombin [46]. Thus, binding to ABE1 facilitates the engagement of a wide range of cleavage sites to the active site of the enzyme leading to cleavage, despite the fact that the residues flanking the site of cleavage reveal no compelling basis for the cleavage specificity of thrombin. On the other hand, ligation of ABE2 is mostly relegated to regulatory ligands such as heparin, which enhances the inactivation of thrombin by antithrombin III, the F2 domain of the F12 propiece, the γ′ region of fibrinogen, and glycoprotein Ib-IX-V [46, 47].

Thrombin also binds thrombomodulin through ABE1 which partly accounts for the procoagulant to anticoagulant switch in its specificity essential for the regulation of coagulation [48]. The binding of thrombin to the cofactor allows it to acquire the ability to activate protein C with a concomitant loss in its ability to cleave most other substrates. The occlusion of ABE1 by thrombomodulin prevents substrate binding by a host of its procoagulant substrates that utilize ABE1, thereby leading to a loss in the ability of thrombin to cleave these substrates [49, 50]. The structure of thrombin bound to a thrombomodulin fragment initially suggested that thrombomodulin would provide an additional surface to which protein C could bind therefore allowing for its cleavage and the anticoagulant function of

activated protein C. Subsequent studies unexpectedly failed to reveal evidence for this newly acquired exosite-dependent substrate recognition mechanism by the thrombin–thrombomodulin complex and instead point to a change in the way the scissile bond in protein C engages the active site of thrombin in complex with thrombomodulin [51]. This idea also likely applies to the ability of thrombomodulin-bound thrombin to activate TAFI and inhibit fibrinolysis [52].

Thrombin Allostery . A second aspect of the switch in specificity between procoagulant and anticoagulant functions of thrombin lies in the concept of proteinase allostery initially proposed as the idea that thrombomodulin likely induces a conformational change in the active site of thrombin to allow the acquisition of protein C activation function [53]. This idea is varyingly supported by a series of studies although it is not obvious from the X-ray structure of the thrombomodulin–thrombin complex [48]. Nevertheless, proteinase allostery originating from the ligation of ABE1 or other sites on the proteinase is likely an important feature underlying the many functions of thrombin that is supported by thermodynamic and NMR studies [54, 55].

A voluminous literature employing mutagenesis, functional and structural studies has accumulated in the past 20 years dealing with the subject of thrombin allostery. A good fraction of it deals with the binding of Na^+ to thrombin to affect its partitioning between a "Slow" form with preferential anticoagulant function and a "Fast" form with preferential procoagulant function. These allosteric changes are proposed to arise from linkage effects grounded in the idea that thrombin is only 50 % saturated with Na^+ in the physiological milieu [56, 57]. More recent measurements at physiological pH indicate a greater binding affinity for Na^+ than initially surmised from studies at pH 8.0 and indicate that thrombin would be near saturably bound to Na^+ in blood [54]. The physiological import of thrombin allostery arising from Na^+-binding and dissociation is now called into question regardless of the number of papers published on the subject.

Primarily as a result of mutagenesis studies and x-ray crystallography, the ideas of Fast (Na^+ bound) versus Slow (Na^+ free) thrombin have given way to a discussion of E* and E states also ascribed with different functions but variously stabilized by a bewildering array of mutations including those in highly conserved disulfide bonds [57, 58]. Accordingly, various thrombin mutants have been prepared with enhanced or preferential anticoagulant function with associated impairment in procoagulant function [59]. While these findings clearly indicate the ability to somehow dissociate the two opposing functions of thrombin, a coherent picture of how this can be achieved in multiple ways, within the context of E and E* thrombins, fails to emerge. This is particularly evident in the proposal that an uncleaved zymogen precursor of thrombin has been ascribed the properties of the E* form.

Is Thrombin Always a Proteinase?

Because proteinase formation results from irreversible cleavage at Arg^{15c} it is implicitly assumed that cleavage irreversibly stabilizes the proteinase. In contrast, early studies with chymotrypsin documented the existence of interconverting zymogen-like and proteinase-like species at near-neutral pH attributable to the protonation or deprotonation of Ile^{16c} [60]. Another notable exception is factor VIIa, which exhibits the characteristics of a zymogen until it binds its cofactor, tissue factor [61]. Investigation along these lines was prompted by the findings that the F12 pro-piece, produced during thrombin formation, bound the zymogen precursor P2 with substantially improved thermodynamics and affinity in comparison to thrombin [27]. In contrast, ligands for ABE1 such as thrombomodulin and hirugen bind thrombin more favorably than the zymogen P2 [38]. Thermodynamic studies were pursued using thrombin and P2 to define the limits of zymogen and proteinase along with a series of mutant variants variably stabilized along the pathway to proteinase formation [54]. The binding isotherms showed large and compensating enthalpic (ΔH) and entropic (ΔS) differences in the binding of F12 to these various forms depending on their increasing proteinase-like character.

As such enthalpy/entropy compensation is expected for ligand to related states that are in reversible equilibrium with each other, the findings surprisingly indicate that zymogen, zymogen-like, proteinase-like and proteinase states can reversibly interconvert along this thermodynamic trajectory. Accordingly, a strong active-site binding ligand could drag the zymogen-like form of thrombin along this thermodynamic trajectory to resemble the proteinase-like form. Conversely, removal of Na^+ while keeping the ionic strength constant could bring back the proteinase along the same trajectory to possess the thermodynamic properties of a zymogen-like form.

These unexpected and new ideas, grounded in thermodynamic measurements, suggest that thrombin can reversibly distribute along a continuum of zymogen-like and proteinase-like states even after cleavage but dependent on the complement of ligands that are bound to it (Fig. 4.11). This reversible distribution lies in the thermodynamically favorable ligation of ABE2 by F12 in the zymogen-like over the proteinase-like form and the converse being true for active site ligands or ligands for ABE1 (Fig. 4.11). Despite its dubious regulatory significance, Na^+-binding is a useful tool to manipulate this equilibrium as it is part of the folded structure of the proteinase but does not bind the zymogen.

These ideas provide a new formal framework for the consideration of allostery in thrombin and perhaps some of the other coagulation proteinases. Clearly, zymogen-like and proteinase-like

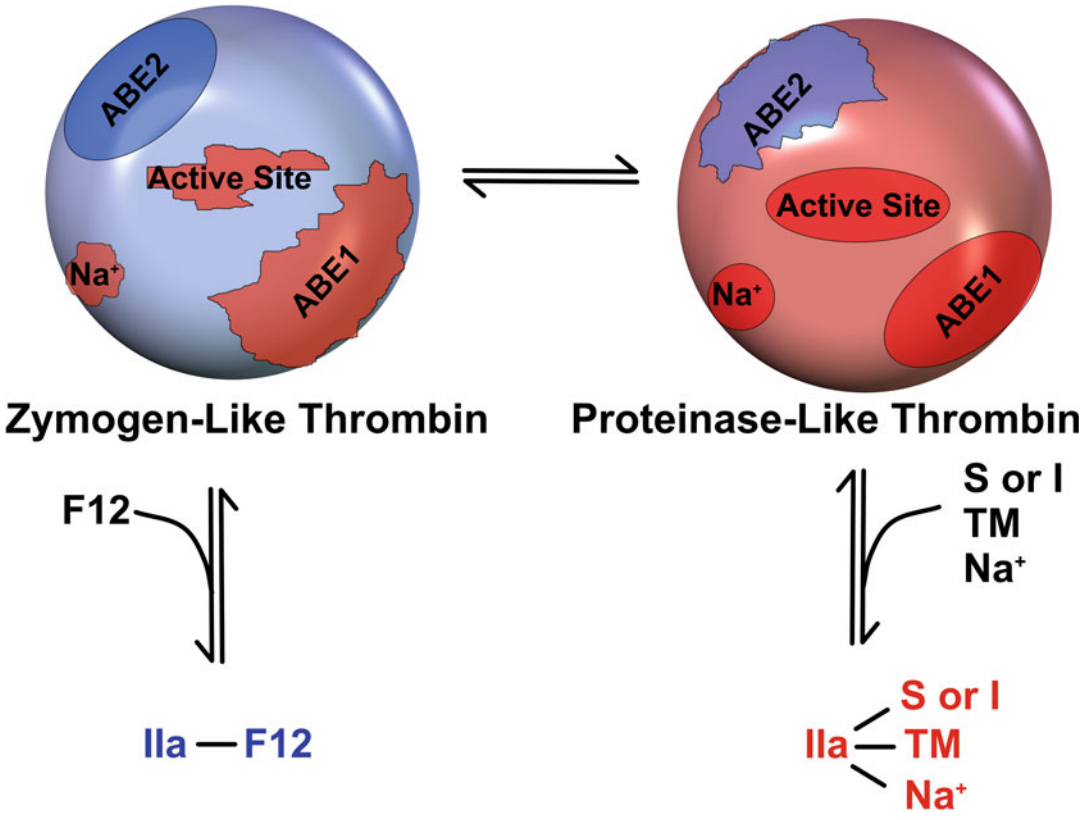

Fig. 4.11 Reversible interconversion of thrombin between zymogen-like and proteinase-like states. In the zymogen-like form, ABE1, the active site and the Na^+ site are illustrated as distorted while ABE2 is optimally functional. In contrast, ABE2 is distorted in the proteinase-like form while the other sites are optimally formed. F12 binds ABE2, while thrombomodulin (TM) binds ABE1. The active site can be ligated by substrates (S) or inhibitors (I). Taken with permission from reference [65]

forms will exhibit variable catalytic function which can be regulated by ligand binding. This framework also provides an explanation for how a myriad of different mutations can seemingly dissociate the opposing functions of thrombin. Any destabilizing mutation will favor zymogen-like forms with reduced activity that can variably be rescued by ABE1-binding ligands depending on their concentration and affinity. As fibrinogen binding is relatively weak, its ability to rescue these destabilized forms will be commensurately weak leading to poor procoagulant function. Conversely, thrombomodulin binds with high affinity to ABE1 and will be far more effective in rescuing protein C activation in the destabilized mutants. This proposal is in line with the distorted structures seen for many of the selectively anticoagulant variants of thrombin [55].

Are Zymogen-Like Forms Biologically Meaningful?

Despite the ability to rationalize thrombin allostery in terms of the ligand-dependent reversible interconversion between zymogen-like and proteinase like forms, there is doubt whether this can be biologically meaningful. This uncertainty arises from the weak interaction between F12 and thrombin and the fact that its binding to ABE2 and favor zymogen-like forms lies at the heart of the ligand-dependent shuttling of thrombin between the two proposed states (Fig. 4.11). Given the relatively weak binding of F12 to thrombin, a significant fraction of the enzyme is unlikely to be regulated in this way [54]. Reversible binding is not an issue in the case of mIIa in which the F12 region is covalently bound to the proteinase domain (Fig. 4.4). The associated prediction that mIIa would be expected to be exceptionally zymogen-like was tested and confirmed in rapid kinetic studies of ligand binding to its active site [62]. These studies revealed the slow equilibration of mIIa between equally populated zymogen-like and proteinase-like states [62]. In accordance with the predictions from studies with thrombin, the distribution between the two forms could be altered in both directions by adding thrombomodulin or by removing Na^+. In rapid kinetic studies using excess prothrombinase to affect near instant cleavage at Arg^{320} by prothrombinase, the zymogen-like form of mIIa was initially produced that only slowly equilibrated with the proteinase-like species (Fig. 4.12). This slow and reversible equilibrium represents the rate-limiting step in thrombin formation because cleavage at Arg^{271} to convert mIIa to thrombin requires its stabilization in the proteinase-like configuration (Fig. 4.8). Consequently, mIIa accumulates in vast excess over levels that would be predicted from the steady state kinetic constants for the sequential cleavage reactions. This represents a major advance in the understanding to prothrombin activation by prothrombinase. The biological significance of this finding lies in the slow equilibration between zymogen-like and proteinase-like forms and the fact that only the proteinase-like form can be processed further to thrombin. The zymogen-like mIIa will be refractory to inhibition by Serpins and be readily available to be washed downstream from its site of vascular damage. Given the fact that ABE1 ligands, such as thrombomodulin, can rescue variants with zymogen-like character, the ability of zymogen-like mIIa to be rescued in this manner and participate in protein C activation may be an important regulatory process in limiting expansive clot growth following vascular damage.

Conclusions

Despite their structural similarity, the proteinases of blood coagulation act on their cognate macromolecular substrates with narrow and defined specificity. This is accomplished by employing a conserved architecture and similar strategies to achieve specificity and enhanced function requisite for a rapid and highly regulated clotting response. Many of these strategies are exemplified in the process of prothrombin activation and by the functions of the resultant thrombin. Mechanistic studies with prothrombinase and thrombin reveal important principles by which regulated function is achieved. These rely on the

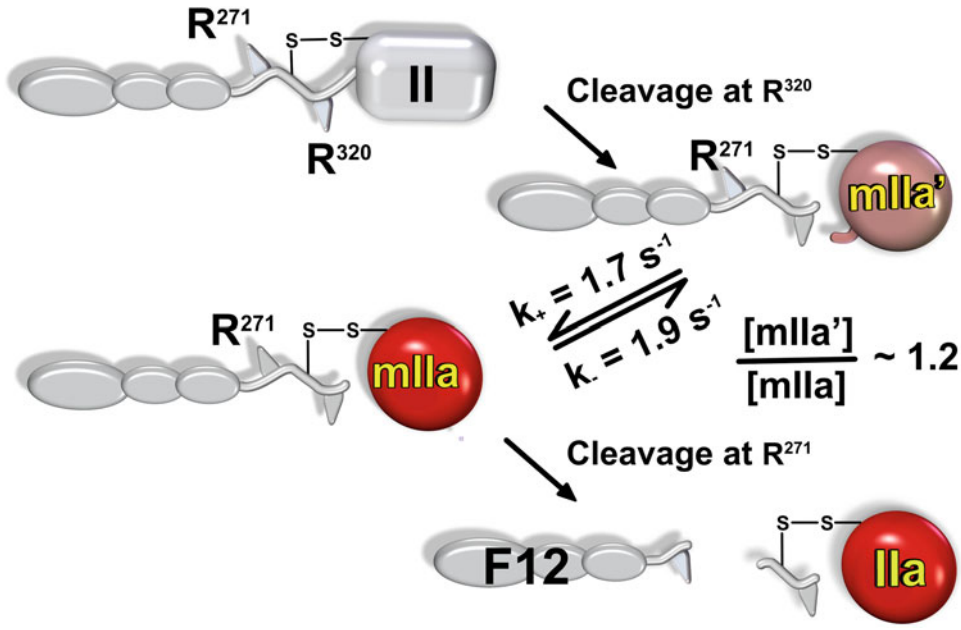

Fig. 4.12 A new rate-limiting step in thrombin formation. Cleavage of prothrombin by prothrombinase at Arg^{320} yields a slowly equilibrating mixture of zymogen-like meizothrombin (mIIa') and proteinase-like meizothrombin (mIIa) that are equally populated. Only the proteinase-like population of mIIa is further processed at Arg^{271} to produce thrombin. This new equilibrium derives from the covalent linkage of the N-terminal F12 region with the catalytic domain. Taken with permission from reference [65]

use of exosite interactions to bind the substrate and achieve very narrow specificity in the case of prothrombinase or pan-specificity in the case of thrombin. The second major mechanistic insight relates to the unexpectedly important role of the zymogen to proteinase transition in regulating prothrombin cleavage and in the process of thrombin allostery affected by its various ligands. Elements of these strategies are evident in the function of the other coagulation enzymes suggesting that they may widely apply and eventually provide an explanation for the diverse functions of the homologous enzymes of coagulation.

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Fibrinogen

5

Eduardo Gonzalez, Ernest E. Moore, and Hunter B. Moore

Introduction

Fibrinogen is an essential protein for hemostasis and circulates at the highest concentration of all the coagulation proteins. Fibrinogen's functions are to: (1) serve as the substrate for fibrin clot formation, (2) bind platelets and support platelet aggregation, (3) provide a template for binding of thrombin as well as proteins of the fibrinolytic system, (4) participate in wound healing. Thrombin catalyzed transformation of fibrinogen into fibrin, the fundamental fabric of blood clots, has intrigued protein scientists for more than a century [1–3]. The intricate regulation of fibrin formation allows for clots to be rigid enough to arrest bleeding and yet degrade for perfusion of the microvasculature.

The objective of this chapter is to review basic and clinical aspects of fibrinogen's structure and functions, focusing on those concepts that bring insight into the pathophysiology of trauma induced coagulopathy (TIC). In an effort to standardize nomenclature, the existing terminology related to fibrinogen structure has been proposed by the International Society on Thrombosis and Haemostasis (ISTH) [4] and will be used throughout this chapter.

Structure

Fibrinogen is a 340 kDa dimeric glycoprotein composed of two identical chains centrally connected by disulfide bonds (Fig. 5.1). Each half consists of three polypeptide chains (A-alpha, B-beta, and gamma), each coded by genes on chromosome 4 (*FGA, FGB, FGG*). This unusual nomenclature of fibrinogen's polypeptide chains arises from the inclusion of fibrinopeptides "A" and "B" that are cleaved by thrombin to yield a fibrin monomer [4]. The *N*-termini of all six chains are joined at the central domain (E-domain) by disulfide bonds, giving way to its dimeric structure [4]. Fibrinogen is 45 nm in length, with globular domains at each end (D-domains) and in the middle (E- domain) [5]. This configuration gives fibrinogen its trinodular structure. A total of 29 disulfide bonds, with all 58 cysteine residues of the protein participating in these interactions, hold the six polypeptide chains of fibrinogen together (Fig. 5.1).

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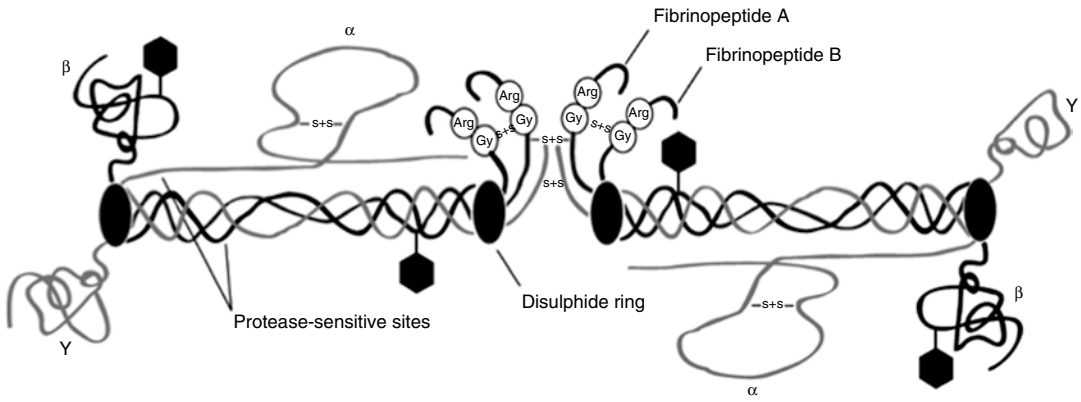

Fig. 5.1 Dimeric structure of fibrinogen formed by homologous alpha (A-alpha), beta (B-beta), and gamma chains. *Hexagons* represent carbohydrates attached to the protein

The gamma chain of fibrinogen has received much attention because of its regulatory role in the binding of thrombin [6, 7] and factor XIII [8, 9]. The *C*-terminal segments of the B-beta and gamma chains fold independently to form the beta- and gamma-nodules, named beta-C and gamma-C respectively [10]. The *C*-terminal segment of the A-alpha chain goes through the D-domain, and folds back to form the four chain coiled coil, named alpha-C. Together, the two alpha-C regions comprise 25 % of the total mass of fibrinogen. The coiled coils are stable and relatively rigid structures whose mechanical properties are likely critical for the overall viscoelastic behavior of fibrin clots. They provide the length needed for fibrin polymerization and serve as surface containing motifs for binding of other proteins.

The presence of carbohydrates on fibrinogen has important functional consequences. Carbohydrates have been found on B-beta and gamma chains, while the A-alpha chain was reported to be devoid of carbohydrates [11]. An example comes from patients with cirrhosis who have hypersialated fibrinogen that forms clots with thin fibers and many branch points [12]. Thin fibers with more branching points are relatively resistant to fibrinolysis [13]. Conversely, if all sialic acid on fibrinogen is removed, clots form with thicker fibers and less branch points [14]. These carbohydrates on fibrinogen modulate clot structure through both charge and mass on fibrin polymerization, particularly lateral aggregation.

Circulating fibrinogen also undergoes variable amounts of nonenzymatic glycation. Patients with uncontrolled diabetes have increased levels of fibrinogen glycation due to the high concentrations of glucose in their blood, but even nondiabetics have several sugar residues attached [15]. Higher levels of glycation appear to result in clots made up of thinner fibers and more branch points [16]. This notion has also been implicated as a mechanism that could explain why fibrinolysis of diabetic clots is significantly slower than that of controls [17].

Synthesis

Fibrinogen is synthesized in the liver [18] with a steady rate of production of 1.7–5.0 g per day and a large reserve capacity of up to 20-fold [19]. Expression of mRNA for fibrinogen polypeptide chains has also been identified in non-hepatic cells such as megakaryocytes and epithelial cells in response to inflammatory mediators [20]. Extrahepatic fibrinogen synthesis is thought to be related to wound healing and extracellular matrix adhesive capacities [21]. It is unknown whether synthesis of fibrinogen outside the liver contributes to the amount of fibrinogen available in plasma. A small pool of fibrinogen (but not the

gamma-chain variant) is stored in platelet alpha granules and appears to be taken up via GPIIb/IIIa receptor mediated endocytosis [22–24].

The polypeptide chains of fibrinogen are assembled in an ordered fashion in the rough endoplasmic reticulum of hepatocytes [25]. Newly synthesized human B-beta chains are secreted and used more rapidly than the other two chains, which accumulate in the hepatocyte. Carbohydrate side chains are added to the B-beta and gamma chains before secretion into the plasma [26]. Gamma chain variants are discussed later in this chapter.

Fibrinogen synthesis is controlled at the level of transcription [27]. The inducible component is mainly influenced by acute phase reactions mediated by interleukin-6 (IL-6) [28]. Glucocorticoids and IL-6 enhance transcription of fibrinogen mRNA; whereas, interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-alpha) suppress fibrinogen synthesis. This is somewhat counterintuitive since IL-1, IL-6, and TNF-alpha are all elevated during inflammation, yet they influence fibrinogen metabolism differently. Interestingly, fibrin(ogen) degradation products stimulate monocytes/macrophages to produce IL-6 [29], further upregulating fibrinogen synthesis. Fibrinogen's behavior as an acute phase reactant implicates additional stimuli and gene regulators that increase its expression; however, the control of basal transcription is required for its induction and the two cannot be separated per-se.

Studies in acute coronary syndromes have demonstrated that plasma fibrinogen can increase up to 20-fold with a peak elevation occurring by 3–5 days following myocardial ischemia and then gradually return to baseline following resolution of inflammation (when IL-6 levels decrease) [28, 30].

Alternative splicing results in a variant gamma chain, which is assembled into a normally occurring fibrinogen variant, referred to as gamma-prime fibrinogen. Approximately 8–15 % of plasma fibrinogen contains a variant gamma chain (gamma-prime) [31]. The ratio of gamma prime fibrinogen to total fibrinogen varies widely during pathologic conditions, including inflammation. The mechanism by which inflammation regulates alternative splicing leading to increase in gamma-prime fibrinogen has not been defined. This variant is associated with clots that are structurally different, with more extensive polymerization. A study of patients undergoing coronary angiography has shown that levels of this variant of fibrinogen were higher in coronary artery disease patients than in patients without coronary artery disease, and that this association was independent of total fibrinogen levels [7]. A case–control study performed in patients with myocardial infarction also reported this association [32]. Although studies like these focusing on arterial thrombosis consistently demonstrate that elevated levels of gamma-prime fibrinogen are associated with cardiovascular disease, the same cannot be said of studies on venous thrombosis and thrombotic microangiopathy [33]. Some studies show a direct correlation of gamma-prime with total fibrinogen levels, whereas other studies show an inverse correlation. Furthermore, there is controversy as to whether gamma-prime fibrinogen is prothrombotic or antithrombotic. Gamma-prime fibrinogen does not lead to thrombosis in rodent models [34]. In vitro, gamma-prime fibrinogen has an inhibitory activity toward thrombin that has shown to decrease its ability to activate coagulation factors and platelets [33]. Furthermore, gamma-prime fibrinogen, compared to other fibrinogen variants, enhances the response of protein C added to plasma, decreasing thrombin generation [35]. On the other hand, gamma-prime fibrinogen has been shown to forms refer to Chap. 14 fibrin clots with an altered clot architecture containing thinner, more numerous fibers, with more branching, that are less permeable, mechanically stronger, and highly resistant to fibrinolysis [36, 37]. Regardless of its effect on thrombosis, gamma-prime fibrinogen has consistently been associated with inflammation [38].

Metablosim

Fibrinogen has a concentration in plasma of approximately 200–400 mg/dL, with a half-life of 4 days and a catabolic rate of approximately 25 % per day [39]. Fibrinogen is subject to three

catabolic pathways that determine its concentration in plasma and consequently its availability to support hemostasis. These pathways are: (1) basic protein turnover, (2) intravascular fibrin formation, (3) intravascular fibrino(geno)lysis. Under physiologic conditions, coagulation and lysis account for <3 % of the total catabolism, and the classic catabolism pathways for most plasma proteins do not apply to fibrinogen [40]. Recently, a circadian rhythm to fibrinogen levels has been reported, with levels being highest in the morning [41, 42].

Cleavage into Fibrin

Central to the clotting mechanism is the spatially restricted, thrombin catalyzed conversion of soluble fibrinogen into an insoluble fibrin matrix. Fibrin polymerization is initiated by the enzymatic cleavage of the fibrinopeptides A and B located in the central E-domain of fibrinogen by thrombin, yielding a fibrin monomer [43]. A set of hydrophobic residues in fibrinopeptide A and B bind to a complementary apolar region on thrombin, hydrolyzing Arg-Gly bonds at the E-domain of the A-alpha and B-beta chains [44]. Fibrinopeptide B is released at a considerably slower rate than A. However, as polymerization proceeds, the rate of fibrinopeptide B release increases, suggesting that either it is preferentially released from polymers or that conformational changes resulting from polymerization facilitate its release [45].

Despite the small size of fibrinopeptides A and B, relative to the fibrinogen protein, their release affects the solubility of the molecule. Unless cleaved by thrombin, these fibrinopeptides mask "knobs" (named knob A and knob B) that are complementary to "holes" (named hole a and hole b) in fibrin that are always exposed [46]. It is thought that these complementary holes exist in the gamma-C and beta-C regions of the D-domains—holes "a" and "b" respectively [47, 48]. Thus, given the trinodular conformation of fibrin(ogen) described earlier in this chapter, two fibrin monomers interact with each other in a half staggered fashion, in such that knob A in the E-domain fits into *hole a* in the D-domain (Fig. 5.2) [49].

Of note, fibrinogen can also bind to thrombin generated fibrin monomers because fibrinogen has holes in the D-domain that are always exposed and can accept fibrin monomer knobs,

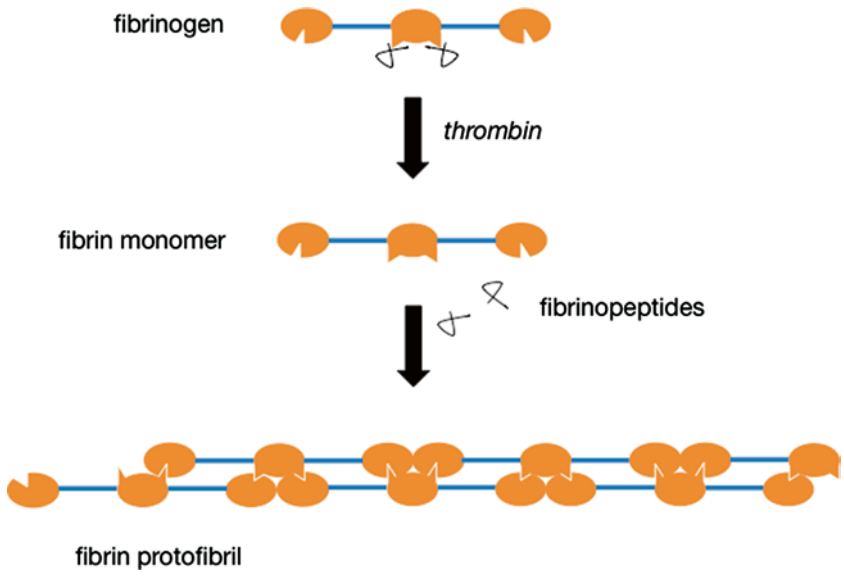

Fig. 5.2 Fibrin polymerization

leading to fibrinogen-fibrin complexes. These complexes represent an important buffer mechanism to prevent polymerization of small amounts of fibrin generated in circulation [50]. This is somewhat counterintuitive, since this concept suggests that increased levels of fibrinogen would be protective from thrombi forming circulation, yet increased levels of fibrinogen have been consistently associated with thrombosis. Functional consequences of the interaction of knob B and *hole b* are less understood. Cleavage of fibrinopeptide A alone produces thinner fibers than those initiated by cleavage of both fibrinopeptides. Based on this notion it has been proposed that knob A—*hole a* interactions are responsible for interactions within the oligomer, while knob B—*hole b* interactions are responsible for lateral aggregation [51]. It is important to note that these knob–hole interactions between fibrin monomers are complementary and non-covalent. Further fibrin clot strength can be gained by introduction of covalent bonds between fibrin molecules, as elicited by factor XIII, which is described later in this section.

Once thrombin cleavage of fibrinopeptides A and B yields the building blocks of clotting, fibrin monomers, dynamics of assembly become important. Fibrin monomers are oligomers, that when assembled through the above described process, reach a certain length at which they are then called protofibrils [52]. When protofibrils associate laterally and gain length, they form fibers. Lateral aggregation is thought to result from the D-domain interaction of two laterally aligned protofibrils [52]. This interaction is thought to be enhanced by the neighboring alpha-C regions given that clots missing these regions produce fibers with less tensile strength [53]. As fibers are formed through lateral aggregation, fiber branching is necessary to produce a three-dimensional network, which in turn leads to a space-filling matrix, point at which a gel, or a clot, exists.[54] This gel point or clotting point is represented clinically by clotting time assays performed on plasma samples [55]. For further description of plasma based assays of clotting time.

Data from X-ray crystallography have shown the presence of four calcium sites in the D-domain of fibrinogen [56]. Calcium concentrations in plasma stabilize fibrin(ogen) by giving it resistance to thermal denaturation, protecting it from reduction of disulfide bonds and from plasmin cleavage of peptide bonds [57–59]. Furthermore, calcium increases the rate and extent of lateral aggregation [60]. Since the dissociation constant for calcium ion binding to fibrinogen is much lower than the free calcium ion concentration in plasma, fibrinogen calcium binding sites should be fully occupied under physiologic conditions [57].

Fibrin clot stiffness can be increased through creation of covalent bonds between the fibrin chains promoted by factor XIII. The affinity of factor XIII for fibrinogen (Kd 10 nM) is such that the majority of circulating factor XIII zymogen circulates with fibrinogen as a complex [9]. Factor XIII binds strongly to the gamma-prime form of fibrinogen, which is most likely the carrier of the zymogen in blood [61]. Interactions of fibrin with factor XIII, thrombin, and calcium generate activated factor XIII (XIIIa) via thrombin cleavage and calcium dependent release of active a2-subunits of factor XIII [62]. Factor XIIIa facilitates clot stability by catalyzing the formation of covalent lysine bonds between fibrin molecules. Factor XIII is not activated until a critical mass of fibrin polymerizes, a delay thought to ensure that the hemostatic plug has a supply of factor XIII as it forms. Plasma concentrations of factor XIII and fibrinogen are approximately 0.07 and 9 μM, respectively [62]. Thus, the molar ratio of factor XIII to fibrinogen in plasma is in the order of 1:100. The generation of activated factor XIII (XIIIa) in plasma can be triggered when as little as 1–2 % of fibrinogen is converted to fibrin polymers. This indicates that factor XIIIa begins stabilizing fibrin polymers before a visible thrombus appears, as the latter requires the conversion of at least 20 % of fibrinogen into fibrin.

During fibrin formation, the globular portions of the alpha-C regions dissociate from the central region so that they are available for

intermolecular interactions [63]. Factor XIIIa introduces a number of covalent bonds within the fibrin clot (Fig. 5.3), of which the first are formed between gamma chains of 2 neighboring fibrin molecules at the protofibril level. Cross-linking of the fibrin alpha chains by XIIIa occurs more slowly than cross-linking of the gamma chains. Nevertheless, the extent of alpha chain crosslinking plays an important role in the regulation of fibrinolysis and viscoelastic properties of fibrin. Most investigators agree that alpha chain cross-links appear to stabilize and promote the association of fibrin protofibrils into fiber bundles with higher tensile strength. Plasmin must degrade fibrin at the alpha chain coiled coil region between the D- and E-domain in order to allow fibrin to release soluble fragments and loose its structure; the extensive alpha chain cross-linking introduced by factor XIIIa interferes with this reaction [64].

Factor XIIIa has also shown to cross-link other proteins to fibrin, including alpha 2-antiplasmin, plasminogen activator inhibitor type 2 (PAI-2), and fibronectin [65, 66]. Factor XIIIa covalently attaches PAI-2 to lysine residues within the alpha chain of fibrin [67]. For further description of these important interactions refer to Chap. 9.

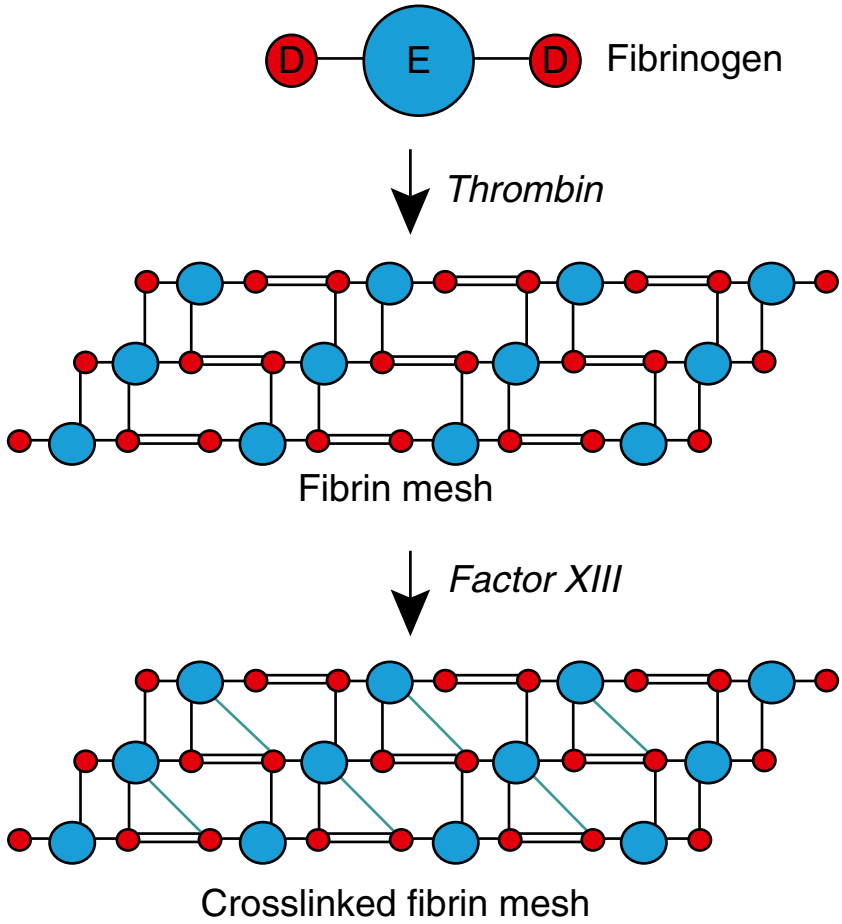

Fig. 5.3 Introduction of covalent bonds between monomers of fibrin by factor XIIIa

Fibrin(ogen) Interaction with Platelets and Other Cells

Fibrin(ogen) binds to integrins and other receptors on platelets and endothelial cells, serving as a bridge between cells and as a scaffold for cell migration. Fibrin also promotes inflammation and angiogenesis through interactions with leukocytes and endothelial cells. Fibrinogen binds to the activated form of the integrin $\alpha_{IIb}\beta_3$ (platelet glycoprotein GPIIb/IIIa) via residues located in the *C*-terminus of gamma chains, bridging platelets due to the dimeric nature of fibrin(ogen) [68, 69]. Figure 5.4 shows fibrin linking two platelets through binding to its GPIIb/IIIa receptor. Endothelial cells adhere to fibrin or surface immobilized fibrinogen via the integrin $\alpha_v\beta_3$, intracellular adhesion molecule type 1 (ICAM-1) (intracellular adhesion molecule 1), and the vascular endothelial cadherin [70]. Fibrin(ogen) binds to leukocytes via the integrin $\alpha_M\beta_2$, localizing leukocytes to the site of injury [71].

Fibrin(ogen) and Fibrinolysis

Both fibrinogen and fibrin serve as templates for the assembly and activation of the fibrinolytic system. Plasminogen, tissue-type plasminogen activator (tPA), and alpha 2-antiplasmin have binding sites on fibrin(ogen). In this context, fibrin(ogen) acts as cofactor for tPA induced plasminogen activation, thus linking fibrin formation to fibrin proteolysis (Fig. 5.5) [72]. Enhancement of tPA induced plasminogen activation into plasmin requires binding of both tPA and plasminogen to specific binding sites on fibrin(ogen) [73, 74]. Furthermore, binding of tPA to fibrin(ogen) protects it from inhibition by plasminogen activator inhibitor type 1 (PAI-1) [75]. Fibrin formation results in approximately 1000-fold enhancement of plasminogen activation into plasmin [76]. Binding of tPA and plasminogen to both fibrinogen and fibrin yields plasmin, with subsequent degradation of both fibrinogen and fibrin. However, the dynamics of

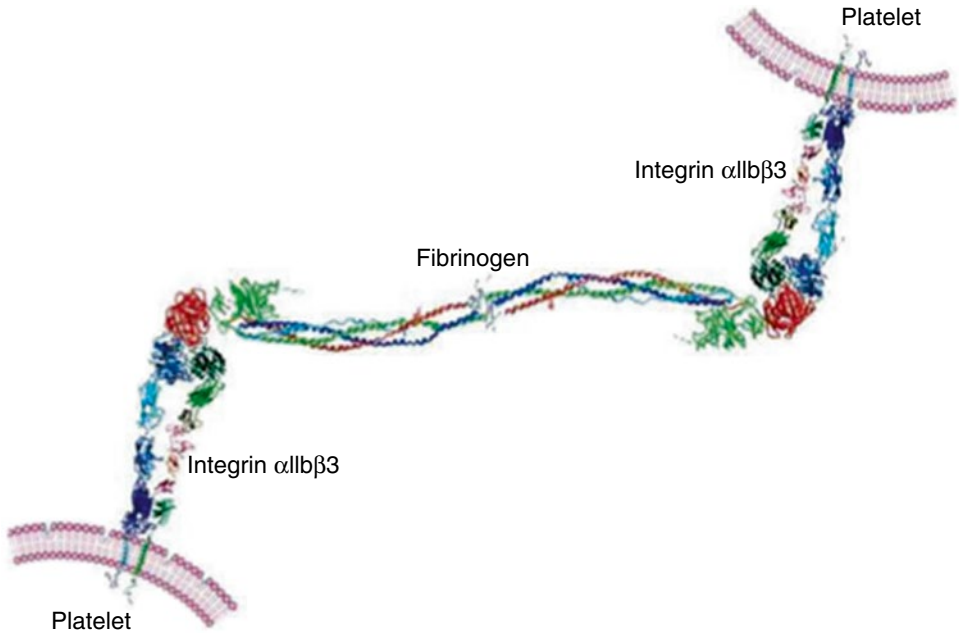

Fig. 5.4 Binding of fibrin(ogen) to two platelets through GP IIb/IIIa (integrin $\alpha_{IIb}\beta_3$) receptor

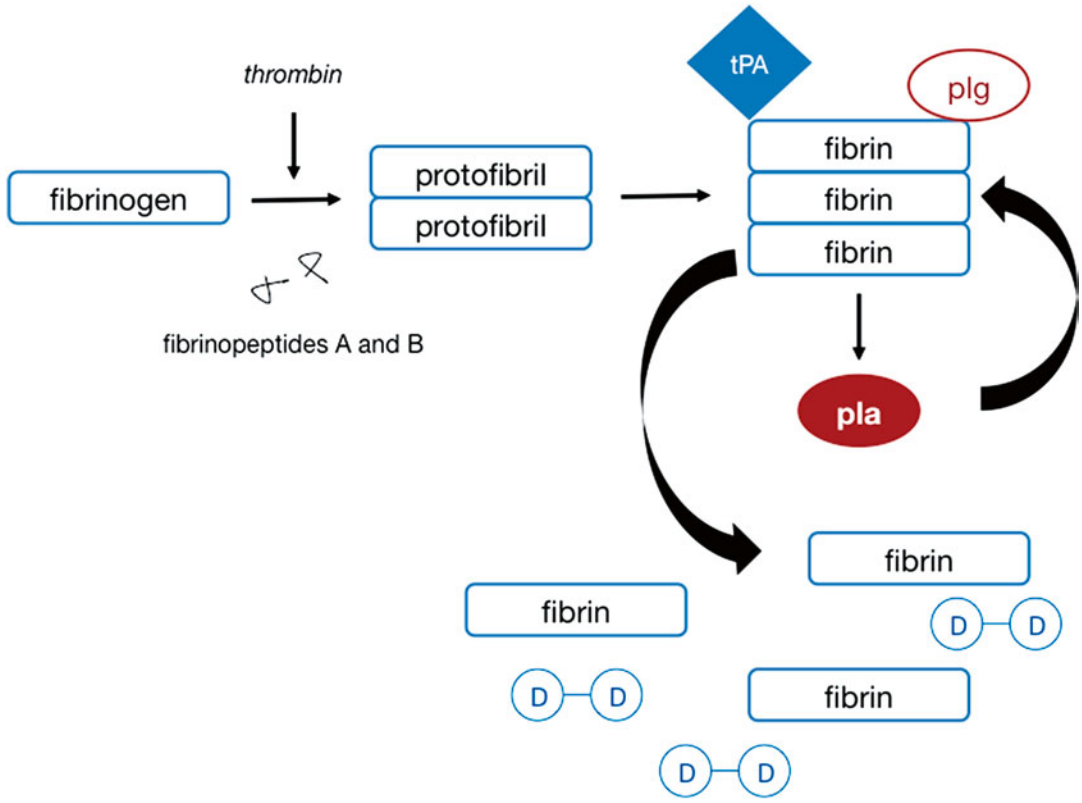

Fig. 5.5 Dual role of fibrin(ogen) in fibrinolysis: co-localizing tissue plasminogen (plg) activator (tPA) and plasminogen, and serving as a substrate for the activity of plasmin (pla)

such plasmin mediated degradation is different for fibrinogen than for fibrin. Plasmin can degrade fibrinogen quicker than fibrin; however, fibrin degradation by plasmin is slower but lasts longer and has more consequences on hemostasis.

The plasminogen-plasmin system is described in detail in Chap. 3. In brief, plasmin has trypsin-like specificity, with an affinity for hydrolysis of lysyl and arginyl bonds; fibrin has 362 lysine and arginine residues, of which plasmin cleaves 50–60 of them. Plasmin degraded fibrinogen can be separated by chromatography into fractions A, B, C, D, and E, of which fragments D and E are the major end products of the original fibrinogen molecule [77]. Fragment D has an anti-polymerizing effect on fibrin by competing for fibrin knobs. In contrast, plasmin degradation of cross-linked fibrin yields different fractions. Cross-linked fibrin degradation mediated by plasmin is much slower than that of fibrinogen or fibrin monomers due to the covalent bonds introduced by factor XIIIa [78]. Prolonged exposure of polymerized, cross-linked fibrin to plasmin in vitro produces a unique fragment (D-dimer), consisting of fragments of the D-domains of adjacent fibrin monomers covalently bound by cross-links between their gamma chain remnants [79]. Consequently, fibrinolysis of fibrinogen yields simple D fragments, while fibrinolysis of fibrin yields D-dimers [78].

Additional binding sites for tPA and plasminogen are present in the alpha-C region of fibrin(ogen) [80]. As plasmin begins to degrade fibrin, more binding sites are exposed by exposure of *C*-terminal lysine residues, amplifying the lytic effect of plasmin on fibrin. It is though that this partial degradation of fibrin, which exposes more sites for binding of plasminogen, and thus generation of plasmin, is how fibrinolysis reaches a critical rate of degradation of fibrin that allows for clot

dissolution. Importantly, tPA and plasminogen binding to fibrin(ogen) is inhibited by other sources of lysine [81]. This is an important notion because certain antifibrinolytic drugs (e.g., aminocaproic acid and tranexamic acid) prevent both tPA and plasminogen binding to fibrin(ogen). These drugs are considered lysine analogues, occupying lysine binding sites on the tPA and plasminogen molecules, which prevents their binding to fibrin(ogen) [82]. These lysine binding sites reside in subsidiary domains of tPA and plasminogen known as kringles [83, 84]. On a related note, thrombin-activated fibrinolysis inhibitor (TAFI) removes *C*-terminal lysine from fibrin(ogen) [85], thus, downregulating fibrinolysis by preventing binding of tPA and plasminogen.

To summarize this section, fibrin(ogen) has two overlapping roles during fibrinolysis. The first involves providing a template for co-localization of tPA and plasminogen to supports its biologic activity; the second is to act as the substrate for plasmin that the first step yields. It appears that the behavior of fibrin(ogen) as a template may be independent of the effectiveness of fibrin(ogen) as a substrate for plasmin [72]. The relationship between fibrin structure and rate of fibrinolysis is not simple and is complicated by physiologic and pathologic factors [86, 87].

Inherited and Acquired Disorders of Fibrinogen

Although describing clinical disorders of fibrinogen is beyond the scope of this chapter, a brief review could provide insights into the pathophysiology of trauma induced coagulopathy. Disorders of fibrinogen take the form of either the production of an abnormal fibrinogen protein (i.e., a qualitative defect; dysfibrinogenemia) or the reduction or lack of production of fibrinogen (i.e., a quantitative defect; hypofibrinogenemia or afibrinogenemia).

Inherited dysfibrinogenemias can result in alterations of fibrinopeptide release, fibrin polymerization, fibrin crosslinking, or fibrinolysis. Inheritance of congenital dysfibrinogenemias is autosomal dominant. These conditions can be expressed as a silent (55 %), hemorrhagic (25 %), or thrombotic phenotype (10–30 %) [88]. Silent (asymptomatic) dysfibrinogenemia is often diagnosed incidentally following abnormal coagulation tests or as part of family screening.

The most common cause of acquired dysfibrinogenemia is liver disease. It is observed in the majority of patients with cirrhosis, acute or chronic hepatitis, and also in those with metastatic disease to the liver [89–91]. Fibrinogen dysfunction in this setting is manifested by prolongation of thrombin and reptilase times. When measured by immunologic methods, fibrinogen levels can be normal. As mentioned earlier in this chapter the abnormal fibrinogen in this setting is characterized by an increased content of sialic acid residues and delayed fibrin polymerization [92]. Removal of the sialic acid from the abnormal fibrinogen normalizes the thrombin time and corrects the polymerization defect [92].

Whether the abnormal fibrinogen seen in liver disease is associated with an increased bleeding risk is difficult to evaluate, since most of these patients have other associated coagulation abnormalities, such as thrombocytopenia and diminished synthesis of other coagulation factors. No increase in thrombotic risk independently associated to fibrinogen in liver disease has been reported.

Other acquired causes of dysfibrinogenemia include renal cell carcinoma [93] and biliary obstruction [94].

Hypofibrinogenemia may occur when there is reduced synthesis or increased turnover of fibrinogen. As an example, patients with hepatic failure or decompensated cirrhosis may have low levels of fibrinogen due to decreased hepatic synthesis or increased turnover (consumption) due to the concomitant presence of disseminated intravascular coagulation.

Fibrinogen can become elevated in response to inflammation or tissue injury (i.e., as an acute phase reactant). For decades, studies have associated elevated plasma fibrinogen levels with cardiovascular disease [95]; however, a mechanism to causality has not been described. Assigning causality to fibrinogen levels is hindered by confounding effects of acute phase inflammatory

stimuli that associate cardiovascular disease with increased fibrinogen levels, and by the absence of a useful fibrinogen lowering agent to test in clinical trials.

Hypofibrinolysis (also termed fibrinolysis shutdown) [96] is also a risk factor for cardiovascular disease and thrombosis and can arise in a number of situations due to abnormal clot structure, high fibrinogen concentrations [97, 98] or because of fibrinogen variants that yield fibrin clots with varying densities [99, 100]. In trauma patients, fibrinolysis shutdown has been associated with increased delayed mortality [96], which is characterized by sepsis, multiple organ dysfunction, and venous thromboembolisms. Studies have implicated fibrinolysis shutdown in the pathophysiology of multiple organ dysfunction [101, 102]. Whether pathologic changes to fibrinogen quantity and function relate to fibrinolysis shutdown remains to be elucidated.

Fibrinogen has a heparin binding site, related to its dimeric structure, which gives fibrinogen high affinity for heparin [103]. It has also been suggested that binding of endogenous heparinoids (endothelial glycocalyx proteoglycans) to fibrin(ogen) may play a role in regulation of fibrin mediated adhesion to surfaces [104]. A recent study in trauma patients requiring intensive unit care and receiving thromboprophylaxis with heparin implicated hyperfibrinogenemia with heparin resistance and failure to achieve protective levels of heparinization [105].

Fibrinogen in Trauma Induced Coagulopathy

Endothelial activation and dysfunction, decreased levels of fibrinogen, impaired thrombin generation, platelet dysfunction, fibrinolysis, endogenous anticoagulants such as protein-C, and antifibrinolytic proteins collectively compose our contemporary, but still partial understanding of the pathophysiology of trauma induced coagulopathy [106]. The role of fibrinogen as a driver of trauma induced coagulopathy has focused on studies quantifying fibrinogen concentration. Investigations over the last decade using functional assays of fibrinogen have provided further insights.

There is increasing awareness regarding the important role of fibrinogen during hemorrhagic shock as a potential target for treatment [107–109]. A longstanding notion that fibrinogen is the first clotting factor to decrease to critically low levels during severe bleeding has prevailed [110, 111]. Such concept stems from several studies reporting that fibrinogen is decreased earlier than other measured coagulation factors in both clinical studies and experimental models of hemorrhagic shock. In a prospective study published in 1995, Hiipala et al. studied 60 patients undergoing elective urologic or abdominal surgery anticipated to have blood loss exceeding 20 % of a calculated blood volume [112]. These investigators quantified fibrinogen (optical density test based on the prothrombin test), platelet count, prothrombin, as well as factors V and VII (one-stage test based on the prothrombin test). This study reported that the critical fibrinogen concentration of 100 mg/dL was reached when the blood loss was 1.42 times the calculated blood volume, compared to critical levels of platelet count ($50{,}000/mm^3$), prothrombin (20 % activity) and factors V (25 % activity) and VII (20 % activity) reached when blood loss was 2.0 times the calculated blood volume. Using a mathematical model to analyze hemostasis during blood loss Singbartl et al. demonstrated that blood loss is highly dependent on fibrinogen levels [113]. For a constant hematocrit (45 %) and platelet count ($225{,}000/mm^3$), varying the fibrinogen concentration to three amounts of 450 mg/dL, 300 mg/dL, and 200 mg/dL resulted in blood loss of 750, 1900 and 3750 ml, respectively.

To investigate the effects of hemorrhage on fibrinogen metabolism, a swine model of hemorrhagic shock (35 % of estimated blood volume) was carried out using isotopic enrichment of fibrinogen [114]. These investigators reported that fibrinogen breakdown was accelerated compared to the control group, with no change in the

rate of fibrinogen synthesis. It is worth mentioning that this was only a moderate model of hemorrhagic shock as there were no changes in pH or temperature compared to the control group. Interestingly, fibrinolysis synthesis was maintained, albeit hemorrhagic shock was only moderate. This suggests that the decrease in fibrinogen may be related to hemorrhage only, and not shock per-se, or to the tissue injury caused by vascular cannulation used to induce hemorrhage. It also suggests that fibrinogen deficiencies may occur with less severe injury and bleeding than expected; however, it is unclear whether such changes have clinical consequences as functional coagulation assessments were not performed in this study. The same author recently published a study achieving more severe shock in swine through a 60 % estimated blood volume loss [115]. In this study the effect of hemorrhage and resuscitation (lactated ringers and normal saline) on fibrinogen metabolism was quantified through isotopic enrichment. The authors reported that hemorrhage and resuscitation compromised albumin synthesis, but not fibrinogen synthesis. Resuscitation with lactated ringers compared to normal saline had no differential impact on albumin or fibrinogen metabolism.

Hypothermia and acidosis are implicated in exacerbating trauma induced coagulopathy. In a study exposing swine to a temperature of 32° C, hypothermia decreased fibrinogen synthesis, with no effect on fibrinogen breakdown [116]. Thrombin generation at the initiation phase was delayed by hypothermia, but there were no changes at the propagation phase. The viscoelastic (thromboelastography) parameter R-time (time to initial clotting) was prolonged and the alpha-angle parameter (rate of clot strength increase) was decreased, whereas the maximum amplitude of clot strength and the lysis parameter LY30 had no significant changes related to hypothermia. Contrasting to these findings, a swine model of acidosis achieved through infusion of hydrochloric acid to a target pH of 7.1 demonstrated acidosis increased fibrinogen breakdown with no effects on fibrinogen synthesis [117]. As these two studies were performed in the absence of hemorrhagic shock, the impact of physiologic (acidosis) and environmental (hypothermia) factors on fibrinogen metabolism must be acknowledged.

In a prospective study were hydroxyethyl starch was used to resuscitate 20 injured patients, Fenger-Eriksen et al. attributed a coagulopathy demonstrated by a decreased maximal clot firmness (MCF; thromboelastometry) to hemodilution by this synthetic colloid [118]. The authors referred to this effect as an in vivo model of hemodilution, demonstrating that fibrinogen (Clauss method) was decreased from baseline in a greater degree than coagulation factors II, VII, VIII, IX, X, XIII (one-stage method with factor deficient plasma) and von Willebrand factor (ristocetin test). These authors concluded that an acquired fibrinogen deficiency seems to be the leading determinant in dilutional coagulopathy. Based on these data it cannot be determined to what degree these findings are attributable to hemodilution or to injury and hemorrhagic shock.

Whole blood functional assays of fibrinogen have been recently developed through variations of viscoelastic assays (thromboelastometry, TEM)(thromboelastography, TEG) [119, 120]. Viscoelastic assays are described in detail in Chaps. 17 and 18. Harr et al. performed the TEG functional fibrinogen assay in severely injured trauma patients [median injury severity score (ISS) =23] in order to differentiate fibrinogen from platelet contribution to clot strength by comparing the maximal clot strength achieved by FF-TEG to standard TEG [119]. The mean contribution of fibrinogen to total (maximal) clot strength was 30 %. The fibrinogen function quantified by FF-TEG correlated strongly (R = 0.87) with fibrinogen measured by the Clauss method, considered the gold standard. Maximal clot strength derived from FF-TEG correlated better than any other TEG parameter. For further details on fibrinogen assays refer to Chap. 15. In a subsequent study, Kornblith et al. also performed FF-TEG on trauma patients (median ISS = 9). The mean contribution of fibrinogen to total (maximal) clot strength was

also 30 %. Patients requiring plasma transfusion had a significantly lower admission fibrinogen contribution to total (maximal) clot strength (26.6 %) than those who did not require a plasma transfusion (30.6 %). As with the previous study, a strong correlation of FF-TEG with fibrinogen (Clauss method) was demonstrated. These authors concluded that lower function of fibrinogen, as a contributor to overall clot strength achieved in whole blood, upon arrival was a predictor of coagulopathy and need for transfusion.

Although decreased fibrinogen levels and changes in its metabolism are present during trauma and hemorrhagic shock, its mechanistic integration into the other hypothesized drivers of trauma induced coagulopathy (i.e., endothelial activation and dysfunction, decreased thrombin generation, activated protein C, platelet dysfunction) is lacking.

Future Directions

Fibrinogen's role in hemostasis has been well characterized as it relates to its interactions with other proteins and cells participating in clot formation. The clinical consequences of these interactions under physiologic and pathologic conditions have not been fully investigated. Regarding trauma induced coagulopathy, fibrinogen is clearly implicated in its pathophysiology, such that clinical trials on early fibrinogen supplementation to bleeding trauma patients in the way of fibrinogen concentrates and cryoprecipitate have been designed and are currently enrolling patients. However, merely the basic aspects of fibrinogen's structure, metabolism, and interactions have been explored during trauma and hemorrhagic shock. For example, the role of gamma-prime fibrinogen, implicated both as a prothrombotic and antithrombotic in other pathologies, has not been investigated in trauma patients. Furthermore, the impact of fibrinogen's structure, posttranslational modifications, and conformational changes as a mechanistic link to hyperfibrinolysis in trauma induced coagulopathy remains to be elucidated.

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Activated Protein C

6

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Molecular Biology of the Activated Protein C Pathway

In normal human hemostasis, the APC pathway attenuates coagulation activation, serving as a critical anticoagulant restraint on the multiple physiologic processes that promote clot formation. Independent of these anticoagulant properties, APC has been shown to have multiple cytoprotective effects as well, acting as an anti-inflammatory agent and preventing endothelial barrier leakage [1, 2]. By these two mechanisms, the APC pathway serves to maintain vascular flow by preventing excessive thrombosis, and also protects cells from damage associated with inflammatory insults, such as sepsis and trauma.

Protein C

Protein C was first named and identified in Sweden in 1976 by Stenflo, who in his investigations of bovine coagulation factors identified "a hitherto unrecognized vitamin K-dependent protein with an unknown function"—the name was derived from its position as the third peak eluted via chromatography: peak C [3]. To be precise, protein C was in fact "re-discovered," as it had been previously described as "autoprothrombin IIa" the decade before, across the Atlantic in the Seegers laboratory in Detroit [4, 5]. As early as the 1960s, this protein was known to have anticoagulant properties, and was thought to have an inhibitory effect on fibrinolysis [6]. Thus the discovery of protein C occurred through ongoing laboratory efforts to define the fundamental biochemistry and dynamics of coagulation in the 1960s and 1970s, prior to any overt awareness of its clinical importance.

Protein C was subsequently isolated and identified as a vitamin K-dependent zymogen precursor of a serine protease, the activation of which is mediated via the binding of thrombin [7]. Once activated, APC achieves its primary anticoagulant function through irreversible proteolytic cleavage of activated factors Va and VIIIa, the major drivers of thrombin formation [8–11]. While these anticoagulant mechanisms were detailed thoroughly by the 1980s, the cytoprotective activity of APC, with respective mechanisms via a specific set of receptors, was not described until decades later [12].

The structure of protein C is homologous to that of the other vitamin K-dependent coagulation proteins, from the level of nucleotide sequence to that of tertiary protein folding. The gene for protein C is located on chromosome 2, spans approximately 11 kB of DNA, and codes for a precursor 461-amino acid polypeptide [13,

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14]. Before secretion, this precursor is modified by removal of a leader signal peptide and a propeptide involved in gamma-carboxylation, yielding a 419-amino acid product with a molecular weight of 62 kDa [15] (Fig. 6.1). An endoprotease cleaves the protein into a two-chain zymogen, with a heavy chain covalently linked to a light chain by a single disulfide bond—the majority of protein C is found in this form [16]. The amino-terminal gamma-carboxyglutamate (Gla) domain of the light chain enables calcium chelation and the binding of membrane complexes, as in homologous vitamin K-dependent clotting factors; this domain also facilitates critical binding to endothelial receptors [17]. Two epidermal growth factor (EGF) domains on the light chain likely mediate interaction with cofactors such as thrombomodulin and Protein S; the activation peptide and serine protease are found on the heavy chain. Posttranslational N-linked glycosylation occurs prior to secretion of the mature glycoprotein. The majority of protein C is synthesized in the liver, circulates at a plasma concentration of 4 mcg/mL (70nM), and has a half-life of 8 h [18].

Activation of the protein C zymogen is achieved via thrombin-mediated cleavage of the heavy chain at Arg169; this releases a 12-residue activation peptide and results in the formation of APC [7]. This reaction is significantly accelerated by endothelial-bound thrombomodulin and the endothelial protein C receptor (EPCR), as described below. Once activated, APC is either released to act as an anticoagulant or remains bound to EPCR, whereby its cytoprotective effects are initiated [19]. As indicated by structural studies, the serine protease domain of the APC molecule is homologous to that of chymotrypsin, and mediates the highly specific inactivation of coagulation factors V and VIII [20]. Circulating APC has a half-life of approximately 20 min, and levels are low in healthy adults, on the order of 1–3 ng/mL, or <40 pM [21]. APC levels are closely correlated to the circulating levels of zymogenic protein C, suggesting that precursor protein C concentration is a limiting factor in the rate of in vivo APC activation [22].

Thrombomodulin

Though thrombin is responsible for the activation of protein C, the kinetics of the thrombin-protein C reaction are extremely unfavorable. Hypothesizing that some additional mechanism must augment this process in vivo, Esmon and Owen found that the activation of protein C was increased 20,000-fold during exposure to endothelium in the coronary microvasculature of an explanted rabbit heart; this effect was confirmed in cultured human endothelial cells [23]. The responsible endothelial cofactor, thrombomodulin, was subsequently isolated, cloned, and named for its ability to convert thrombin from its canonical procoagulant action to that of a functional anticoagulant via the activation of protein C [24].

Thrombomodulin is a transmembrane protein that is found predominantly on the surface of endothelial cells; it is coded by a gene on chromosome 20, has a molecular mass of approximately 70 kDa, and contains a lectin-like domain, six EGF domains, and a serine/threonine-rich domain near the membrane that serves as a site for extensive glycosylation [25]. Thrombin and protein C bind at the EGF domains, which are essential for effective activation of protein C [26]. Once bound to thrombomodulin, thrombin undergoes conformational changes that affect its substrate specificity, further shifting the balance from the procoagulant thrombin-fibrin pathway to the anticoagulant APC pathway [27, 28]. In the ser/thr domain, the binding of glycosaminoglycans like chondroitin sulfate can significantly increase the binding affinity for thrombin, further enable the activation of protein C, and accelerate the inhibition of thrombin by proteases [29, 30]. Thrombin bound to thrombomodulin is inactivated much more rapidly than free thrombin [31].

Thrombomodulin expression differs significantly by tissue type, and given a constant endothelial concentration, it has much higher activity in the microvasculature than in larger vessels, due to several 100-fold increase in endothelial surface area to blood ratio in capillaries [32, 33]. It thus serves to prevent the formation of thrombi in small peripheral vessels, where thrombin activity might otherwise favor clot formation. In

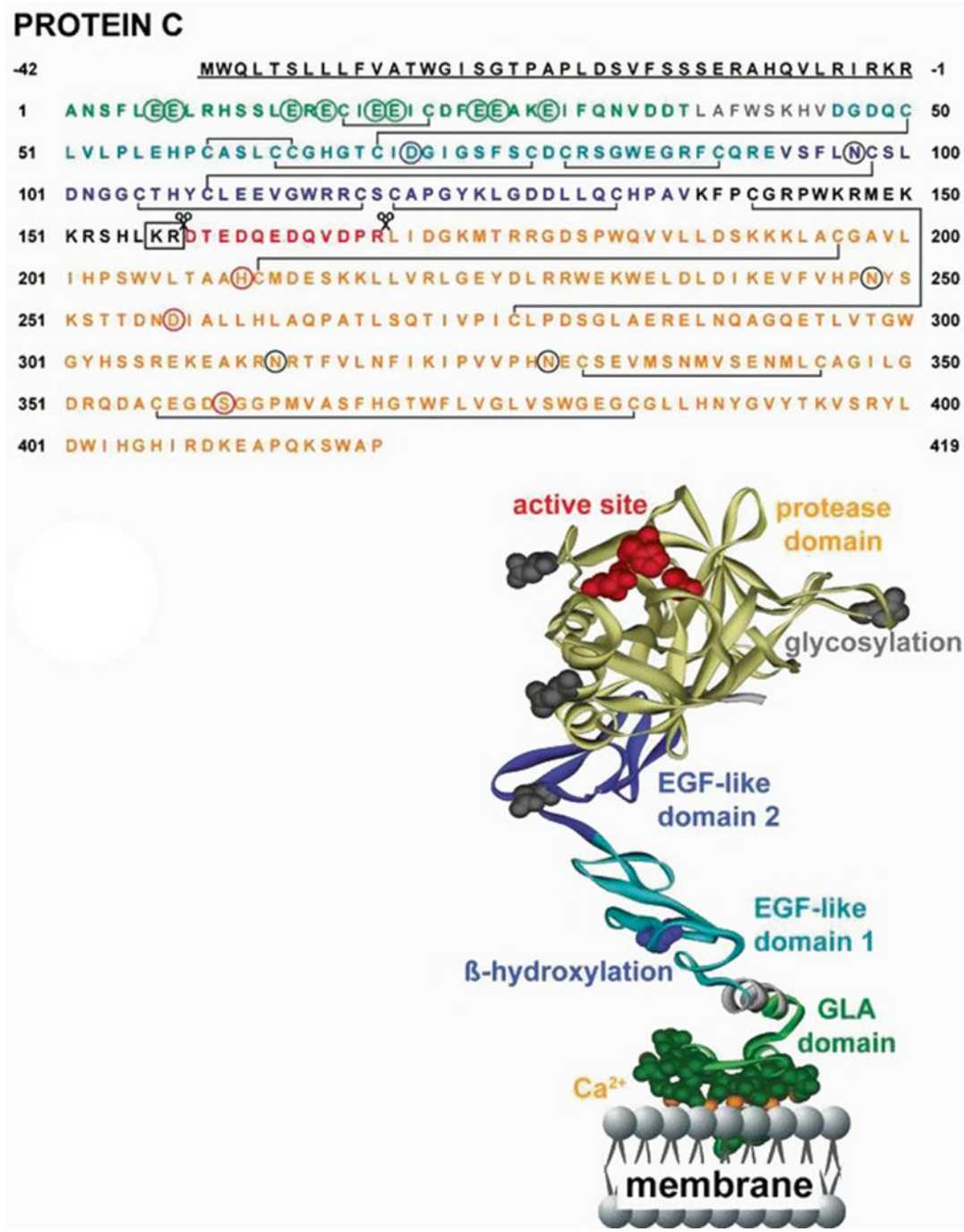

Fig. 6.1 Amino acid sequence of protein C and ribbon polypeptide scheme of APC. Amino acids are numbered from the amino-terminus of the mature protein with the signal peptide sequence underlined. Specific domains are *color coded* as indicated in the *ribbon cartoon. Green circles* depict γ-carboxylation, *blue circles* depict β-hydroxylation, and *black circles* represent sites of N-linked glycosylation. Sites of proteolytic cleavage during posttranslational processing and protein C activation are indicated by the scissors. The dipeptide that is proteolytically removed during the posttranslational processing of most protein C molecules in the liver is identified by the box. The serine, aspartic acid, and histidine residues that constitute the active site triad are identified by *red circles*. (Reproduced from Mosnier, Griffin; Protein C, Protein S, Thrombomodulin, and the Endothelial Protein C Receptor Pathways; in Marder et al, Hemostasis and thrombosis: Basic principles and clinical practice: 6th Edition, Lippincott Williams & Wilkins, 2013, p. 300–14, with permission.)

mice, deletion of the thrombomodulin gene is fatal at the embryonic stage [34].

Thrombomodulin has been found to circulate in plasma in soluble form, presumably following cleavage of the membrane-binding and cytoplasmic domains [35]. Elevated levels of soluble thrombomodulin have been associated with certain pathologic states, including atherosclerosis, sepsis, and inflammatory conditions [36, 37]. The functional relevance of the circulating pool is unclear: while it likely represents a marker of endothelial damage, especially when elevated above normal levels [38], studies have also shown that it also retains function as a cofactor in the activation of protein C [39]. The overall effect of such circulating thrombomodulin on coagulation remains unclear. Interestingly, more recent data indicate that soluble thrombomodulin released during endothelial stress states may also have a protective role in inflammatory conditions, exerting an antiapoptotic effect on target cells [40].

Protein S

Protein S was discovered by DiScipio and Davie at the University of Washington in 1979 and was named for its city of discovery, Seattle—it was first identified as a vitamin K-dependent plasma protein with unknown function [41]. Shortly thereafter, the newly discovered glycoprotein was found to be a potent cofactor of APC in the inactivation of factor Va [42].

Protein S is a 77 kDa glycoprotein coded by a gene on chromosome 3; after removal of a leader peptide, it is secreted as a single-chain polypeptide of 635 amino acids with three carbohydrate side chains [43, 44]. Like homologous vitamin K-dependent glycoprotein relatives, it has a Gla domain that binds calcium and facilitates binding to phospholipid membranes. Following a thrombin-sensitive domain, four EGF-like domains are found—these have been implicated in the protein's cofactor activity [45]. Unlike other vitamin K-dependent proteins, protein S is not a serine protease, and circulates in active form; however, a C-terminal sex hormone binding globulin (SHBG)-like domain facilitates binding to C4b binding protein (C4BP), a regulatory glycoprotein of the complement system [46]. Approximately 60 % of the circulating plasma protein S is bound to C4BP, and this bound form has reduced APC cofactor activity, especially in its interaction with factor Va; the remaining free 40 % (~130 nM, or about 25 μg/mL) of plasma protein S is available to function as active cofactor [47, 48]. Though primarily synthesized in the liver and endothelium, protein S has been shown to be produced by multiple other cell types, including activated platelets [49].

Early investigations identified the role of protein S in facilitating binding of the activated protein C complex to the phospholipid membrane, enabling the inactivation of factor Va [50]. Multiple protein S domains have been proven necessary for effective cofactor augmentation of APC activity, with recent studies implicating the first EGF-like domain as the critical site of interaction with APC [45]; a Gla domain has also proven key to effective interaction and potentiation [51]. Via these mechanisms, Protein S helps to bind APC to the phospholipid membrane, in proximity to Va and VIIIa, and also orients the structure of the enzyme-substrate complex to optimize proteolysis [52], increasing inactivation of Va up to 20-fold [53], and inactivation of VIIIa at least threefold [54].

Independent of its role as a cofactor for APC, protein S can function as a direct inhibitor of thrombin formation via a host of proposed mechanisms, including direct binding and blockade of coagulation factors and potentiation of tissue factor pathway inhibitor (TFPI) [55, 56]. More recent studies have also identified a separate cytoprotective effect, whereby protein S facilitates the clearance of apoptotic cells via specific cell surface receptors [57].

Endothelial Protein C Receptor (EPCR)

In an ongoing search to better understand the endothelium-mediated process of protein C activation, and the suspected role of APC in

modulating inflammatory responses, the Esmon lab identified, cloned, and characterized the endothelial protein C receptor (EPCR) in 1994 [58]. Subsequent studies demonstrated that EPCR increased protein C activation fivefold in vitro [59]; in vivo primate studies indicated an approximate 20-fold enhancement of protein C activation via EPCR [60].

EPCR shares structural homology with the major histocompatibility class I (MHC I) or CD1 superfamily of transmembrane receptors; the 46,000 kDa mature glycoprotein contains 221 amino acids and is coded by a gene on chromosome 20q [61]. In the site where MHC molecules contain a presented antigen, EPCR is tightly bound to a phospholipid, the removal of which eliminates binding to protein C [62]. EPCR binds to Gla domains of protein C and APC with similar affinity [17]. By binding the zymogen protein C and presenting it to the thrombin-thrombomodulin enzymatic complex, EPCR serves to reduce the required substrate (protein C) concentration for effective activation, which is otherwise far greater than the normal plasma concentration of protein C [63]. In addition, it is via binding to EPCR that APC exerts its cytoprotective effects, through cleavage of the PAR-1 receptor (discussed below) [12]. Though originally identified on human endothelium, EPCR has been localized to several other cell types, including hematopoietic, epithelial, and neuronal cells; it has also been shown to have multiple ligands other than protein C and seems to play a complex role in the pathogenesis of inflammation, cancer, and severe malaria [64]. Unlike thrombomodulin, which is found at highest concentrations in the microvasculature, EPCR is more densely concentrated in larger blood vessels [33].

A soluble form of EPCR, which circulates in normal human plasma at an approximate concentration of 100 ng/mL (~2.5nM), has been shown to bind protein C with an affinity similar to its membrane-bound form [65]. This soluble form seems to inhibit the anticoagulant properties of APC in vitro both by blocking membrane binding and by inducing structural changes in the APC binding site [66]. Soluble EPCR has been shown to be increased in the settings of sepsis and systemic lupus erythematosus [67], and also appears to be elevated in trauma patients with critical injuries [68]. EPCR release from the endothelium has been related to tissue necrosis factor alpha (TNF-α) and circulating thrombin [69]. Like thrombomodulin, the meaning of elevated sEPCR in these human disease states remains unknown, possibly representing endothelial membrane shedding and nonfunction or increased expression and function. However, based on murine models, the effect of such soluble EPCR on systemic coagulation dynamics may well be insignificant at relevant physiologic levels [70].

Pathway Activation, Regulation, Inactivation

Based on our current understanding of these essential pathway elements, the activation of protein C proceeds as follows: as thrombin is generated, much of it binds to endothelial thrombomodulin, which forms an enzymatic complex on the endothelial surface. Zymogenic protein C binds to EPCR at the Gla domain, and is thereby optimally oriented to the thrombin-thrombomodulin complex for activation. Thrombin then cleaves the zymogen at the Arg169-Leu170 bond, producing the functional protease APC. This APC can release from EPCR to bind protein S in the anticoagulant pathway, or it can remain bound to EPCR, whereby its cytoprotective actions are mediated [71]; these activities are described in further detail in subsequent sections.

One known stimulant of protein C activation is platelet factor 4, a cationic alpha-granule protein release by activated platelets in the presence of damaged endothelium. Initially found to increase protein C activation in vitro by up to 25-fold in a Gla-domain-dependent manner [72], platelet factor 4 infusion also produced elevated APC levels in a primate in vivo model, presumably by enhancing the affinity of protein C for the thrombin-thrombomodulin complex [73]. Earlier studies showed that protein C activation was also accelerated by factor Va, both in solution and on

endothelial cells [74]. Negatively charged endothelial phospholipids, which can be exposed after thrombin-induced cell activation, significantly increase APC formation as well; this effect may be mediated via a calcium-dependent interaction with protein C [75].

By definition, the generation of APC is limited by both zymogen availability, measured by circulating protein C level [76], and thrombin generation. For similar reasons, given the strong dependence of protein C activation on both thrombomodulin and EPCR, changes in the expression or activity of these elements represent a key means of altering overall APC production. For example, inflammatory processes reduce protein C activation by a host of mechanisms: TNF can downregulate thrombomodulin and EPCR transcription [58, 77], homocysteine has been shown to directly inhibit thrombomodulin and reduce its cell surface expression [78], and atherosclerosis decreases both EPCR and thrombomodulin expression on the endothelial surface [79]. Proteolytic release of thrombomodulin from the cell surface is another mechanism for downregulation and has been associated with neutrophil elastase [80]. Interestingly, heat shock has an opposing effect, increasing the transcription of thrombomodulin in human endothelial cells, a process that can overcome the downregulation induced by TNF-α [81]. The association of these receptors with caveolae may represent another means of regulating protein C activation [82]. As mentioned previously, the distribution of thrombomodulin and EPCR in specific vessel types also has implications for the differential regulation of coagulation throughout the vasculature.

Thrombin itself has been shown to have divergent effects on the key elements of the APC pathway. In vitro studies of human endothelial cells have shown that thrombin binding induces endocytosis of the thrombin-thrombomodulin complex, which may represent a mechanism for both thrombin clearance and degradation, and downregulation of protein C activation [83]. Conversely, and likely via independent receptors, thrombin can induce an increase in thrombomodulin transcription [84]. Though thrombin may be associated with endothelial shedding of EPCR, it also serves to increase EPCR transcription in animal models [69, 85]. Though difficult to assess in vivo, these results suggest that a surge of thrombin, as seen in traumatic injury, might well lead to significant increases in EPCR and thrombomodulin transcription and expression.

One area of key interest, especially with regard to traumatic coagulopathy, is the effect of hypoxemia on protein C activation. Earlier in vitro studies using bovine aortic endothelium showed that hypoxemia led to marked selective decreases in the expression of thrombomodulin over several hours, which were reversible with re-oxygenation [86]. Similar effects were found in human endothelium in vitro [87]. This would seem to indicate that shock, and its attendant hypoxemia, would lead to an overall decrease in the activation of protein C. Nonetheless, several clinical studies have demonstrated the opposite: ischemia leads to increased protein C activation in vivo. This has been established in pigs, where selective coronary artery occlusion leads to a rapid increase in protein C activation that facilitates recovery from ischemic insult [88], and in humans, where brain vasculature APC levels rise within seconds of carotid artery occlusion [89]. Another clinical study, in the setting of cardiopulmonary bypass, demonstrated significant increases in activated protein C levels in the minutes following aortic unclamping, with the degree of APC increase correlated to improvements in postoperative cardiovascular function [90]. These clinical results correlate to more recent in vitro findings from our laboratory, where hypoxic insult leads to rapid non-transcription-dependent increases in human endothelial thrombomodulin, EPCR, and protein C activation (data submitted for publication).

Inactivation of protein C is mediated by protein C inhibitor (PCI), a member of the family of serine protease inhibitors (serpins) [91]; like other serpins, PCI forms a covalent bond with its target, which leads to both APC degradation and conformational change of the inhibitor [92]. Other known inhibitors of APC include α_1-antitrypsin and α_2-macroglobulin; in

concert with PCI, these inhibitors result in the relatively long half-life of APC (approximately 20 min) [93, 94].

Anticoagulant Actions of APC

Inactivation of Coagulation Factors

Once activated and released from EPCR, activated protein C achieves its primary anticoagulant effect by proteolytic inactivation of activated coagulation factors Va and VIIIa. This activity is dependent upon APC Gla domain binding to negatively charged phospholipid membranes [95]. Given that factor Va enhances the activity of the prothrombinase complex greater than 10,000-fold, APC-mediated inactivation of Va effectively ceases thrombin production [96, 97]. Proteolytic cleavage of Va at Arg506 is the more rapid reaction, but only results in partial inactivation, whereas cleavage at Arg306 leads to complete inactivation [10, 98]. As described previously, protein S serves as a potent cofactor in this cleavage, optimizing APC orientation for binding to both the phospholipid membrane and the targeted substrate; protein S can potentiate the more definitive Arg306 cleavage up to 20-fold [52, 53]. Overall, APC serves to shut down Va activity, which prevents the formation of thrombin via the extrinsic pathway, severely delaying primary clot formation.

The inactivation of factor VIIIa resembles that of Va, with APC-induced cleavage occurring at Arg336 and Arg562 [99]. Unlike factor Va, cleavage at either site in VIIIa induces functional inactivation [54]. Both protein S and inactivated factor V serve as cofactors for APC in the inactivation of VIIIa, which has a relatively short half-life regardless of inactivation, but may be protected by binding to other factors [100]. When active, and prior to inactivation by APC, factor VIIIa can enhance the formation of activated factor Xa via the intrinsic pathway up to 200,000-fold [101]. Thus APC serves to downregulate both the rapid thrombin formation produced by the extrinsic pathway, through cleavage of factor Va, and the ongoing secondary thrombin formed by the intrinsic pathway, through cleavage of factor VIIIa [97].

Effects on Fibrinolysis

In addition to these direct anticoagulant effects, APC has long been associated with increases in fibrinolysis. Early published characterizations of "autoprothrombin IIa" by its discoverers demonstrated profound induction of fibrinolysis, as assayed by thromboelastography, in both canine and human blood in vitro; an in vivo canine model of APC infusion yielded similar results [6]. These investigators suspected a possible indirect effect on plasminogen, which was confirmed in subsequent studies identifying a secondary messenger mechanism by which APC produced increases in plasminogen activator [102, 103] (Fig. 6.2). The direct effect of APC on plasminogen activator inhibitor 1 (PAI-1) was shown in both whole blood clot lysis assays [104, 105] and studies of cultured endothelium [106, 107]. The relevance of this mechanism was questioned when it could not be replicated in the primate setting [108], and proceeded slowly in a purified system with human proteins. Then, in 2001, Rezaie showed that the glycoprotein vitronectin acts as a stabilizing cofactor to dramatically accelerate the reactivity of PAI-1 with APC in humans [109]; based on these findings, it appears that in vivo APC binds to and inactivates PAI-1, itself a serpin, in a reaction potentiated by vitronectin, with resulting de-inhibition of fibrinolysis. Of note, this also implicates PAI-1 as another significant inhibitor of APC.

Thrombin activatable fibrinolysis inhibitor (TAFI), another key mediator of fibrinolysis, is intimately tied to the APC pathway: its major physiologic activator is the thrombin-thrombomodulin complex [110]. A carboxypeptidase enzyme, activated TAFI inhibits fibrinolysis by removing lysine residues from fibrin, preventing the activation of plasminogen and the activity of plasmin [111, 112]. Before Rezaie demonstrated the key role of vitronectin in potentiating the interaction of PAI-1 and APC, TAFI was identified as a possible means by which APC

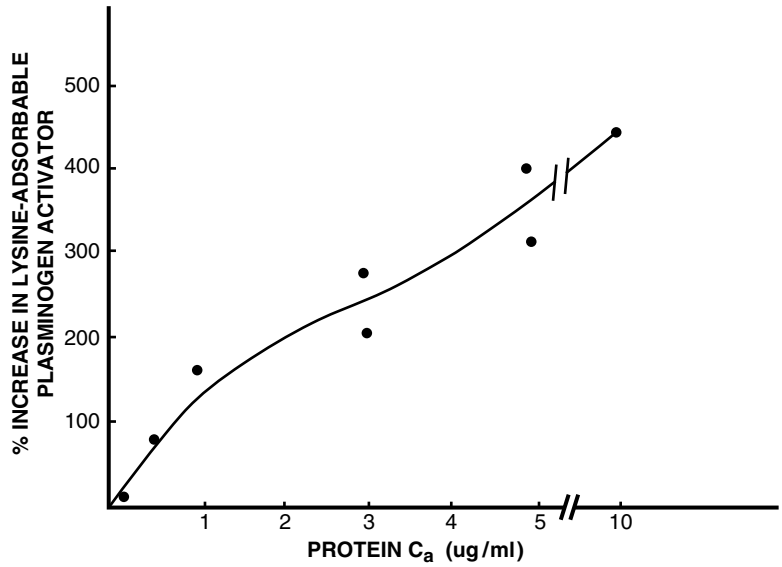

Fig. 6.2 Relationship between level of activated protein C administered and lysine-absorbable plasminogen activator level generated. A single dog was administered increasing doses of APC, ranging from 0.1 to 10 μg protein C per milliliter plasma volume on 8 different days. The plasminogen activator levels were measured before and 5 min after APC, and the percentage increase in plasminogen activator was plotted as a function of APC dose administered. Similar changes in plasminogen activator levels following APC infusion were observed in four other dogs. (Reproduced from Comp, Esmon; Generation of fibrinolytic activity by infusion of activated protein C into dogs. Journal of Clinical Investigation 1981 Nov;68(5):1221–8, with permission.)

could promote fibrinolysis: by its overall anticoagulant effect, APC reduces thrombin formation, limiting the key substrate that activates TAFI and leading to reduced inhibition of fibrinolysis [113, 114]. The concentration of thrombomodulin may serve to regulate the overall effects of TAFI and APC on fibrinolysis, perhaps in a tissue-specific pattern based on thrombomodulin distribution within the vasculature [115]. The substrate preference of thrombomodulin may also be regulated by platelet factor 4, which when released from activated platelets promotes protein C activation and inhibits TAFI activation, leading to a profibrinolytic state [116].

Cytoprotective Actions of APC

In the past two decades, the understanding of APC's direct effect on cells has expanded immensely, with notable implications from the level of molecular biology to that of clinical care. Through these discoveries, APC has emerged as a critical linchpin connecting the complex pathways of coagulation and inflammation.

Initially, investigators theorized that APC's anti-inflammatory effects were primarily related to decreased production of pro-inflammatory thrombin. Indeed, thrombin has been shown to exert multiple inflammatory actions on cells, primarily via GTP-binding protein-coupled protease-activated receptors (PARs) [117], and APC likely plays an indirect role in mitigating such activity through its anticoagulation pathway. Early awareness of a more direct relationship between APC and inflammation was postulated when thrombin infusion actually led to increased survival in a canine sepsis model; infusion was also associated with anticoagulation and enhanced fibrinolytic activity, presumably due to thrombin-induced activation of protein C [118]. This prompted a directed investigation of APC's effects in a primate model of sepsis, wherein APC prevented the lethal effects of

high-dose *Escherichia coli* infusion in baboons, and APC blockade exacerbated the effects of sublethal *E. coli* challenge [119]. These studies, conducted in the late 1980s, paved the way for the eventual randomized controlled trials of recombinant human APC therapy for sepsis two decades later.

Based on the growing body of animal data indicating an APC-induced anti-inflammatory effect beyond mere attenuation of thrombin production, Joyce and colleagues performed transcriptional profiling that showed recombinant human APC modulates human endothelial cell gene expression, with resulting inhibition of major apoptotic and inflammatory pathways [120]. Shortly thereafter, APC was shown to directly signal cells through the PAR-1 receptor; this interaction was found to be dependent on APC-to-EPCR binding, and affected the gene expression of several specific intracellular regulators of apoptosis and inflammation [12]. Since that discovery, a series of experiments have identified a pleiotropic array of candidate receptors and pathways for APC signaling, on multiple cell types including endothelium and immune cells (as reviewed in [121]); elucidating these pathways remains an area of active investigation.

Recognizing that novel APC receptors and pathways are the subject of ongoing research efforts, at this time the paradigmatic model for APC's cytoprotective effects focuses on EPCR and PAR-1: after activation, APC bound to EPCR serves to induce a specific noncanonical N-terminal cleavage and activation of PAR-1, which results in a series of intracellular processes distinct from those induced by thrombin-PAR-1 binding [122]. Given its relatively long half-life of 20 min, APC that remains bound to EPCR has ample time to exert its direct effects on the endothelial cell. These effects depend to some extent on the cell type, and include the regulation of gene expression via suppression of nuclear transcription factor activity, as discussed [120]. In endothelial cells, APC PAR-1 signaling inhibits vascular adhesion molecule expression and the release of pro-inflammatory molecules [123]. Endothelial barrier function is also enhanced in vitro by APC, an effect that is dependent upon PAR-1 signaling and mediated via the sphingosine kinase pathway and sphingosine 1-phosphate receptor [124]. In leukocytes, APC prevents adhesion molecule expression and reduces the release of pro-inflammatory mediators like TNF-α, potentially attenuating the cytokine storm that characterizes sepsis and post-injury inflammation [125, 126]. APC has been shown to inhibit apoptosis in an EPCR- and PAR-1-dependent manner [127], and in hypoxic stress states, APC downregulates proapoptotic proteins like p53 and Bax in human brain endothelium [128]. Though many of these mechanisms have been delineated in vitro, they have been supported by ongoing animal experiments, particularly those that employ the use of genetic knockouts (e.g., to assess the necessity of EPCR and PAR-1 in APC cytoprotective signaling) and domain-specific targeted mutants of APC [129]. These studies have taken place while large-scale human trials of recombinant APC for therapeutic use in sepsis have been conducted, analyzed, and debated, as discussed below.

Independent of the PAR-1 direct cell-signaling pathway, APC is known to attenuate inflammation in an indirect fashion via its inactivation of extracellular histones. APC cleaves histones and reduces their cytotoxic effects in vitro, and in a mouse model APC co-infused with lethal doses of histones prevented death [130]. By thus dampening the cytotoxicity wrought by extracellular histones, APC can play an indirect cytoprotective role in the setting of sepsis or other major inflammatory states, such as trauma.

Clinical Relevance of the Protein C Pathway

Protein C was discovered and characterized in a laboratory, long before any awareness of its clinical significance; it would be decades before clinical reports emerged detailing the manifestations of protein C pathway abnormalities. In more recent years, with the use of domain-specific APC mutants and genetically manipulated animal models, the overall understanding of the significance of this pathway in hemostasis and

inflammation has expanded immensely. Multiple studies have shown that genetic deficiencies of key components of the APC pathway, from protein C [131] to thrombomodulin [34] to EPCR [132], can lead to early thrombosis, hemorrhage, and embryonic or perinatal lethality. These findings underscore the essential role that APC plays in maintaining the organism's hemostatic balance.

The first human data on a clinical syndrome related to protein C was reported in 1981, when Griffin and colleagues identified a 22-year-old man with multiple thromboembolic complications and a family history of recurrent thrombotic disease. In the patient and two affected family members, significantly depressed levels of protein C were identified [133]. Two years later, a Dutch study described congenital protein C deficiency in three unrelated families with high rates of thrombophlebitis, and through pedigree analysis posited that the disorder was transmitted in autosomal dominant fashion, with incomplete penetrance [134]. Shortly thereafter, independent investigators reported increased thrombosis in patients with hereditary protein S deficiency [135, 136]. Since these initial discoveries, many genetic mutations have been identified that lead to both quantitative and qualitative protein C or protein S deficiencies; the vast majority of identified patients are heterozygous, and do manifest higher rates of venous thrombosis [137]. Homozygous protein C deficiency occurs much more rarely, most likely due to fetal demise, and manifests as neonatal purpura fulminans, a highly morbid condition that can be treated with protein C concentrate [138, 139]. Similar pathophysiology is observed in acquired protein C deficiency, the classic example being the occurrence of skin necrosis following initiation of warfarin anticoagulation therapy, when the relatively short half-life of protein C leads to an initial predominance of longer half-life procoagulant factors, with resulting thrombosis in the microvasculature [140].

Disappointed by the lack of identifiable protein C (or S) deficiency in many patients with apparently familial thrombosis syndromes, Swedish researchers set out to find alternative genetic mechanisms, and in 1993 identified a heritable resistance to APC [141]; a simultaneous Dutch study arrived at similar conclusions [142]. This resistance phenotype was found to be far more prevalent than any of the previously known deficiencies and is now known to be the most common cause of inherited thrombophilia [143]. The following year, three groups (including University Hospital in Leiden) showed that APC resistance is characterized by a single-nucleotide substitution mutation in Factor V, wherein the arginine at active APC-cleavage site Arg506 is replaced with a glutamine, preventing efficient inactivation of factor Va by APC [144]. Factor V Leiden remains the best characterized heritable thrombophilia and is found in heterozygous form in up to 5 % of Caucasians; it is thought to have arisen from a single ancestor 21,000–34,000 years ago and may have persisted due to the relative benefits of hypercoagulability (namely decreased hemorrhagic death) in the premodern setting [145].

Since the turn of the twenty-first century, the anti-inflammatory actions of APC have garnered great interest, and perhaps even greater scrutiny, in human sepsis trials. Following upon the remarkable protective effects of APC observed in baboon sepsis studies, a large international prospective multicenter trial was conducted to assess the efficacy and safety of activated drotrecogin alfa (recombinant human APC, rhAPC) treatment of sepsis in humans. This randomized, placebo-controlled phase 3 study was discontinued early when a significant mortality benefit was found in rhAPC-treated patients: these patients had a reduction in the relative risk of death at 28 days of 19 %, with an absolute reduction of 6 % [146]. This finding represented the first (and to date, only) successful non-antimicrobial pharmacologic therapy for severe sepsis, and its mortality benefit across subgroups was hailed as a breakthrough in critical care medicine [147]. However, subsequent investigations in less severely ill and pediatric populations failed to show a similar benefit and raised questions about the risk of bleeding complications [148, 149]. A follow-up multicenter trial failed to show any benefit to rhAPC treatment,

and as a result it was withdrawn by the manufacturer [150]. However, the actual comparability of this study to the original trial has been questioned, as has its statistical power in the setting of a lower overall mortality rate; subsequent meta-analyses have suggested that there may in fact be a significant overall therapeutic benefit to rhAPC [151, 152].

Spurred by the initial success of rhAPC in sepsis and an emerging awareness of APC's cytoprotective mechanisms, several animal and human studies have been conducted to assess further potential therapeutic applications. Animal studies indicated that APC administration might attenuate lung injury, but these results were not replicated in a human multicenter randomized controlled trial [153, 154]. Anti-inflammatory effects combined with promotion of angiogenesis make APC a promising therapeutic for chronic wound healing [155, 156]. Multiple animal studies have indicated a neuroprotective role for APC, especially in the setting of ischemia [128, 157]. These findings have prompted human trials of a cytoprotective-selective APC variant in the treatment of ischemic stroke, which are currently underway [158]. The ability to deliver such domain-specific agents at much higher doses than those used in prior rhAPC trials (since risk of bleeding can be nearly eliminated by inactivation of anticoagulant function) may also prompt a re-evaluation of possible therapeutic applications in sepsis.

APC and Trauma-Induced Coagulopathy

Though posttraumatic coagulation abnormalities had been classically attributed to coagulation factor consumption or iatrogenic interventions (e.g., the "vicious triad" of hemodilution, hypothermia, and acidosis), in 2003 two independent cohort studies reported that roughly 25 % of trauma patients present with coagulopathy on admission [159, 160]. Those with impaired coagulation had increased transfusion requirements, increased rates of organ dysfunction, and a fourfold increased risk of mortality. Coagulopathy corresponded to injury severity, but its effect on mortality was independent of injury severity score (ISS). Unable to determine the causal mechanism of this newly identified "endogenous" coagulopathy, the authors posited that it may "be an indicator of loss of regulation of the local inflammatory response" [160].

Seeking to better understand this endogenous coagulopathy, our group studied 208 trauma patients at San Francisco General Hospital, hypothesizing that the combination of tissue injury and hypoperfusion might lead to a pathologic activation of the APC pathway. Measuring tissue hypoperfusion by base deficit, they found that coagulopathy only occurred in the presence of significant base deficit (>6 mEq/L), regardless of total thrombin formed (by prothrombin fragments 1 + 2 levels) [161] (Fig. 6.3). Combined with the finding of normal fibrinogen levels in all patients, this argued against a coagulation factor consumption mechanism, as would be seen in a more typical disseminated intravascular coagulation (DIC) pathophysiology. Increasing degree of shock (base deficit) was also associated with decreasing levels of zymogenic protein C and increasing levels of soluble thrombomodulin; though protein C also decreased with increasing thrombin formation, it only did so in the presence of increased base deficit. Decreasing levels of protein C correlated with increased PT and PTT, decreased PAI-1 activity, and increased D-dimers, indicating an overall hypocoagulable and fibrinolytic state (Fig. 6.4). Transfusion requirements, complications, and mortality were all associated with decreased protein C zymogen and increased soluble thrombomodulin.

This first study linking APC and TIC was not without limitations, primary among them the lack of direct measurement of the activated form of protein C. Also, as discussed previously, the significance of soluble thrombomodulin remains unknown and may not be a direct assay of overall thrombomodulin activity. The data was also collected from a single time-point blood draw, upon admission. Perhaps most importantly, the findings represented a compelling correlation but fell short of definitive causal evidence. Nevertheless, the hypothesis and results generated considerable

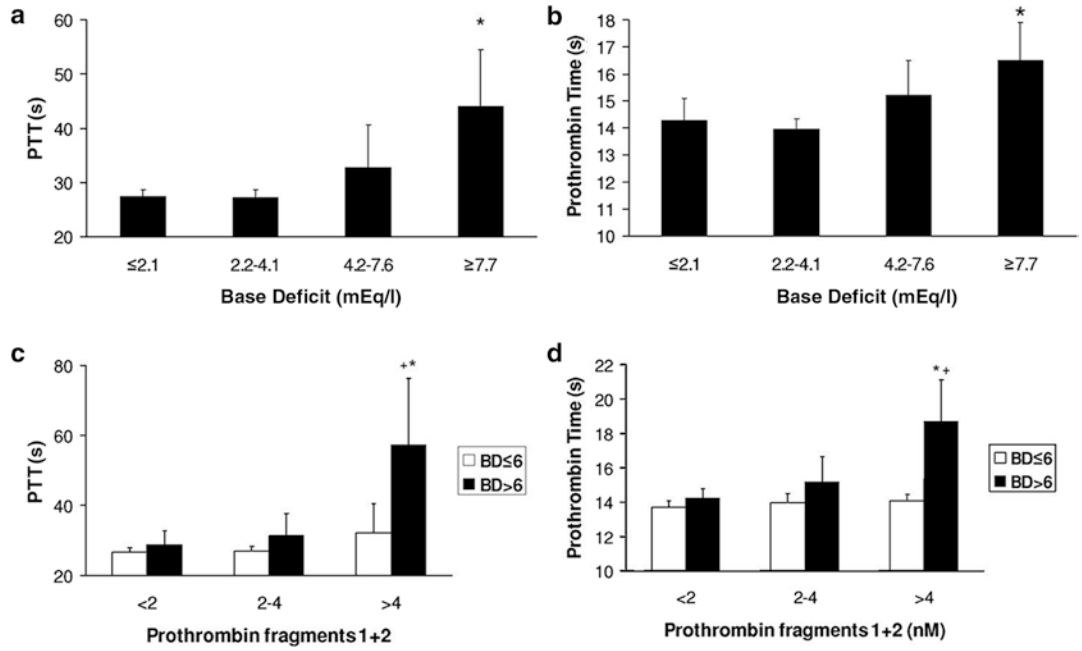

Fig. 6.3 Effects of tissue hypoperfusion on coagulation. (**a**) Increasing partial thromboplastin time with increasing BD. (**b**) Increasing prothrombin time with increasing BD. BD in quartiles. (**c**) Partial thromboplastin time is prolonged only in the presence of a raised BD. While the BD remains low (*white bars*), increasing thrombin generation has no effect on the prothrombin time. With a raised BD (*black bars*), prolongation of the prothrombin time is seen with increasing thrombin generation. PF1 + 2 in tertiles. (**d**) Prothrombin time is prolonged only in the presence of a raised BD (as **c**). PF1 + 2 in tertiles. (Reproduced from Brohi et al, Acute traumatic coagulopathy: initiated by hypoperfusion: modulated through the protein C pathway? Annals of Surgery 2007; 245(5):812–8, with permission.)

discourse, especially regarding the therapeutic implication that reversal of shock might be more critical than coagulation factor repletion in trauma resuscitation [162].

In a clinical trauma study published the following year, increases in soluble thrombomodulin were shown to be correlated to decreased utilization of fibrinogen, decreased levels of zymogenic protein C, and increased TAFI, indicating that soluble thrombomodulin levels may well correlate to increased overall activity [163]. In an attempt to clarify the mechanisms of fibrinolysis in this population, tPA was shown to be increased in the presence of decreased PAI-1 levels, which in turn correlated to D-dimer levels, whereas TAFI levels had no significant correlation with D-dimer. This suggested that the primary effect on fibrinolysis was mediated via decreased PAI-1 (and thus increased tPA and plasmin), rather than protein C competing with TAFI for thrombomodulin binding sites (and thus decreasing overall TAFI activity).

Three years later, in a study of 203 severely injured trauma patients, the definitive evidence of protein C activation was established [164]. In this study, activated protein C levels were measured using an enzyme capture assay and were found to correlate to decreasing levels of zymogen protein C, as expected. Patients with severe injury and shock had significantly elevated levels of APC (Fig. 6.5), and APC was shown to correlate with the degree of coagulopathy in linear regression, wherein a log increase in APC resulted in a 2.4 s increase in PT. APC was inversely related to levels of factors Va and VIIIa, and directly related to increases in tPA and D-dimer. With regard to outcomes, the degree of protein C activation (by APC:PC ratio on admission) correlated to

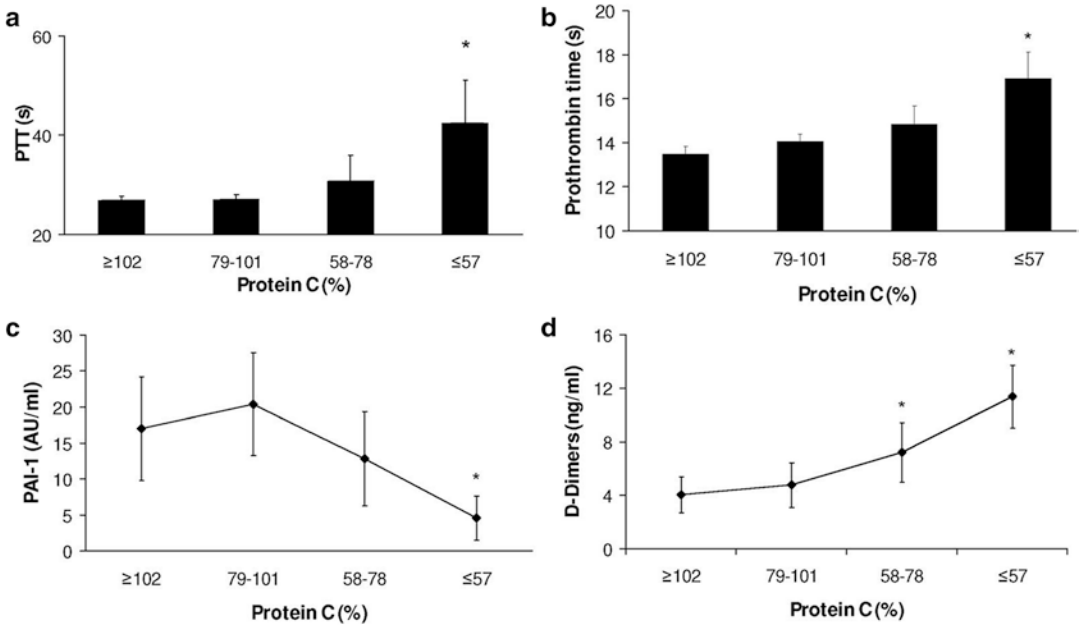

Fig. 6.4 Indirect evidence for activation of protein C. (**a**) Partial thromboplastin time is prolonged as protein C falls. Protein C in quartiles. (**b**) Prothrombin time is prolonged as protein C falls. Protein C in quartiles. (**c**) Plasma plasminogen activator inhibitor-1 (PAI-1) levels fall as protein C falls. (**d**) Plasma D-dimer levels rise as protein C levels decrease. (Reproduced from Brohi et al, Acute traumatic coagulopathy: initiated by hypoperfusion: modulated through the protein C pathway? Annals of Surgery 2007; 245(5):812–8, with permission.)

increased odds of ventilator-associated pneumonia (VAP), lung injury, and multi-organ failure, with a 2.1-fold increase in the odds of mortality. An analysis of longitudinal protein C levels following admission showed that increased APC with failure to recover normal zymogen levels correlated with increased risk of VAP.

These findings, as noted, were arrived at using prospectively collected data from a single institution. To investigate the mechanistic drivers of TIC in a larger multicenter context, an analysis was conducted using the prospective observational major trauma transfusion (PROMMTT) study. Assessing nearly 1200 patients from ten centers, shock and base deficit were found to be predictive of admission coagulopathy [165]. Analysis of coagulation factors identified activation of protein C (decreased protein C zymogen, increased APC) and depletion of coagulation factors, notably factors V and VIII, to be predictive of coagulopathy.

Since results from human trauma studies, no matter how compelling, remain by design correlative and observational, a traumatic hemorrhage model was developed in mice to investigate hypothesized mechanisms. In this model, tissue injury combined with hypoperfusion led to reproducible coagulopathy. A directed antibody (mAb 1591) was used to block the anticoagulant domain of APC in these mice; pretreatment with this antibody prevented traumatic coagulopathy [166] (Fig. 6.6). Though limited by its context in a murine model, this finding provided the mechanistic evidence that coagulopathy in the setting of traumatic shock is caused by APC. Interestingly, 100 % of mice pretreated with an antibody that blocked both the anticoagulant and cytoprotective domains of APC (mAb 1609) died within 45 min of shock initiation, and were found to have diffuse arteriolar thrombosis in the lungs, accompanied by perivascular and alveolar hemorrhage; this underscored the key role that APC plays in mitigating systemic inflammation. In a parallel rat model of penetrating traumatic shock, coagulation measured by thromboelastography

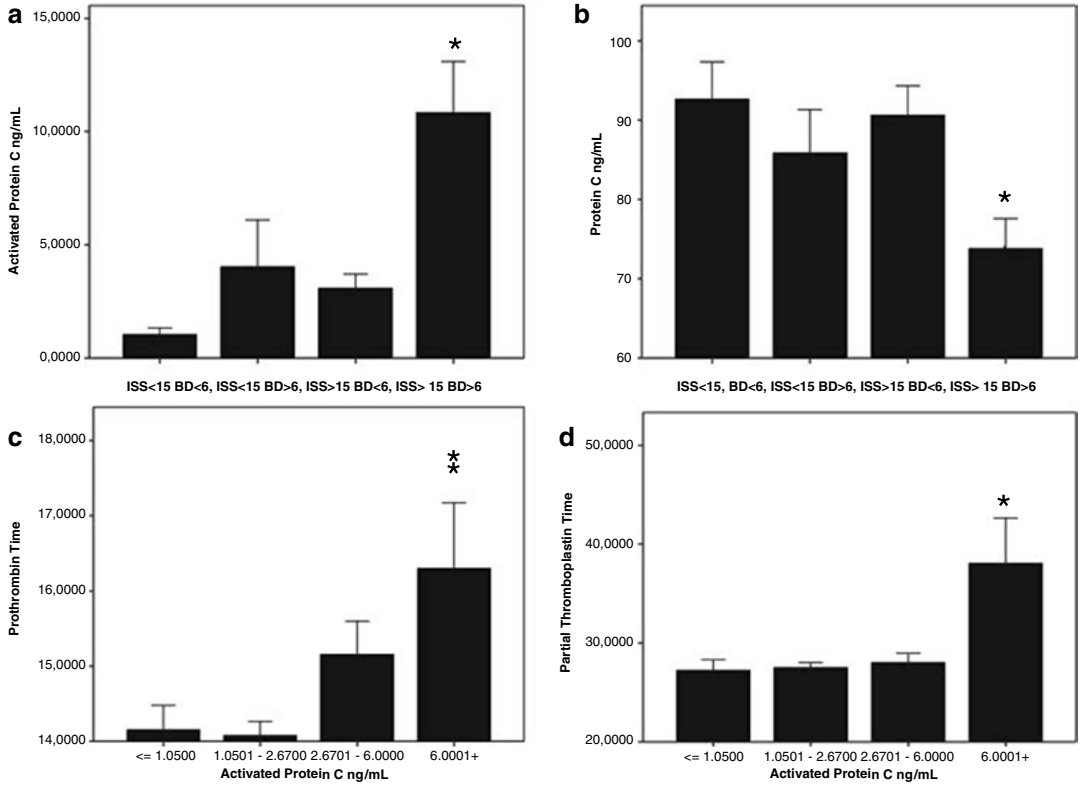

Fig. 6.5 Tissue injury and shock result in a systemic activation of protein C pathway associated with coagulopathy in trauma patients. Patients were divided into groups using previously described definitions of injury severity based on Injury Severity Score and Shock based on Base Deficit. This resulted in four groups; minimal injury, no shock (ISS < 15, BD < 6); minimal injury, shock (ISS > 15, BD > 6); severe injury, no shock (ISS > 15, BD < 6); and severe injury and shock (ISS > 15, BD > 6). Plasma levels of activated protein C (aPC) and protein C (PC) were assayed. Patients with severe injury and shock had elevation of plasma levels of aPC (*Panel A*) and a concomitant decrease in levels of PC (*Panel B*). aPC levels were then divided into quartiles. Patients with the highest quartile of plasma levels of aPC had elevated PT (*Panel C*) and PTT (*Panel D*). All $P < .05$ by Kruskal–Wallis rank test. (Reproduced from Cohen et al, Critical role of activated protein C in early coagulopathy and later organ failure, infection and death in trauma patients; Annals of Surgery 2012; 255(2):379–85, with permission.)

indicated that the resulting TIC was due to impaired thrombin formation rather than later clotting dynamics, favoring the mechanism of Va and VIIIa inactivation by APC [167]

Based on this mechanistic data and the human clinical results, our group posited that TIC represents an evolved but maladaptive response to severe injury. In the setting of severe trauma and tissue hypoperfusion, an excess of protective anti-inflammatory APC is released in an attempt to prevent local microvascular thrombosis and mitigate cellular dysfunction (Fig. 6.7). This view is supported by data from a trauma cohort demonstrating that poor outcomes associated with increased levels of inflammatory histones are abrogated by simultaneous increases in endogenous APC, implying a protective effect of APC in the setting of widespread inflammation [168]. According to this hypothesis, APC may be activated as part of an innate response to inflammatory injury, one that results in an "inadvertent" anticoagulant and profibrinolytic effect, leading to the development of a clinically significant coagulopathy. The subsequent depletion of the overactivated protein C system exposes the individual to eventual organ injury and infection.

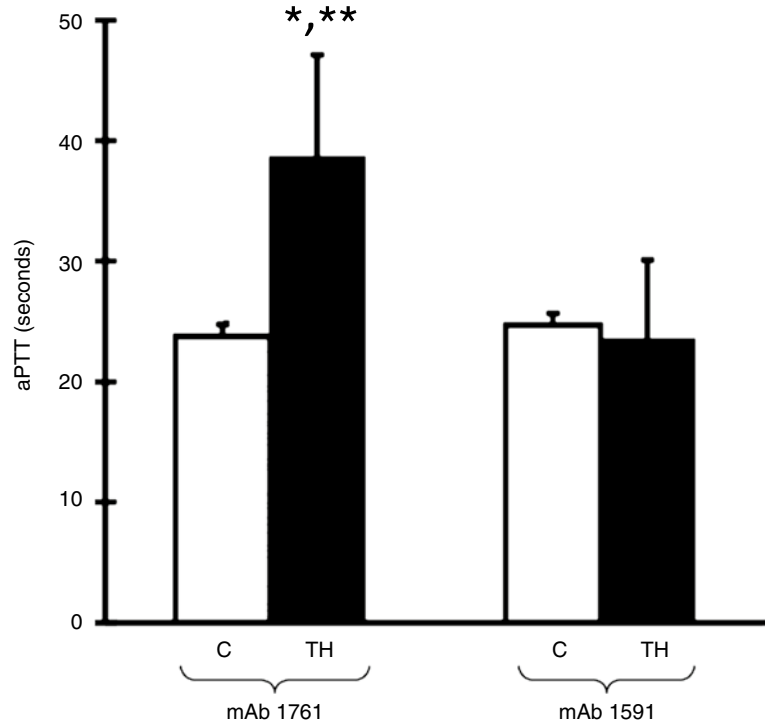

Fig. 6.6 Inhibition of the anticoagulant function of PC prevents the development of acute traumatic coagulopathy in mice. Mice were pretreated with a mAb that inhibits the anticoagulant function of PC (mAb 1591) or with an isotype control mAb (mAb 1761). After 10 min, the mice then underwent TH. Activated partial thromboplastin time values were measured after 60 min of hemorrhagic shock. (Reproduced from Chesebro et al, Increase in activated protein C mediates acute traumatic coagulopathy in mice; Shock 2009; 32:6 p 659, with permission.)

Several key questions remain regarding this overall hypothesis. One critical mechanistic element relates to the actual driver of protein C activation in the setting of traumatic shock. While thrombin is readily available in the setting of significant tissue injury, the upregulation of thrombomodulin in hypoperfused states remains a matter of debate. As discussed previously, in vitro studies have indicated that hypoxemia may decrease the expression of thrombomodulin [86, 87], but multiple studies have demonstrated that ischemia actually leads to increased protein C activation in vivo [88–90]. In order to address this question directly, our lab recently conducted an in vitro study of endothelial hypoxemic insult, finding that such stimuli led to rapid non-transcription-dependent *increases* in human endothelial thrombomodulin, EPCR, and protein C activation (data submitted for publication).

Another area of active interest is that of the upstream drivers of APC-induced TIC.

The activation of protein C is known to be activated by thrombin, but the mechanisms of thrombin production in trauma have not been fully characterized. Though the central roles of tissue factor and the extrinsic pathway in hemostasis are long established [169], recent findings have raised the possibility that alternative inflammatory mediators may drive changes in coagulation dynamics, possibly via the intrinsic pathway [170–172]. In order to elucidate which pathway predominates in coagulation activation after trauma, and which then drives coagulopathic changes in the setting of shock, targeted antibody blockades were employed in the previously described murine model of traumatic shock [173]. These data indicate that coagulation activation following traumatic hemorrhage is driven primarily by the (extrinsic) tissue factor pathway, and that the resulting increase in thrombin production is responsible for the activation of protein C seen in TIC.

A recent in vitro study added increasing levels of APC to healthy subjects' whole blood and concluded that the relatively APC-resistant population of Va on platelets effectively negated any

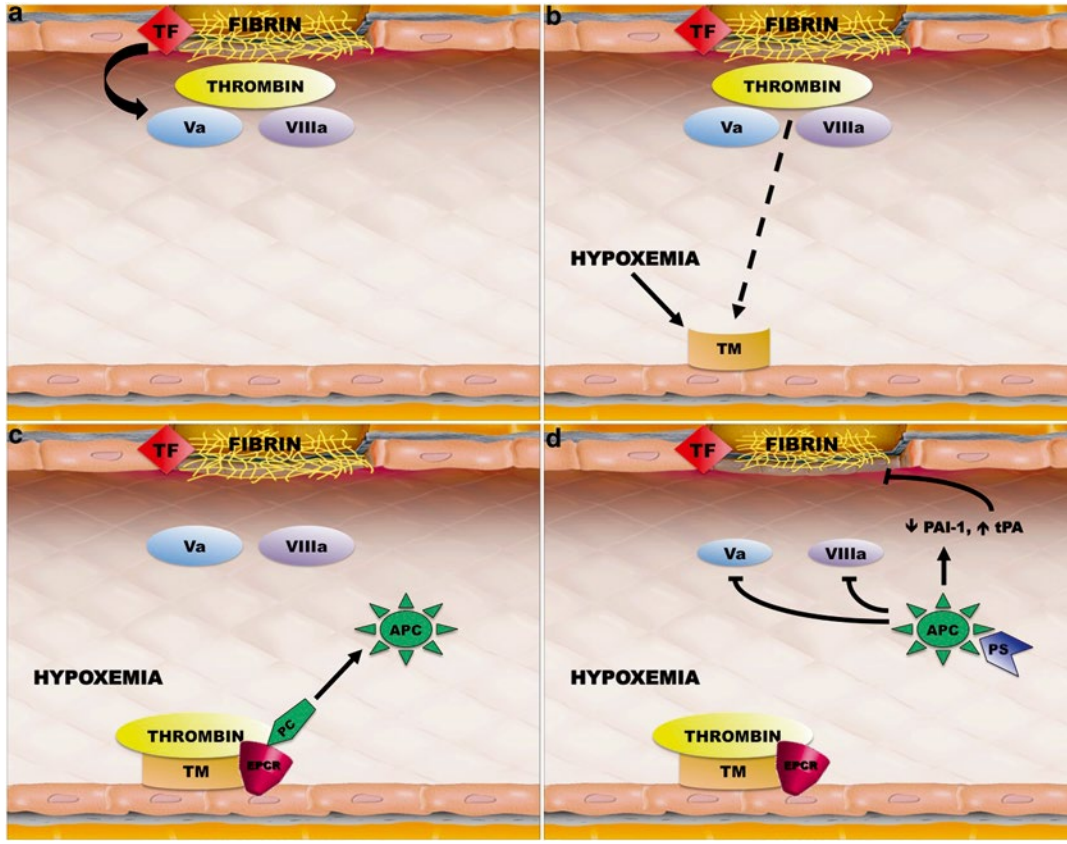

Fig. 6.7 The activated protein C pathway in trauma-induced coagulopathy. (**a**) Tissue injury leads to exposed tissue factor (TF), which drives thrombin formation via the coagulation cascade, leading to the production of fibrin clot. (**b**) Hypoxemia leads to increased endothelial thrombomodulin (TM) expression, which diverts thrombin from clot formation. (**c**) Thrombin bound to thrombomodulin activates zymogenic protein C (PC) in a reaction potentiated by EPCR. (**d**) In actions potentiated by protein S (PS), activated protein C (APC) inactivates Va and VIIIa by proteolytic cleavage and disinhibits fibrinolysis by inhibiting PAI-1, leading to an overall hypocoagulable state

hypocoagulable effects from APC at or above reported in vivo concentrations [174]. Though the authors surmised that these findings precluded the role of APC in TIC, the study did not incorporate an endothelial element, nor did it acknowledge the fact that tissue-specific concentrations of APC in injured areas may well differ from those measured at a site of remote venipuncture [175]. Their conclusions also contradicted rotational thromboelastometry (ROTEM) data from healthy subjects collected a decade before, in order to assess the potential coagulation effects of rhAPC administration; this study demonstrated a significant increase in clotting time at relatively low doses of rhAPC [176]. Another recently presented in vitro "spiking" study did show a dose-dependent effect of APC on coagulation dynamics, with hypocoagulability by ROTEM at increasing doses (data presented at 2014 American Association for the Surgery of Trauma Annual Meeting, submitted for publication). By definition, such studies are limited by their ex vivo context, and though they may provide heuristic insights as to the mechanisms of TIC, they should be interpreted with caution.

It should be noted that throughout this discussion, attention has been focused on the APC pathway as a potential causal agent in TIC—however, this approach should not be misunderstood as an attempt to characterize APC as the

only, or even the paramount, driver of TIC. As ongoing study from myriad research groups has shown over the past decade, and as this larger volume indicates, TIC represents a complicated and likely heterogeneous pathophysiology, one that incorporates multiple pathways of inflammation, coagulation, and especially fibrinolysis [177]. The application of more sophisticated statistical methods, such as principle components analysis, to understanding TIC dynamics in clinical populations indicates that APC-induced coagulopathy may represent one phenotype among many [178].

Future Directions and Therapeutic Implications

Perhaps the most intriguing question raised by this body of research centers around its therapeutic implications: if APC is indeed a major causal driver of TIC, how might this pathway be manipulated to alter the course of coagulopathy, and subsequent morbidity and mortality, in trauma? Moving forward beyond the characterization stage, this question will continue to animate efforts toward the development of translational applications.

Based on the results of mechanistic murine studies, one approach might be to develop a human antibody similar to murine mAb 1591, which effectively blocked APC's anticoagulant domain and prevented coagulopathy in the setting of traumatic shock [166]. Though blocking APC anticoagulation in trauma might in fact attenuate coagulation abnormalities, the potentially prothrombotic complications of such a therapy would be concerning, and extensive preclinical testing would be required. Also, determining the appropriate patients for therapy and defining the optimal time after injury for administration represent significant challenges.

One promising area of APC investigation is that of domain-specific variants. A more thorough understanding of APC's structure and related functional implications has led to the development of engineered APC that is selective for specific functions. As noted, following the concerns of bleeding complications and limited clinical benefit related to rhAPC, variants with diminished anticoagulant but intact cytoprotective function have been engineered [178]. Site-specific mutagenesis has preserved EPCR binding and PAR-1 cleavage while nearly eliminating proteolytic coagulation factor inactivation altogether [179]. While this has obvious appeal in the treatment of sepsis and related inflammatory conditions, including the ability to deliver much higher doses than were tolerated in the original rhAPC trials, in theory such variants could provide a potent therapeutic option in the setting of trauma. By administering a cytoprotective-selective variant following significant injury with attendant shock, a system otherwise depleted of APC could be buffered against inflammatory sequelae such as nosocomial infection and organ damage. Again, the optimal patient selection and timing of such therapeutics would need to be carefully studied. Lastly, the use of variants can be complimented by genetic knockouts and overexpressors (e.g., of EPCR) in animal models; this will enrich our growing understanding of the complex APC pathway dynamics in the setting of traumatic shock.

Though the initial clinical and mechanistic characterizations of APC in TIC have been reviewed here, the translation of such knowledge into potential interventional approaches remains in its most nascent stages. The promise of incorporating this increased awareness of APC's role into an integrated understanding of TIC, in order to better treat our most critically injured patients, represents a research priority now and for the foreseeable future.

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The Endothelium

7

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Introduction

Trauma-induced coagulopathy (TIC) develops in severely injured patients shortly after the time of injury [1, 2]. Upon arrival to the emergency department (ED) 25–35 % of these severely injured patients are coagulopathic by conventional coagulation assays—a condition associated with a fourfold increase in mortality [3–7]. Given that TIC begins before hemodilution and hypothermia, there is emerging consensus that that it is initiated by endogenous mechanisms that have been identified minutes post-injury [7–14].

More than a century ago, Cannon described that hemorrhagic shock induces an immediate activation of the sympathoadrenal system [15, 16], resulting in instantaneous release of and rise in circulating levels of catecholamines [15–21]. The trauma-induced catecholamine surge exerts widespread dose-dependent effects on metabolism and the vascular system [17, 18, 22, 23], with redistribution of blood flow, hemoconcentration, platelet marginalization, and activation and release of procoagulant and fibrinolytic factors from the endothelium [24]. Though this "fight-or-flight" response is an evolutionary adaptation, it may become maladaptive and contribute to organ damage [8, 22, 23]. Thus, in high concentrations, catecholamines directly damage the vascular endothelium leading to endothelial cell swelling, necrosis, and progressive de-endothelialization [25–28]. Concurrent with this, high catecholamine levels paradoxically induce hypocoagulability [21, 29].

The Endothelium as an Organ

The vascular endothelium comprises a single layer of cells that line the inner of blood vessels and lymphatics in each and every organ, covering a total surface area of 4000–7000 m^2 with a total weight of 1 kg [30]. The properties of endothelial cells vary considerably between different sites of the vasculature (arteries, veins, capillaries, different organs) and from one moment to another, reflecting the capacity of the endothelium to respond to the unique needs of the underlying specific tissue [31–34]. It is beyond the scope of this section to review the literature of endothelial

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cell heterogeneity but the reader is referred to several excellent recent reviews on this topic [31–42].

The luminal surface of the endothelial cells is covered by a 0.2–1.0 μm thick negatively charged carbohydrate-rich surface layer, the endothelial glycocalyx, that also represents a large structure in the vascular system by containing a fixed non-circulating plasma volume of approximately 1 l in adults [43, 44], corresponding to one third of the vascular plasma volume [45]. The glycocalyx is an antiadhesive and anticoagulant structure that protects the endothelial cells and maintains vascular barrier function [45–48]. It is bound to the underlying endothelial cells through various backbone molecules like proteoglycans (the most abundant being syndecan-1 and glypican) and glycoproteins [49]. The proteoglycans have long glycosaminoglycan side chains comprised mainly of heparan sulfate thathasheparin-likefunctions[50].Theproteoglycan-glycosaminoglycan mesh binds and incorporates various soluble molecules derived from the plasma and endothelium, with the highest amounts of plasma-derived constituents towards the luminal surface [49].

The endothelium, i.e., the entity comprising both endothelial cells and glycocalyx, traverses each and every organ in the body and thereby establishes a unique dialogue between the underlying tissue and flowing blood. Through gatekeeper, biosensor, and input/output device functions the endothelium controls the delicate balance between the vascular system and the different organs in the body [30]. The endothelium plays an important role in vasomotor control, permeability and vascular integrity, blood cell adhesion and trafficking, immune surveillance, inflammation, and angiogenesis, and it is instrumental for balancing hemostasis through its release, expression, and support of systems and elements that either promote or inhibit hemostasis [30, 32, 33, 36, 51–53] (Table 7.1).

Table 7.1 Systems and elements released, expressed, and/or supported by the endothelium that either promote or inhibit hemostasis [30, 32, 33, 36, 51–53]

Pro-hemostatic factors	Fibrin, coagulation factors, and kallikrein (binding sites)
	Tissue factor (TF)
	von Willebrand factor (vWF)
	Plasminogen activator inhibitor (PAI)-1 and PAI-2
	Thrombin activatable fibrinolysis inhibitor (TAFI) activation
	Platelet-activating factor (PAF)
	Thromboxane (Tx)A_2
	Endothelin-1
Anticoagulant factors	Glycocalyx
	Antithrombin (AT)
	Thrombomodulin/endothelial protein C receptor (EPCR) (protein C system, described in detail in Chap. 2)
	Tissue factor pathway inhibitor (TFPI)
	Tissue- and urokinase-type plasminogen activator (tPA, uPA)
	uPA receptor (uPAR)
	Plasminogen (binding sites)
	Prostacyclin
	NO
	Ecto-ADPase
	RNAse

Endothelial Damage

There is emerging evidence that the change from a normal quiescent endothelium to endothelial cell activation, glycocalyx damage, and ultimate endothelial cell injury reflects a progression from reversible to irreversible endothelial damage [54–56]. *Endothelial activation* is associated with increased or de novo expression of leukocyte adhesion molecules, Weibel-Palate body exocytosis, cytokine and growth factor production, and upregulation of HLA molecules; together resulting in discrete increases in whole blood thrombin generation and FVIII and vWF levels [24, 54, 57].

Glycocalyx damage can range from discrete disturbances in the composition of the most luminal layer to excessive destruction and degradation with loss of the entire glycocalyx [45]. Upon shedding, the glycocalyx glycosaminoglycans retain their anticoagulant (heparin-like) activity [50] and promote measurable hypocoagulability in the flowing blood through endogenous autoheparinization [50, 58–60]. Rehm et al. provided the first evidence for an acute destruction of the endothelial glycocalyx in patients undergoing vascular surgery associated with ischemia/reperfusion injury [61]. This study reported that syndecan-1 and heparan sulfate levels increased after the ischemia/reperfusion injury during vascular surgery and that this increase correlated to shedding of glycocalyx as detected by electron microscopy. Despite 40 to 60-fold increases in syndecan-1 post-ischemia, the levels of the classical endothelial adhesion molecules ICAM-1 and VCAM-1 did not change [61], emphasizing the occurrence of selective glycocalyx disruption.

Glycocalyx damage is associated with a change in endothelial cell phenotype from antithrombotic to prothrombotic [54] and pathophysiologic sequels like platelet activation, accelerated inflammation, capillary leakage, and loss of vascular responsiveness [45, 62].

Endothelial cell damage is evidenced by progressive endothelial swelling, necrosis, and vascular de-endothelialization [25, 28]. Endothelial cell injury results in shedding of thrombomodulin [54, 63–65], which further promotes a prothrombotic endothelial phenotype [54] and concurrent anticoagulation of the flowing blood through retained anticoagulant properties of thrombomodulin in soluble form [66]. Soluble thrombomodulin inhibits thrombin generation dose-dependently [66] and activated protein C acts as an anticoagulant (inhibiting factors Va and VIIIa) and profibrinolytic factor [67, 68] (described in detail in Chap. 2). The net effect of endothelial cell damage is extensive exposure of TF and collagen, endothelial "stripping" (with release of adherent cells and glycocalyx), all facilitating hemostasis and thrombus formation at the vascular wall. Concurrent with this, the flowing blood becomes progressively more hypocoagulable with high levels of thrombomodulin, activated Protein C, tPA, and soluble heparin-like substances.

Sympathoadrenal Activation and Endothelial Damage as Drivers of TIC

It is counterintuitive that the most severely injured bleeding patients become progressively more hypocoagulable. However, we propose that progressive hypocoagulability reflects an evolutionary adaptation aiming at counterbalancing the procoagulant state that results from systemic endothelial damage. This adaptive physiology would thereby increase the odds of survival by maintaining a patent microvasculature during hemorrhagic shock [8] (Fig. 7.1). Crucial to this hypothesis is the notion that hemostasis occurs in a vascular system that consists of whole blood *and* blood vessels in a delicate balance with each other. Thus, we infer that coagulopathy should be interpreted with emphasis on the *balance and interaction* between the flowing blood (the fluid phase of the vascular system) and the endothelium (solid phase of the vascular system). This is reflected in how the state of the fluid phase, including its cellular elements, is critically related to the state of the endothelium. The degree of endothelial damage, as a surrogate of tissue injury and shock, results in a progressively more procoagulant endothelium, which in turn promotes a gradient of increasing anticoagulation

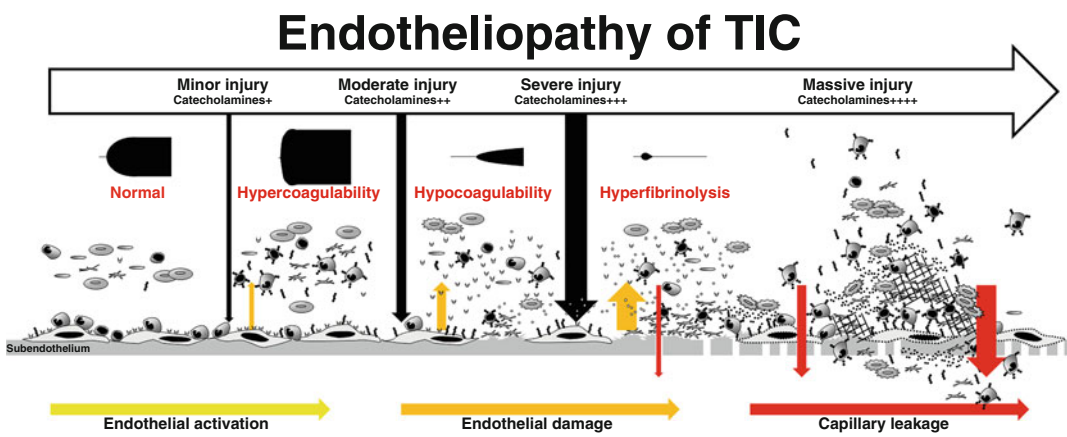

Fig. 7.1 Endotheliopathy of trauma-induced coagulopathy (TIC). Relationships between hemorrhagic shock severity, catecholamine release, coagulopathy, endothelial activation, endothelial damage, and capillary leakage. Increased levels of catecholamines and other injurious "hits" (*black arrows*) result in progressive endothelial activation (*yellow arrows*), damage (*orange arrows*), capillary leakage (*red arrows*), and progressive coagulopathy (TEG silhouettes changing from normal, hypercoagulable, hypocoagulable, and hyperfibrinolytic, *red text*). This endotheliopathy determines the degree of microvascular (dys)function and oxygen delivery to vital organs, hereby driving (multiple) organ failure

towards the fluid phase [8]. It is hypothesized that excessive trauma-induced sympathoadrenal activation is a driver of TIC as the ensuing catecholamine surge rapidly and potently influences the fluid phase (blood) and solid phase (endothelium) of the vascular system in a direction promoting hypocoagulability (coagulopathy) of the flowing blood.

Sympathoadrenal Activation Effects on Coagulopathy and Endothelium

In severely injured trauma patients, high plasma adrenaline levels are an independent predictor of prolonged APTT that correlates with injury severity [69]. Furthermore, plasma adrenaline levels are also independently associated with circulating syndecan-1 [69], a biomarker of endothelial glycocalyx degradation [61], indicating that the trauma-induced adrenaline surge directly damage the glycocalyx [69]. The notion that high circulating adrenaline levels directly damage the glycocalyx is supported by a recent study demonstrating that chemical sympathectomy attenuates glycocalyx shedding and coagulopathy in rats with acute traumatic coagulopathy [70].

The endothelial glycocalyx represents a large structure that contains significant amounts of heparin-like substances [45, 49] that can induce endogenous auto-heparinization [50, 58, 59, 61, 71, 72]. This has been prospectively validated in trauma by investigating admission blood samples in critically injured trauma patients with thrombelastography (TEG), with concurrent analysis of both standard kaolin-TEG and heparinase-TEG [60]. The study demonstrated that in the 5 % of the patients with the highest degree of endogenous heparinization, as evidenced by reversibility of prolonged time to clotting by heparinase-TEG, the syndecan-1 level was increased fourfold. These patients also had higher injury severity and transfusion requirements, as well as more severe coagulopathy evidenced by hyperfibrinolysis on TEG and a prolonged international normalized ration of prothrombin time (INR) [60].

Based on the above observations, the neurohumoral response, and in particular the sympathoadrenal activation, is considered a major driver of TIC. Importantly, the released catecholamines influence the endothelium and flowing blood in opposite directions with an overall aim to induce local hemostasis while at the same time preserving perfusion/oxygen delivery and minimizing the

risk of systemic intravascular coagulation. We thus infer that this switch from hyper- to hypocoagulability in the flowing blood represents an evolutionary adaptation rendering the trauma patient with optimal ability to survive [8] (Fig. 7.1).

Sympathoadrenal Activation and Endothelial Damage as Predictors of Trauma Mortality

Non-surviving trauma patients have higher adrenaline levels compared to survivors and the circulating adrenaline level is an independent predictor of mortality [69]. In this regard, it is notable that several retrospective studies of trauma, sepsis, and surgical patients have reported a paradoxical survival benefits for patients receiving β-blockers as part of their regular medication at the time of the injurious hit [73–77], despite these patients being older and suffering from more comorbidities compared to patients not receiving β-blockers. Furthermore, randomized clinical studies comparing β-blockers with placebo in trauma, sepsis, and patients suffering from acute ischemic heart disease are in line with these findings [78, 79]. It is tempting to speculate that the beneficial effects of β-blockers observed in many acute critically ill patients may in part be due to protective effects on the endothelium.

With regard to the predictive value of endothelial damage, we and others have demonstrated high circulating levels in trauma patients of angiopoietin-2 [80], syndecan-1 [56, 81, 82] and soluble thrombomodulin [67, 81], indicators of endothelial activation, glycocalyx degradation and endothelial cell damage, respectively, with strong associations between high levels of these biomarkers and poor outcomes [56, 67, 80, 81]. It is notable that early resuscitation with plasma is associated with a survival benefit in prospective studies of trauma patients [83] (described in detail in Chap. 4) given that plasma seems to rejuvenate and revert the endothelial glycocalyx damage following hemorrhagic shock in rodents [55]. Since endothelial damage seems a central component of the pathogenesis of multiple organ failure [84], it is tempting to speculate that early administration of plasma to trauma patients may, in addition to supporting hemostasis, rejuvenate the damaged endothelium [55] and attenuate its procoagulant phenotype resulting in improved microcirculation, reduced capillary leakage, and improved patient outcome [55, 56, 85].

Coagulopathy of Acute Critical Illness from an Evolutionary Perspective

As outlined above, the hemostatic capacity of the flowing blood is regulated by the endothelium through its active role in balancing the fluid (blood) and solid (endothelium) phases of the vascular system (Table 7.1). Consequently, injurious hits inflicted on the endothelium, either directly from tissue trauma or indirectly from downstream effects of an injury [86–89], induce coagulopathy.

In the case of minor to moderate trauma, the endothelium is not systemically damaged and the anticoagulant systems normally located at the endothelial surface remain unchanged or become upregulated in the cell suspension near the vascular endothelium (i.e., around the platelets) rather than being diffusible in the flowing blood [90] (Fig. 7.1, Minor to moderate injury). With increasing injury severity, the anticoagulant systems and elements related to the glycocalyx and the endothelium are released to the fluid phase where they now exert their anticoagulant activity [58, 66, 67, 91–93] (Fig. 7.1, Severe to massive injury). In parallel, the degradation and release of the anticoagulant systems and elements normally residing at/in the endothelium unravels the endothelial integrins, which together with the progressive disruption of the endothelium increases the procoagulant/prothrombotic properties of the solid phase of the vascular system.

Active release of endothelial derived molecules in severely injured hemorrhaging trauma patients promotes progressive hypocoagulability in the flowing blood [7–14, 69, 81, 94] through induction of endogenous anticoagulation, hyperfibrinolysis, and auto-heparinization [8, 10, 50, 60,

67, 81]. Progressive coagulopathy may provide a survival benefit in severely injured hemorrhaging trauma patients [8], explained by an evolutionary adaption of a universal response by the organism to severe injurious hits [95, 96]. It has been known for more than a century that acute critical illness immediately activates the sympathoadrenal system [15–21] with ensuing excessive release of catecholamines that induces widespread, dose-dependent effects on the vascular system [8, 17, 22] including the endothelium [24]. Thus, high levels of circulating catecholamines may, through their opposite directed effects on the endothelium [8, 25, 26] and flowing blood [8, 21, 24, 29, 94, 97, 98], ensure oxygen supply to vital organs in acute critical illness by balancing the clotting ability of the flowing blood to the endothelial level of anti-/procoagulation in the microcirculation. Hereby, the evolutionary rational for the coagulopathy observed in different populations of acute critically ill patients may reflect that evolution has prioritized tissue oxygenation above hemostasis.

This is further supported by the notion that several endothelial derived molecules that promote hypocoagulability in the flowing blood also exert potent anti-inflammatory, cytoprotective [45, 49, 88, 99–101], and antimicrobial [71] functions that may ultimately generate at survival advantage in injured individuals [102]. Also, in addition to progressive hypocoagulability, severe trauma is associated with enhanced vascular permeability which, in part, may result from downstream effects of glycocalyx degradation [45, 49] and excess of molecules that promote capillary leakage such as angiopoietin-2 [103, 104]. In a context without resuscitation (from the evolutionary perspective), acute increases in vascular permeability may generate a survival advantage since a rapid shift of volume from the intra- to the extravascular compartment in a hemorrhaging organism may both lower blood pressure and contain fluid within the body for latter mobilization if the organism survives, which seems favorable as compared to bleeding out a large un-replaceable intravascular volume. Such response (or a more exaggerated one) may, however, not generate the same survival benefit in a context with aggressive volume resuscitation and life support in severely injured individuals, and this may explain the consistent finding that the highest levels of several endothelial derived molecules are negatively associated with outcome in trauma [56, 67, 80–82].

In conclusion, TIC is linked to endothelial damage with the trauma-induced catecholamine surge driving progressive hypocoagulability to balance the flowing blood and the endothelium in opposite directions with an overall aim to induce local hemostasis while at the same time preserving perfusion and oxygen delivery. This may represent an evolutionary adaptation rendering the trauma patient with optimal ability to survive suggesting that, although coagulopathy is bad, the alternative may be worse.

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Platelets

8

Scott L. Diamond

Introduction

Trauma-induced coagulopathy (***TIC***) leaves certain patients at risk for uncontrolled bleeding. Hemorrhagic shock is a significant cofactor in TIC when combined with traumatic tissue damage. The acute loss of blood volume is the direct cause of hypotension, hypothermia, hypoxia, and acidosis (Fig. 8.1). Following injury, thrombin generation in the systemic circulation distal of released tissue factor (TF) and/or contact activators can cause: (1) fibrinogen consumption, (2) endothelial release of tPA and consequent fibrin(ogen)olysis [1–3], (3) protein C activation and plasminogen activation with consequent Factor V (FV), FVIII and PAI-1 consumption, and (4) platelet activation and/or platelet dysfunction [4]. Platelet release of soluble CD40 ligand (sCD40L) [5] and other cytokines is associated with endothelial dysfunction, neutrophil activation, and complement activation. Endothelial dysfunction is linked to elevated levels of circulating soluble thrombomodulin (sTM), VEGF-Receptor-1 [5], histone-DNA [6], as well as syndecan-1 and heparin sulfate release, both of which are anticoagulants [7, 8]. These molecular and cellular events lead to increased endothelial permeability and bleeding. Acute reductions in blood volume and hematocrit are also associated with depressed nitric oxide function, while inducible nitric oxide synthase (iNOS) may be elevated locally in injured tissue [9, 10]. Recently, the massive release of digestive proteases has been proposed as a major mediator of pathogenesis during shock [11]. These diverse and multifactorial processes can result in serious platelet dysfunction in the trauma patient.

Successful hemostasis requires the rapid accumulation of platelets to seal up a site of blood leakage. Flowing platelets are captured via GPIb to wall bound von Willebrand Factor (VWF), allowing for GPVI activation by collagen and intracellular calcium mobilization. Elevated calcium helps drive: (1) dense granule release of ADP and serotonin, (2) alpha granule release of VWF and other proteins along with P-selectin display, (3) activation of $\alpha_{IIb}\beta_3$ for binding VWF and fibrin(ogen) and activation of $\alpha_2\beta_1$ for binding collagen, (4) cyclooxygenase-1 dependent synthesis of thromboxane, and (5) scramblase-dependent exposure of phosphatidylserine for coagulation factor binding. Direct imaging of laser-injured arterioles in mice with healthy blood has revealed that the first layer of arriving platelets can seal a small wound in ~30 s. Within ~1 min, a dense, stable, and contracted

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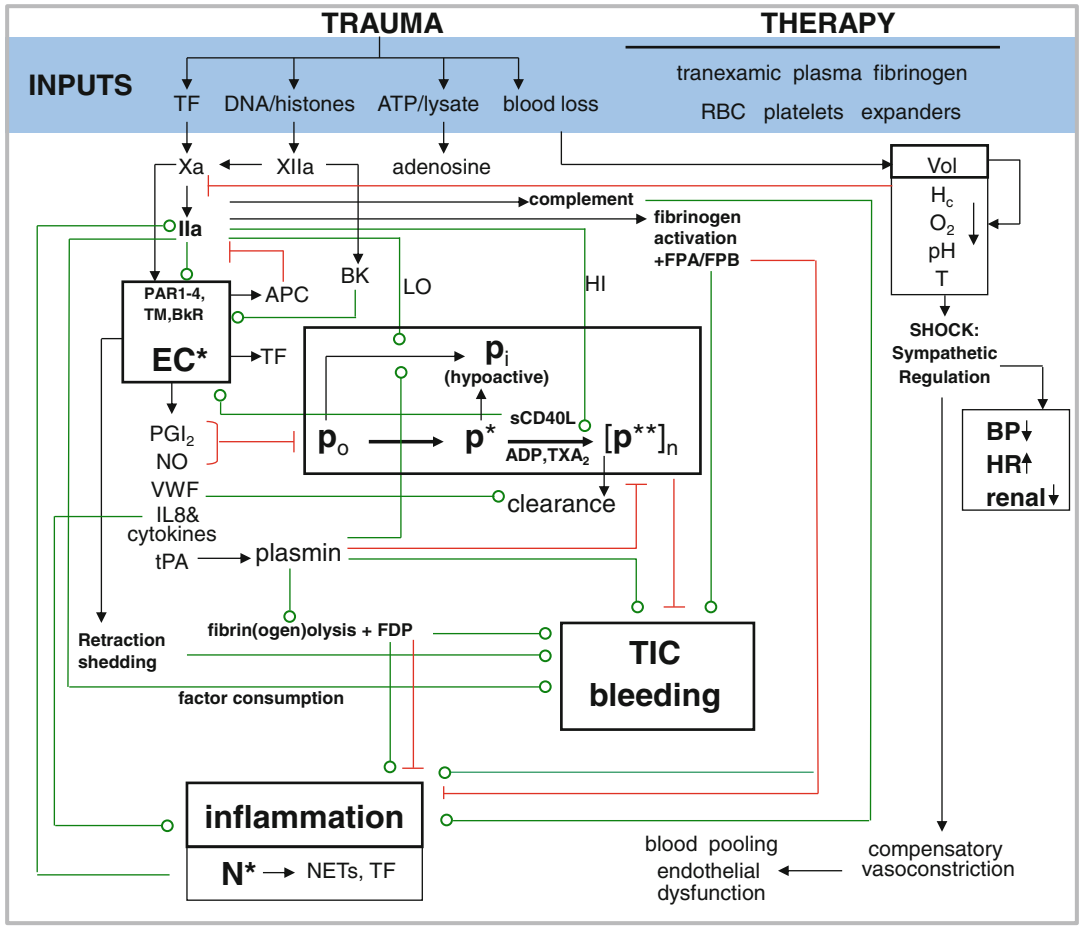

Fig. 8.1 Systems Biology wiring diagram of trauma-induced coagulopathy (TIC) showing interactions between platelets (p), endothelial cells (EC), neutrophils (N), and various coagulation or blood factors [tissue factor TF, FXa, FXIIa, thrombin (FIIa), activated protein C (APC), bradykinin (BK)]. (*, activated; *green line*: potentiated; *red line*: attenuated)

core of activated, P-selectin$^+$ platelets is formed that is rich in thrombin and fibrin, while an outer shell contains less activated, P-selectin$^-$ platelets that are exposed to locally released ADP and thromboxane (TXA_2) [12–15]. During defective hemostasis in the context of severe hemophilia, microfluidic studies of severe hemophilic blood (<1 % critical factor level) indicate that low sub-nM levels of thrombin are critical for activating platelets to promote hemostasis, while higher ~100 nM levels of thrombin in a clot are required to generate fibrin for clot stabilization [16]. Direct measurements of platelet clot permeability (fibrin-free) vs. platelet-fibrin clot permeability also indicate that dense retracted platelet masses (fibrin-free) have a sufficiently low permeability to mediate hemostasis, while the fibrin within a hemostatic clot has only a modest effect on clot permeability [17]. This work with hemophiliac blood and measurements of clot permeability indicate that hemostasis is achieved via activated platelets as the primary sealant with fibrin serving as a secondary stabilization of the platelet plug. This chapter focuses on how molecular level events during trauma alter platelet function so as to interfere with successful hemostasis, thus placing a patient at risk for TIC.

Multifactorial Processes in Trauma That Impact Platelet Function

External triggers that activate platelets are very well studied from arterial and venous thrombosis research and most importantly include collagen and thrombin that work in conjunction with platelet released ADP and thromboxane. However, the precise roles of these platelet activation pathways during TIC, and their distinctions from classic DIC (disseminated intravascular coagulopathy), are less well defined. Interestingly, the TIC patient may be as likely to display platelet dysfunction as opposed to severe platelet consumption (<50,000 platelet/μL) [3, 4, 18].

With respect to TIC, the molecular definitions of the *desensitized*, *stunned*, *inactive*, *post-activated*, *dysfunctional*, *degranulated*, or *exhausted* platelet are still emerging. Table 8.1 lists many of the known activators and inhibitors to which platelets can respond via receptor-mediated signaling. A resting platelet will display hypoactivity if exposed to inhibitory modulators such as PGI_2, PGE_2, adenosine, or NO. In contrast, a platelet exposed to low level stimulation of agonists may display receptor desensitization when exposed subsequently to higher levels of stimulation (e.g., $P2Y_1$ desensitization by low-dose ADP via receptor endocytosis) [19]. Furthermore, circulating platelets may experience complex combinations or sequential exposures of agonists to trigger novel modes of activation as indicated by shape change, degranulation, and/or receptor display or activation or shedding. However, it is less clear how such partially activated platelet remains in the circulation since they should presumably aggregate and be cleared. Also, platelet mitochondria dysfunction is poorly understood in the context of TIC. Additionally, numerous antiplatelet and anticoagulant pharmacological agents, alcohol, and/or drugs of abuse may cause platelet hypoactivity. Also, the metabolic state or pharmacological state of the elderly patient may be relevant to platelet dysfunction during TIC [20]. Finally, the *platelet storage lesion* has been well studied in transfusion medicine and is also relevant to the treatment of the trauma patient as a further example of platelet dysfunction. As platelets age with room temperature storage, they release soluble CD40L [21, 22] and display mitochondrial dysfunction [23] and altered procoagulant function.

Table 8.1 Agonists that bind platelet receptors to cause platelet activation or inhibition

Activators	*Receptors*
Collagen	GPVI, $\alpha_{2b}\beta_1$
Thrombin	PAR1, PAR4
Elastase/cathepsin G	PAR1
ADP	$P2Y_1$, $P2Y_{12}$
ATP	$P2X_1$
Thromboxane (TXA_2)	TPα
Histamine	H2
Dopamine	D2
Serotonin	$5HT_{2A}$
Histone/DNA	TLR4
Endotoxin	TLR4
HMGB1	TLR4
Epinephrine	α_{2A}
oxLDL	CD36
vWF	GPIbα
Vasopressin	V1
Succinate	SUCNR1
Inhibitors	*Receptor*
PGI_2	IP
PGD_2	DP
PGE_2	IP, EP_{1-4}
NO	Guanylate cyclase
Adenosine	A_{2A}
Acidosis	SOCE (orai1/TRPC6?)
(*Pharmacological*)	
Clopidogrel	$P2Y_{12}$ inhibitor
Aspirin	COX1 inhibitor
Ethanol	Nonspecific
Dipyridamole	PDE
Cilostazol	PDE3

Prevailing changes in **blood biology**, **independent of coagulation**, **fibrinolysis or complement**, can alter platelet function. Acidosis, hypoxia, and low hematocrit are linked to TIC and their direct molecular actions on platelets are diverse. Low hematocrit, especially with the use of plasma expanders, will reduce platelet concentrations in the RBC-free plasma layer near the vessel wall. Platelet margination to the vessel wall requires RBC and is critical for hemostasis.

Thus, the clinical use of RBC transfusion can have direct effects on both oxygenation (hemoglobin) and platelet margination (biorheology). The effects of hypoxia on platelet function have been studied in vitro. Recent research has shown that hypoxic conditions allow for an RBC-dependent reduction of nitrite to release NO that then inhibits platelets [24]. The molecular effects of acidosis on platelet function are not as extensively studied as expected given the role of acidosis in bleeding. An early 1977 study of umbilical vein platelets found reduced aggregation with ADP as pH was reduced to 7.0 with lactic acid or CO_2 [25]. Platelet store operated calcium entry (SOCE) and aggregation were found to be reduced at pH 6.9 [26], suggesting an acid-dependent inhibition of the platelet Orai-1/STIM1 complex. Consistent with acid-impaired SOCE, Etulain et al. [27] found that PAC1 and fibrinogen binding to the active $\alpha_{IIb}\beta_3$ platelet receptor were reduced at a pH of 6.5 in thrombin-stimulated platelets as were calcium mobilization, aggregation, adhesion, TXB_2 and ATP release, and phosphatidylserine (PS) exposure. Intracellular lactic acidosis as a result of hypoxia may reduce SERCA-mediated refilling of the platelet dense tubular system (DTS) resulting in an elevation of intracellular calcium [28]. Platelets have an Na/H exchanger, but its role in acidosis-induced platelet dysfunction may be secondary to the direct effects of low extracellular pH on SOCE [29].

During trauma, changes in **blood biochemistry due to a prevailing procoagulant state**, **fibrinolytic state**, **or complement assembly** can also alter platelet function. While fibrinogen consumption by thrombin or fibrinogenolysis by tPA will reduce the potential to make fibrin, platelet aggregation and adhesion requires only very low levels of fibrinogen. For example, washed platelets can release sufficient fibrinogen to mediate robust aggregation and hypofibrinogenic patients with ~1–10 % normal fibrinogen do not present a severe bleeding phenotype in the absence of trauma.

Thrombin-mediated cleavage of platelet protease-activated receptor PAR1 and PAR4 in the trauma patient would certainly be expected to cause platelet activation via G_q signaling and subsequent calcium mobilization. Distinct from thrombin generated and protected within a hemostatic clot, intravascular generation of thrombin may result in a transient or low level thrombin stimulation of the platelet, possibly in conjunction with elevated exposure to endothelial derived inhibitors of platelet function (PGI_2 or NO). Such a complex state may be difficult to recreate or assay in vitro. Changes in **endothelial function** driven by perturbed blood chemistry can alter platelet function. During trauma, the generation of circulating triggers of coagulation (either TF or activators of FXIIa) leads to thrombin generation. Thrombin is a potent trigger of endothelial retraction and acute tPA release, leading to a fibrinolytic state. For example, direct injection of Factor Xa on PS/PC in a primate model triggered massive tPA release and a subsequent lytic state [2, 30]. The action of plasmin on platelets is generally associated with both platelet activation [31] and subsequent surface proteolysis, events observed during thrombolytic therapy. Release of VWF by activated endothelium would also be expected during trauma, potentially driving platelet consumption.

Neutrophil activation and platelet activation are often intertwined since the two cell types can form heterotypic adhesions under flow via PSGL1/P-selectin, activate each other, and respond to some of the same stimuli. Neutrophil-platelet crosstalk has been explored in detail in the context of sepsis, acute lung injury, intimal hyperplasia, and thrombosis. Platelets can activate neutrophils via release of sCD40L. Strong inflammatory signals that cause neutrophil extracellular traps (NETs) can lead to thrombin generation and subsequent platelet activation [32]. Also, neutrophil histones in NETS lead to platelet activation. Proteases released from activated neutrophils such as human neutrophil elastase (HNE) or cathepsin G can activate platelets via PAR cleavage [33]. Also, neutrophils and monocytes can express tissue factor which may lead to local thrombin production. **Toll-like receptors** (**TLR**) are present in platelets and neutrophils and can be activated by inflammatory or infectious agents, particularly during sepsis. Recent mouse

studies have shown a role of platelet TLR4 pathways in hemorrhagic shock [34], but the activating ligands of TLR4 may be multiple in the context of sterile inflammation or trauma. Cohen et al. [35] showed that high mobility group box nuclear protein 1 (HMGB1) was elevated in trauma patients, particularly in non-survivors and those with acute lung or renal injury. While HMGB1 is a known activator of platelet TLR4, the extent of platelet TLR4 activation in trauma patients requires future investigation.

Pharmacological effects on platelet function are well understood. Numerous trauma patients, particularly in the elderly population, can be expected to take aspirin, $P2Y_{12}$ inhibitors, and anticoagulants targeting FXa, thrombin, and vitamin K-dependent coagulation factors. Other drugs such as phosphodiesterase PDE5 inhibitors (i.e., sildenafil) have been linked to bleeding events and are known to inhibit platelet function by preventing cGMP breakdown to GMP, thus potentiating NO-induced signaling. Ethanol is a potent platelet inhibitor at the level of routine human consumption. Interestingly, a non-COX1 dependent action of high-dose aspirin has been shown to occur via TACE cleavage of GPIbα and GPV [36].

Intracellular and Autocrinic Mechanisms of Platelet Hypoactivity

This section focuses on how intracellular signaling pathways within platelets or autocrinic mechanisms cause platelets to become dysfunctional, inhibited, downregulated, or hypoactive. Both **cAMP and cGMP pathways** are well studied in platelets. PGI_2 or PGE_2 stimulation of the IP receptor linked to G_s results in adenylate cyclase activation, generation of cAMP, and activation of protein kinase A (PKA). VASP phosphorylation is important marker of PKA activation. PKA can also phosphorylate CalDAG-GEFI to inhibit Rap1b activation [37], thus preventing integrin activation. In contrast, ADP binding to $P2Y_{12}$ results in G_i activation which in turn inhibits adenylate cyclase, thus the antithrombotic efficacy of $P2Y_{12}$ inhibitors in downregulating platelets by protecting adenylate cyclase activity. The platelet inhibitor NO is a membrane permeable activator of guanylate cyclase which produces cGMP to activate PKG in platelets.

The ability to turn off G protein-coupled receptors is an important modality of downregulation. Platelets contain **regulator of G protein signaling** (**RGS**) proteins that act to turn off G protein signaling by accelerating the hydrolysis of Gα•GTP to Gα•GDP by enhancing the GTPase activity of the G_α subunit. Platelet have detectable mRNA for RGS 1,2,3,6,9,10,16,18, and 19 with RGS2 and RGS18 being the most studied in platelets to date [38, 39]. RGS proteins can turn off platelet-activating signals propagated through G_q or G_i as well as turn off platelet-inhibiting signals propagated through G_s. Little is known about the state of platelet RGS proteins in the context of TIC.

Collagen multimerization of GPVI leads to phosphorylation of immunoreceptor tyrosine-based activation motif (ITAM) domains of the associated FCRγ. Signaling from ITAM ultimately drives strong activation of phospholipase Cγ (PLCγ) to activate platelets. PECAM-1 contains **immunoreceptor tyrosine-based inhibition motif** (**ITIM**) domains that can turn off ITAM signaling following PECAM-1 homodimerization, presumably requiring platelet-platelet contact [40]. ITIM-based signaling drives activation of SH2 domain-containing tyrosine phosphatases SHP-1 and SHP-2 to turn off GPVI signaling or downregulate sensitivity to collagen. Other ITIM domains found in platelet include those of G6B-b [41, 42] and leukocyte immunoglobulin-like receptor subfamily B (LILRB). Angiopoietin-like protein 2 (ANGPTLE2) is released by activated platelets and binds LILRB, potentially as an autocrinic downregulatory mechanism via ITIM signaling [43]. The levels of platelet ITIM phosphorylation, SHP-1/2 activity, or circulating ANGPTL2 during TIC are currently unknown.

Proteases can alter platelet function, particularly related to the shedding of surface proteins [44] such as P-selectin, GPIbα, GPV, GPVI, and CD40L. GPIbα, GPV, and Semaphorin 4D are

shed by TNFα converting enzyme (TACE/ADAM17) which can be elicited by oxidative stress or serotonin [45–47]. In contrast, GPVI is shed by ADAM10. Stimulation of platelets with thrombin or collagen related peptide (CRP) causes GPIbα shedding [48]. Additionally, GPIbα can be internalized by endocytosis following platelet activation. The shedding of GPIbα and GPVI appears to be differentially regulated. For example, serotonin can trigger GPIbα shedding via ADAM17, while GPVI remains unshed [46]. Both shed GPIbα (soluble glycocalicin) and shed GPVI (soluble sGPVI) may be useful biomarkers of platelet activation during trauma. P-selectin is displayed on the activated platelet surface during α-granule release. The displayed P-selectin can be endocytosed or released with platelet microparticles or shed as soluble P-selectin from platelets. The P-selectin sheddase is likely a metalloproteinase. CD40L is displayed on activated platelets and can be shed as soluble CD40L (sCD40L) by matrix metalloproteinase MMP-2 [49] or MMP-9 [50]. Serum levels of sCD40L are elevated in various context of trauma as a biomarker of platelet activation. Elevated levels of sCD40L in stored platelets for transfusion have been linked with inflammatory complications with transfusion, including transfusion-related acute lung injury (TRALI).

Detection of Platelet Function

Clotting time assays are not predictive of TIC risk, while overall clot strength as measured by rheometry (TEG or ROTEM) appears a more useful metric of bleeding risk and TIC. However, TEG is not always fully sensitive in detecting fibrinolytic states during trauma and its utility to readout platelet function remains nuanced. To date, point-of-care diagnostics and validated biomarkers of platelet and endothelial dysfunction during trauma are not available. Platelet hypoactivity, distinct from activation, is poorly understood or defined during TIC and remains a focus of current research.

Rheometry (TEG and ROTEM) is a dynamic overall clot strength measurement that combines thrombin generation, fibrin polymerization (and lysis), and platelet binding and contractile activity on fibrin, with secondary influences of hematocrit. The signal is an overall measurement of clot strength that is likely correlated with hemostatic potential in a patient. However, the emergent and overall metric of clot strength, as read out by rheometry, creates a difficult task of scoring individual defects in thrombin production, fibrinogen level, and platelet. Rheometry of blood from trauma patients can be rapidly initiated by strong triggering agents such as TF and contact activators. Experimental acidification to 6.95 of otherwise healthy blood results in a reduction in clot strength [51]. Combined acidosis and hypothermia (pH 7.0 and 30 °C) can especially impair clot strength [52]. In some assay formats, the platelet is uncoupled pharmacologically with abciximab and cytochalasin D from the fibrin network so as to allow measurement of residual fibrin strength in the clot and the distinct platelet contribution [53]. TEG has detected platelet dysfunction in traumatic brain injury (TBI) patients [54].

Platelet aggregometry or **whole blood impedance aggregometry** allows a direct measure of platelet function, especially in the absence of fibrin. Platelet aggregometry requires centrifugation of platelet-rich plasma making this standard laboratory assay difficult for point-of-care implementation. Platelet aggregometry has detected platelet dysfunction in animal models of traumatic brain injury [55] and in patients. Impedance aggregometry detected platelet dysfunction in 45 % of trauma patients on admission while 91 % of trauma patients had platelet dysfunction at some point during their ICU stay [4]. By impedance aggregometry, patients with platelet hypofunction to strong agonists such as arachidonic acid or collagen were found to have the greatest risk of early and late mortality.

Microfluidic assay allows individual evaluation of platelet activation by collagen, subsequent platelet-platelet aggregation mediated by activated $\alpha_{IIb}\beta_3$-fibrinogen and potentiated by ADP and thromboxane via $P2Y_1/P2Y_{12}$ and TP receptors, thrombin production by surface linked TF [56] and thrombin production distal of con-

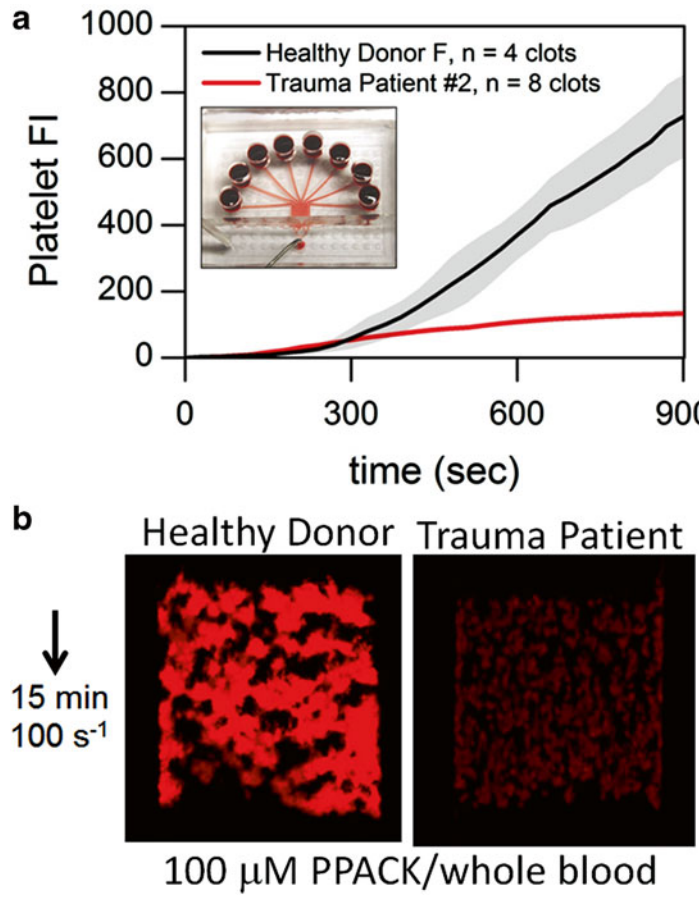

Fig. 8.2 Detection of platelet dysfunction under flow conditions using microfluidics. (**a**) Platelet accumulation for flow on collagen at 100 s^{-1} for whole perfusion using blood from a healthy donor or a trauma patient. (**b**) Net platelet accumulation at 15 min. Citrated whole blood was recalcified with 100 µM PPACK to inhibit thrombin immediately prior to perfusion. For experimental details, see [62]

tact activation [57], fibrin polymerization, fibrinolysis, occlusion time, and clot stability [58]. The microfluidic assay has been multiplexed to run under 24 different assay conditions and can be run in constant flow rate mode or constant pressure drop mode [59]. The use of fluorescent antibodies and a fluorescent thrombin biosensor allows independent and instantaneous monitoring of platelets, fibrin, and thrombin. The action of various drugs such as ASA, P2Y1 and $P2Y_{12}$ antagonists can also be evaluated [60]. The activity of ASA in humans is easily observed as a defect in thromboxane-mediated platelet accumulation on a surface. This secondary aggregation response can be normalized by the primary response to give a metric that does not require reference to an external sample [61]. The placement of a recalcified citrated whole blood sample into a microfluidic assay can reveal a serious defect in platelet function when that blood is obtained from a trauma patient prior to transfusion (Fig. 8.2).

Summary

Platelet hypoactivity has been detected in trauma patient blood using clot strength, aggregometry, and microfluidic assays. The molecular drivers of this hypoactivity are potentially numerous and diverse in an individual patient and likely heterogeneous across a patient population. The trauma patient blood may contain diverse soluble species in the plasma (thrombin, tPA, ADP, PGI_2, PGE_2, NO, low pH and O_2, aspirin, $P2Y_{12}$ and PDE inhibitors, ethanol, etc.) that drive platelet hypo-

activity. Compounding this intrapatient complexity is the variability of platelet transfusion products and the lack of point-of-care functional testing technology to score those products immediately prior to use. A number of intracellular signaling and/or autocrinic shedding pathways may be triggered within platelets of the trauma patient that cause hypoactivity. The roles of PKA/PKG, ITIM, RGS signaling as well as receptor internalization or shedding are amenable to laboratory study and may help define distinct classes of platelet hypoactivity in the trauma patient. Finally, establishing the predictive value of point-of-care platelet functional assays for directing treatment or diagnosing TIC risks remains a significant opportunity in the future of trauma patient care.

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Fibrinolysis

9

Hunter B. Moore, Ernest E. Moore,
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Historical Perspective

The association between fibrinolysis, trauma, and death has been recognized for over 200 years. John Hunter during his wartime experiences at the end of the 1790s appreciated incoagulability from victims of lethal gunshot wounds [1]. Nearly a century later it was discovered that whole blood could dissolve formed clots and the term "fibrinolysis" was proposed. Blood removed from corpses from sudden death proved to be a useful source of blood for transfusions, as their blood would initially clot, but then re-liquefy and not require an anticoagulant. Macfarlane et al.[1] identified that the commonality of these early observations was rapid death resulting in reversal of clot formation.

During the 1960s a number of investigators developed the hypothesis that fibrinolysis was a physiologic process to counterbalance thrombosis and was perpetually active. Stafford's [2] review on fibrinolysis and hemostasis in 1964 established a logical understanding on how fibrinolysis becomes pathologic: "a general assumption has been developed that clotting is not episodic but a continuous process which is normally never allowed to progress to a physical end point." The two pathologic ends of the fibrinolysis spectrum (excessive clot degradation: hyperfibrinolysis, and impaired clot degradation: fibrinolysis shutdown) were clinically described during this era of heightened interest in fibrinolysis. Pathologic hyperfibrinolysis was defined by Starzl et al. using thrombelastography (TEG) during the early operative phase of liver transplantation [3], while Colonel Hardaway in 1965 described fibrinolysis shutdown following trauma and prolonged hemorrhagic shock [4]. Recently it has been identified that trauma patients can present with a spectrum of fibrinolysis within 12 h of injury [5] (Fig. 9.1).

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Challenges of Measuring Fibrinolysis

Systemic Versus Local Fibrinolysis

The regional distribution of specialized endothelial cells contributory to the fibrinolytic system is reflective of the importance of localization of this process [6]. The primary driver of intravascular fibrinolysis, tissue plasminogen activator (tPA), is released from precapillary arteriole and postvenular endothelial cells. This allows for a rapid

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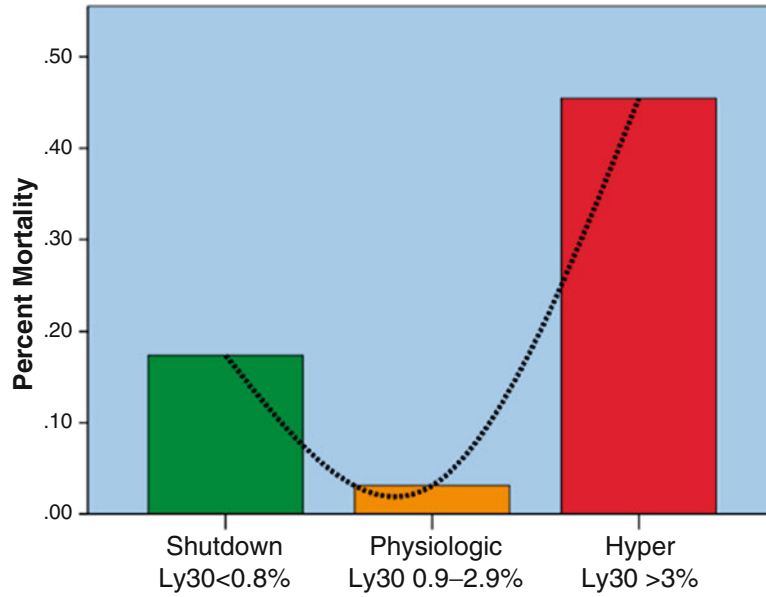

Fig. 9.1 Viscoelastic measurements of 200 critically injured patients (injury severity score >15) identified the spectrum of fibrinolysis. Patients with a moderate (physiologic) level of fibrinolysis had the lowest mortality. Those patients with fibrinolysis above this range (hyperfibrinolysis) had nearly a 50 % mortality, while those patients with impaired fibrinolysis (shutdown) had nearly a fourfold rate mortality compared to the physiologic range. These data implicate a protective role of physiologic fibrinolysis following severe injury

response to prevent low flow occlusion of small vessels. The body is continuously clearing fibrin deposition, and after trauma resulting in thrombin generation from tissue injury this maintenance process is increased to keep the microvasculature distal from injury patent. This explains why the D-dimer assay is nonspecific. Local fibrinolysis, which is a physiologic process, will release fibrin degradation products systemically (Fig. 9.2). However, this does not necessarily correlate with an increase in systemic fibrinolysis that leads to bleeding (Fig. 9.3). Elevation of D-dimer can be a physiologic reaction to trauma, and correlation to systemic hyperfibrinolysis and coagulopathic bleeding requires additional assays.

Plasma Markers of Fibrinolysis Have Limitations

The limitations of the "gold standard" assay of fibrinolysis, the euglobulin lysis test (plasma based), have been discussed since 1962, with emphasis on the importance of developing a whole blood assay that includes the important contributions of platelets [7]. This is further emphasized by the lack of ability to show reversal of fibrinolysis with certain antifibrinolytic medications using the euglobulin lysis test—these medications are precipitated out of the plasma during preparation of this assay [8]. Perhaps the most important component of whole blood that is removed during the euglobulin lysis test is the platelet. Platelets provide a structural framework to resist fibrinolysis. During primary hemostasis platelets aggregate and form adhesions between themselves and the surrounding environment including collagen, and von Willebrand factor. This creates a unique microenvironment in which fibrin polymerization can accelerate by providing a phospholipid membrane for thrombin burst and regionalization of clotting components away from the circulating vascular system [9]. This area is further enriched with prothrombotic and antifibrinolytic proteins released during platelet degranulation [10]. In vitro addition of lysed platelets has been shown to reverse tPA enriched whole blood fibrinolysis [11]. Because platelets play an important role in the regulation of fibrinolysis, non-whole blood assays require careful evaluation as they have been cleared of important inhibitors of the fibrinolytic system.

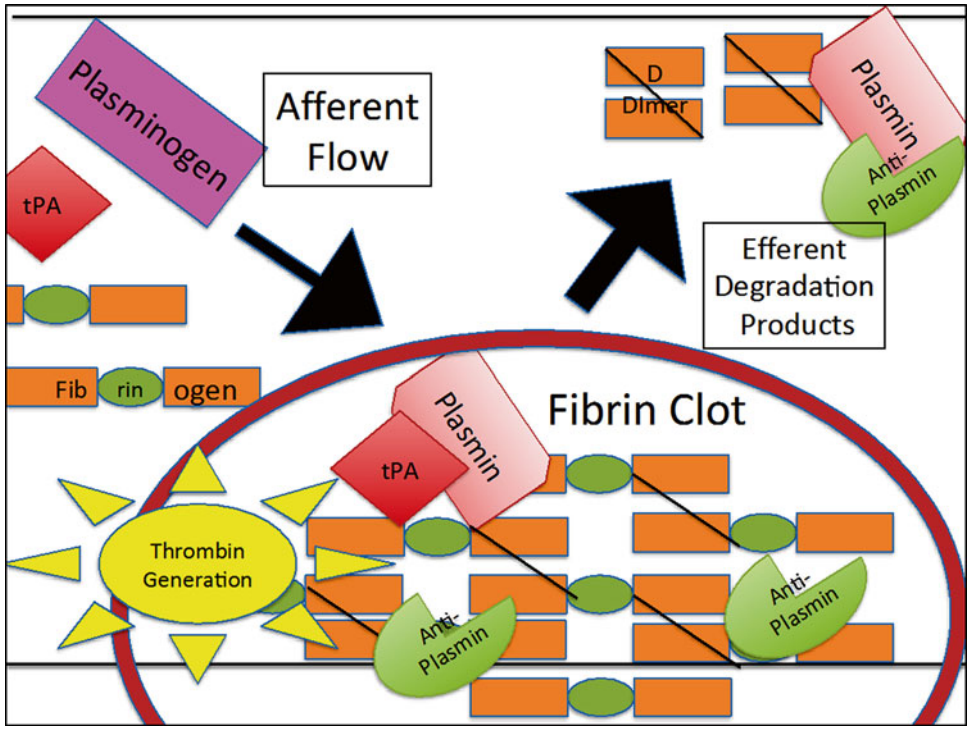

Fig. 9.2 Afferent flow to an area of injury provides fibrinogen which is acted upon by a local thrombin burst creating a fibrin seal which becomes cross-linked by factor XIII that also integrates antiplasmin proteins. Circulating tPA and plasminogen enter the clot and bind fibrin on site-specific lysine residues. This results in the cleavage of plasminogen into plasmin. Plasmin actively cleaves polymerized cross-linked fibrin and releases degradation products including D-dimer. As the clot degrades antiplasmin proteins are uncovered and directly bind plasmin resulting in the formation of plasmin antiplasmin complexes, which are released into systemic circulation

Viscoelastic Assays and Fibrinolysis

The viscoelastic assays, thrombelastography (TEG) and rotational thromboelastometry (ROTEM), provide clinically useful measures of fibrinolysis (results within 1 h: Fig. 9.4). Thresholds between 3 and 15 % LY30 (percent degradation of clot 30 min after reaching maximum amplitude) have been identified as indicating increased risk for massive transfusion and mortality [12–14]. The hyperfibrinolytic patient is relatively easy to identify with these assays, although the optimal assay activator and the percent lysis threshold within the abovementioned range remain to be defined. For further description of viscoelastic assays, please refer to Chaps. 17 and 18 (TEG-Gonzalez, ROTEM-Gorlinger). Evaluating the other end of the spectrum of the fibrinolytic system, fibrinolysis shutdown, is more challenging. Appreciation of the two extremes of the fibrinolytic system in response to trauma and surgery dates back to the 1960s [15]. Recently this spectrum of fibrinolysis has been identified in injured patients using TEG [5]. Raza et al. proposed limitations of viscoelastic assays to detect fibrinolysis [16]. In this study, they identified a patient population without overt hyperfibrinolysis measured by ROTEM but with increased plasmin/antiplasmin (PAP) levels. These patients with high PAP compared to patients with low PAP lacking hyperfibrinolysis by ROTEM had worse outcomes and higher mortality. PAPs are reflective of a local environment of fibrinolysis, and elevation of PAP suggests that plasminogen is being activated into plasmin and is concomitantly being inhibited, but does not reflect systemic activity of plasmin. Thus PAP levels are difficult to interpret as they can reflect

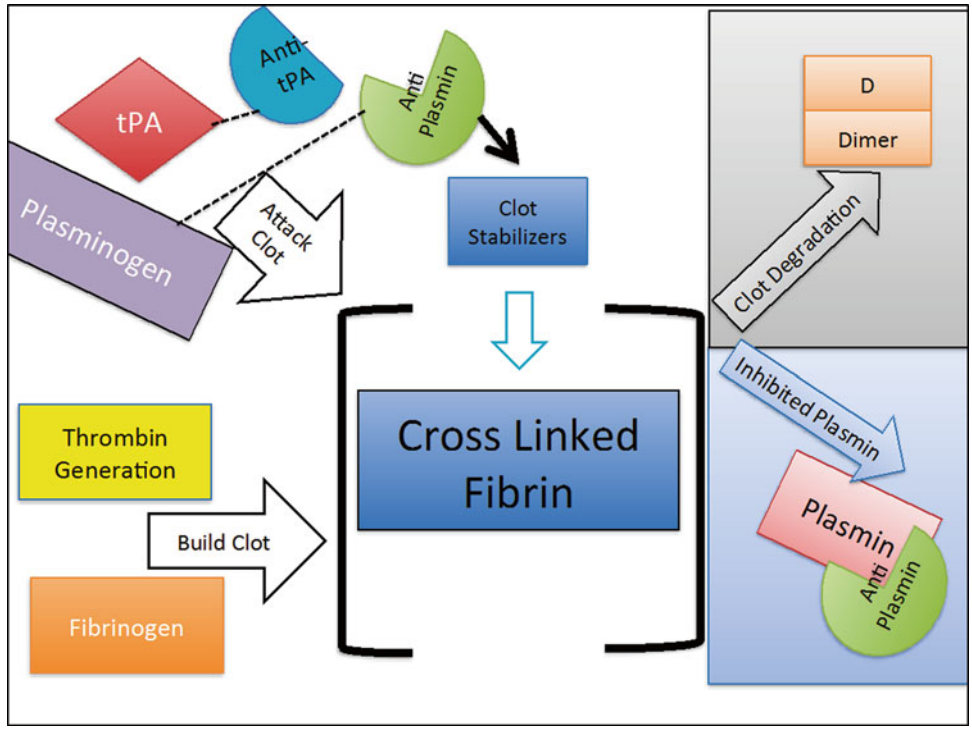

Fig. 9.3 Measurement of D-dimer and plasmin–antiplasmin (PAP) complexes is the result of the degradation of fibrin clot. Therefore the systemic circulating levels of these plasma proteins are dependent on the patients clot burden. The elevation of these products can be due to increased activity of the plasmin system, increased clot burden from extensive tissue injury or intravascular thrombosis, or decreased clearance of these products. They do not necessarily reflect the systemic activity of the fibrinolytic system in circulation. Microvascular beds can have elevated fibrinolytic activity in efforts to keep vascularture patent, a increasing D-Dimer and PAP levels, but not resulting in systemic hyperfibrinolysis. Acute pulmonary embolism is an example of an elevated D-Dimer level but not systemic hyperfibrinolysis. An acute occlusion of the pulmonary vasculature results in a rapid release of local tPA to clear fibrin clot in the pulmonary vasculature and an elevation in D-dimer. Measurement of systemic fibrinolysis would be within a normal range as the circulating regulator proteins would buffer the local surge tPA released from the pulmonary system preventing systemic hyperfibrinolysis. In polysystem trauma patients it is not a mystery why these protein levels have poor specificity for identifying the hyperfibrinolytic phenotype, as the body is actively building and degrading clot to optimize tissue perfusion. This only becomes pathologic when activity becomes unregulated and results in excessive clot degradation in areas of vascular injury resulting in refractory bleeding

systemic fibrinolysis shutdown or local suppression of fibrinolysis in an area of active fibrin breakdown, similar to D-dimer.

Viscoelastic assays are also limited to a no flow environment in which high levels of thrombin are rapidly generated, particularly when a potent activator is used, such as tissue factor. Maximum platelet activation can occur and, therefore, suppress fibrinolytic activity. Conversely, maximal platelet activation can result in platelet contraction followed by relaxation producing a pattern overestimating fibrinolytic activity. Furthermore, the low values of LY30 (or high values of Ll30 for ROTEM) seen during fibrinolysis shutdown, particularly when an activator (commonly tissue factor and/or kaolin) is used, make quantification of fibrinolysis shutdown difficult with conventional viscoelastic assays. Alternative measures to quantify the fibrinolysis resistance of whole blood are to challenge the whole blood sample with profibrinolytics while performing the assay. The tissue plasminogen activator challenge in ROTEM and TEG may be a novel assay to quantify fibrinolysis resistance (shutdown).

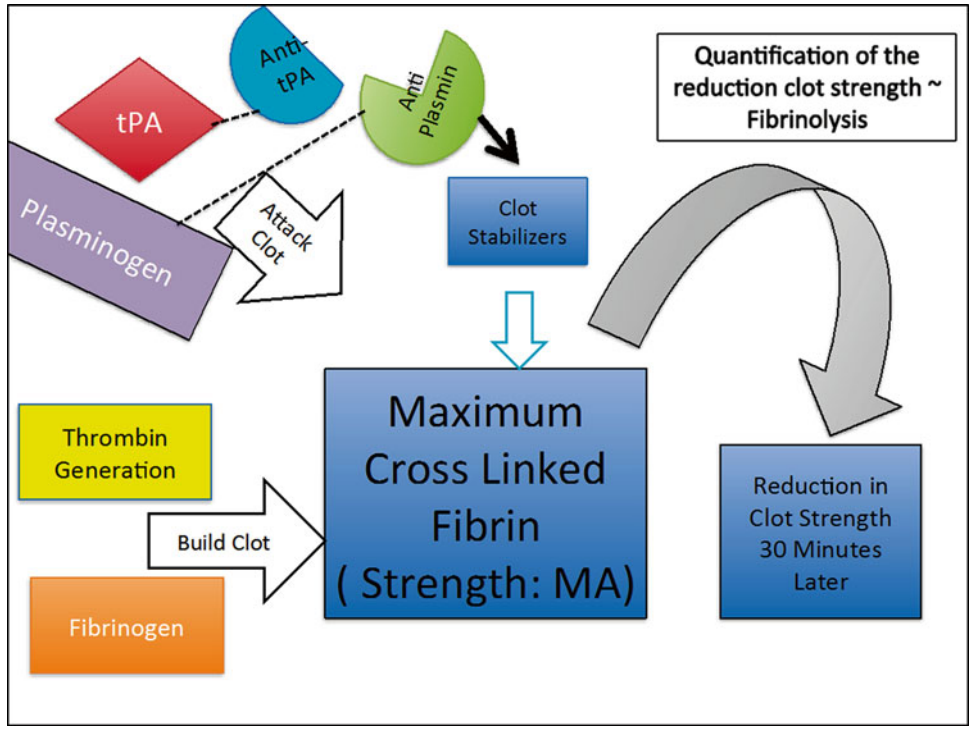

Fig. 9.4 Thrombelastography measures the progression of clot formation to maximum strength from the systemic blood of a patient in an in vitro environment. There are numerous contributing factors to clot strength including thrombin generation, platelet aggregation/degranulation, and clot stabilization such as factor XIII that are indirectly measured by this assay. In this in vitro environment the clot will degrade after reaching maximum clot strength. The reduction in clot strength after 30 min represents clot weakening from fibrinolytic activity and to some degree platelet relaxation. This is dictated by the balance of pro- and antifibrinolytic proteins at the tPA and plasmin level. In addition, clot strengthening and modification can occur which render fibrin clot more resistant to plasmin degradation (e.g., TAFI cleaves lysine domains on fibrin). This measurement of clot weakening after 30 min is reflective of the patient's current systemic fibrinolytic function, and increases specificity for identifying systemic pathologic hyperfibrinolysis. The strength of this assay includes all circulating factors that impact coagulation and fibrinolysis. Limitations of this assay are low flow environment and lack of endothelial surface that can modify local fibrinolytic activity. There are currently no perfect assays for measuring fibrinolysis, but thrombelastography provides the most clinically relevant assay at this time to detect pathologic systemic hyperfibrinolysis

Drivers of Pathologic Fibrinolysis

Fibrinolysis May Not Be Linked to Coagulation Changes in TIC

Coagulation changes after trauma are described to have two components: (1) impairment of blood clot formation (hypocoagulation) and (2) increased rate of clot degradation (hyperfibrinolysis) [17]. While one in four patients with severe injury has coagulation abnormalities within 30 min of injury, the prevalence of hyperfibrinolysis is considerably less [12–14]. As the assessment of coagulation changes postinjury has become refined, hypocoagulation and hyperfibrinolysis appear mechanistically distinct. Principal component analysis suggests that hyperfibrinolysis does not correlate with impaired clot formation [18, 19].

An interesting observation from human studies identified that blood taken from patients who had nontraumatic cardiac arrest had a high prevalence of hyperfibrinolysis [20]. There is compelling evidence that hypoperfusion drives hyperfibrinolysis, although others have argued that tissue factor from tissue injury may be contributory to over activation of systemic fibrinoly-

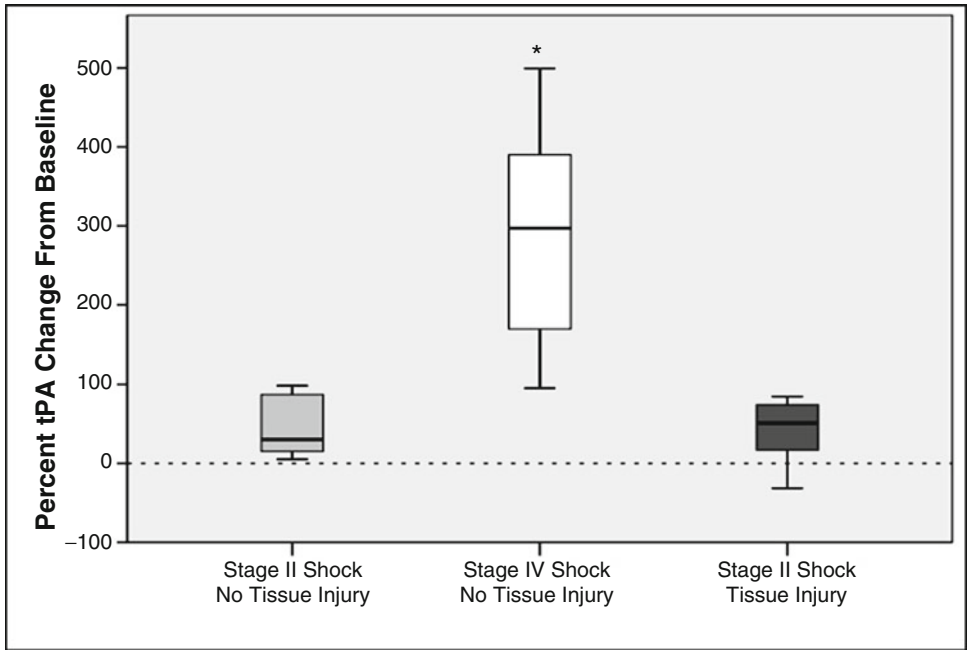

Fig. 9.5 In a rodent model evaluating tissue injury versus deep shock driving fibrinolysis, it was identified that tissue plasminogen activator is markedly elevated in response to shock, whereas tissue injury mildly increased changes and was comparable to control animals (stage II shock no tissue injury)

sis based on animal work [21]. Trauma patients have variable degrees of tissue injury and shock, and these collectively determine the different phenotypes of coagulation abnormalities. There may be unique patterns of injury that correlate to accelerated or inhibited fibrinolysis. Our group's recent animal work demonstrated that tPA levels were markedly increased in animals undergoing hemorrhagic shock, while tissue injury did not elevate tPA (Fig. 9.5). Interestingly, traumatic brain injury (with no herniation) appears to promote fibrinolysis shutdown [22, 23].

Currently, the only established risk factor for hyperfibrinolysis is inadequate tissue perfusion, which is supported by numerous retrospective studies identifying base deficit and systolic blood pressure upon arrival as being independently associated with the development of hyperfibrinolysis [13, 17, 24, 25]. This notion is further strengthened by the fact that patients in the CRASH II trial who had the greatest survival benefit from receiving tranexamic acid had a systolic blood pressure of less than 75 mmHg [26]. This is not a new concept, as investigators in the 1950s attributed fibrinolysis activation to ischemia and anoxia [27]. Shock may also be causing metabolic disturbances increasing fibrinolysis. It has been demonstrated that the taurocholic acid increased fibrinolysis in vitro and is markedly elevated following shock in rodents [28]. The drivers of fibrinolysis in trauma remain an active area of research.

Hyperfibrinolysis

Pathologic hyperfibrinolysis was appreciated by Starzl et al. in 1963 [3] using TEG during the anhepatic phase of liver transplant surgery. In the original series of human transplants, these authors advocated for empiric antifibrinolytics to reduce bleeding. However, 6 years later, when evaluating the coagulopathy of liver transplant, Starzl's group [29] retracted their statement advocating empiric antifibrinolytics when they appreciated increased mortality from venous thromboembolisms (VTE). Recognizing the

potential for spontaneous resolution of excessive fibrinolysis is an important concept. During transplantation, removal of the liver results in hyperfibrinolysis, which corrects itself once the grafted organ is perfused. The likely driver of fibrinolysis in liver transplant surgery is the removal of the liver, which has the ability to rapidly clear tPA through receptor-mediated processes [30].

Like most receptor-mediated processes, hepatic clearance of tPA can be saturated [31]. In trauma patients who develop TEG detectable hyperfibrinolysis, tPA levels on admission are elevated compared to those without TEG detected fibrinolysis [32]. The question in trauma remains whether hyperfibrinolysis is the result of excessive tPA generation or decreased tPA clearance. It is likely that both contribute. Historic literature suggested systemic hyperfibrinolysis occurs early after injury, but then proceeds to shutdown after resuscitation [15, 33], suggesting that after an acute phase of profibrinolysis the body compensates by inhibition of the system.

The elevation of tPA in the plasma of injured patients does not always correlate to overall fibrinolysis activity. The cognate direct inhibitor of tPA, plasminogen activator inhibitor 1 (PAI-1), has been found to decrease in patients with TIC [34] and hyperfibrinolysis [32]. The acute decrease in PAI-1 has been attributed to activation of protein C; however this relative drop in PAI-1 may simply be an increase in tPA causing a complex of tPA and PAI-1 decreasing the relative amount of free PAI-1. This is an important notion that must be considered when interpreting studies measuring tPA in plasma. Ideally, free tPA, free PAI-1, and the tPA/PAI-1 complex should be measured simultaneously in order to assess the total quantity of these proteins and fully characterize the imbalance that occurs during hyperfibrinolysis and fibrinolysis shutdown. PAI-1 is an acute phase reaction protein, and plasma levels do not peak for hours after hemorrhagic shock in animal models [35].

There are a number of additional proteins present in plasma that inhibit fibrinolysis by either inhibiting tPA or inhibiting plasmin. Some of these proteins are normally in relatively high abundance in the plasma including alpha 2 macroglobulin, alpha-1 antitrypsin, C-1 esterase inhibitor, and histidine-rich glycoprotein. As described in Chap. 3 (PAP-Booth) these are back-up inhibitors of the fibrinolytic system, which will regulate fibrinolysis when tPA is in excess in the circulation. These proteins, in addition to being potent downregulators of fibrinolysis, including alpha 2 antiplasmin (A2AP) and thrombin-activatable fibrinolysis inhibitor (TAFI), which are directly released from platelets, ensure a tight regulation of the system. Plasma can buffer the effects of tPA, as is evident with previous experiments in which exogenous tPA mixed in whole blood from healthy volunteers requires supra-physiologic concentrations to reproduce hyperfibrinolysis [36].

Fibrinolysis Shutdown

Impairment of the fibrinolytic system has detrimental effects on patient outcomes. The mortality rate of patients with fibrinolysis shutdown has been reported to be nearly four times greater than patients with a physiologic level of fibrinolysis [5]. Death is mostly attributable to organ failure. The term fibrinolysis shutdown was first used in 1969 [15] in a review on fibrinolysis describing the effects of electroplexy, myocardial infarction, and elective surgery. Animal work prior to this time suggested microemboli in visceral small vessels lead to irreversible shock [37] that was later found to be survivable through treatment with a profibrinolytic [38, 39]. Colonel Hardaway translated these findings to humans and implicated trauma and shock in producing microvascular occlusion [40]. Confusion arises as the nomenclature of describing impaired removal of vascular thrombi is referred to as either the syndrome of disseminated intravascular coagulation (DIC) or pathologic impairment of fibrinolysis (shutdown). Refer to Chap. 13 (DIC-Gando) on DIC as some investigators consider DIC to have two distinct phenotypes; i.e., hyperfibrinolysis and fibrinolysis shutdown, which is somewhat difficult to reconcile as diffuse intravascular thrombi should not exist in the presence of sys-

temic hyperfibrinolysis. Considering the major components of TIC (hypocoagulation and hyperfibrinolysis) are not necessarily linked, this section will focus on impairment of the fibrinolytic system, and not the clinical syndrome of DIC.

It is well known that trauma patients are prone to thrombotic events. When screening for postinjury venous thrombosis, the prevalence has been reported to approach 60 % [41]. There is evidence that thrombi in the pulmonary vasculature exist in nearly 1 in 4 seriously injured patients within 48 h of injury [42]. Furthermore, microvascular thrombi are implicated in organ dysfunction [43, 44]. Therefore it is intuitive that maintaining adequate fibrinolysis to clear the microvasculature of excessive fibrin deposition would be beneficial. The importance of inhibition of fibrinolysis at the site of injury remains essential during the acute phase of trauma, but systemic fibrinolysis shutdown is detrimental.

Inhibition of fibrinolysis measured by TEG in patients with severe sepsis has been attributed to elevated levels of PAI-1 [45]. Lipopolysaccharide administration to animals increases PAI-1 levels over several hours [46] and similar observations have been made in humans [47]. Acute lung injury models using mustard gas have implicated PAI-1 in addition to other antifibrinolytics (TAFI, and A2AP) as culprits in progression to pulmonary failure [48]. Fibrinolysis inhibition (shutdown) is more common after trauma than hyperfibrinolysis. In 1991 Enderson et al. described that the majority of polytrauma patients in their study had elevated D-dimers and decreased fibrinolytic activity [49]. Raza et al. in 2013 showed minimal fibrinolysis activity measured by ROTEM and high plasmin antiplasmin complexes in 57 % of their patients [16]. Most recently we demonstrated that 65 % of severely injured patients had suppressed fibrinolytic activity measured by TEG [5]. In all these studies, decreased fibrinolysis activity was associated with increased mortality, although the mechanisms remain to be defined. Identifying such mechanisms could lead to the investigation of treating fibrinolysis shutdown, holding promise on novel approaches to organ failure and thromboembolic events after injury.

Treatment Strategies to Attenuate Progression to Pathologic Fibrinolysis

In managing trauma patients at risk for hyperfibrinolysis, it is important to first assess if there is active bleeding. Hemorrhage control should remain the priority for all trauma patients, as progression to shock will increase the likelihood of hyperfibrinolysis and ultimately death. While hemorrhage control is in progress, resuscitation should be designed to be neutral on the fibrinolysis system until the patient's fibrinolytic phenotype has been defined. Currently, viscoelastic assays are clinically useful for detecting fibrinolytic activity in order to phenotype critically injured patients.

High volumes of prehospital crystalloid have been associated with hyperfibrinolysis [13] and supported by in vitro studies in which fibrinolysis was exacerbated by hemodilution of whole blood with saline and colloids [36]. Colloids may be problematic in resuscitating a patient at risk for hyperfibrinolysis as they have been implicated in impaired fibrin polymerization [50]. The role of permissive hypotension has a role in the prehospital setting as dilution of antifibrinolytics (platelets and plasma) may lead to a hyperfibrinolytic phenotype. This may partially explain the benefit of delayed fluid administration reported by Bickell et al. in patients with penetrating torso trauma [51]. However, patients with inadequate perfusion are also at risk for increased tPA production and becoming hyperfibrinolytic. It is well known that patients in profound shock benefit from prehospital saline [52]. However, the optimal prehospital fluid remains to be established. In our recent in vitro and animal studies, plasma resuscitation was found to attenuate hyperfibrinolysis. Plasma-first resuscitation may prove to be the optimal resuscitation fluid for trauma patients in hemorrhagic shock, and randomized prospective studies are underway. A case example of the beneficial

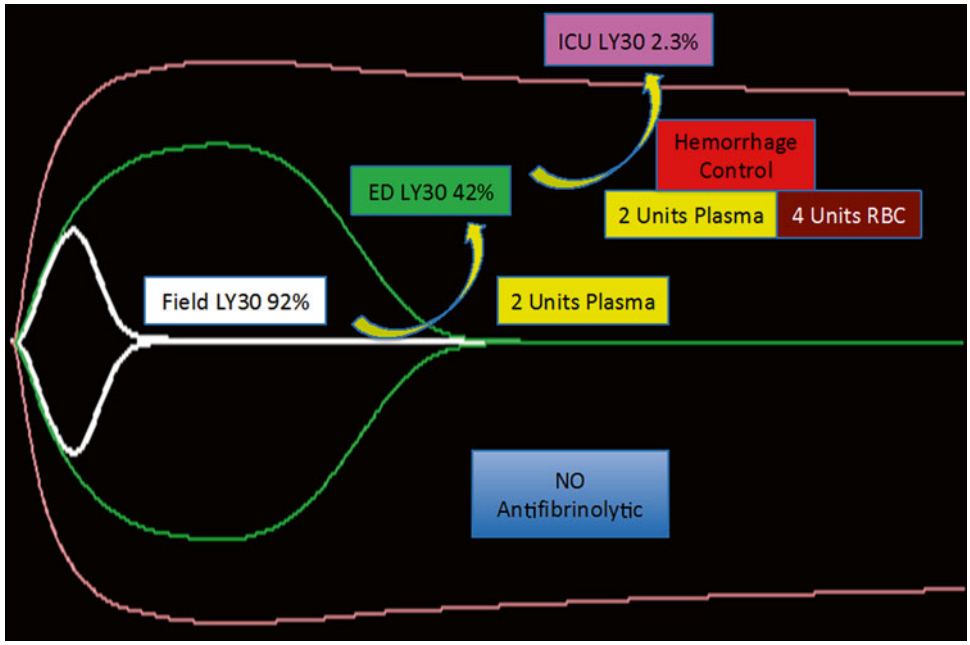

Fig. 9.6 Plasma-first resuscitation reversed a hyperfibrinolytic patient found by the paramedics with significant injuries, unresponsive, and profound shock (SBP 70 mmHg). This patient received two units of plasma en route to the hospital and increased his systolic blood pressure to 100 mmHg. His LY30 was reduced from 92 % in the field to 42 % upon arrival to the emergency department. The patient went emergently to the operating room where hemorrhage control was obtained. Receiving an additional 2 units of plasma and 4 units of RBC the patient was transferred safely to the intensive care unit where his LY30 had returned to a physiologic range of 2.3 %. He would be discharged from the hospital after a week and a few days with a full recovery. The patients required no antifibrinolytic medication and based on his field (prehospital) TEG had a >90 % predicted mortality

effect of plasma-first resuscitation in a hyperfibrinolytic patient is provided in Fig. 9.6.

The use of empiric antifibrinolytics in trauma patients warrants careful evaluation. Antifibrinolytic medications and specific recommendations for their use are reviewed in detail in Chap. 25 (Antifibrinolytics—Gruen). While the randomized CRASH II trial suggested a modest benefit in survival, the study inclusion criteria have been criticized [26], and the administration of tranexamic acid (TXA) 3 h after injury was associated with increased mortality [53]. The retrospective observational MATTERS study also suggested a survival benefit of TXA in the military setting, at an increased rate of VTE [54]. There is also the confounder of the high rate of cryoprecipitate transfusions in patients who received TXA in this retrospective study, and many of these patients were resuscitated with colloids, which have the potential to increase the risk of hyperfibrinolysis. None of these studies quantified fibrinolysis in patients being treated with TXA. In a recent retrospective study from the United States, TXA use was associated with nearly twofold increased mortality in propensity-matched trauma patients [55]. However, there is no disagreement that antifibrinolytic agents should be used in a targeted fashion, administered to those in whom a hyperfibrinolytic phenotype is detected; it is its empiric use that remains controversial.

Blood products may also contribute to fibrinolysis inhibition. As previously discussed, platelets contain a number of potent antifibrinolytic proteins. While transfusion of platelets can be life saving during hemorrhagic shock, they have also been associated with an increased risk for organ failure [56], which may be attributed to fibrinolysis

shutdown. Cryoprecipitate may also contribute to fibrinolysis impairment as suggested by the MATTERs II trial [57]. Cryoprecipitate not only contains fibrinogen [58] but also factor XIII [59] and fibronectin [60], all of which make the clot more resistant to fibrinolysis.

Hardaway suggested the potential role for a profibrinolytic in patients with fibrinolysis shutdown and at risk for organ dysfunction [61]. Patients with profound acute respiratory failure 48 h from trauma tolerated a tPA infusion with no adverse bleeding events and marked improvement in pulmonary function. Ongoing research using the TEG tPA challenge assay in trauma patients in the intensive care unit has identified significant resistance to fibrinolysis. The majority of these patients by hospital day 2 are resistant to supraphysiologic levels of tPA, even if some of them presented with a hyperfibrinolytic phenotype during the acute phase. Thus, therapeutic windows exist both for the management of bleeding from hyperfibrinolysis and for fibrinolysis shutdown during the recovery phase of trauma patients.

Clinical Assessment of Fibrinolysis

The optimal test to determine systemic fibrinolysis remains to be established. The euglobulin lysis test has been considered as the gold standard, but the important role of platelets in regulating fibrinolysis indicates that a whole blood assay is required. While D-dimers and PAP levels are sensitive in identifying the existence of fibrinolysis, they do not accurately reflect the current status of systemic fibrinolytic capacity that leads to bleeding. At this moment, TEG and ROTEM appear to be the optimal tests to identify the fibrinolysis status of the injured patient. Retrospective studies from Denver [12] and Houston [13] suggest that a 3 % LY30 threshold should be considered for treating hyperfibrinolysis with an antifibrinolytic medication; beyond this cut-off, the risk of massive transfusion and death is significant. However, both of these studies used different activators (kaolin and tissue factor) to perform their TEG assays. Preliminary data from our group supports that using no activator in TEG yields the best detection of fibrinolysis. TEG initiated with an activator (kaolin and tissue factor) was not found to cause an appreciable increase in LY30 when adding physiologic levels of exogenous tPA in healthy volunteers, whereas a non activated (native) TEG detected a significant change. These data also suggests that activation of the sample with exogenous tissue factor, while providing results quicker, makes the assay more prone to underestimating fibrinolysis. Therefore, future research with prospective validation is needed to define the optimal threshold for treating hyperfibrinolysis.

Conclusion

The fibrinolytic response to trauma can vary from shutdown to hyperactivity. Trauma's two components of tissue damage and hemorrhagic shock modified by the patients genetic predisposition and medical comorbidities lead to a complex non-uniformed derangement of the fibrinolytic system following severe injury (Fig. 9.7). Fibrinolysis shutdown is the most common of these two phenotypes following severe injury, which has a reported prevalence greater than 50 %. Hyperfibrinolysis is relatively rare but associated with high mortality. Resuscitation fluids and blood products can alter the fibrinolytic phenotype. The goal of resuscitation should be neutral on the fibrinolytic system until a phenotype can be determined. There is consensus for the use of antifibrinolytics in patients with confirmed hyperfibrinolysis or profound shock, while empiric use of these medications remains controversial and requires further investigation. The role of profibrinolytics may be on the horizon to treat patients in fibrinolysis shutdown and organ failure in the intensive care unit. There are many questions to be answered in regard to the mechanisms that drive these processes.

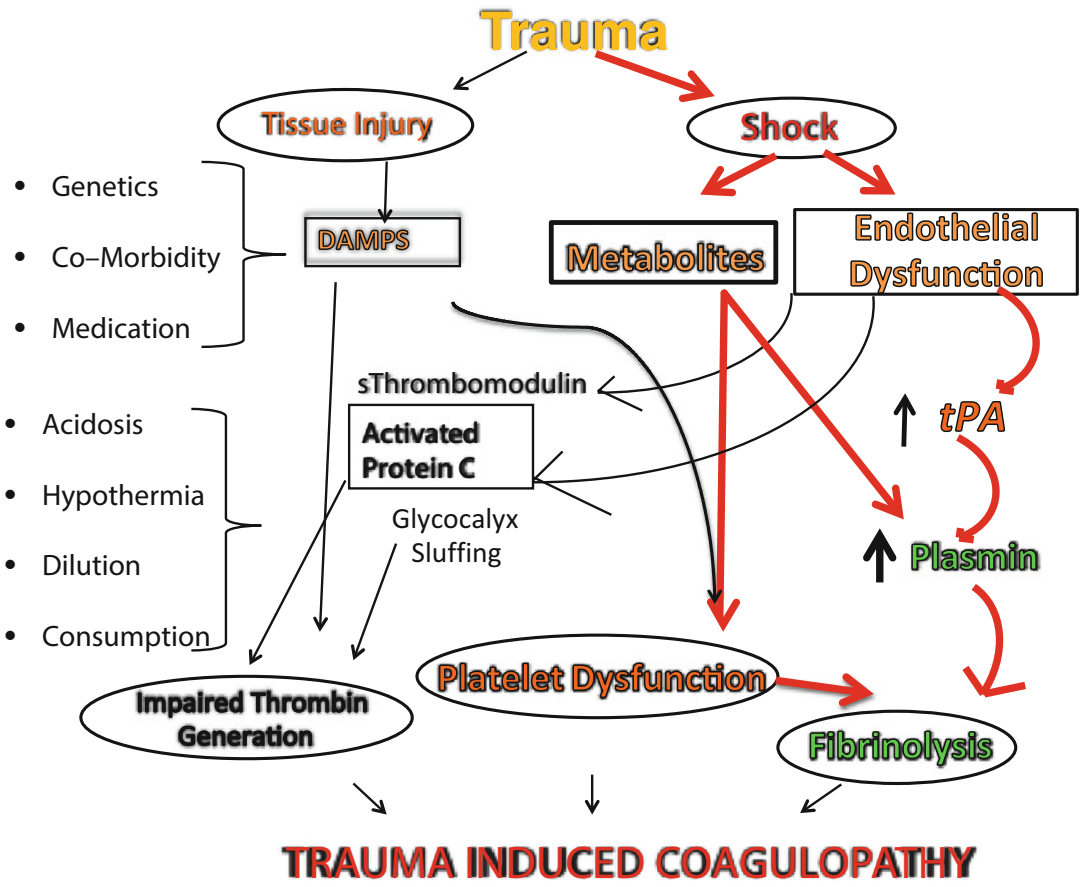

Fig. 9.7 Trauma-induced coagulopathy is a combination of tissue injury and shock resulting in impaired thrombin generation, platelet dysfunction, and hyperfibrinolysis. There are numerous contributing factors that impact the patient's ultimate derangement in coagulation. Patients can have a combination or isolated abnormality of these three pathways resulting in uncontrolled bleeding and death. There is evidence that impaired thrombin generation and hyperfibrinolysis are not driven by the same factors. Our current understanding of pathologic hyperfibrinolysis is that this appears to be predominantly driven by systemic hypoperfusion from hemorrhagic shock. Identifying what drives each of these processes caters to individualized resuscitation to optimally treat unique phenotypes of TIC

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Neutrophils, Inflammation, and Innate Immunity in Trauma-Induced Coagulopathy

10

Christopher D. Barrett and Michael B. Yaffe

Introduction

It should come as no surprise that, as we learn more about the innate immune and coagulation systems, we continue to discover additional ways that the two are intimately and inseparably linked [1]. Reducing it to the simplest terms, one can imagine that it would be biologically advantageous to have a hemostatic, antimicrobial (inflammatory), pro-regenerative response following traumatic injury. Of particular interest in mediating cross-talk between the coagulation and immune systems is the polymorphonuclear leukocyte (PMN), or neutrophil, so named for its neutral staining characteristics on Wright Stain compared to the other two granulocyte populations (basophils and eosinophils) [2]. Neutrophil antibacterial properties have been known to surgeons for two and a half centuries, where during the Seven Years' War in 1761 the British surgeon John Hunter noted a delay in blood spoilage when the "buff" colored layer from one blood sample was added to another blood sample [3]. Centuries later this innate immune cell, with its respiratory burst (via the NADPH oxidase complex) and toxic protein granule products, is now known to do far more than just provide antibacterial defenses. In the modern era, neutrophils have been shown to play a key physiologic role in response to tissue damage and wound healing, coagulation, and endothelial barrier function and injury [4–12]. Unfortunately, loss of tight control of neutrophil function can also have serious pathophysiologic consequences, with aberrantly activated neutrophils contributing to diseases such as the acute respiratory distress syndrome (ARDS), transfusion-related lung injury (TRALI), ischemia-reperfusion injury, sepsis, and more recently the coagulopathy associated with traumatic injury [12–17].

Following traumatic injury, areas of tissue damage and the associated injured vascular endothelium lead to exposure of the extracellular matrix (ECM) and a shift in function and expression of coagulation regulatory molecules on the endothelium (reviewed elsewhere in this text). Additionally, injured endothelial cells and the type I and type II activation of surrounding

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un-injured endothelial cells in response to inflammatory cytokines and ischemia-reperfusion lead to increased expression of cell surface adhesion molecules [15, 18–20]. The exposed subendothelium and upregulated endothelial adhesion molecules, including P-selectin, E-selectin, ICAM-1, and VCAM-1, act to recruit neutrophils, platelets, and other leukocytes (e.g., macrophages) to the site of injury, in order to provide antibacterial defenses, promote tissue remodeling, and release tethered growth factors and cytokines/chemokines that promote inflammatory responses, wound healing, and hemostasis [4, 5, 21–26]. In the case of traumatic coagulopathy, the associated severe injury results in the release of large amounts of inflammatory cytokines and damage-associated molecular patterns (DAMPs), which causes priming and systemic activation of neutrophils. In an indiscriminate fashion, these activated neutrophils release significant amounts of extracellular ROS via the NADPH oxidase complex, release an array of toxic and proteolytic enzymes via degranulation, and extrude their DNA contents decorated in histones and granule proteins to effect further endothelial injury, propagate coagulation through increased thrombin generation and decreased anticoagulant protein activity, and cause end-organ damage.

Neutrophil Localization in Injury and Inflammation

Neutrophils themselves exist in two populations outside the bone marrow, the marginated pool and the circulating pool, each comprising about half of the total peripheral blood PMNs [27]. Neutrophils within the circulation interact with the endothelium via the leukocyte adhesion cascade in the post-capillary and collecting venules primarily via PSGL-1 and integrins (αβ-heterodimers), although other proteins and ligands like L-selectin, CD44, and ESL-1 likely also play a role [21]. In homeostatic conditions, neutrophils will tether and roll along the endothelium in a selectin-ligand dependent manner (the main neutrophil ligand being PSGL-1), with very high association and dissociation constants, but will not become adherent or transmigrate across the endothelial barrier [28]. Upon stimulation by increased selectin-mediated signaling, chemokine activation of G-protein-coupled receptors, and increased inflammatory cytokines and chemotactic factors, there is a change in conformation of integrins (LFA-1, VLA-4) on the cell surface from a bent low-affinity to an extended low-affinity state, followed by transition to a high-affinity extended conformation for their ligands, primarily ICAM-1 and VCAM-1 [28–32]. The final transition of these integrins to their extended high-affinity state may, at least in part, be dependent on shear stress via a "catch bond" phenomena [33]. This binding interaction of the PMN integrins with their endothelial ligands leads to arrest of rolling and tight adhesion of the neutrophil to the endothelial surface, whereupon the neutrophil can transmigrate across the endothelial barrier in response to further chemotactic stimuli in a paracellular or transcellular manner. This initial migration out of the circulation is then followed by subsequent chemotaxis into the underlying extravascular tissue [28, 34–36]. This process of neutrophil adhesion and transmigration across the endothelial barrier is thought to contribute to microvascular hyperpermeability under certain circumstances [37]. Under normal circumstances, PMN transmigration is considered to be a polarized unidirectional process with very few neutrophils returning to the circulating granulocyte pool. In mice it has been shown that reverse transmigration back into circulation can potentially turn localized inflammation into systemic inflammation with secondary organ damage [38].

In the case of traumatic injury with a denuded endothelium and exposed subendothelial matrix, neutrophil localization is primarily platelet-dependent, with a similar process of transmigration occurring when neutrophils adhere to activated adherent platelets [39]. Platelets bind at the site of exposed ECM in a vWF/GPIb dependent manner and recruit PMNs following platelet activation, initially via P-selectin/PSGL-1 binding and subsequently by binding of the neutrophil β_2 integrin Mac-1 directly to platelet GPIb and possibly also to fibrin(ogen) that is adherent to platelet $\alpha IIb\beta_3$ integrin (GPIIbIIIa) within the

developing thrombus [39–48]. If the injury is severe enough (e.g., severe traumatic injury or sepsis), however, the ability of neutrophils to localize via adhesion and transmigration becomes impaired by the overwhelming inflammatory signaling response. Neutrophils become "paralyzed" in such states, rendering them unable not only to tightly adhere in a spatially localized manner but also to chemotax towards sites of infection and injury with increased membrane rigidity and capillary sequestration [34, 49]. Recent studies have shown that traumatic injury causes release of distinct human damage-associated molecular patterns (DAMPs), including mitochondrial formyl-peptides and mitochondrial DNA, whose release can activate the same G-protein-coupled formyl peptide receptor-1 (FPR1) and TLR9 as their bacterial formyl-peptide and DNA counterparts, with FPR1 and TLR9 being expressed on both neutrophils and endothelial cells [50–54]. These DAMPs are likely responsible for the clinical similarities of shock and inflammation following trauma and sepsis, including the systemic neutrophil responses. In these states of critical illness there is reduced adhesion molecule expression secondary to upregulation of iNOS, HO-1, and PPARγ, early priming and systemic activation of neutrophils that is followed later by desensitization to future priming and activation from prolonged stimulus exposure, and CXCR2 downregulation by TLR2 and TLR4 agonism [34, 55]. These states of critical illness, inclusive of significant trauma, have the potential to lead to high levels of nonlocalized systemic neutrophil activation with subsequent innate immune compromise secondary to paralysis and neutrophil desensitization.

Neutrophil NADPH Oxidase Priming and Highly Reactive Oxygen Species Production

One of the most important tools available to neutrophils following traumatic or infectious insults to the host is their ability to produce highly reactive oxygen species (ROS) via the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex. At rest, the NADPH oxidase components are not fully assembled, with gp91phox and p22phox making up a membrane protein called flavocytochrome b_{558}, a transmembrane αβ-heterodimer with two non-identical coordinated heme prosthetic groups that make up the electron transfer system across the membrane to produce superoxide anion via electron transfer to O_2 [56, 57]. The other proteins p40phox, p47phox, p67phox, and Rac-2 GTPase remain in the cytosol away from the flavocytochrome b_{558} complex and exist in several uncomplexed and partially complexed states [58, 67]. Following stimulation of neutrophils with an agonist such as LPS, TNF-α, PAF, or fMLP (amongst many others), the neutrophil becomes "primed" for subsequent activation of the NADPH oxidase complex in response to a second stimulus [59–67]. The key feature of priming is that the response primed neutrophils have to a given second stimulus is exponentially greater than that of unprimed neutrophils to the same stimulus, lending a critical mechanism of regulation for when and under what conditions neutrophils generate ROS.

On a molecular level, priming occurs via multiple mechanisms and depends on the priming agent, but the two most commonly implicated mechanisms involve (a) p47phox phosphorylation with subsequent conformational changes that allow for additional phosphorylation and binding site exposure to facilitate both the binding of p47phox to the p40phox:p67phox complex and later binding to flavocytochrome b_{558} at the plasma membrane during activation, and (b) direct mobilization of specific and gelatinase granules containing flavocytochrome b_{558} to the cell membrane to increase the number of potential NADPH oxidase complexes that can be assembled [58, 63]. Upon exposure to a second stimulus, the primed neutrophil now assembles the holoenzyme NADPH oxidase complexes and proceeds to generate a large amount of superoxide anion at the plasma membrane and within phagosomes that leads to several toxic ROS species, a process that can lead to extensive tissue damage and endothelial cell activation, injury, and cell death [64–67].

The underlying signaling pathways for priming involve several key mediators which also depend on the priming agent. Brown and colleagues [67] showed that TNF-α priming of human neutrophils for subsequent respiratory burst activation was strictly dependent on p38 MAPK phosphorylation of p47phox and p67phox, whereas PAF priming was independent of p38 MAPK activity, and resulted in only p67phox phosphorylation, with Sheppard et al. subsequently finding that PAF priming led to p67phox translocation to the plasma membrane [68]. Additionally, Brown et al. showed that regardless of the priming agent, fMLP activation for ROS production by primed neutrophils was reduced by 70 % when p38 MAPK was inhibited using the specific p38 MAPK small molecule inhibitor SB203580, whereas complement-opsonized zymosan (OPZ) activation of primed neutrophils was independent of p38 MAPK and instead appeared to involve the Erk1/2 MAPK pathway. Subsequent elegant work by Dang et al. [69] using human PMN showed that Ser345 on p47phox, which lies within a MAPK consensus sequence (-PXSP-), is the phosphorylation target for priming by TNF-α via p38 MAPK activation and by GM-CSF via Erk1/2 activation, with both kinase pathways converging on phosphorylation of Ser345. Addition of a direct p38 MAPK inhibitor (SB203580) or indirect Erk1/2 inhibitors (PD98059 and UO126 for inhibition of MEK1/2 upstream) abrogated priming by TNF-α and GM-CSF, respectively. Further, evidence for in vivo relevance was observed through increased ROS production in response to fMLP along with increases in phospho-Ser345-p47phox, phospho-p38 MAPK, and phospho-Erk1/2 in synovial PMN from human rheumatoid arthritis (RA) patients compared to circulating PMN from the same RA patients and healthy human controls, and competitive substrate assays using cell-permeable TAT-Ser345 showed inhibition of both basal and fMLP-stimulated ROS production of these PMN [69].

The importance of phosphorylation at Ser345 on p47phox for human neutrophil priming by TNF-α was further clarified by El-Benna and colleagues, who showed that the *cis-trans* prolyl isomerase Pin1 binds to phospho-Ser345 form of p47phox and induces conformational changes that allow for the additional required serine phosphorylations by PKC to prime PMN [70], as well as facilitate binding of p47phox to the other cytoplasmic subunits p40phox and p67phox. It is thought that these conformational changes and additional phosphorylation events lead to exposure of cryptic SH3 domains in p47phox that in nonprimed (nonphosphorylated) states remain bound to a proline-rich autoinhibitory region within p47phox itself. Thus, phosphorylation of p47phox liberates the SH3 domains to bind the proline-rich region of the cytosolic tail on the p22phox component of the membrane-bound cytochrome, and provides a targeting mechanism for p47phox and its associated cytosolic oxidase components to bind at the membrane for holoenzyme assembly during activation. Other signaling pathways that prime and activate the NADPH oxidase complex result in p47phox phosphorylation on different serine residues, such as Akt phosphorylation of Ser304 and Ser328 in response to fMLP, IL-8, and GRO-α activation of PI3K by binding their respective GPCRs [71–73]. Whether or not priming via phosphorylation of cytosolic oxidase components leads to pre-assembly of the holoenzyme NADPH oxidase complex at the plasma membrane is a topic of debate, and it is likely that this may be the case with some priming agents but not with others. Yaffe and colleagues have shown using membrane fractionation techniques that priming of human neutrophils with TNF-α does not lead to translocation of p47phox or p67phox to the plasma membrane despite causing phosphorylation of both, while stimulation with the nonphysiologic direct PKC activator phorbol myristate acetate (PMA) does cause membrane translocation of p47phox and p67phox. This observation argues against a role for oxidase pre-assembly at the plasma membrane in p38 MAPK-dependent TNF-α priming of human neutrophils. Others have shown that LPS-induced priming of human PMN is a p38 MAPK-dependent process resulting from cytochrome b_{558} shuttling to the plasma membrane via specific granules along with limited p47phox translocation, while Sheppard et al. showed that priming by PAF resulted in p67phox membrane translocation [68, 74, 75].

Direct evidence for the role of neutrophil-derived ROS in traumatic coagulopathy is limited, but there are numerous studies that suggest this is the case. Jacobi et al. showed that human PMNs from hemodialysis patients exist in a highly primed state and that co-culture with human umbilical vein endothelial cells (HUVEC) leads to significant amounts of HUVEC apoptosis, tissue factor and IL-8 production, and immediate increases in HUVEC cell surface P-selectin compared to healthy human controls [64]. Importantly, in those experiments a membrane barrier was used to prevent direct PMN-HUVEC adhesion, and further showed that the observed effects on HUVEC were abrogated by addition of superoxide dismutase (SOD) and catalase. These observations directly implicate neutrophil-derived ROS and other soluble products of primed and activated PMN in causing endothelial cell activation and procoagulant states in addition to mediating endothelial cell injury and death. These findings are supported by earlier studies that showed rat pulmonary artery endothelial cell injury and death in response to activated PMN could be prevented with early addition of catalase, and at later time points showed significant synergistic endothelial cell killing by PMN-derived ROS and proteases [65]. More recently, neutrophils from human polytrauma patients were shown to cause significant amounts of endothelial progenitor cell (EPC) killing compared to healthy human controls that could be prevented by pretreatment of PMN with the NADPH oxidase inhibitor diphenyliodonium chloride. In contrast, however, inhibition of neutrophil elastase did not show any protective effect [76]. Given the important role of EPCs in tissue and vascular repair following traumatic injury, it is thought that the excessive neutrophil priming and activation seen in severe traumatic injury may prevent wound healing and vascular repair through killing of EPCs. Additionally, it is known that the formation of neutrophil extracellular traps (NETs) requires ROS generation by the NADPH oxidase complex and that NETs propagate coagulation through thrombin generation, cleavage of TFPI, and direct endothelial damage (discussed in more detail below) [6, 7, 9, 77]. The above findings are supported by work from our lab (unpublished) that has demonstrated a robust priming of human neutrophils by human serum (i.e., the liquid product of clotted whole blood) for ROS production in response to formylated peptides, an in vitro approximation of what neutrophils may see following traumatic injury with generation of serum and release of human mitochondrial-derived formylated peptides (prototypic DAMPs). Interestingly, we have found that the liquid product of clotted plasma (i.e., the liquid product of clotting the cell-free liquid fraction of blood) is equally potent at priming human PMN for ROS generation in response to formylated peptides, and further have observed only a partial reduction in priming potential by platelet depletion prior to activation of the clotting cascade. Taken together, mounting evidence suggests that ROS from activated human neutrophils in traumatic injury is directly responsible for endothelial cell activation, injury, and death, altered coagulation and adhesion molecule expression, and worsening coagulopathy in the severely injured patient. Further, there may be a significant yet currently undefined role for plasma products of coagulation in priming neutrophils for ROS generation in traumatic injury, providing a feedforward mechanism for both coagulation and inflammation that may help drive trauma-induced coagulopathy.

Neutrophil Granule Proteins and Coagulation

Another key component of neutrophil effector responses to inflammatory stimuli and injury is the release of enzymes and proteases from neutrophil granules. These enzymes and proteases are likely to be direct contributors to the coagulopathic state, further propagating the deleterious feedforward cycle of traumatic coagulopathy and inflammation. Many of the same stimuli responsible for neutrophil priming and ROS generation also lead to degranulation of primary (azurophilic), secondary (specific), and tertiary (gelatinase) granules and secretory vesicles in a stimulus-dependent fashion, with more potent stimuli being required for primary granule release given their more toxic contents and less

Table 10.1 Neutrophil agonists that regulate chemotaxis, priming, and/or activation in either solution phase or adherent neutrophils

Agent	Chemotaxis	Priming	Activation (ROS production, degranulation)
N-formyl peptides (fMLP)	+	+	+
Mitochondrial DNA	+	+	+
PAF	+	+	+
PF4	+	+	
FPB	+		
LTB_4	+	+	+
Gro-α	+	+	+
IgG/IgM			+
LPS		+	
C5a	+		
G-CSF	+	+	+
GM-CSF	+	+	
TNF-α	+	+	+
IFN-γ		+	
IL-1β	+	+	+
IL-6	+		
IL-8	+	+	+

Adapted from references [50, 59–62, 79, 125–136]

potent stimuli required for the sequentially less toxic granule release (Tables 10.1, 10.2, and 10.3) [78, 79]. The function of granule products varies widely, including catalyzing HOCl production from ROS (myeloperoxidase/MPO), regulation of the coagulation system (elastase, cathepsin G), direct microbicidal activity (defensins, lysozyme), ECM remodeling via proteolysis and cytokine/chemokine processing (matrix metalloproteinases/MMPs, elastase, other serine proteases), and neutrophil priming via specific and gelatinase granule secretion (flavocytochrome b_{558}) [80, 81].

A critical recent discovery is the observation that secretion of neutrophil elastase and cathepsin G from azurophilic granules leads to cleavage of tissue factor pathway inhibitor (TFPI) and subsequent tissue factor (TF)- and factor XII-dependent activation of coagulation [6]. In a study by Massberg et al., they observed in vivo that fibrin deposition following vascular injury was significantly reduced in mice deficient in neutrophil elastase and cathepsin G due to deficient cleavage of TFPI and that the process of TFPI cleavage by neutrophil serine proteases in WT animals was dependent on NETs to co-localize these proteases with TFPI. Treatment of WT mice with H2A-H2B-DNA-specific antibody suppressed coagulation and fibrin formation but did not lead to additional reduction in fibrin formation in the elastase −/−, cathepsin G −/− mice. Further, they showed in vitro that DNase was also able to abrogate the increased TF activity and TFPI degradation by neutrophil serine proteases observed in reconstituted nucleosome coagulation assays. These findings showed in a substantial way that neutrophils are direct regulators of the natural anticoagulant system and that neutrophil dysfunction can have powerful consequences on coagulation and hemostasis.

In addition to proteolytic cleavage of the natural anticoagulant TFPI, it is known that neutrophil elastase, MMP-8, and MMP-9 can promote coagulation by cleaving proteoglycans in exposed blood vessel walls that model injured or denuded blood vessels such as those seen in trauma [82]. By degrading part of the proteoglycan matrix,

Table 10.2 Intramembranous proteins found within neutrophil granules and secretory vesicles

Azurophilic (primary) granules	Specific (secondary) granules	Gelatinase (tertiary) granules	Secretory vesicles
Membrane	Membrane	Membrane	Membrane
Granulophysin (CD63)	MAC-1 (CD11b/CD18)	MAC-1 (CD11b/CD18)	MAC-1 (CD11b/CD18)
CD68	Cytochrome b_{558}	Cytochrome b_{558}	LFA-1 (CD11a/CD18)
V-type H^+-ATPase	TNF-R	DAG lipase	Cytochrome b_{558}
	fMLP-R	fMLP-R	fMLP-R
	u-PA receptor	u-PA receptor	u-PA receptor
	SCAMP	SCAMP	SCAMP
	Laminin-R	Laminin-R	CR1
	Thrombospondin-R	V-type H^+-ATPase	C1q-R
	Vitronectin-R	VAMP-2	DAF
	Fibronectin-R		Alkaline phosphatase
	VAMP-2		VAMP-2
	G-protein$_\alpha$-subunit		CD14
	CD66		CD16
	CD67		V-type H^+-ATPase
	CD15 antigens		Neutral Endopeptidase (CD10)
	NB 1 antigen		Aminopeptidase N (CD13)
	Rap1		Tyrosine phosphatase (CD45)
	Rap2		

Adapted from references [79, 137]

Table 10.3 Soluble proteins found within neutrophil granules and secretory vesicles

Azurophilic (primary) granules	Specific (secondary) granules	Gelatinase (tertiary) granules	Secretory vesicles
Matrix	Matrix	Matrix	Matrix
Myeloperoxidase	Collagenase (MMP-8)	Gelatinase	Tetranectin
Defensins	Gelatinase	Lysozyme	Other plasma proteins
Bactericidal/permeability-increasing protein	Lactoferrin	β_2-Microglobulin	
Cathepsins	Lysozyme	Acetyltransferase	
Elastase	u-PA	Tetranectin	
Lysozyme	β_2-Microglobulin		
Proteinase 3	hCAP-18		
Azurocidin (heparin-binding protein, CAP37)	Histaminase		
α_1-antitrypsin	Heparanase		
α-mannosidase	NGAL		
Acid β-glycerophosphatase	Sialidase		
Acid mucopolysaccharide	SGP28		
β-Glycerophosphatase	B_{12}-Binding protein		
β-Glucuronidase			
N-Acetyl-β-glucosaminidase			
Sialidase			
Ubiquitin-protein			

Adapted from references [79, 137]

vWF binding sites on vessel wall collagen are exposed and this leads to a dramatic increase in platelet adherence under flow conditions through vWF-GpIb binding interactions, which can further amplify neutrophil recruitment and activation. This procoagulant effect seen using formyl peptide-activated human neutrophils, PMN supernatants, and purified neutrophil elastase, MMP-8, and MMP9 could be completely mitigated by a combination of serine- and metalloprotease inhibitors. Beyond the procoagulant activity of neutrophil proteases in the subendothelial matrix, co-culture models with neutrophils and multiple different human endothelial cell lines have shown endothelial cell damage and killing by neutrophil elastase, with augmentation by ROS observed in many of them [10, 83, 84]. Neutrophil elastase and cathepsin G have shown a potent ability to cleave VE-cadherin and disrupt endothelial cell monolayers in vitro, providing a possible mechanism for the vascular permeability and shock seen in trauma patients with severe inflammatory responses despite adequate resuscitation [11]. Another mechanism by which neutrophil elastase may contribute to vascular hyperpermeability and hypotension in traumatic injury is through production of E-kinin from high molecular weight kininogen, which has been demonstrated in a rat model, and is likely mediated by binding of the GPCR bradykinin β_2 receptor [85], and in humans a correlation between plasma levels of neutrophil elastase and pulmonary vascular permeability has been demonstrated in patients with severe pneumonia [86].

Another important granule protein, myeloperoxidase (MPO), is widely accepted as a critical effector enzyme for microbial killing through production of hypochlorous acid from H_2O_2 and also has diverse effects on endothelial cell function and coagulation [87]. In human patients, serum MPO levels strongly correlate with endothelial dysfunction as measured by brachial artery dilation studies, and MPO is known to sequester vasoactive NO by using it to produce pro-inflammatory reactive nitrogen species [88, 89]. Support for MPO's direct vasoactive role is further demonstrated in MPO-deficient mice that exhibit resistance to endotoxin-mediated vasomotor dysfunction [90]. In vitro studies have shown direct injury to endothelial cells by MPO-bearing neutrophilic microparticles, released upon neutrophil activation, with loss of endothelial cell membrane integrity and concomitant morphologic changes consistent with endothelial damage, and others have shown direct endothelial cell cytolysis resulting from MPO-generated HOCl [91, 92]. NETs are coated with MPO, and the ability of NETs to cause direct endothelial cell cytotoxicity can be partially inhibited by the MPO inhibitor dihydrolipoic acid [9]. MPO is also capable of endothelial barrier transcytosis independent of neutrophil emigration in a heparin-dependent mechanism and has been implicated as a catalyst in ECM protein tyrosine nitration, including nitration of fibrinogen and fibronectin, leading to more rapid fibrin clot formation and factor XIII-dependent fibrin crosslinking with a resulting prothrombotic state [93, 94]. MPO endothelial transcytosis lends further support for the ability of neutrophil activation to cause diffuse nonspecific vascular inflammation and thrombosis, and the observed heparin dependence of MPO-endothelial cell interactions provides an explanation for the anti-inflammatory effects of heparin [94, 95]. Taken together, it is likely that MPO from activated neutrophils in traumatic injury causes direct, systemic endothelial and subendothelial damage, vasomotor dysfunction, and thrombosis that significantly contributes to trauma-induced coagulopathy.

Neutrophil Extracellular Traps: A Link Between Innate Immunity and Coagulation

In recent times, much interest has been directed towards the phenomenon of neutrophil extracellular traps, (NETs), where neutrophils extrude their nuclear DNA decorated with histones and toxic granule contents (e.g., myeloperoxidase, elastase, defensins, serine proteases) in a process

termed NETosis [96]. It seems apparent that NETs are yet another mechanism by which neutrophils provide innate immunity, releasing a toxic web that can trap and kill bacteria [6, 97]. Additionally, NETs have now been shown to propagate coagulation and thrombus formation by generating thrombin in a platelet-dependent and platelet-independent manner, and facilitate tissue factor pathway inhibitor cleavage by serine proteases bound to NETs leading to TF and factor XII-dependent coagulation [6, 7]. NET formation is dependent on several key prerequisites, including ROS production, migration of granule contents to the nucleus, and in certain circumstances requiring Mac-1 outside-in signaling [96, 98]. Stimulation of platelet TLR4 by bacterial LPS has been shown to rapidly induce NETosis via increased platelet binding to adherent neutrophils, and there are many other non-infectious agonists of TLR4 such as HMGB1 that are released in traumatic injury and have been implicated in NET formation [7, 97, 99]. It is not surprising, then, that NETs have been shown to occur in other non-infectious scenarios in both humans and mice (e.g., autoimmune vasculitis, TRALI, ventilator-associated lung injury), and there are numerous other stimuli for NETosis beyond TLR4 stimulation (e.g., ANCA antibody) [98, 100]. NETs form in the microvascular system such as liver sinusoids and pulmonary microvasculature where beyond capturing bacteria they can cause microvascular thrombosis and ischemia, lead to direct endothelial and epithelial damage, and cause end-organ injury, and there may also be a role for NETs in the pathogenesis of both arterial and venous thrombosis [8, 9, 96, 97]. As it relates to trauma, the role of NETs in the injury inflammatory response has been postulated based on the increased understanding of the role of NETs in disease, and just recently McIlroy et al. demonstrated in vivo that NETs are formed in human patients following traumatic injury and subsequent surgery [101, 102]. Thus following traumatic injury, intravascular NET formation is likely to participate in physiologic hemostasis, and loss of regulation of NETosis may be a major driver in tipping the balance towards pathologic coagulopathy.

Finally it is worth pointing out that in 2007, Brohi et al. [103] showed that one of the hallmarks of TIC following severe traumatic injury is an early increase in activated protein C (aPC) production due to tissue hypoperfusion, with later development of a prothrombotic state that likely develops secondary to exhaustion of the protein C anticoagulant pathway. This fits well with the concept of NETosis, whereby severe trauma leads to high levels of microvascular NET formation and subsequent endothelial damage, thrombus generation, and end-organ and tissue ischemia that may be responsible for the hypoperfusion-associated aPC generation observed by Brohi and colleagues. Furthermore, NETs also provide a procoagulant scaffold to facilitate the observed late thrombogenic state seen in patients who developed TIC. Given all the known and potential points of intersection between traumatic injury, NET formation, and coagulation it is all but certain that NETs play a major role in the pathogenesis of TIC.

Early Activation of Innate Immunity in Trauma: Danger, Damage-Associated Molecular Patterns, and the Late Immunosuppression of Critical Illness

The innate immune system is the body's first-line defense against invading pathogens and other dangerous processes to the host such as tissue damage. In order to provide a rapid response to these threats, the innate immune system has evolved mechanisms to recognize common "danger" signals occurring in nature from *both* self and non-self, a theory originally championed by Polly Matzinger that has gained traction amongst many in the trauma community. This "danger signal" model may offer key insights into entities such as the systemic inflammatory response syndrome (SIRS or "sterile sepsis") often seen following severe trauma [50, 104, 105], where high levels of danger signals may be inadvertently released. Of particular interest in traumatic injury are the human damage-associated molecular

patterns, (DAMPs), including mitochondrial formylated peptides and mitochondrial DNA (mtDNA), whose release is observed following traumatic injury and can activate the same G-protein-coupled formyl peptide receptor-1 (FPR1) and TLR9 as their bacterial formylated peptide and DNA counterparts [50]. It has been shown that FPR1 and TLR9 are expressed on both neutrophils and endothelial cells, which supports the notion that human DAMPs play a significant role in the similarities of shock and inflammation observed following trauma and sepsis [50–54]. Human trauma and femur fracture patients have thousands-fold higher plasma mtDNA levels than healthy human controls, and mitochondrial products from trauma patients and femoral fracture reamings cause increased IL-8, MMP-8, and MMP-9 release in human PMN that can be inhibited by FPR1 blockade and additionally show potent neutrophil chemotactic activity [50, 106]. Mice rapidly develop peritonitis in response to mitochondrial DAMPs (MTD), while rat models consistently demonstrate end-organ neutrophil infiltration and pro-inflammatory cytokine production in the liver and lungs as well as significant increases in plasma concentrations of TNF-α, IL-6, and IL-10 in response to MTDs with implication of the p38 MAPK, p44/42 MAPK, and NF-KB pathways through TLR9 and FPR1 signaling [50, 106, 107]. Importantly, these responses are absent in both nuclear DNA and vehicle controls. Trauma and hemorrhagic shock (T/HS) models of rats have provided additional evidence that significant amounts of mtDNA get released in states of shock, and there is some limited evidence that plasma mtDNA levels can help quantify inflammatory tissue injury and differentiate sepsis from sterile SIRS in non-human primates [108, 109].

Another important DAMP in neutrophil function under increasing investigation is the high-mobility group box 1 protein (HMGB1), originally thought to function only as a nuclear protein responsible for transcription regulation by bending DNA to facilitate binding of other regulatory protein complexes, including those from the nuclear hormone receptor family, V(D)J recombinases, and p53-p73 transcriptional complexes [110]. More recently, HMGB1 has been found to act as a prototypic DAMP when released into the extracellular compartment in response to cellular stress by immune cells, hepatocytes, platelets, and endothelial cells in addition to passive release by necrotic cells [111]. HMGB1 is now known to bind and activate the receptor for advanced glycation end products (RAGE) and several pattern recognition receptors including TLR2, TLR4, and TLR9 depending on the scenario, with HMGB1 levels being significantly elevated in human trauma patients [111, 112]. In a murine hemorrhagic shock/resuscitation (HS/R) model HMGB1 has been shown to activate the neutrophil NADPH oxidase via TLR4 signaling through both MyD88-IRAK4-p38 MAPK and MyD88-IRAK4-Akt pathways with subsequent ROS production [113]. HMGB1 is also a potent neutrophil chemotactic agent in its reduced form when in a heterocomplex with CXCL12 via CXCR4 signaling [114–116]. Hemorrhagic shock in mice causes HMGB1 levels to increase in plasma and lung tissue, with increased pulmonary sequestration of PMN and increased lung permeability observed [117]. Treatment with anti-HMGB1 antibodies prior to hemorrhage prevents neutrophil accumulation in the lungs and reduces lung permeability, demonstrating a role for HMGB1 in neutrophil-mediated acute lung injury following hemorrhagic shock. Similarly, the effects of HMGB1-mediated gut barrier dysfunction in another in vivo murine HS/R model could be abrogated with anti-HMGB1 antibody and showed a survival benefit, while a murine femur fracture model further demonstrated a critical role for HMGB1/TLR4 signaling in systemic inflammation and remote end-organ (liver) injury in trauma, presumably through neutrophil-mediated effector mechanisms [118, 119].

While primed and activated by DAMPs and inflammatory cytokines early following traumatic injury, the ability of neutrophils to continue responding appropriately to an insult by transmigrating to sites of injury or infection can be overwhelmed in states of ongoing systemic critical illness and injury with incipient immunosuppression. Underlying mechanisms of innate

immunodysfunction include reduced adhesion molecule expression secondary to upregulation of iNOS, HO-1, and PPARγ, and desensitization to future priming and activation in a mechanism that depends on GPCR phosphorylation, CXCR2 downregulation and desensitization by FPR1 and TLR2/TLR4 agonism, ongoing CXC stimulation (e.g., IL-8, GRO-α), and BLT1 (LTB_4 receptor) suppression amongst others [34, 50, 55, 120]. Neutrophils become "paralyzed" in such states, rendering them unable to adhere or efficiently chemotax towards sites of infection and injury with increased membrane rigidity and capillary sequestration [34, 49, 120]. In tandem, it has long been recognized that patients with severe trauma and sepsis develop suppressed adaptive immune responses including decreased antigen presentation, macrophage paralysis, diminished T-cell proliferation responses, increased lymphocyte and dendritic cell apoptosis, and a shift from TH1 to TH2 lymphocyte predominance [121]. While the original literature designated this response as a late "compensatory anti-inflammatory response syndrome" (CARS), this paradigm has been challenged recently with increasing evidence to suggest that the adaptive immunosuppressive response occurs both early and simultaneously with increased innate immune inflammatory responses, a notion rooted in decades-earlier observations that critically ill ICU/trauma patients often demonstrate anergy that is strongly associated with sepsis-related mortality [122, 123]. Genome-wide expression analysis of severe blunt trauma patient leukocytes has demonstrated early simultaneous increased expression of systemic inflammatory response, innate immune response, and compensatory anti-inflammatory response genes with concomitant suppression of adaptive immune genes [122]. In lieu of these and other similar observations, a new clinical entity termed "persistent inflammation, immunosuppression, and catabolism syndrome" (PICS) has been introduced that, in part, has resulted from improved surgical and critical care techniques with increased survival of patients early after trauma, sepsis, and organ failure [121]. Subsequent studies have seemingly validated this entity, with genomic analysis showing persistently elevated inflammatory gene expression and decreased adaptive immune gene expression in patients with complicated clinical trajectories following severe blunt trauma [124]. This gene expression data correlated with clinical evidence of persistent inflammation, immunosuppression, and protein depletion, supporting PICS as a predominant clinical phenotype in prolonged critical illness following trauma [124].

In summary, human DAMPs such as MTDs and HMGB1 offer numerous explanations for the "septic" phenotype observed in non-infected trauma victims, with many of their downstream effects being executed by neutrophils and the innate immune system through recognition of these host "danger" signals. At the same time, adaptive immunity is suppressed and patients are at high risk for infectious complications and poor long-term outcomes. DAMPs and their downstream targets, especially neutrophils, are likely an excellent therapeutic target for novel pharmaceutical interventions in trauma and TIC.

Summary Conclusions

In keeping with a focus on traumatic coagulopathy, one can envision from this brief review of neutrophil biology and its cross-talk with the coagulation system how a loss of regulation and negative feedback in neutrophil-mediated responses may play a critical role in propagating inflammation and coagulopathy following traumatic injury. Through DAMP release with subsequent priming, neutrophil activation with release of toxic ROS and proteases, and NETosis, the neutrophil provides an array of mechanisms by which the innate immune inflammatory response and coagulation system can communicate and lead to unintended endothelial, tissue, and end-organ injury. With further endothelial and tissue injury come additional demands on the coagulation system and further priming and activation of neutrophils. Without proper regulation and return to homeostasis, this positive feedback loop (Fig. 10.1) drives worsening coagulopathy and diffuse inflammatory injury that can have deadly consequences in trauma-induced coagulopathy.

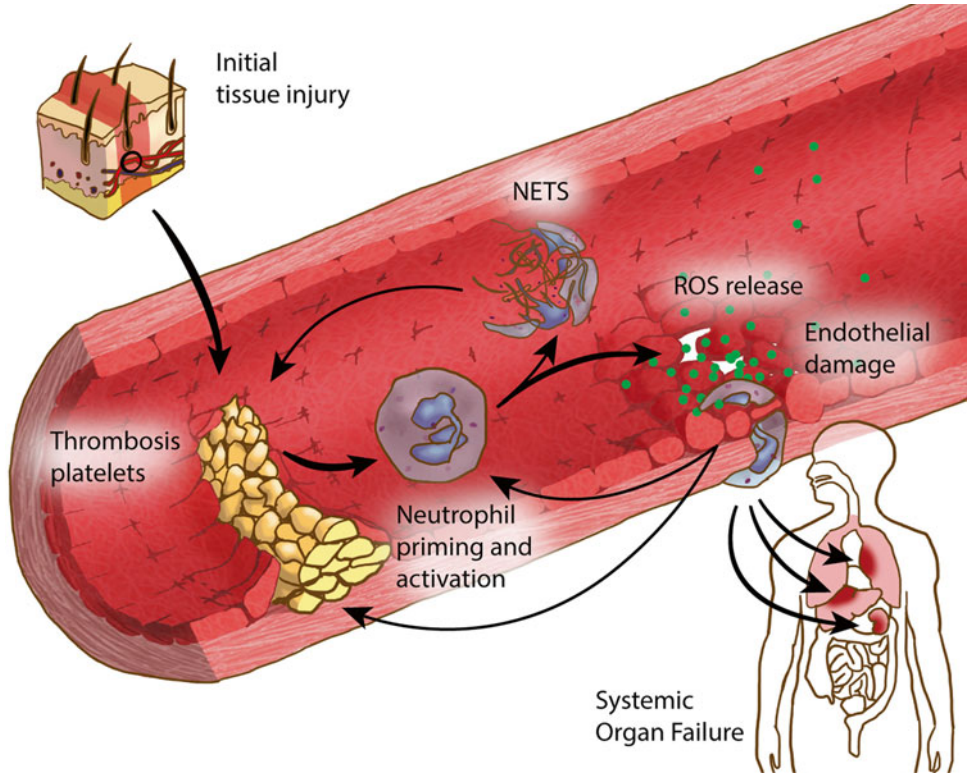

Fig. 10.1 Positive feedback loop between tissue injury, neutrophil activation, thrombosis, and endothelial damage in traumatic coagulopathy. See text for details. Illustration by Iris Fung, MIT Class of 2017

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DAMPs: Damage-Associated Molecular Pattern Molecules in Hemostasis

11

Charles T. Esmon

Damage-associated molecular pattern molecules (DAMPs) can initiate immune responses in non-infectious injury, making them particularly likely to be involved in trauma. In general, they can also trigger blood coagulation. DAMPs are generally intracellular components that are released in response to cell injury, cell activation, or cell death. They include several different classes of molecules. Those that are shown to participate in modulating coagulation are summarized in Table 11.1.

Simple Overview of Coagulation Control and the Links Between Thrombosis, Disseminated Intravascular Coagulation, and Coagulopathy

Many things modulate coagulation—activation of platelets, cellular injury leading to expression of negatively charged phospholipids on the outer leaflet of the membrane, induction of tissue factor mRNA, and function and suppression of natural anticoagulants. In addition, activation of neutrophils under appropriate conditions leads to the formation of extracellular traps that are intimately involved in clot formation.

Whether a coagulation trigger leads to occlusive thrombosis, disseminated intravascular coagulation (DIC), or a consumptive coagulopathy may relate to where the trigger is initiated and on which cells. Under normal circumstances, much of coagulation occurs on adherent platelets at the site of injury [19]. However, in experiments in which artificial membranes are infused (perhaps mimicking damaged cell debris), along with factor Xa (a potent procoagulant), the primary response is DIC and consumptive coagulopathy [20, 21]. A potential mechanism for generating nonadherent procoagulant membranes was suggested by the observation that histones, at the levels detected in trauma patients, lead to the expression of procoagulant phospholipids on red cells that support thrombin generation effectively [22]. On top of this, injury, especially to certain organs like the brain, leads to massive release of fibrinolytic agents such as tissue plasminogen activator that disrupt the clot structure, inactivate coagulation factors and may aid in the generation of coagulopathy [23–27]. Shared features of all of tissue injury are the induction of procoagulant enzymes, cellular activation/death and in the case of thrombosis/hemostasis, normally a localized area of injury.

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Table 11.1 Examples of DAMPs shown to participate in modulating coagulation

DAMPs	References
Histones	[1–5]
HMGB1	[6–9]
Polyphosphates	[10–14]
DNA	[15, 16]
RNA	[17]
Small molecules like ATP	[18]

HMGB1 high mobility group box 1

Inflammatory cytokines such as tumor necrosis factor-α, interleukin 1-β, and interleukin 6 are often elicited by various DAMPs [3, 7, 16, 28–30]. In turn, these cytokines elevate tissue factor levels, stimulating coagulation, impair anticoagulant mechanisms, particularly impairing the microvascular circulation, and usually modulate the fibrinolytic system through elevation of plasminogen activator inhibitor 1 [31–34]. Considering these links, inflammation induced by trauma is almost certain to contribute to coagulation abnormalities in patients ranging from consumptive coagulopathy to thrombosis and contributing to organ failure.

This chapter focuses on the DAMPs that are most likely to have major impacts on coagulation.

Probable Role of DAMPs in Trauma

Sterile trauma initiates a potent proinflammatory response [25]. Histones and high mobility group box 1 protein, HMGB1, are known to induce inflammatory cytokines like TNF [8, 9]. Histones, particularly histone H4, interact with toll-like receptors (TLR) 2, 4, and 9, inducing cytokine elaboration and tissue injury including cell death [2, 3, 16]. The histones also induce a major calcium flux that contributes to cell activation and death [2].

HMGB1 and Histones

HMGB1 triggers inflammatory processes by interacting with several receptors including TLR2, TLR4, and RAGE (receptor for advanced glycation endproducts) [35, 36].

Particularly relevant to severe trauma, the levels of extracellular histones rise higher in trauma than any other disease studied. In several other clinical conditions, it has been shown that inhibition of histone toxicity improves outcome, at least in mice [2–3, 16]. The histone levels seen in trauma patients are sufficient to initiate coagulation leading to a DIC-like response [2, 37]. In mice injected with lipopolysaccharide (LPS), a model of acute inflammation, coagulation is triggered leading to DIC and a coagulopathy in some respects similar to what is seen in trauma. One means of measuring the extent of coagulation is determining the level of the thrombin–antithrombin complexes in the plasma. Antibodies to histones prevent death from the LPS treatment and also reduce the circulating thrombin–antithrombin complex levels to approximately 50 % of the LPS-treated animals without histone blockade.

Histones and nucleosomes (DNA-histone fragments of chromatin) activate platelets and potentiate coagulation further [4, 11]. Histones bind to polyphosphate secreted from the activated platelet and directly facilitate thrombin formation through a pathway dependent on factor XI but not on factor XII or tissue factor [11]. Plasma experiments suggest that the histones make platelets about 20 times more procoagulant than other agonists [11].

In addition to stimulating thrombin formation and platelet activation the histones shut down a key anticoagulant mechanism, the protein C anticoagulant pathway, by binding to thrombomodulin, the trigger for the pathway, and inhibiting the necessary binding of thrombin to thrombomodulin [1]. This is likely

particularly relevant in the microcirculation where this pathway is critical in preventing microvascular thrombosis, an event that could contribute to organ failure [38]. Particularly relevant, Cheng Hock-Toh's laboratory has shown that the circulating histone levels in the plasma of the trauma patient are sufficient to kill endothelium [37]. Consistent with earlier results [2], they also showed that these levels could induce lung injury in mice and that the elevation of histone levels positively correlated in patients with lung failure [37].

The mechanisms of HMGB1 and histone modulation of blood coagulation are not restricted to the mechanisms above. It is well known that the inflammatory cytokines like interleukin 1 and TNF can induce tissue factor expression on monocytes and thereby initiate a potent stimulus to coagulation [39]. Therefore, both of the nuclear components would aid in stimulating an uncontrolled DIC-like state.

Polyphosphate

Polyphosphate is a simple organic molecule composed of hundreds of covalently linked phosphate molecules. Polyphosphate is found in bacteria and also in platelet dense granules and mast cells. The bacterial polyphosphate is larger than the platelet polyphosphate which is relevant to coagulation since larger polyphosphates initiate the activation of factor XII more effectively [40]. The polyphosphate released during platelet activation binds histones and this complex is orders of magnitude more potent than simple platelet activation alone [11]. The mechanisms of the histone–polyphosphate participation in coagulation remain unclear but it likely involves factor XI. The stimulation of coagulation in platelet-rich plasma does not appear to require either factor XII or tissue factor suggesting a unique pathway to clot formation [11].

DNA and RNA

Extracellular DNA released from activated neutrophils or damaged cells activate TLR9 which in turn leads to inflammatory responses such as TNFα and interleukin 6 formation. In hypoxia and organ transplant, this can contribute to cell death [5].

ATP

ATP released from damaged cells has the potential to initiate a DIC-like response by binding to the P2X7 receptor leading to the generation of tissue factor [18] that occurs in conjunction with microparticle release. Depending on the adhesion receptors on the microparticles, they could either contribute to consumptive coagulopathy or thrombosis.

Summary

The functions of the DAMPs in coagulopathy are illustrated in Fig. 11.1. Most DAMPs induce cytokine formation through toll-like receptors or other surface receptors. These in turn activate tissue factor gene transcription and tissue factor triggers coagulation responses. ATP released from damaged cells activates tissue factor gene transcription directly. Histones released either as neutrophil extracellular traps [41] or as nucleosomes from broken cells activate platelets and generate potent procoagulant responses by interacting with polyphosphate. Histones also induce major calcium influx into endothelium and kill endothelium. The calcium influx likely leads to vasodilation [42, 43].

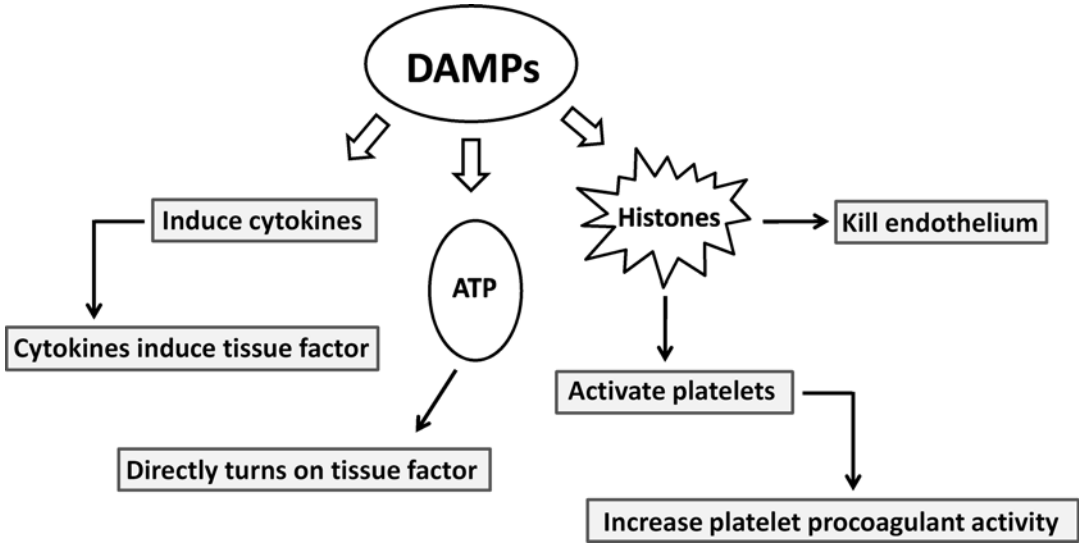

Fig. 11.1 DAMPs consist of many different molecules, several of which that are most directly involved in modulating coagulation are derived from nucleus of the cell. The other components are described in the text. The scheme also depicts the ability of cytokines to modulate coagulation. Taken together various DAMPs modulate platelet function and fibrinolysis

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The Complement System and Coagulation

12

Narcis I. Popescu and Florea Lupu

Introduction

One of the biggest challenges faced by multicellular organisms is the invasion of foreign pathogens; hence complex mechanisms that recognize, contain, and clear infectious agents have been evolutionarily developed. The response to invading pathogens is highly orchestrated and involves multiple immune, coagulation, and inflammatory pathways. Depending on the localization and the strength of the detected infectious signal, one or more of these pathways are sequentially initiated. In a simplified view, soluble or cellular receptors of the immune system are responsible for the recognition of the foreign agents, then the coagulation system isolates the threat from systemic distribution, and molecular and cellular immune effectors destroy and clear the pathogens. In addition, inflammatory mediators are responsible for fine quantitative tuning of the immune and coagulation responses to the level of pathogen threat, and ultimately initiate the healing events after the clearance of infectious agents. Central to this physiologic response is a network of blood serine proteases grouped into several proteolytic cascades—the complement, coagulation, and fibrinolytic systems—which share a similar overall architecture and have developed from a common ancestral pathway. For an optimal response to foreign agents, fine integration of these cascades needs to be achieved through multiple cross-talk mechanisms. Dysregulation of these networks, where the explosive potential of the proteolytic cascades is redirected towards self-structures, contributes to the pathophysiology of human diseases that have a strong inflammatory component. In this section we review the architecture of the complement system, the interactions with the coagulation pathway, and its role in hemostasis and development of prothrombotic human diseases.

Complement Organization and Function

The complement system is one of the earliest immune mechanisms developed during the evolution of animal kingdom [1, 2]. Its primary role is the rapid identification and clearance of microbial intruders [3, 4]. As shown in Fig. 12.1, complement system includes a large number of components, both soluble and cellular effectors, and an array of regulatory proteins that modulate the activation and propagation of the complement reactions [5]. Three distinct pathways of complement activation have been characterized to date: classical, alternative, and lectin pathways

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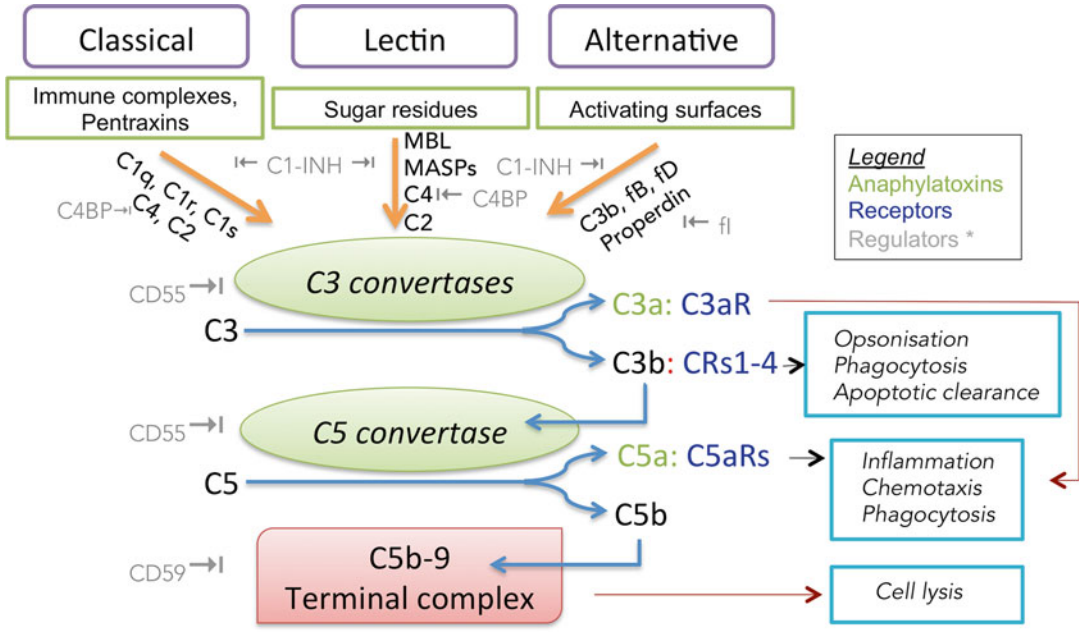

Fig. 12.1 Molecular pathways and mechanisms controlling complement activation and its physiological and pathological effects

(CP, AP, and LP, respectively). All three pathways share a similar overall architecture. Preformed complement components, present as zymogens, are activated in response to initiating events to generate effector serine proteases that cleave and activate other downstream proteases. This evolutionary design of cascaded biochemical reactions allows a fast response to intruding pathogens without the need of de novo synthesis. Moreover, this permits an explosive amplification of the signal since any upstream enzyme can proteolytically cleave multiple downstream zymogen substrates, which become proteases themselves. The proteolytic cascade underlying blood coagulation shares a similar design and is detailed in another chapter of this book. The three activation pathways converge at distinct points in the complement cascade, ultimately leading to the deposition of C5b on pathogenic surfaces. C5b deposition initiates the terminal complement pathway, the sequential formation of a multimeric membrane-spanning complex with cytolytic antimicrobial function. In contrast to the activating pathways, the terminal pathway does not require proteolysis of complement components. Additionally, small peptide fragments released during the proteolytic activation of complement components interact with cell-associated receptors and initiate downstream signaling events that modulate the immune and inflammatory responses. The activation and propagation of the complement responses are tightly controlled by multiple soluble and cell-associated regulators, which ensure that the complement is primed to act on intruding pathogens but does not attack self-structures.

Analysis of complement deficiencies in human diseases [6] revealed that the primary role of the complement system is the rapid recognition and removal of intruding pathogens. In addition, targeted manipulations of the complement system in experimental models, genetic associative studies, and, more recently, the use of selective inhibitors against complement effectors have revealed new and sometimes unexpected roles for the complement system. As such, the complement components play a direct role in the phagocytosis of opsonized pathogens by macrophages and dendritic cells [7]; initiate and direct adaptive immune responses [4]; contribute to the nonin-

flammatory clearance of apoptotic cells and the removal of antibodies against self-antigens [8]; and shape the natural antibody repertoire [9]. In tantalizing recent developments, the complement's potential to clear cellular processes has been harnessed for synaptic remodeling and maintenance of homeostasis within the central nervous system [10, 11]. Conversely, excessive complement activation can have deleterious systemic effects associated with increasing numbers of human pathologies with strong inflammatory components, such as autoimmune diseases [12], sepsis [13], and thrombotic microangiopathies [14], among others.

Complement Activation Pathways

The complement system (Fig. 12.1) can be activated through three distinct pathways: classical, lectin, and alternative pathway [15]. All three pathways contribute to an optimal complement response, although they recognize different pathogen-associated molecular patterns (PAMPs), are initiated by distinct pattern recognition molecules (PRMs) or structures, and use different molecular components. Furthermore, the same PAMPs can activate multiple PRMs, leading to redundant activation of complement pathways. The three activation pathways intersect at distinct points throughout the proteolytic cascade, either the C3 or C5 convertase complexes, and elicit similar downstream biologic effects.

The classical pathway of complement activation, albeit being the first characterized, is probably the newest developed evolutionarily [1] and takes advantage of the antibody recognition of foreign antigens. Multimeric IgM immunoglobulins, or clusters of IgG immunoglobulins, interact with the C1 complement component and initiate complement activation. C1 is a pentameric complex containing one C1q subunit and two each of the C1r and C1s subunits. C1 interaction with immunoglobulin-antigen complexes induces a conformational change in the C1q PRM, followed by C1r autoactivation and C1s transactivation [16]. C1s is the effector protease of the classical pathway initiating complex, and cleaves the complement protein C4 into the C4a and C4b fragments. C4b has enhanced reactivity and binds to surfaces in the vicinity of the complement activation site leading to their opsonization. The complement protein C2 binds C4b and is cleaved by C1s, generating the classical pathway C3 convertase complex C4b2a. C4b2a localizes the proteolytic function of the C2a subunit to surfaces opsonized by C4b. This C3 convertase subsequently cleaves complement protein C3, which is responsible for amplification and downstream effects of the complement activation.

The classical pathway can also be initiated by antibody-independent mechanisms induced by acute-phase PRMs such as the pentraxins PTX3, C-reactive protein (CRP), and serum amyloid P (SAP) bound to specific PAMPs [17]. Upon interaction with C1, they induce a conformational change in C1q that supports activation of C1r and C1s, subsequent proteolytic cleavage of C4 and C2, and generation of the C3 convertase C4b2a.

The lectin pathway of complement activation is initiated by carbohydrate structures on the pathogen surface or acetylated patterns from damaged self. These PAMPs are recognized by PRMs from the collectin family, such as mannose-binding lectin (MBL) [18], ficolins (ficolin-1, -2, and/or -3) [19], and other collectins (CL-L1, CL-K1, CL-P1) [20], usually found in complex with three serine proteases, termed MBL-associated serine proteases (MASP-1, -2, and -3). Similar to the classical pathway, PAMP recognition by collectin PRMs leads to autoactivation of MASP-1, and/or the alternatively spliced isoform MASP-3 [21], which in turn transactivates MASP-2 [22]. MASP-2 is the critical protease from the lectin pathway-initiating complex since it is the only one that can cleave C4 required for the C3 convertase complex [23]. C2 bound to C4b at the site of activation is cleaved by both MASP-1 and MASP-2 [24]. This generates the C4b2a complex, identical to the C3 convertase initiated by the classical pathway.

The alternative pathway is evolutionary the oldest and the least specific pathway of complement activation. The alternative pathway is thought to occur via a tick-over mechanism in

which C3b is constantly generated in plasma in sufficient amounts to interact with factor B (FB) and generate a fluid-phase C3 convertase [25]. The initial C3b can be formed through multiple mechanisms such as spontaneous hydrolysis of C3 [26, 27], via C3 convertases formed by the other two complement-activating pathways, or by vascular proteases such as coagulation proteases FIIa, FIXa, FXa, and FXIa, and plasmin [28, 29]. FB then binds C3b and is activated by the serine protease factor D (FD) resulting in the formation of the alternative pathway-initiating complex C3bBb, which retains proteolytic activity towards C3. This initiating complex is stabilized on activating surfaces by properdin (P), and forms the C3 convertase C3bBbP. Through a positive feedback amplification more C3b is generated which opsonizes the target surface, can form new alternative pathway-initiating complexes with FB, or can attach to the C3bBb and form the C3bBb3b convertase with proteolytic activity towards C5. It has been estimated that the alternative pathway amplification, and implicitly the C3bBb3b C5 convertase, accounts for the overwhelming majority of C5 activation even when the complement cascade is initiated by the classical or lectin pathways [30].

Regardless of the molecular pathway involved, activation of the complement system leads to the proteolytic cleavage of C3 and generation of two biologically active fragments, C3a and C3b. C3a, a small 77 residue polypeptide, is one of the complement anaphylatoxins that modulates downstream immune and inflammatory responses through engagement of C3a receptors (C3aR) present on multiple cells [31]. C3b is the larger fragment released by C3 cleavage and functions as an opsonin, tagging molecular structures for subsequent immune response and clearance. During C3 activation, a highly reactive thioester bond is exposed on the C3b fragment, which allows the covalent attachment to vicinal proteins and carbohydrates through ester or amide linkages [32]. In contrast to native C3, C3b also exposes binding sites for other complement components: FB and properdin, which contribute to the generation of the alternative pathway C3 convertase in the presence of FD; complement protein C5, critical for downstream terminal complement pathway; cellular complement receptors important for opsonin-induced phagocytosis; and negative regulators (the protease factor I, FI) and cofactor proteins (factor H, FH; membrane cofactor protein, MCP; decay-accelerating factor, DAF) that inactivate C3b. Despite showing enhanced specificity for binding to carbohydrates, C4b and IgG [32], C3b cannot discriminate between self and non-self structures and can opsonize host cells as well. The local balance between negative regulators (FI, DAF) and downstream effectors (C5, FB, P) controls the outcome of C3b deposition, resulting in either amplification or inactivation of the complement cascade. Host cells express cofactors on their surface, which enhance C3b proteolytic inactivation by FI, and thus are normally protected from complement attack. On pathogen surfaces however, C3b inactivation is slower in the absence of cofactors, which leads to local amplification of the complement cascade and further deposition of C3b on the multimeric C3 convertases. The resulting convertase complexes, either C4b2a3b or C3bBb3b, shift their substrate preference towards C5 conversion, and initiate the terminal complement pathway.

Terminal Complement Pathway

The proteolytic cleavage of C5 is the first step of the terminal effector pathway responsible for the generation of the terminal complement complex on microbial surfaces. Proteolytic activation of C5 generates a small anaphylatoxin, C5a, and the larger fragment C5b, which initiates the formation of the terminal complement complex (TCC, or membrane attack complex, MAC). C5b generated at activation sites sequentially binds to complement proteins C6 and C7, forming a stable C5b-7 trimer that exposes lipid-binding sites through which the complex inserts itself into membranes. C5b-7 recruits complement protein C8 and generates the C5b-8 tetramer, which in turn promotes binding and polymerization of multiple C9 subunits into a tubular structure forming the C5b-9 complex. C5b-9 is the TCC,

or MAC, responsible for the cytolytic activity of the complement [15, 33].

Similar to C3b deposition, the TCC intermediaries, C5b-7 to C5b-9, do not discriminate between self and non-self surfaces and have the potential to bind to host cells as well. C5 can also be proteolytically cleaved by vascular proteases such as thrombin at sites of vascular injury, leading to the formation of the C5a anaphylatoxin and fluid phase C5b-7/8/9. Multiple mechanisms have evolved to protect against damaging complement attacks on host cells, such as the expression of cell surface CD59 [34, 35] and/or the interaction of soluble C5b-9 (sC5b-9) with circulating vitronectin or clusterin which blocks its membrane insertion. Nonetheless, non-lytic concentrations of C5b-7/8/9 are able to activate endothelial cells, lymphocytes, and smooth muscle cells [36], and thus contribute to an integrated complement-coagulation response at sites of injury, as detailed later in this section.

Complement Receptors

Complement activation exerts multiple downstream biological effects, not only through its terminal effector arm, the cytolytic C5b-9 complex, but also through complement receptors that engage intermediaries generated throughout the cascade. There are two main groups of complement receptors: opsonin receptors, which recognize C4b and C3b opsonins and their proteolytically inactivated products, and anaphylatoxin receptors.

The opsonin receptors, such as complement receptors CR1, CR2, CR3, CR4, the complement receptor of immunoglobulin family (CRIg), and C1q receptors [3, 5, 37], usually promote phagocytosis of complement-opsonized structures. On specialized cells these receptors induce complementary signaling events that contribute to the adaptive immunity and/or inflammatory response. CR1 (CD35) is widely expressed and versatile, recognizing all major complement opsonins, C1q, C3b, and C4b [38], as well as their inactivated forms iC3b and iC4b. CR1 is exposed on the surface of erythrocytes, neutrophils, monocyte/macrophages, and subsets of B and T lymphocytes and follicular dendritic cells (FDCs) [5]. On professional phagocytes, CR1 stimulates phagocytosis of opsonized structures, while on erythrocytes CR1 is critical for the clearance of circulating immune complexes [39]. CR1 is a negative regulator of complement convertases on host cells, and also functions as a cofactor for FI-mediated proteolytic inactivation of C3b and C4b [40]. Furthermore, CR1 modulation of CR2-dependent signaling events in B lymphocytes and FDCs [41] is critical for optimal adaptive immune responses [42].

CR2 (CD21) is a complement receptor expressed mainly on immune cells such as B lymphocytes, FDCs, thymocytes, and some peripheral T cells [5]. CR2 binds C3b and the proteolytically inactivated forms iC3b, C3dg, and C3d on opsonized surfaces and regulate downstream adaptive immune responses. In addition, CR2-mediated events play a crucial role for the clonal selection of B-1 lymphocytes and development of the natural antibody repertoire, regulation of B cell tolerance, and maintenance of long-term B cell memory [4, 43, 44].

CR3 (CD11b/CD18) and CR4 (CD11c/CD18) are complement receptors from the β2 integrin family present mainly on neutrophils, NK cells, monocytes, activated T lymphocytes, and specialized liver macrophage Kupffer cells [45]. CR3 and CR4 enhance phagocytosis of iC3b-opsonized particles [3, 5]. These β2 integrin receptors also have complement-independent functions in leukocyte interaction with vascular cells and integrin-mediated signaling. The complement receptor of the immunoglobulin family (CRIg) is expressed by Kupffer cells; interacts with C3b, iC3b, and C3c fragments; inhibits AP C3/C5 convertase activity; and plays a major role in the clearance of C3-opsonized particles [46, 47].

The anaphylatoxin receptors recognize small peptides released during complement activation, namely C3a, C4a, and C5a [31]. Three receptors belonging to this class have been characterized so far: the C3a receptor (C3aR) and two C5a receptors, C5aR1 (C5aR, CD88) and C5aR2 (C5L2) [37]. To date, no specific receptor for C4a has

been identified, although in experimental models, but not humans, C4a binds to C3aR [48]. C3aR and C5aR1 receptors are G-protein-coupled receptors (GPCR) expressed by a wide variety of cells. C5aR2 has a similar structure with C5aR1, is usually co-expressed with C5aR1, but is uncoupled from cytosolic G-proteins. The function of C5aR2 is not yet clear but it could be a modulator of C5a-dependent signaling events [31, 49, 50].

Anaphylatoxin receptors have pleiotropic proinflammatory effects. Anaphylatoxins are strong chemoattractants for monocytes/macrophages, neutrophils, activated B and T lymphocytes, basophils, and mast cells [50]. They are powerful vasodilators of small blood vessels and promote leukocyte adhesion to endothelium, as well as immune cell recruitment and cell migration towards sites of infection, where they trigger oxidative burst in macrophages, neutrophils and eosinophils, degranulation of neutrophils, and release of soluble mediators from basophils, mast cells, and platelets [31, 49, 50]. In addition, anaphylatoxin receptors initiate multiple cell signaling pathways, including PI3k/Akt, MAP kinase, phospholipase C, and NFkB pathways, inducing a proinflammatory transcriptome and production of multiple chemokines and cytokines such as TNFα, IL-1β, IL-6 among others [49]. Excessive C5aR signaling however can have deleterious effects, such as paralysis of neutrophil immune functions [28], thymocyte apoptosis [51], and/or induction of consumptive coagulopathy [52, 53].

Complement Regulation

Similar to other proteolytic cascade systems, such as the coagulation, the optimal complement response is maintained through a fine balance between activators and inhibitors of the complement system. This design ensures that the complement cascade is primed to react to invading pathogens while limiting its deleterious effects on host cells, which are usually protected through surface expression of negative complement regulators. Complement regulation acts at multiple steps throughout the complement cascade, and includes inhibitors of complement activation and/or propagation convertases, proteases that inactivate complement effectors, cofactors, and allosteric modulators, as well as clearance receptors [3–5]. Sometimes the same regulator can act through multiple mechanisms, such as the CR1 complement receptor discussed above, which is a competitive inhibitor of complement convertases, an allosteric cofactor for proteolytic inactivation of opsonins, and a clearance receptor of opsonized surfaces.

Initiation of the complement cascade is maintained silent by the C1 esterase inhibitor (C1-INH), an abundant vascular serpin inhibitor of proteases from both CP- and LP-initiating complexes. C1-INH has a broad spectrum towards vascular serine proteases, inhibiting also coagulation and fibrinolytic enzymes such as FXIIa, FXIa, kallikrein, and plasmin, and concomitantly regulating multiple hematologic responses [54]. If the complement cascade progresses, the C3b and C4b opsonins are proteolytically cleaved by complement FI, the main protease that inactivates complement cascade progression. FI cleaves C3b and C4b opsonins either in solution or on activated surfaces, and its enzymatic activity can be accelerated by soluble and cell surface cofactors. Complement factor H (FH) is a soluble decay accelerator of the AP C3 convertase (C3bBb) and a cofactor of FI-mediated proteolysis of C3b, while C4BP, C4b-binding protein, is a soluble decay accelerator of the CP/LP C3 convertases and a cofactor of FI-mediated proteolysis of C4b. FH and C4BP protective function is also concentrated on host cell surface through specific interactions with carbohydrate structures such as sialic acid and glycosaminoglycans. In addition, complement receptors (CR1, CR2, C2 receptor inhibitor trispanning (CRIT)), DAF (CD55), and the cell surface cofactor MCP (CD46) can bind C2, C3b, and/or C4b, restricting their availability for the formation of C3/C5 convertases and enhancing FI-mediated opsonin proteolysis on the surface of host cells. Furthermore, the surface-exposed CD59 blocks C5b-9 formation on

host cells, while vitronectin and clusterin bind fluid-phase C5b-9 and prevent its insertion into host cells. Finally, circulating carboxypeptidases convert complement anaphylatoxins into their desarginated forms (C3a-desArg and C5a-desArg), which either impair or shift the signaling response mediated by C3aR/C5aR1 receptors [31].

Integrated Hematologic Response to Injury

Vascular trauma, defined as the disturbance of vascular homeostasis, could be initiated by multiple types of injuries: infections, biomechanical injuries, and/or exacerbated inflammation (Fig. 12.2). Despite their initiating diversity, they are all resolved through similar effector pathways involving immune (complement), hemostatic (coagulation/fibrinolysis), and inflammatory responses. Due to their concerted action, these pathways are closely intertwined and are co-regulated by common molecular mechanisms. Together they form a dynamic integrated response to injury aimed at stabilizing the damaged site, resolving the injury, and repairing the organism. While here we refer to vascular disturbances for ease of exemplification, extravascular injuries also require a hematologic response for the recruitment of molecular and cellular immune effectors, their transport across the vascular wall, and the coordinated protection against the loss of vessel integrity at extravasation sites.

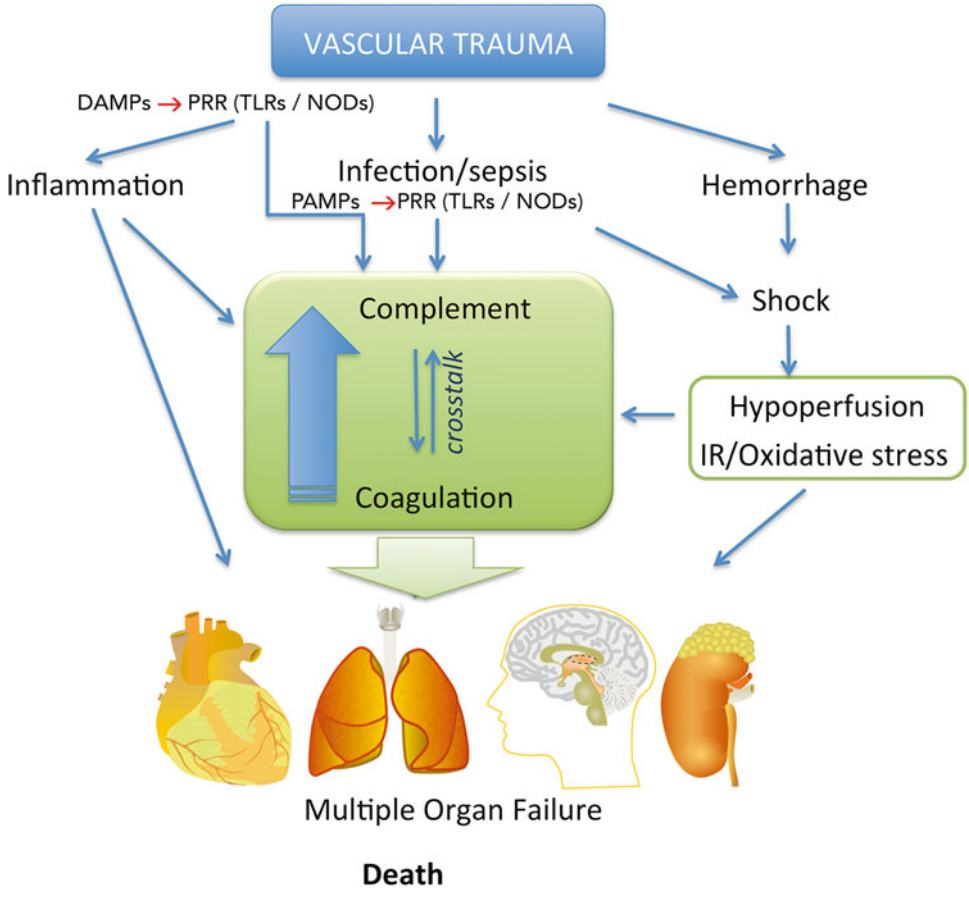

Fig. 12.2 Role of the complement-coagulation cross talk in the pathophysiology of sepsis and trauma

The optimal hematologic response has several characteristics: (1) it is designed to *act locally* at sites of injury; (2) it is *discrete* and *progressive*; and (3) it is *reversible*. The first two characteristics are a direct consequence of the abundance of negative regulators/inhibitors of the initiation pathways; the reversibility of the hematologic response is ensured by balancing proteolytic cascades (fibrinolysis) and/or tissue-remodeling pathways, and is needed to restore the functionality of the organism. The hematologic response is initiated either by the complement system at sites of infection or by the coagulation system at sites of vessel wall damage. At these sites, the presence of PAMPs, or danger-associated molecular patterns (DAMPs), enhances the proteolytic activity of initiating proteases and tips the balance towards initiation/amplification of the hematologic response. The proteolytic response is maintained for as long as the initiation/amplification factors exceed the counteracting effect of the inhibitors; hence the response is *discrete* and does not use all components present systemically. If enough PAMPs and/or DAMPs are detected, the proteolytic cascade *progresses* to the amplification phase, which further recruits molecular and cellular effectors and engages complementary response systems, such as inflammation. After the infection and/or the vessel damage is resolved, remodeling mechanisms, such as the fibrinolytic system, are activated and *reverse* the affected areas to their prior functionality. At the same time, clearance of DAMPs allows the intrinsic inhibitors to *reverse* the balance towards repression of the proteolytic potential of the hematologic response. In contrast, when the hematologic response looses one or more of its characteristics, such as systemic and/or exacerbated activation due to failure of regulatory mechanisms, the explosive proteolytic potential can have damaging effects on the host, leading to multiple organ failure and death. In this section we focus on the interplay between the proteolytic cascades that usually initiate the hematologic response to injury, namely the cross talk between the complement system and coagulation.

Architectural Similarities Between the Coagulation and Complement Systems

The coagulation system is detailed in another chapter of this book; therefore we only highlight its similarities with the complement here. The coagulation system is closely related to the immune proteolytic system (complement) evolutionarily, architecturally, and functionally, and they are intimately connected during the initiation of the hematologic response. Evolutionary analysis revealed that the coagulation cascade has derived from a proto-immune proteolytic system, similar to the complement system, through acquisition of unique structures and specialized functions [55]. Molecular analysis of the invertebrate immune system from the *Limulus* species revealed a proteolytic system with both opsonic (complement) and clotting (coagulation) functions [56, 57]. This is thought to represent the ancestral proteolytic cascade before the divergence of the two systems. Interestingly, the current FDA-approved test for endotoxin/pyrogen detection (Limulus amoebocyte lysate, LAL, test) takes advantage of this dual functionality. In the LAL test, lysates of Limulus hematocytes (amoebocytes) containing the proto-immune cascade are incubated with the test sample, whereby the endotoxin initiates the lectin complement pathway and leads to opsonization as well as clot formation, which is the endpoint readout of the assay.

Analogous to the complement system, coagulation is organized as a cascade of serine proteases that are present in the vasculature in zymogen form. Coagulation can be initiated by the intrinsic or contact pathway, or the extrinsic, TF-dependent pathway. Similar to complement activation, the contact pathway is initiated by the conformational autoactivation of factor XII on exogenous surfaces [58], such as negatively charged polyanions [59] and/or microbial surfaces [60]. In contrast, the extrinsic pathway is initiated upon exposure of a danger-associated molecule, tissue factor (TF), to circulating factor VII(a) [61]. TF is the main physiologic initiator

of blood coagulation and is normally restricted from exposure to blood. It is constitutively expressed in mural and adventitial layers of the vessel wall; thus exposure of TF to circulating coagulation factors is usually associated with the loss of vessel wall (endothelial) integrity. Due to its critical role in blood clotting, TF is an important node for the functional convergence of complement and coagulation systems [13, 62, 63], and inflammatory mediators regulate its vascular expression, as detailed below.

Similar to the complement system, the two blood clotting pathways converge on the activation of factor Xa (FXa), which, in the presence of cofactor Va (FVa), generates thrombin (FIIa) through proteolytic cleavage of zymogen prothrombin. Thrombin is the final effector protease of the clotting cascade, which converts soluble fibrinogen into insoluble fibrin that provides the matrix for blood clots. Both FXa and thrombin are involved in positive feedback amplification of the clotting cascade as well as molecular communication with the complement system through C3 and C5 activation, and inflammatory responses through engagement of protease-activated receptors (PARs) in the vasculature.

Like the complement cascade, the proteolytic potential of the coagulation cascade is repressed by multiple regulatory mechanisms. Coagulation only progresses if the local activator signals overcome the negative regulators. C1-INH, the circulating inhibitor of the classical and lectin complement activation pathway, also controls the initiation of the contact clotting pathway. Thus C1-INH represents a direct molecular bridge between the complement and coagulation cascades, ensuring their concomitant regulation. The initiation of blood clotting through the extrinsic pathway is controlled by tissue factor pathway inhibitor (TFPI), a serpin present on endothelial cells, and activated platelets, the main cellular entities associated with the propagation of the clotting cascade [64]. TFPI, similar to surface-associated complement regulatory proteins, ensures that coagulation is not initiated on undisturbed host cells. Antithrombin III (ATIII) is the main soluble inhibitor of the amplification/termination phase of blood coagulation. While considered to be the main thrombin inhibitor, ATIII exhibits broad substrate specificity, inhibiting upstream coagulation factors (FIXa, FXa, FXIa, FXIIa) [65, 66] as well as MASP-1/2 proteases of the lectin pathway of complement activation [67]. Other regulators of blood coagulation, such as the protein C (PC)—thrombomodulin (TM) axis, are discussed in detail in other chapters of this book.

Cross Talk Between the Complement and Coagulation Cascades

The molecular interactions between the complement and coagulation cascades are multiple and complex, and the cross talk can either be direct, whereby components of one cascade interact with members of the complementary system, or indirect, through engagement of inflammatory responses and cellular mediators. Inflammatory responses seem to be the preferred communication conduit for the integration of hematologic responses to vascular trauma, controlling the level of activation as well as the progression of the response.

Complement system activation induces a prothrombotic environment by augmenting the procoagulant function of clotting factors or decreasing the anticoagulant regulatory mechanisms. Some of the underlying molecular mechanisms are highlighted in Fig. 12.3. At sites of infection, activation of complement initiating proteases from the classical and/or lectin pathways primes a clotting response through the direct engagement of clotting factors or recruitment and activation of common cellular effectors, such as monocytes, platelets, and endothelial cells. For example, MASP-1, activated on microbial surfaces by an MBL-dependent mechanism, promotes a local prothrombotic environment through activation of prothrombin [68]. The resulting thrombin can prime the coagulation cascade through activation of cofactors (FV and FVIII) and activates surrounding vascular cells through PAR signaling. In addition, MASP-1-

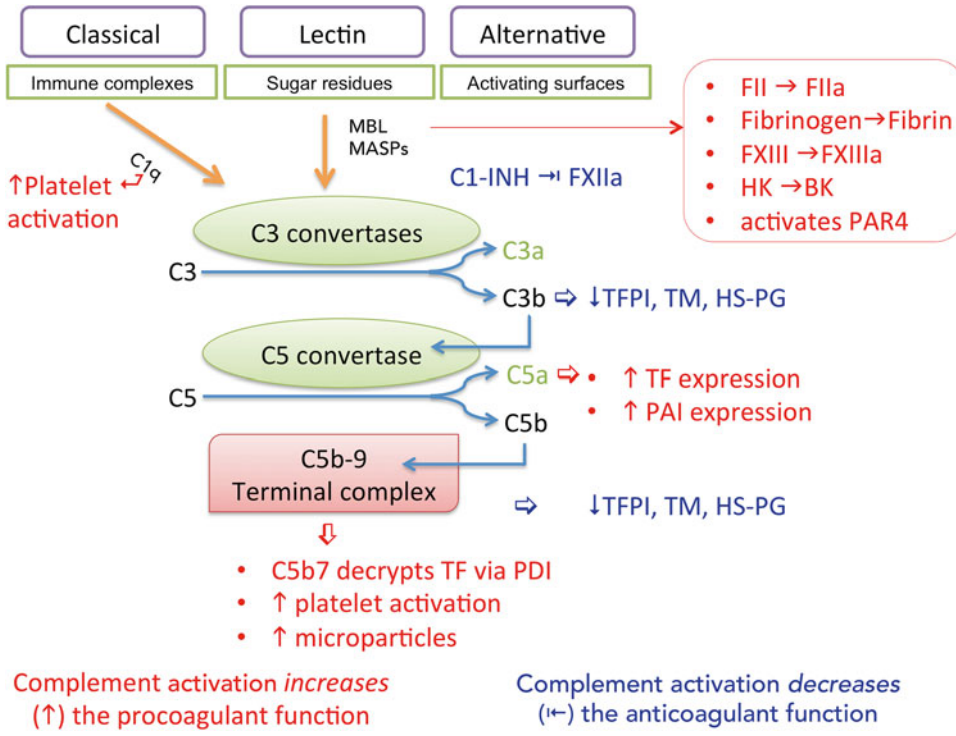

Fig. 12.3 Interactions between complement proteins and the hemostatic system

mediated proteolysis of thrombin substrates, such as plasma transglutaminase factor XIII (FXIII), fibrinogen, and thrombin-activatable fibrinolysis inhibitor (TAFI), could influence the stability and the structure of the clot formed at sites of infection [24, 68, 69]. Activated FXIII (FXIIIa) enhances the cross-linking of fibrin fibrils within the clot [70], while TAFI inhibits fibrin-dependent fibrinolytic events initiated by tissue plasminogen activator (t-PA) [21]. MASP-1 does not cleave fibrinogen at similar sites as thrombin [69], and as a result it does not generate fibrin clots directly; however, MASP-1 proteolysis of fibrinogen releases the chemotactic fibrinopeptide B, which recruits cellular immune effectors without the pleiotropic effects of the proinflammatory anaphylatoxins released by complement [71]. In addition, MASP-1 can activate PAR4 on endothelial cells, leading to a distinct proinflammatory phenotype that favors recruitment of neutrophils over monocytes at the site of infection [72, 73]. Furthermore, MASP-1 activation of the kinin-kallikrein system can promote clotting through the contact pathway and induce proinflammatory effects through generation of bradykinin (BK).

Complement effectors induce pleiotropic proinflammatory responses in vascular cells that emphasize the production of prothrombotic factors (TF, PAI-1) and reduction in regulatory molecules (TFPI, TM, heparan sulfate proteoglycans, HS-PG) [74]. Since the most explosive clotting reactions are initiated by TF, amplification of the complement cascade at sites of microbial injury also targets transcriptional activation of TF on monocytes [75], neutrophils [76], and possibly platelets [77, 78] and endothelium [79]. Complement induction of TF is mediated by the potent C3a and C5a anaphylatoxins, which, either alone or in conjunction with Toll-like receptors (TLRs) [80], activate multiple downstream signaling events leading to activation of the NFkB pathway and subsequent cytokine and TF production. Complement-induced proinflam-

matory cytokines (TNFα, IL-6, IL-1β, CD40L, and others) can, in turn, sustain TF transcriptional activation on vascular cells [81]. The procoagulant activity of TF exposed on vascular surfaces can be further enhanced by complement effectors, such as the TCC intermediate C5b-7 or the C5b-9 membrane attack complex, through exposure of anionic phospholipids or PDI-dependent decrypting mechanisms [82, 83].

Endothelial cells and platelets are cellular effectors of the hematologic response with important functions in both hemostasis and innate immunity. Under physiologic conditions endothelium displays a thromboresistant phenotype through expression of anticoagulant, profibrinolytic, and vasomodulator molecules [84]. Endothelial regulators control the initiation, amplification, and termination phases of the clotting cascade: endothelial TFPI is the main regulator of TF-initiated blood clotting [85]; ATIII adsorbed on surface heparin sulfate peptidoglycans (HS-PG) inhibits the amplification phase of coagulation [66], while endothelial TM initiates thrombin-dependent activation of protein C and regulates the termination phase of blood clotting [86]. TM is also a negative regulator of complement activation on endothelial surface [87]. In response to vascular injury, the endothelium is activated either through direct engagement of pattern recognition receptors (TLRs) by microbial PAMPs or indirectly, through immune (complement), inflammatory (cytokines), or hemostatic (FVIIa, FXa, thrombin) mediators. Complement induces local endothelial activation through multiple C1q receptors, C3b receptors (CR1 and CR3), and anaphylatoxin receptors (C3aR and C5aR) [88]. Complement-induced endothelial activation is characterized by the loss of anticoagulant regulators HS-PG, TFPI, and TM from endothelial surface [86, 89, 90]; release of procoagulant von Willebrand factor from Weibel-Palade bodies [91]; and rapid exposure of the leukocyte adhesion molecule P-selectin [91]. Furthermore, anaphylatoxin receptors initiate the transcriptional activation of a proinflammatory response with progressive expression of cell adhesion molecules (E-selectin, ICAM-1, VCAM-1), cytokines/chemokines (IL-1β, IL6, IL8, RANTES), and related receptors (VEGFC, IL-18R) [92, 93]. Finally, endothelium is also a target of the terminal complement complex. Sublytic concentrations of the C5b-9 membrane attack complex or the soluble, inactive, form of the complex (sC5b-9 complexed with clusterin or vitronectin), could enhance the expression of adhesion molecules, and induce TF procoagulant activity on endothelial surface [94] and anionic phospholipid exposure with subsequent enhancement of prothrombinase (FXa/FVa) activity and release of prothrombotic microparticles [95].

Platelets are cellular entities situated at the interface between coagulation, inflammation, and immunity, where they play critical roles in hemostasis, immune surveillance, and host response to microbial invasion [96]. Platelets activated in response to vascular trauma rapidly expose adhesion molecules, which enable their localization at injury sites, and release prothrombotic, proinflammatory, and antimicrobial mediators [97, 98]. Activated platelet supports a bidirectional communication with multiple vascular proteolytic cascades. Platelets can either be activated by or can activate and/or amplify the complement, coagulation, and kinin-kallikrein systems. Platelets enable the recruitment of immune cells and modulate the response of vascular cells at sites of injury, but they can also mediate systemic responses through release of inflammatory mediators and bioactive platelet microparticles [99]. At sites of infection, platelets are activated through direct recognition of microbial PAMPs by TLRs [100, 101], engagement of complement receptors [77], and/or the terminal complement complex C5b-9 [102, 103]. Complement activates platelets through the alternative [104] and/or classical pathways [105], and initiates a cross-activation/amplification step. Accordingly, activated platelets stimulate complement activation through exposure of P-selectin [104] and release of stored complement factors and regulators [105–107], which in turn can promote additional platelet activation. Complement-induced platelet activation is characterized by integrin-dependent platelet aggregation, degranulation, and exposure of P-selectin, as well as exposure of a prothrombotic phospholipid surface

[103, 108], with direct effects on local clotting reactions. P-selectin is a molecular convergence node for the innate immune, coagulation, and inflammatory responses at injury sites. It acts as a C3b opsonin receptor on the surface of platelets, and possibly endothelium, amplifying complement activation through the alternative pathway [104]. P-selectin also promotes leukocyte recruitment [109], and induces monocyte TF expression [110], phosphatidylserine exposure, and subsequent prothrombotic activity on the recruited monocytes [111]. In addition, P-selectin could recruit circulating monocyte-derived TF microparticles [112], further concentrating clotting initiators at sites of injury and promoting thrombus growth. Other prothrombotic responses induced by activated platelets are discussed in another chapter of the current book.

The complement-coagulation cross talk is bidirectional, and complement activation is amplified in prothrombotic environments (Fig. 12.4). Coagulation proteases have broad specificities and can activate complement factors directly. For example, FXIIa, the coagulation protease initiating the contact pathway, can also initiate the classical pathway of the complement system through proteolytic activation of C1r/s proteases [113]. Recently, thrombin has been shown to activate C3 and C5 in experimental murine models, leading to the generation of bioactive C3a and C5a anaphylatoxins [28, 114]. In addition, both the coagulation and complement pathways share common regulators as detailed above. C1-INH is the main soluble inhibitor of both clotting factor FXIIa and complement proteases C1r/s and MASP-1/2. Furthermore, surface-exposed TM and HS-PGs, negative regulators of clotting reactions on undisturbed endothelium, also inhibit complement activation/amplification on endothelial surface [115, 116]. TAFI, a carboxypeptidase that modifies fibrin making it less susceptible to plasmin proteolysis, also desarginates C3a and C5a [117] and

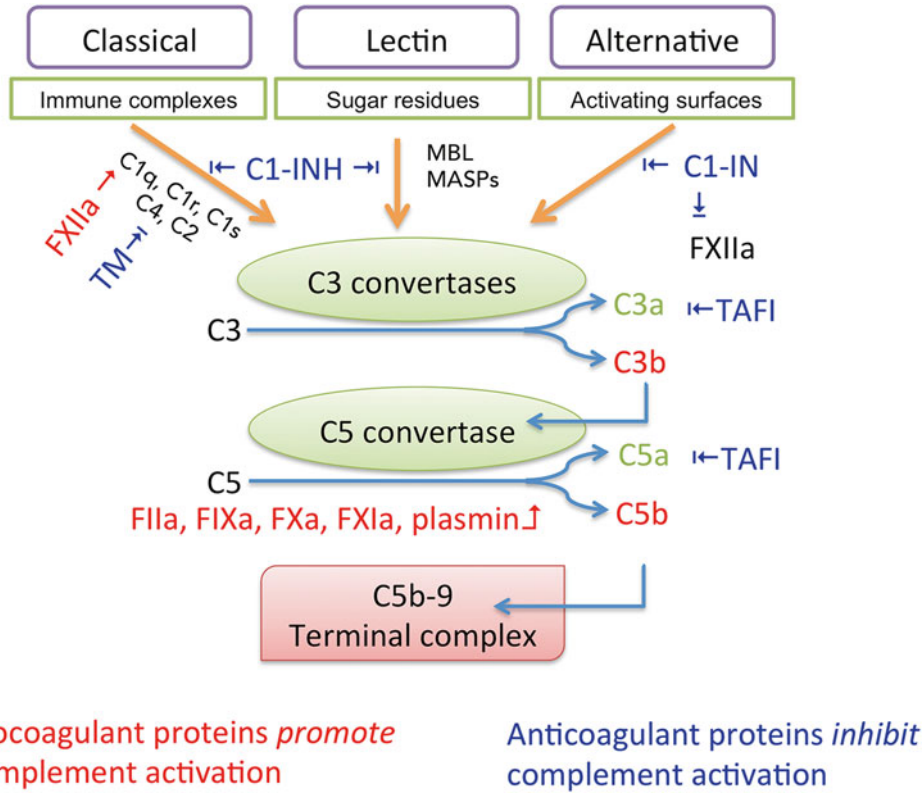

Fig. 12.4 Effects of the coagulation proteins on the complement pathways

reduces their proinflammatory effects. Finally, thrombin activation of platelets at sites of vascular injury also induces complement activation on platelet surface through the exposure of P-selectin and the release of chondroitin sulfate [106]. While the physiologic role of coagulation-induced complement activation is not always clear, complement activation and generation of TCC intermediates are necessary for an optimal hemostatic response since C6-deficient mice exhibit prolonged tail bleeding times [118].

Prothrombotic Manifestations of Complement Deficiencies

The synergistic activities of complement, coagulation, and inflammation are required for an optimal and balanced hematologic response to vascular trauma. An inadequate response can occur either because of an inefficient activation or due to improper regulation of the effector pathways, and can have deleterious outcomes for the host organism. Human pathologies associated with deficiencies of complement highlight its essential roles in the innate and adaptive immunity. Deficiencies of factors from the three complement activation pathways are associated with both an increased risk of recurrent infections, mainly bacterial, and the development of autoimmune disorders with a strong inflammatory component such as systemic lupus erythematosus (SLE) or SLE-like pathologies [6, 119]. Deficiencies of terminal complement pathway components are strongly associated with recurrent infections with Gram-negative bacteria, such as *Neisseria*, due to impaired antimicrobial function [120], but not autoimmune diseases. In general, deficiencies of complement effectors express phenotypically as autosomal recessive [119] and, due to redundancy within the immune response, are compatible with life. However, the enhanced susceptibility to microbial infections increases the risk of systemic dissemination and sepsis, with subsequent prothrombotic manifestations such as disseminated intravascular coagulation (DIC) [121].

Deficiencies of complement regulatory proteins can impair the hematologic response through excessive activation of complement and/or hemostatic pathways. Deficiency of C1-INH, the main classical and lectin pathway inhibitor, leads to hereditary angioedema, an autosomal dominant pathology characterized by extravascular edema due to excessive production of bradykinin [122]. While the pathophysiology of the angioedema is probably complement independent and due to excessive activation of contact coagulation pathway and/or kinin-kallikrein system also controlled by C1-INH, low levels of C2 and C4 have been detected in these patients, suggesting unbalanced complement activation [120].

Impaired regulation of the alternative complement pathway due to deficiencies in FH, FI, and/or MCP leads to multiple pathophysiologies such as the atypical hemolytic uremic syndrome (aHUS) and membranoproliferative glomerulonephritis (MPGNs) [5, 123]. The uncontrolled AP C3 convertase depletes plasma C3 [123, 124], and induces exacerbated C3b deposition mainly in the kidney, and subsequent thromboinflammatory responses resulting in glomerular thrombotic microangiopathy, thrombocytopenia, and microangiopathic hemolytic anemia. Pharmacologic inhibition of complement effectors, such as eculizumab blockade of C5 activation [125], has proven successful in aHUS [126] and MGPNs patients [127], indicating that thrombotic complications develop downstream of complement activation in these diseases.

Similarly, deficiencies of membrane-bound complement regulators may expose host cells to the damaging effects of complement activation. DAF (CD55) and CD59 are glycophosphatidylinositol (GPI)-anchored cell-surface-negative regulators of complement convertases and the C5b-9 TCC, respectively. Mutations in the PIG-A gene that encodes the GPI-anchoring enzyme lead to the development of paroxysmal nocturnal hemoglobinuria (PNH), a complement-mediated disease characterized by the absence of CD55/CD59 on vascular cells [128]. The absence of these protective mechanisms allows AP-initiated complement cascade to progress on the surface of

vascular cells resulting in intravascular hemolysis [125, 128]. Thrombophilia is a common clinical manifestation associated with PNH, with thromboembolism being the leading cause of mortality in PNH patients [129]. While the prothrombotic mechanisms are not completely understood, eculizumab inhibition of downstream complement effectors also reduces the incidence of thrombotic complications in PNH [130].

Complement-Coagulation Interplay in the Pathophysiology of Sepsis

Sepsis is an acute, progressive, systemic inflammatory response syndrome (SIRS) induced by an impaired hematologic response to infection [121, 131, 132], and a frequent complication of trauma. Severe sepsis and/or trauma complicated with multiple organ dysfunction syndrome (MODS) are leading causes of death in intensive therapy units worldwide [133]. Sepsis is initiated by the detection of pathogenic PAMPs within the vasculature, which trigger complement, coagulation, and inflammatory responses (Fig. 12.2). The excessive concomitant activation of multiple hematologic systems overcomes the regulatory networks in place and damages the host's own tissues and organs, ultimately leading to multiple organ failure and death [121]. In addition, survivors can exhibit sepsis-induced long-term sequelae such as diffuse pulmonary fibrosis [134] and cognitive impairment due to structural brain alterations [135], which affect their quality of life and long-term mortality [136]. Despite extensive research efforts, the pathophysiology of sepsis is not completely understood and no treatments are currently available for sepsis. Experimental inhibition of inflammatory, coagulation, and complement cascade effectors have proven unsuccessful in sepsis trials [132], and as a result new approaches are needed to characterize the systemic hematologic response and identify interacting nodes that control multiple pathways.

During initial stages of sepsis, complement activation is critical for the immune response to invading pathogens [137]. Complement activation can occur through all three initiating pathways, leading to C3 activation and formation of the terminal C5b-9 complex [74, 138]. Inhibition of complement activation at this stage negatively affects the outcome in sepsis due to impaired microbial clearance. Complement activation also has downstream proinflammatory and prothrombotic effects. The anaphylatoxins C3a and C5a are the main complement-induced proinflammatory mediators as detailed above, affecting the permeability of blood vessels, leukocyte recruitment and activation, cytokine/chemokine expression, as well as TF expression on monocytes and endothelium [53, 139]. The exacerbated activation of the complement cascade however reflected by the increased plasma levels of C3a, C4a, and C5a anaphylatoxins [140] has been associated with multiple organ failure and enhanced mortality in septic patients [139]. In contrast, delayed complement inhibition ameliorates physiologic parameters, attenuates sepsis-induced inflammation, and protects against multiple organ failure in a nonhuman primate model [74].

Sepsis activates coagulation mainly through the TF-dependent pathway [141], although systemic activation of the contact pathway also occurs in patients and experimental models [142]. Coagulation activation is tightly interconnected with inflammation [143], and promotes DIC, the most important marker of poor prognosis in sepsis [121]. Sepsis induces a thrombophilic phenotype through enhanced expression of TF on vascular cells [144, 145] and depression of negative regulatory mechanisms, such as TFPI, APC, TM, and ATIII [143, 146, 147]. Complement activation can contribute to sepsis-associated thrombophilia through multiple mechanisms, like C5a-dependent inflammatory reactions [139], TF expression by immune cells [76], TF decryption [82], platelet activation [108], and enhanced exposure of anionic phospholipid surfaces [148] among others. Inhibition of complement effectors using compstatin, a C3 convertase inhibitor [74], or C5-blocking reagents [33, 52]

reduced thrombosis in experimental septic models. Compstatin inhibition also preserves endothelial properties, reduces vascular leakage, decreases inflammation and leukocyte recruitment, and attenuates organ injury [74]. These studies reveal the clinical importance of the complement-coagulation interplay during the development of sepsis and provide proof-of-concept strategies for concurrent therapeutic control of multiple hematologic pathways in sepsis.

Conclusions

The current knowledge reviewed above highlights the tight interplay between the complement and coagulation pathways on the pathophysiology of sepsis and trauma. The close cross talk between the two pathways leads to their reciprocal amplification creating a vicious cycle that contributes to MOF and death. Since activation of the complement system occurs early during organ failure progression, use of complement inhibitors to prevent MOF is a logical approach. Complement therapeutics armory under development or in clinical use include (1) enhancing the expression of negative regulators (CD55, CD59, soluble complement receptor-1) and supplementing the natural complement inhibitors (C1 inhibitor); (2) blocking antibodies, peptides, aptamers, or small molecules that bind to key proteins and prevent the formation of C3 and C5 convertases; (3) antibodies that neutralize complement-derived anaphylatoxins C3a and C5a; and (4) antibodies or small molecules that interfere with signaling of C3a and C5a via their specific receptors. Nevertheless, blockade of the complement cascade may impair the host defense responses against pathogens, thus increasing the risk of secondary infections and affecting the healing of the wounds. Therefore, identification of the appropriate therapeutic window and development of approaches that may control complement activation while preserving its protective and reparative functions are important challenges that need to be addressed.

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Disseminated Intravascular Coagulation

13

Satoshi Gando

Historical Perspective

Disseminated intravascular coagulation (DIC) was first described clinically in a case of sepsis following trauma and was termed as "DIC syndromes" in 1961 [1]. On the basis of autopsy evidence, MacKay lists trauma as one of the most common causes of DIC and he discusses the pathogenesis of DIC based on a thorough understanding of the clinical courses of coagulation changes seen after trauma [2]. As early as the 1970s, Flute [3] correctly pointed out that trauma stimulates blood coagulation and fibrinolysis concurrently, and that fibrinolysis is the compensatory mechanism for fibrin formation in the blood. When this compensating mechanism fails, DIC may cause microvascular thrombosis with bleeding and tissue necrosis resulting from a defibrination syndrome. The pathogenesis of DIC in trauma is considered to be due to the entry of thromboplastic materials, such as the products of tissue damage, into the circulation, which in turn lead to platelet aggregation, coagulation activation, and fibrin deposition. If sufficiently severe to induce the consumption of hemostatic components, systemic intravascular coagulation results [2–4]. So, even by the early 1970s, the definition, pathogenesis, clinical aspects, and treatment of DIC as well as the clinical conditions associated with it, such as trauma, had already been described.

Until the early 1980s, whole blood was transfused, which provided adequate amounts of most coagulation factors. During massive transfusion of packed red blood cells (PRBC) the dilution of coagulation factors occurs long before thrombocytopenia develops [5]. Trauma specialists are well aware of the fact that injury and hemorrhagic shock, not hemodilution, induce the early coagulopathy of trauma [6–10]. In 1985, Ordog et al. clearly demonstrated that coagulation abnormalities during hemorrhagic shock are attributable to the trauma itself, independent of fluid and blood replacement; this was reported to most likely be DIC [2, 6, 11]. In the late 1980s, the use of whole blood was almost completely replaced by PRBC, which have no coagulation factors. Around a decade later, Hiippala [12] revealed that hypofibrinogenemia develops first, is followed by other coagulation factor deficits and later by thrombocytopenia. Therefore, the use of plasma transfusion became the primary intervention for abnormal bleeding [12]. Despite these changes in transfusion strategies and the importance of Hiippala's report, the incorrect notion that plasma should be transfused late in the resuscitation process continued to be advocated until recently [13]. This partly explains the

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prevailing notion of dilutional coagulopathy and the lack of acceptance of an endogenously induced DIC according to trauma or shock itself [14]. In view of this historical perspective, although multiple factors contribute to trauma induced coagulopathy, it is DIC induced consumption coagulopathy, and not dilution coagulopathy, that is important pathogenetically, with hypothermia and acidosis subsequently modifying the processes of DIC.

Definition and Diagnosis of DIC

The Scientific and Standardization Committee (SSC) on DIC of the International Society on Thrombosis and Haemostasis (ISTH) defines DIC as an acquired syndrome characterized by the intravascular activation of coagulation with loss of localization arising from different causes. It can originate from and cause damage to the microvasculature, which if sufficiently severe, can produce organ dysfunction [15]. The most important aspects of this definition are the "activation of coagulation with loss of localization" and "damage to the microvasculature," referring in turn to thrombin generation in the circulation and to extensive damage to endothelial cells, which results in insufficient coagulation control. Figure 13.1 shows the basic concept of DIC in which circulating blood is hypercoagulable due to systemic thrombin generation due to insufficient anticoagulation mechanisms, but is hypocoagulable outside the vessels and difficult to clot following injury due to consumption coagulopathy [16].

The DIC diagnostic criteria of ISTH and the Japanese Association for Acute Medicine (JAAM) have been prospectively validated in critically ill patients, including those with trauma [16–18]. The JAAM DIC scoring system has proven diagnostic validity for DIC in the early phase of trauma and has better diagnostic sensitivity than the ISTH scoring system. In addition, the JAAM DIC score on admission to the emergency department (ED) can independently predict death and the need for massive transfusion in trauma patients [19–21]. These features of the JAAM diagnostic criteria may be dependent on the deletion of fibrinogen as a scoring criteria, inclusion of sensitive systemic inflammation criteria, and the addition of a dynamic component such as decreasing rate of platelet count. The ISTH scoring system includes a table of the "clinical conditions that may be associated with DIC" as a mandatory clause, and restricts the use of the scoring algorithm in patients without underlying diseases. JAAM presents the same table while also adding another table with the title of "clinical conditions that should be carefully

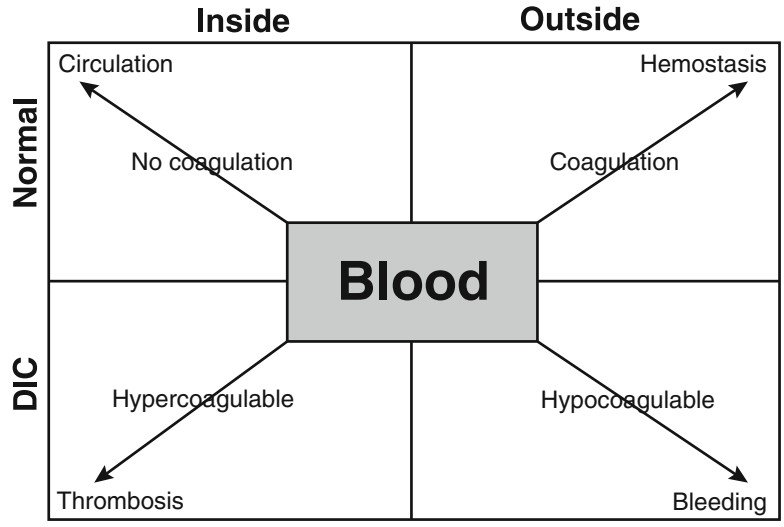

Fig. 13.1 The properties of blood inside and outside the vessels under normal conditions and DIC. Reprinted with modifications, with permission from Clinics in DIC by Matsuda T; Shinkoh-Igaku Shuppan Co., Ltd., Tokyo, 1983

Table 13.1 The scoring system for overt disseminated intravascular coagulation (DIC) proposed by the International Society on Thrombosis and Haemostasis (ISTH)

Clinical conditions that may be associated with overt DIC	
• Sepsis/severe infection (any microorganism)	
• Trauma (e.g., polytrauma, neurotrauma, fat embolism)	
• Organ dysfunction (e.g., severe pancreatitis)	
• Malignancy	
– solid tumors	
– myeloproliferative/lymphoproliferative malignancies	
• Obstetric calamities	
– amniotic fluid embolism	
– abruptio placentae	
• Vascular abnormalities	
– Kasabach–Merritt syndrome	
– large vascular aneurysms	
• Severe hepatic failure	
• Severe toxic or immunologic reactions	
– snakebite	
– recreational drugs	
– transfusion reactions	
– transplant rejection	
1. Risk assessment: Does the patient have a underlying disorder known to be associated with overt DIC?	
If yes: proceed; If no: do not use this algorithm;	
2. Order global coagulation tests (platelet count, prothrombin time, soluble fibrin monomers, or fibrin degradation products)	
3. Score global coagulation test results	
	Score
• Platelet counts (10^9/L)	
<50	2
≥50 < 100	1
≥100	0
• Elevated fibrin-related marker (e.g., soluble fibrin monomers/fibrin degradation products)	
Strong increase	3
Moderate increase	2
No increase	0
• Prolonged prothrombin time (s)	
≥6	2
≥3 < 6	1
<3	0
• Fibrinogen level (g/mL)	
<100	1
≥100	0
4. Calculate score	
5. If >5: compatible with overt DIC; repeat scoring daily	
If <5: suggestive (not affirmative) for non-overt DIC; repeat next 1–2 days.	

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ruled out" in order to increase the specificity of the scoring system. Tables 13.1 and 13.2 show the ISTH and JAAM DIC scoring systems, respectively [15, 18].

Phenotypes and Time Courses of DIC

DIC can be subdivided into the fibrinolytic (hemorrhagic) and antifibrinolytic (thrombotic) phenotypes [14, 22, 23]. DIC in the early phase of trauma manifests a fibrinolytic phenotype, which contributes to coagulopathic bleeding and is associated with a poor prognosis [14, 18]. DIC in the late phase of trauma has a thrombotic phenotype that also affects prognosis as it leads to organ dysfunction [14, 16, 24]. The synergistic activation of primary and secondary fibrin(ogen)olysis by tissue-type plasminogen activator (tPA) is considered to be the cause of DIC with the fibrinolytic phenotype [22, 23], while plasminogen activator inhibitor-1 (PAI-1) mediated inhibition of fibrinolysis is considered to be the cause of DIC with the thrombotic phenotype [16, 23, 24]. Activation of coagulation and an ineffective anticoagulation system are common to both phenotypes (Fig. 13.2) [25]. For further description of fibrinolysis mechanisms seen in trauma induced coagulopathy refer to Chap. 9 (Fibrinolysis-Moore).

Use of the DIC diagnostic criteria distinguishes the pathological reaction of DIC from physiological hemostasis and wound healing [14, 16, 26]. Figure 13.3 (left) shows normal changes in hemostasis and wound healing, while Fig. 13.3 (right) shows the abnormal hemostatic responses associated with DIC from immediately after trauma to the late phase of trauma [14, 16, 26].

Table 13.2 The scoring system for disseminated intravascular coagulation (DIC) by the Japanese Association for Acute Medicine (JAAM)

1. Clinical conditions that may be associated with DIC
(1) Sepsis/severe infection (any microorganism)
(2) Trauma/burn/surgery
(3) Vascular abnormalities
– large vascular aneurysms
– giant hemangioma
– vasculitis
(4) Severe toxic or immunological reactions
– snakebite
– recreational drugs
– transfusion reactions
– transplant rejection
(5) Malignancy (except bone marrow suppression)
(6) Obstetric calamities
(7) Conditions that may be associated with SIRS
– organ destruction (e.g., severe pancreatitis)
– severe hepatic failure
– ischemia/hypoxia/shock
– heat stroke/malignant syndrome
– fat embolism
– rhabdomyolysis
– other
(8) Other
2. Clinical conditions that should be carefully ruled out
A. Thrombocytopenia
(1) Dilution and abnormal distribution
Massive blood loss and transfusion, massive infusion
(2) Increased platelet destruction
ITP, TTP/HUS, HIT, drugs, viral infection, alloimmune destruction, APS, HELLP, extracorporeal circulation
(3) Decreased platelet production
Viral infection, drugs, radiation, nutritional deficiency (vitamin B12, folic acid), disorders of hematopoiesis, liver disease, HPS
(4) Spurious decrease
EDTA-dependent agglutinins, insufficient anticoagulation of blood samples
(5) Other
Hypothermia, artificial devices in the vessel
B. Prolonged prothrombin time
Anticoagulation therapy, anticoagulant in blood samples, vitamin K deficiency, liver cirrhosis, massive blood loss and transfusion
C. Elevated FDP
Thrombosis, hemostasis and wound healing, hematoma, pleural effusion, ascites, anticoagulant in blood samples, antifibrinolytic therapy
D. Other
3. The diagnostic algorithm for SIRS
(1) Temperature >38 °C or <36 °C
(2) Heart rate >90 beats/min
(3) Respiratory rate >20 breaths/min or $PaCO_2$ < 32 Torr (<4.3 kPa)
(4) White blood cell >12,000 cells/mm^3, <4000 cells/mm^3, or 10 % immature (band) forms
4. The diagnostic algorithm

	Score
SIRS criteria	
≥3	1
0–2	0
Platelet counts (10^9/L)	
<80 or more than 50 % decrease within 24 h	3
≥80 < 120 or more than 30 % decrease within 24 h	1
≥120	0
Prothrombin time (value of patient/normal value)	
≥1.2	1
<1.2	0
Fibrin/fibrinogen degradation products (mg/L)	
≥25	3
≥10 < 25	1
<10	0
Diagnosis	
Four points or more	DIC

SIRS systemic inflammatory response syndrome, *ITP* idiopathic thrombocytopenic purpura, *TTP* thrombotic thrombocytopenic purpura, *HUS* hemolytic uremic syndrome, *HIT* heparin-induced thrombocytopenia, *APS* antiphospholipid syndrome, *HELLP* hemolysis, elevated liver enzymes, and low platelet, *HPS* hemophagocytic syndrome, *EDTA* ethylenediaminetetraacetic acid, *FDP* fibrin/fibrinogen degradation products

Reprinted with permission [18]

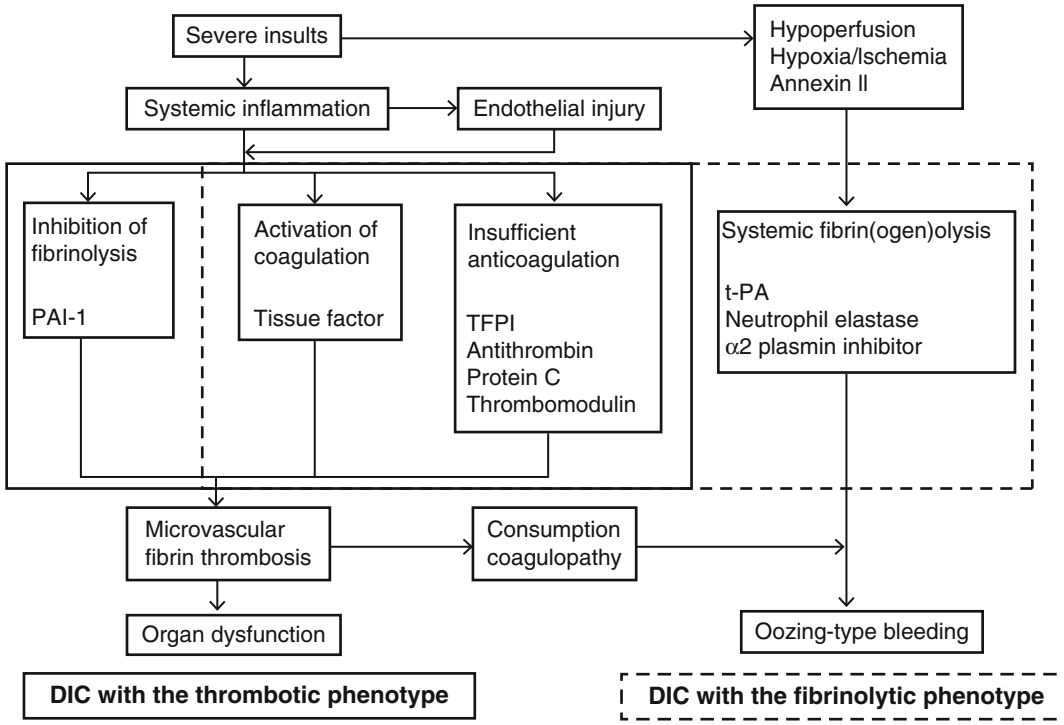

Fig. 13.2 The two phenotypes of DIC. Although the activation of the tissue-factor dependent pathway as the initial step of the coagulation cascade and the presence of insufficient anticoagulation systems are the same, DIC can be subdivided into the fibrinolytic (*broken line*) and thrombotic (*straight line*) phenotypes. In DIC with the fibrinolytic phenotype, DIC and systemic fibrin(ogen) olysis coexist. Annexin II expression on the promyelocytes increases the t-PA activity in patients with acute promyelocytic leukemia. Microvascular thrombosis- and shock-induced hypoxia/ischemia in endothelial cells accelerate t-PA release from endothelial Weibel–Palade bodies. Neutrophil elastase-derived fibrinolysis and consumption-induced α2-plasmin inhibitor α2 plasmin inhibitor depression enhance fibrin(ogen)olysis. Reprinted from reference [25]

Pathogenesis of DIC

Innate Immunity, Inflammation, and Coagulation at the Injury Site

Trauma can produce a systemic inflammatory response syndrome (SIRS) characterized by pro-inflammatory cytokine release and activation of leukocytes and endothelial cells, processes which are understood as innate immunity [27]. Close interactions between innate immunity, inflammation, and coagulation have been recognized [28, 29]. Innate immune cells have evolved cell-specific prothrombotic pathways that are activated after insults and operate in intact blood vessels to protect the host from nonself (pathogen-associated molecular patterns, PAMPs) and altered-self (damage-associated molecular patterns, DAMPs); this concept is referred to as immunothrombosis [30]. During the responses to PAMPs and DAMPs, monocytes and their microparticles express tissue factor, which activates the extrinsic coagulation pathway [31, 32]. Neutrophils are recruited to the sites of inflammation and are activated [33], and then release neutrophil extracellular traps (NETs), which are comprised of a matrix of DNA, histones, nucleosomes, and neutrophil elastase, thereby promoting thrombosis [34]. Histones induce platelet activation and also promote thrombin generation

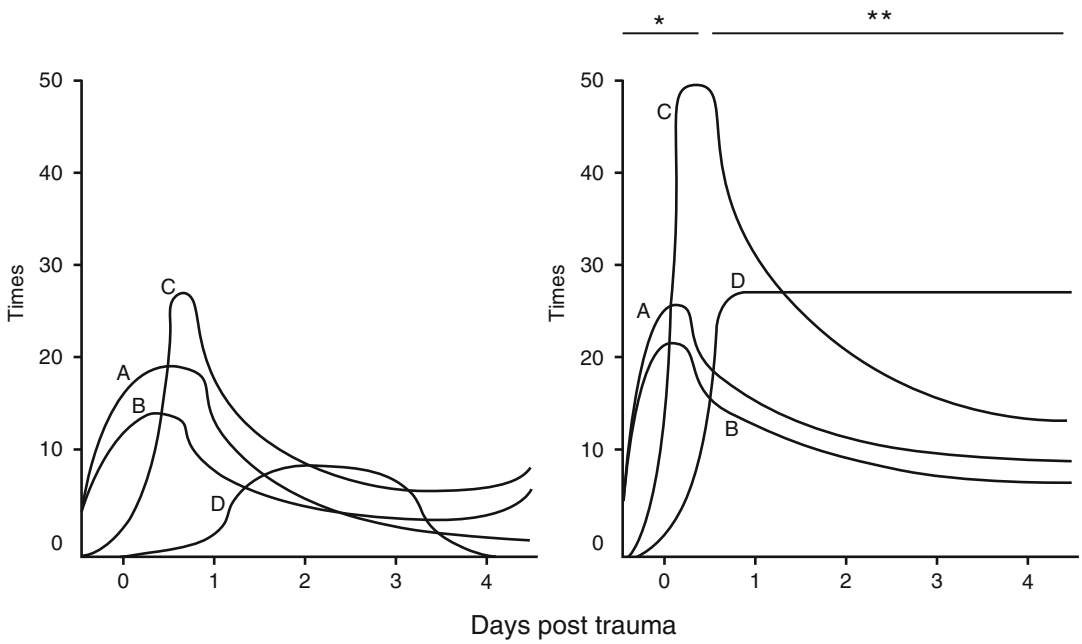

Fig. 13.3 Schematic diagrams of the variations in thrombin activity (*A*, measured by fibrinopeptide A-FPA), plasmin activity (*B*, fibrinopeptide B β15-42-FPBβ15-42), fibrin formation, and secondary fibrinolysis (*C*, D-dimer) from day 0 (in the emergency department) to day 4. *Left*, normal changes in hemostasis and wound healing. There are three phases of fibrinolysis: early activation, impairment (*D*, PAI-1: fibrinolysis shutdown), and reactivation. Normally, both thrombin activity and PAI-1 are completely shut off by days 3–5 after trauma, followed by the reactivation of fibrinolysis. *Right*, pathological changes in DIC. Massive thrombin activation persists until day 4 after trauma; increased activation of plasmin as well as excessive fibrinolysis are present on day 0 (* DIC with the fibrinolytic phenotype), followed by impairment of fibrinolysis due to persistent elevation of PAI-1 released from endothelial cells with transcription (** DIC with the thrombotic phenotype). Reprinted with permission [26]

both by the recruitment of platelet and the impairment of thrombomodulin-dependent protein C activation [35–37]. NETs can also activate the intrinsic coagulation pathway by activating FXII to form FXIIa [38], which then promotes the activation of complement pathways. The generated C3a and C5a further promote thrombosis and platelet activation [39]. In addition, extracellular RNA derived from damaged cells constitutes a procoagulant cofactor for the activation of the FXII/FXI coagulation pathway [40]. The neutrophil elastase that is present on NETs induces the degradation and inactivation of tissue factor pathway inhibitor (TFPI) [41, 42] and the thrombomodulin expressed on the endothelium [43, 44]. These processes further promote thrombin generation. For further description of DAMPs as well as of neutrophils and the innate immune system during injury, please refer to Chap. 11 (DAMPs-Esmon) and Chap. 10 (Neutrophils-Yaffe), respectively.

Activation of coagulation and insufficient control of it lead to immunothrombosis at injury sites with inflammation, impeding the dissemination and tissue invasion of PAMP and DAMPs as well as pathogens and damaged cells themselves [30, 42]. In trauma, thrombin escaping into the circulation from the injury sites is controlled by antithrombin, TFPI, and thrombomodulin present in intact endothelial cells, as depicted in the cell-based model of hemostasis [45]. However, when systemic inflammation caused by both extensive injury and shock overwhelms these control mechanisms that restrict hemostasis and inflammation locally, DIC ensues [45]; Figs. 13.4 and 13.5 outline these processes.

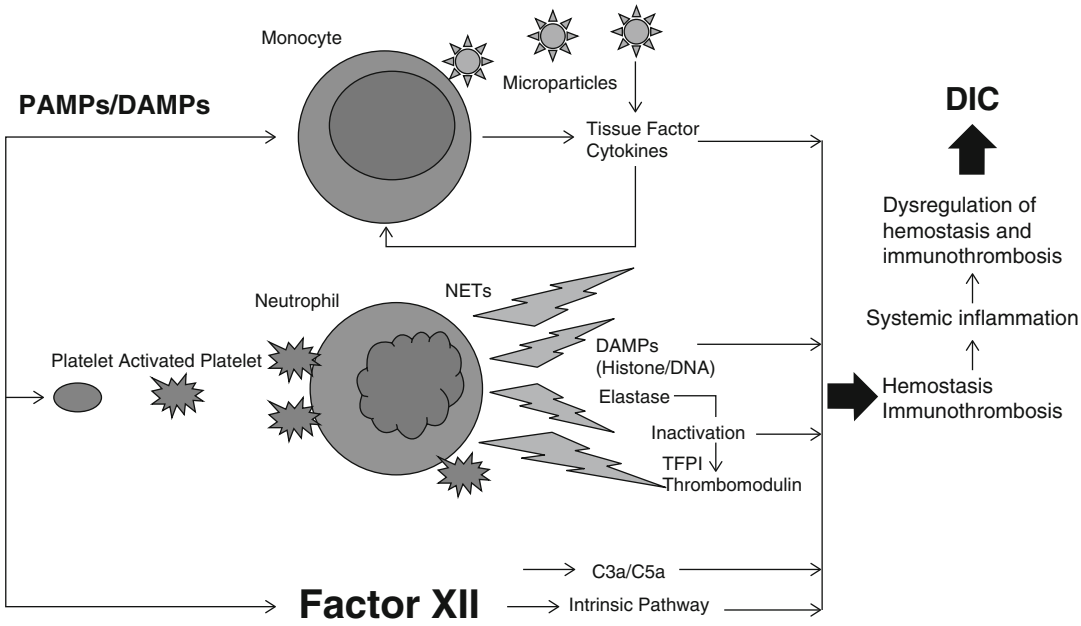

Fig. 13.4 The pathophysiological processes of local hemostasis, immunothrombosis, and systemic DIC. Tissue injury promotes local hemostasis and wound healing. Tissue injury also induces microvascular fibrin thrombosis, or immunothrombosis, to protect the host from DAMPs and to restrict DAMPs only to the injured vascular compartment. DIC results when local hemostasis and immunothrombosis are no longer able to anchor thrombin or to restrict the spread DAMPs at the injured site. Reprinted from reference [25]

Systemic Activation of Coagulation

The major DAMPs released into the extracellular environment following tissue injury are histones, mitochondrial DNA, nucleosomes, and high mobility group box 1 (HMGB1) [46]. Mitochondrial DNA at levels thousands times higher than those measured in healthy volunteers have been observed at a median 93 min after trauma [47]. Elevated levels of mitochondrial DNA were associated with SIRS and correlated with Injury Severity Score (ISS) in trauma patients [48, 49]. Elevated levels of histones and HMGB1 have been seen in severely injured trauma patients within 30 min of trauma or immediately after arrival to the ED [50–52]. Toxic levels of histones insult cells, leading to endothelial injury (elevation of soluble thrombomodulin), platelet aggregation, coagulation activation [increase in thrombin–antithrombin complex (TAT)], interleukin-6 (IL-6) release, and NETs formation [52]. These processes result in edema, microvascular thrombosis, and neutrophil accumulation in the lungs. The HMGB1 released by damaged and inflammatory cells at the injury site promotes the development of microvascular thrombosis [53]. An important point is that HMGB1 inhibits the anticoagulant protein C pathway mediated by the thrombin–thrombomodulin complex, and stimulates tissue factor expression on monocytes. Histones also reduce the cofactor activity of both soluble and endothelial thrombomodulin, impairing protein C activation, and thereby stimulating plasma thrombin generation [37]. For further description of the protein C pathway please refer to Chap. 6 (Protein C-Cohen).

Tumor necrosis factor α (TNF-α) and IL-6 are elevated immediately after histone infusion [52, 54]. In fact, TNF-α and IL-1β are increased on arrival to the ED in trauma patients with DIC [55], with an IL-6 surge occurring within 2 h from injury [56]. This early release of IL-6 suggests it is most likely released from pre-synthesized

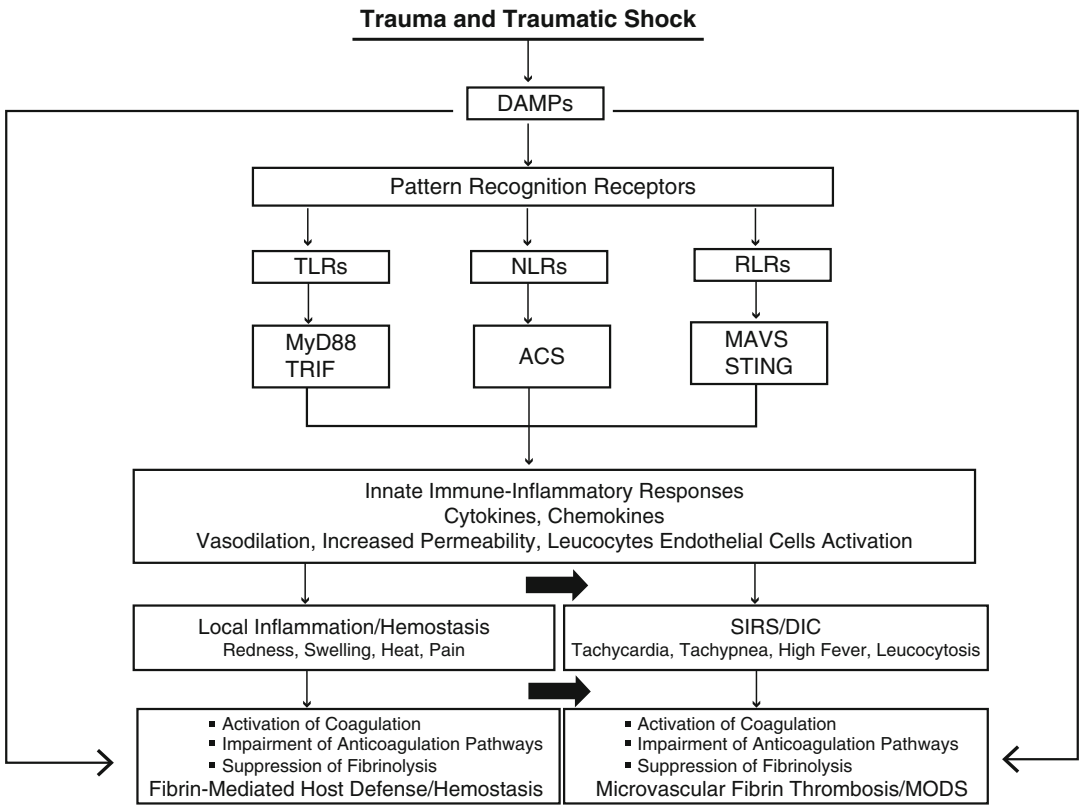

Fig. 13.5 Trauma elicits nonspecific innate immune inflammatory responses that limit and repair tissue damage after insults. The figure depicts a simplified schematic representation of the engagement of pattern recognition receptors by DAMPs and their signaling through the adaptor proteins. This cascade promotes the transcription of several pro-inflammatory cytokines and chemokines, leading to local and systemic inflammatory responses. Local inflammation begins as an adaptive host response, serving to promote host defense and physiologic hemostasis and wound healing. Spillover of the inflammatory cytokines into the circulation elicits SIRS, which activates systemic coagulation, suppresses fibrinolysis, and overwhelms the anticoagulant control mechanisms that restrict hemostasis locally, giving rise to DIC. Importantly, DAMPs themselves activate coagulation and impair anticoagulation pathways through endothelial damage (reprinted, with modifications, with permission from Gando S, et al. Infectious and non-infectious insults and body responses. Jp J Acute Med 2011;35:747–52). *ACS* apoptosis-associated speck-like protein containing caspase recruit domain, *DAMPs* damage-associated molecular patterns, *DIC* disseminated intravascular coagulation, *MAVS* mitochondrial antiviral signaling, *MODS* multiple organ dysfunction syndrome, *MyD88* myeloid differentiation factor 88, *NLRs* nucleotide-binding oligomerization domain containing receptors, *RLRs* retinoic acid inducible gene-I-like receptors, *SIRS* systemic inflammatory response syndrome, *STING* stimulator of interferon gene, *TRIF* toll/IL-1 receptor homology domain-containing adaptor inducing interferon beta, *TLRs* toll-like receptors

stores [52]. IL-6 is the most important driver of tissue factor expression on monocytes and endothelial cells [57]. TNF-α and IL-1 also have been shown to elicit tissue factor formation and expression on the surface of monocytes and endothelial cells [58]. These inflammatory cytokines subsequently block the protein C anticoagulant pathway by downregulating thrombomodulin and the endothelial protein C receptor (EPCR) on the endothelium [58]. Furthermore, these inflammatory cytokines activate neutrophils and endothelial cells, and the activated neutrophils release neutrophil elastase, which can cleave thrombomodulin, leading to the release of soluble thrombomodulin from the endothelium in a less active form [43, 44, 58, 59].

Although, the mechanisms involved in microparticle formation in-vivo remain essentially

unknown, blood contains microparticles derived from a variety of cell types, including platelets, monocytes, and endothelial cells [60]. All microparticles are pro-coagulant because they provide a membrane surface for assembly of coagulation proteases [61]. In severe trauma, activated platelets enhance the microparticle formation associated with platelet and leucocyte interaction [62]. Increased microparticle formation and thrombin generation are observed immediately after trauma and correlate with ISS [63].

These lines of evidence clearly indicate that the release of DAMPs from injured cells and tissues, the DAMP induced release of inflammatory cytokines, and microparticle formation synergistically hamper the mechanisms controlling coagulation by protein C pathways and activate coagulation, leading to SIRS and systemic thrombin generation, namely DIC, immediately after trauma [30, 64].

Impairment of Anticoagulation Pathways and Endothelial Injury

TFPI

The highly activated tissue factor-dependent pathway is not sufficiently prevented by normal TFPI levels in DIC patients after trauma because neutrophil elastase cleaves TFPI within the polypeptide that links the first and second Kunitz domains [65]. This impairs the ability of TFPI to neutralize both FXa and the tissue factor/FVIIa complex. This finding suggests that tissue factor and tissue factor/FVIIa complex are continuously formed at a rate that normal TFPI inhibition cannot match in DIC patients after trauma [65, 66].

Thrombomodulin and Endothelium

Higher levels of neutrophil elastase and soluble thrombomodulin have been confirmed in patients with DIC and in patients with severe traumatic injuries [52, 67, 68]. Soluble thrombomodulin can be formed by the limited proteolysis of endothelial cell membrane thrombomodulin by neutrophil elastase without any evidence of active secretion [43, 44]. The amount of soluble thrombomodulin correlates with the degree of endothelial injury [44]. Moreover, early elevation of TNF-α and IL-1β in DIC patients after trauma causes thrombomodulin downregulation in the endothelium [43, 44, 55]. Traumatic shock-induced hypoxia leads to a reduction in thrombomodulin and the suppression of thrombomodulin mRNA in the endothelium [69, 70]. Therefore, the high soluble thrombomodulin levels in DIC patients suggest a loss of functional thrombomodulin in the endothelium. In addition, soluble thrombomodulin has only 20 % of the activity of thrombomodulin bound to the endothelium [71]. Taken together, these results suggest that endothelial injury and functional loss of both soluble and endothelial thrombomodulin occurs in DIC after trauma.

Protein S and Protein C

Low levels of protein C activity have been repeatedly confirmed from the early to late phases of DIC [16, 18, 20, 67]. The thrombin–thrombomodulin complex activates protein C to generate activated protein C. For activated protein C to function more effectively, it must form a complex with both protein S and EPCR. The anticoagulant activity of protein S is neutralized by the formation of a complex with complement C4b binding protein (C4bBP). Increased levels of C4bBP as a consequence of the acute phase reaction following inflammatory insults cause a relative protein S deficiency, which contributes to a procoagulant state and lethal DIC [72]. Lower levels of protein S activity associated with thrombin generation (prothrombin fragment 1 + 2, PF1 + 2) have been demonstrated in trauma patients immediately after arrival at the ED [9]. For further description of the protein C pathway please refer to Chap. 6 (Protein C-Cohen).

Activated protein C is immediately inactivated by protease inhibitors, such as the protein C inhibitors, α1-antitrypsin, α2-antiplasmin, and α2-macroglobulin. In cases of DIC, lower protein C and protein S levels, relative protein S deficiency, and impaired functions of both soluble and endothelial thrombomodulin are all implicated in the insufficient conversion of protein C to activated protein C and the inability of activated protein C to function normally. Increases in activated protein C levels do not indicate a shut-off of thrombin generation. In fact, the elevated

activated protein C levels (~10 ng/mL) did not reach a concentration sufficient to inhibit thrombin generation (70–80 ng/mL) in severely injured trauma patients with tissue hypoperfusion [73, 74].

Antithrombin

Antithrombin inactivates thrombin and inhibits several proteases in both the extrinsic and intrinsic coagulation pathways, including FIXa, FXa, FXIa, and FXIIa. For further description of antithrombin please refer to Chap. 2 (TAT-Bock). Thus, a reduction in antithrombin can markedly influence the coagulation processes and is a potential risk factor for thrombosis [75]. Insufficient levels of antithrombin, compared with the potential for thrombin generation in the prothrombin complex concentrate, induced DIC characteristics in a pig model of coagulopathy with blunt liver injury [76]. The severity of injury and tissue hypoperfusion are major contributors to the reduction in antithrombin in trauma [77, 78]. Low antithrombin levels are associated with thromboembolic complications, which can develop from DIC [79]. Extremely low levels have been observed in cases of trauma patients immediately after arrival at the ED [8, 21] and for several days thereafter [8, 21, 80].

Two studies showed that a decreased ability to localize hemostasis at the wound site and subsequently generate thrombin systemically results from decreased antithrombin levels in patients with DIC and coagulopathy immediately after trauma [81, 82]. Similarly, a multiple regression analysis demonstrated that low antithrombin levels are an independent determinant of high soluble fibrin levels and a marker of thrombin generation in trauma patients with DIC [80].

These findings also indicate there is much lower availability of the TFPI, antithrombin/glycosaminoglycan, and thrombomodulin/protein C systems for the regulation of thrombin generation and activation in DIC patients. Moreover, higher soluble thrombomodulin levels suggest the presence of extensive damage to microvasculature endothelium. Figure 13.6 summarizes these changes.

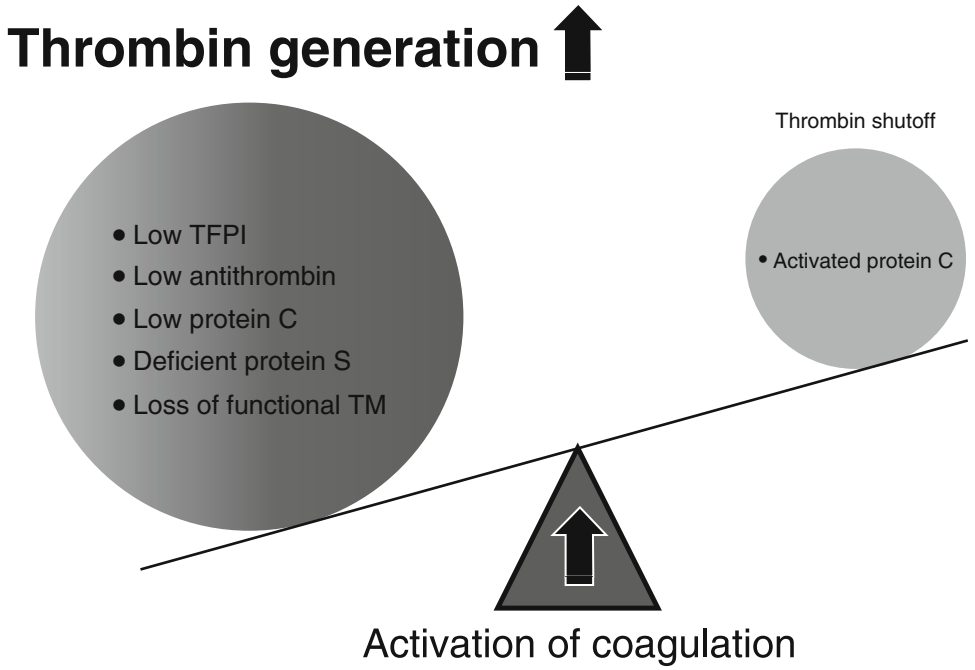

Fig. 13.6 The balance between thrombin generation and inhibition. DIC occurs when there is an imbalance between thrombin generation and inhibition. Insufficient coagulation control mechanisms contribute to massive thrombin generation in the circulation, which overwhelms activated protein C mediated inhibition of thrombin generation. Reprinted from reference [25]

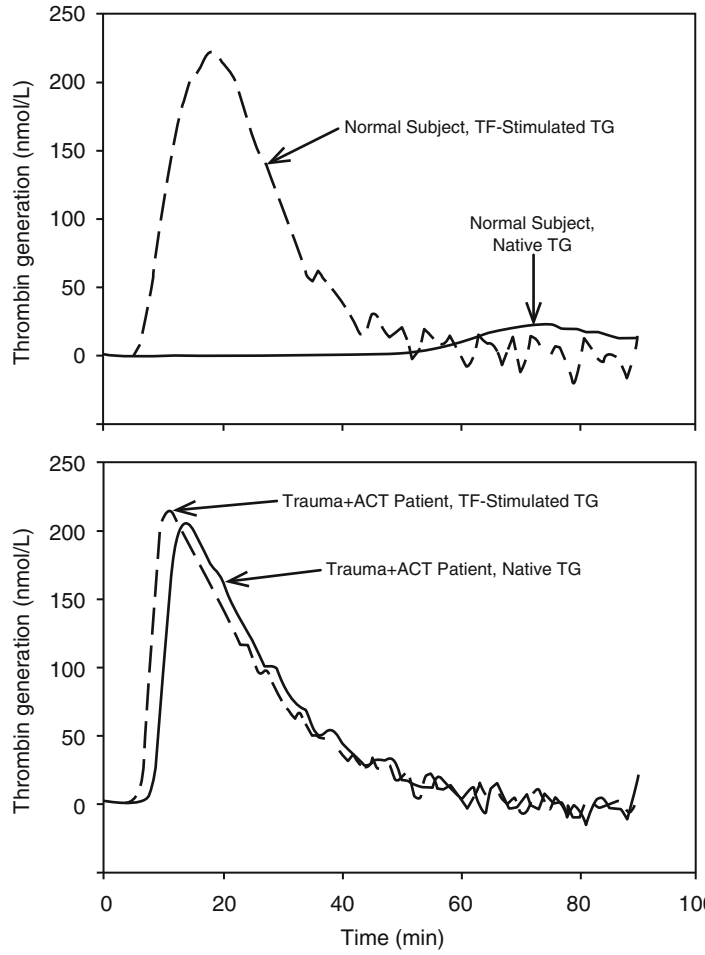

Fig. 13.7 Native and tissue factor (TF) stimulated thrombin generation (TG) curves in a normal subject and in a patient with acute coagulopathy of trauma. Native TG: no added tissue factor or phospholipid, contact activation blocked. TF stimulated: sample activated with TF and phospholipid. In normal subjects, little or no thrombin was generated during native TG. In contrast, in patients with acute coagulopathy of trauma, native and TF stimulated TG curves were often similar. This indicates that plasma from those with trauma induced coagulopathy has circulating pro-coagulant activity that can spontaneously initiate coagulation throughout the vascular system, not just at the injured site. Note: The term "acute coagulopathy trauma" used in this study [81] was changed to "DIC" in a subsequent study [82]. Modified with permission [81]

Thrombin Generation in the Systemic Circulation

Soluble fibrin and fibrinopeptide A are regarded as accurate markers of thrombin generation and activity because both are formed as a result of the direct action of thrombin on fibrinogen, which leads to fibrin formation. Extremely elevated levels of fibrinopeptide A have been noted in trauma patients with DIC immediately after arrival at the ED [8]. In addition, higher levels of these molecular markers of thrombin generation in the early phase of trauma have been repeatedly confirmed [9, 14, 67, 68]. In two studies, Dunbar and Chandler observed excessive non-wound-related thrombin generation in trauma patients with both DIC and trauma induced coagulopathy immediately after arrival at the hospital (Fig. 13.7) [81, 82]. Their first study showed marked systemic thrombin generation due to circulating procoagulants that initiate thrombin generation systemically, as well as reduced ability to localize hemostasis at the wound site due to the loss of antithrombin. Their second study found that tissue factor activity accounted for approximately 80 % of all procoagulant activity. Importantly, the term "acute coagulopathy of trauma" in the first study was changed to "DIC" in this second one. Reports showing a significant correlation between tissue factor and the markers of thrombin generation, and microparticle formation by activated platelets support these results [62, 83].

The overall function of the thrombomodulin/protein C anticoagulant pathway can be precisely

evaluated by measuring prothrombinase activity [84]. Prothrombinase is a complex comprising FXa, FVa, phospholipids, and Ca^{2+} and it is the major determinant of thrombin generation from prothrombin. Prothrombinase activity, measured as the thrombin generation rate, decreases in proportion to the amount of thrombin-thrombomodulin complex formation of activated protein C and the subsequent inactivation of FVa [84, 85]. DIC patients after trauma have shown normal prothrombinase activity associated with higher levels of soluble fibrin [80]. These findings suggest that the inhibition of the prothrombinase activity caused by activated protein C-mediated anticoagulation does not overwhelm the activation of the tissue factor systemic thrombin generation or its activation in trauma patients with DIC. This imbalance between thrombin generation (soluble fibrin) and its inhibition (prothrombinase activity) is due to insufficiency in the other anticoagulant mechanisms, such as TFPI and antithrombin, as well as impaired thrombomodulin function due to endothelial injury [80].

Consumption Coagulopathy

The consumptive processes in DIC reflect the multiple actions of thrombin. Increased thrombin generation accounts for decreases in platelets, fibrinogen, FII, FV, FVIII, and FXIII in acute consumption, and the rapid clearance of activated clotting factors in vivo accounts for decreases in other clotting factors such as FIX and FX [22]. Thrombin also induces the release of tPA from endothelial cells, leading to plasmin generation. If plasmin is formed sufficiently in the circulation, it degrades fibrinogen, FV, and FVIII. These lines of evidence support the rapid consumption of thrombin-sensitive hemostatic factors, including platelets, fibrinogen, and Factors V, VIII, and XIII. In pre-DIC and DIC, sensitive and rapid decreases in the levels of FV and FVIII have been observed as a result of thrombin-mediated protein C activation [86, 87]. In DIC due to trauma, platelets are sometimes consumed slowly due to marginalization in blood vessels and release from storage in organs such as the spleen, liver, and lungs [12, 14, 16]. FVIII is known to paradoxically increase in response to clinical insults, including trauma, due to release of von Willebrand factor (VWF) from the endothelial Weibel–Palade bodies [88] and the acute phase behavior of FVIII. VWF immediately interacts with FVIII, prolonging the plasma half-life of FVIII [89]. The consumption of coagulation factors prolongs both the prothrombin time (PT) and activated partial thromboplastin time (PTT); however, the PTT is sometimes normal or even shortened because of the interactions between FVIII with VWF in spite of a prolonged PT in DIC patients.

In cases of trauma with DIC, prolonged PT reflects a decrease in FV, to a lesser extent decreases in Factors II, VII, and X, and decreases in fibrinogen levels immediately to several days after trauma [8, 14, 16, 18, 19, 55, 67, 80, 83]. Prolonged PTT, which reflects a decrease in Factors V, VIII, and fibrinogen, has also been confirmed immediately after trauma in patients who develop DIC [8]. The FVII antigen has been demonstrated to be consumed at a relatively slow speed for about 8 h in a rabbit model of DIC [90]. Importantly, this translated into a FVIIa level increase to 120 % within 2 h after DIC induction, before declining thereafter. Furthermore, in patients studied upon arrival to the ED [91] FXIII and α2-plasmin inhibitor levels where shown to be markedly decreased in DIC.

Activation and Suppression of Fibrinolysis

DIC and pathological systemic fibrin(ogen)olysis sometimes coexist following an insult such as trauma and is referred to as DIC with the fibrinolytic phenotype [22, 23]. Tissue hypoperfusion causes tPA release from the endothelial Weibel–Palade bodies, which leads to systemic fibrin(ogen)olysis in addition to DIC with secondary fibrinolysis [22, 23, 88]. Increased fibrinolysis, as well as the activation of coagulation in trauma, has long been recognized [3, 92], as it was confirmed in a study of severely injured patients, 40 % of whom had a PT international normalized ratio >1.2 [93].

This study demonstrated increased thrombin generation with concomitant fibrinogen and antithrombin consumption, as well as increased tPA levels, plasmin generation, and fibrinolysis along with α2-plasmin inhibitor consumption, all of which coincided with DIC with the fibrinolytic phenotype. *The most important point in the pathogenesis of fibrin(ogen)olysis in the early phase of trauma is that there is a several hour time difference between the immediate release of tPA from the endothelium and the later expression of PAI-1 mRNA, leading to an extreme imbalance in these molecules* [94–96]. In support of this imbalance, the levels of PAI-1 antigen and activity were found to be almost identical in patients with and without DIC immediately after trauma, while the levels of tPA and plasmin and α2-plasmin inhibitor complex, a marker of plasmin generation, were significantly increased only in DIC patients [8, 16, 55, 80].

In addition to plasmin, neutrophil elastase mediated fibrinolysis is also involved in the pathogenesis of fibrin(ogen)olysis in DIC with the fibrinolytic phenotype [67]. The lower levels of α2-plasmin inhibitor, FXIII, and fibronectin in DIC patients suggest that there is insufficient inhibition of plasmin, impaired cross linking of fibrin, and delayed wound healing, leading to fragile fibrin formation associated with coagulopathic bleeding [8, 91]. A study showing tissue factor driven fibrin(ogen)olysis without tissue hypoperfusion suggests that secondary fibrinolysis caused by tPA release driven by a massive amount of fibrin formation may also have a role in DIC with the fibrinolytic phenotype [97]. Importantly, thrombomodulin mediated thrombin-activatable fibrinolysis inhibitor (TAFI) activation does not appear to have an important role in the pathogenesis of fibrin(ogen)olysis immediately after trauma [67], which indirectly implicates thrombomodulin/protein C pathway impairment.

Fibrinolysis driven by immediate tPA release is usually followed by PAI-1 suppression of fibrinolysis. After achieving hemostasis, the activation of coagulation and PAI-1 disappears completely and fibrinolytic reactivation occurs to degrade excess fibrin clots during physiological wound healing [8, 14, 16, 26]. However, persistent PAI-1 elevation continues until day 5 after injury in DIC patients, which is referred to as DIC with the thrombotic phenotype [8, 14, 16, 26, 55, 95]. When uncontrolled, DIC with the fibrinolytic phenotype in the early phase of trauma progresses to DIC with the thrombotic phenotype in the late phase of trauma [98]. DIC severity and the presence of organ dysfunction briefly after injury, but with complicating sepsis, are involved in the pathogenesis of this continuous progression. During the thrombotic stage, increased fibrinolysis, reflected by elevated D-dimer levels, cannot match the massive fibrin formation, leading to microvascular thrombosis, hypoperfusion and impaired oxygen delivery, which in turn give rise to multiple organ dysfunction syndrome [24, 95, 99]. Figure 13.3 illustrates these processes [26].

DIC and Microvascular Thrombosis

Trauma and Hemorrhagic Shock

Histological evidence of microvascular thrombosis in DIC, especially in DIC with the thrombotic phenotype, has been reported by clinical, experimental, and autopsy studies [99]. Evidence of DIC with the fibrinolytic phenotype is rarely available in humans and was extensively debated during the 1960s and 1970s [100]. These debates on the inconsistency of thrombus formation had come about because of the existence of hyperfibrin(ogen)olysis in the early phase of trauma and hemorrhagic shock. However, fibrin thrombosis [101], vein thrombi formation [102], platelet aggregation, and emboli formation [103, 104] were repeatedly confirmed in hemorrhagic shock and trauma. Subsequently, platelet and fibrin thrombosis became more evident during antifibrinolytic therapy using tranexamic acid in a dog model of hemorrhagic shock [105] (Fig. 13.8). It should be emphasized that the authors of a report expressing negative opinions about DIC, did in fact conclude that some fibrin thrombi were observed in their histological study [106]. Importantly, signs of inflammation, microthrombus and embolus formation

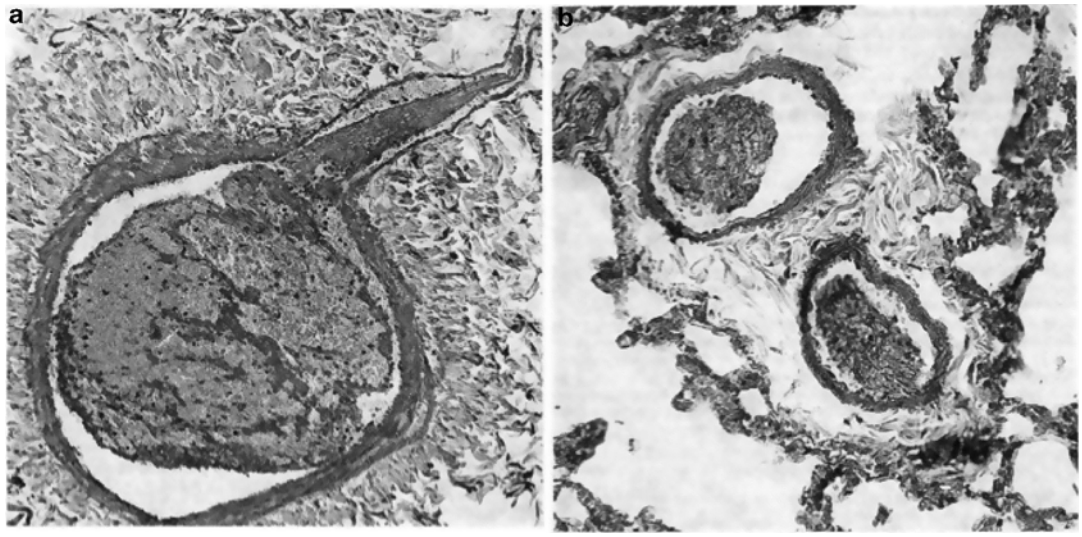

Fig. 13.8 The inhibition of fibrinolysis by tranexamic acid revealed microvascular thrombosis and thromboemboli formation in large vessels in a dog model of hemorrhagic shock. (**a**) Section of a branch of the portal vein almost completely filled by a mixed thrombus consisting of platelets and fibrin threads in bundles. (**b**) Section from lung vessels with thrombotic masses consisting of both platelets and fibrin thread. PTAH-staining. Modified with permission [105]

have also been observed within 24 h of injury in human studies [107, 108].

Isolated Traumatic Brain Injury

Publications have demonstrated that the coagulopathy of isolated traumatic brain injury (iTBI) coincides with the definition of DIC proposed by the ISTH; namely intravascular activation of coagulation, with loss of localization of coagulation, and damage to the microvasculature [15, 109, 110]. Therefore, coagulofibrinolytic changes after iTBI may have some overlap with those observed in trauma patients without brain injury; if sufficiently severe, they give rise to DIC [111].

Microthrombi are frequently present in the brains of iTBI patients who died within 24 h of injury, and were found to be associated with marked changes in platelet count, coagulation, and fibrinolysis markers (Fig. 13.9) [109]. More importantly, systemic microthrombi were seen in the spinal cord, liver, lungs, kidneys, colon, and pituitary gland, indicating the presence of DIC in 88 % of patients after autopsy [109]. Stein et al. examined brain tissue from several sources, including animal models and patients with contused brain tissue removed during surgical decompression, and found a high correlation between the severity of coagulopathy and the presence of intravascular thrombosis, confirming the association between intravascular thrombosis and DIC [110, 112]. These investigators also demonstrated a strong link between intravascular thrombosis and the area of ischemic changes and neuronal death [110, 113]. An animal experiment confirmed that the immediate posttraumatic decrease in peri-contusional blood flow is caused by platelet activation and subsequent microthrombosis in the cerebral circulation [114]. These lines of evidence clearly indicate that a DIC processes occurring immediately after iTBI can contribute to secondary brain injury.

Fat Embolism Syndrome

Fat embolism syndrome (FES) typically occurs 12–36 h after long bone and pelvic fractures, although fulminant cases immediately after

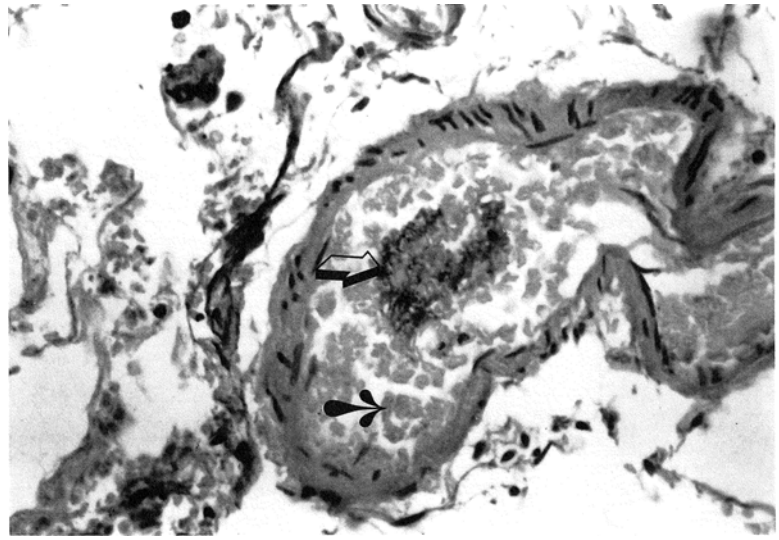

Fig. 13.9 Microthrombi in the meningeal vessels were observed in patients with iTBI. Fibrin stains brown (*white arrow*), containing with red blood cells, which are colorless to faint blue (*black arrow*) (Immunoperoxidase stain with antifibrinogen specific antiserum and hematoxylin counterstain, ×400). Modified with permission [109]

injury have also been reported. Several lines of evidence indicate the presence of DIC in patients with FES [115–117].

Saldeen et al. observed morphological changes in pulmonary, cerebral, and systemic fat emboli that are associated with pathophysiological characteristics of DIC [118]. Furthermore, fibrin thrombosis in pulmonary vessels has been confirmed more often in patients with FES than in other post-traumatic cases, especially in those in whom FES was considered to be the only explanatory cause of death [119]. The presence of fibrin thrombi in lung vessels was also confirmed in patients with FES and acute respiratory distress syndrome [120]. Hyaline microvascular thrombosis and aggregates of platelets, indicating fibrin thrombi and intravascular coagulation, have been reported in both the cerebral arteries and veins in post-traumatic FES patients [121]. Histopathological analysis of FES in a living body revealed intravascular thrombus formation, with the thrombus consisting of fibrin as well as erythrocytes and leukocytes with lipid granules [122].

Animal Models of DIC

Noble–Collip drum-induced polytrauma without significant hemorrhage has been used to mimic lethal traumatic injury [123]. The model reproduces typical DIC with the fibrinolytic phenotype, with animals exhibiting a decreased platelet count, prolonged PT and PTT, decreased fibrinogen and antithrombin levels, and elevated fibrin/fibrinogen degradation products (FDP) levels [123–125]. Furthermore, elevated tPA levels, shortened euglobulin lysis time, and decreased α2-plasmin inhibitor levels indicate immediate activation of the fibrinolytic system [124, 125]. Decreases in the levels of FXII, prekallikrein, and CH50 suggest the activation of both the intrinsic coagulation pathway and the complement system [125]. Immediately after Noble–Collip drum trauma, tissue factor increases in the circulation, and its mRNA expression has been observed in various organs, indicating the activation of the extrinsic coagulation pathway [126]. The Noble–Collip drum model also exhibits a spontaneous thrombin burst measured by a thrombin generation assay. Systemic thrombin generation accelerates as a result of insufficient control by antithrombin. These data support those of a previous study [81]. Moreover, tPA release driven by hypoperfusion of the endothelium leads to hyperfibrin(ogen)olysis. Meanwhile, there was no evidence of activated protein C-mediated shutdown of thrombin generation in the systemic circulation [127].

A systematic review addressed the question: "what are relevant experimental models with

which to study early traumatic coagulopathy?" [128]. In this review, a tissue factor induced DIC model was reported to provide "grade A" evidence, representing a key model for traumatic coagulopathy. This model demonstrated that a massive amount of tissue factor also induces DIC associated with fibrin(ogen)olysis without tissue hypoperfusion [97]. This suggests that trauma itself could give rise to DIC without tissue hypoperfusion [67]. Tissue factor activation of coagulation leads to the generalized consumption of not only platelet and coagulation factors including fibrinogen, but also the inhibitory feedback factors involved in controlling coagulation and fibrinolysis, namely, antithrombin and α2-plasmin inhibitor, respectively [81, 91, 97].

Management of DIC

The cornerstone of DIC management is specific and vigorous treatment of the underlying disorder, that is, injury itself and hemorrhagic shock [14, 129]. There are clear differences in the treatment of DIC with the fibrinolytic phenotype in the early phase of trauma and DIC with the thrombotic phenotype in the late phase of trauma. Management of the latter DIC type is the same as for typical DIC; anticoagulants, platelet and plasma substitution, and coagulation inhibitor concentrates have been proposed [129]. Our discussion will follow with, treatment of fibrinolytic phenotype DIC through a novel approach.

After careful deliberation of "one concept and six considerations" for hemostatic changes during the early phase of trauma proposed by the SSC on DIC of the ISTH [130], trauma and hemorrhagic shock should be managed through damage control resuscitation, which integrates hemostatic resuscitation, damage control surgery, and in certain cases permissive hypotension [131]. Guidlienes by Spahn et al. review the physiological targets of resuscitation and suggest dosing of fluids, blood products, and pharmacological agents in bleeding trauma patients [132]. Anticoagulants are contraindicated for DIC with the fibrinolytic phenotype. Substitution therapies with plasma transfusion, platelet transfusion, and fibrinogen concentrate or cryoprecipitate transfusion maintain normal platelet counts and function, adequate levels of coagulation factors and endogenous anticoagulants including antithrombin and protein C [14, 129, 130]. Guidance for treatment of DIC published by the ISTH recommends the transfusion of plasma, which includes anticoagulant factors such as protein C, protein S, and antithrombin [129]. For further description of plasma transfusion please refer to Chap. 20. The use of agents that are capable of restoring dysfunctional anticoagulant pathways in DIC patients with sepsis has been extensively studied. However, there has been no study on the use of anticoagulant factor concentrates such as activated protein C, recombinant human thrombomodulin, and antithrombin in trauma induced DIC. Supranormal levels of anticoagulant factors foster bleeding. At present, plasma transfusion to maintain normal levels of protein C and antithrombin may be a reasonable strategy for treating DIC after trauma.

Tranexamic acid can reduce the risk of death in bleeding trauma patients [129, 133] and should be given as early as possible because any delay in administration after trauma reduces its efficacy and may actually be harmful [134]. These studies provide the theoretical basis for antifibrinolytic therapy in DIC with the fibrinolytic phenotype in the early phase of trauma [129]. Since the publication of the CRASH-2 results, there has been considerable discussion about how tranexamic acid should be used in practice. Some authors suggest limiting tranexamic acid use to specific patient subgroups, such as those with low blood pressure or laboratory evidence of hyperfibrinolysis [135, 136]. *A theoretical argument has been published, stating that biological insight into how the treatment works is more relevant when applying research results to patient care rather than the application of statistical reasoning* [137]. When administering tranexamic acid in the trauma setting, it is important to realize that the challenge for clinicians is not to identify a specific subgroup of patients but to detect those patients that will die from coagulopathic bleeding [137].

Table 13.3 Classification of trauma-induced coagulopathy

Trauma induced coagulopathy
1. Physiological changes
• Hemostasis and wound healing
2. Pathological changes
• Endogenously induced major pathologies
– Disseminated Intravascular Coagulation (DIC)[a]
• Activation of coagulation
• Insufficient anticoagulant mechanisms
• Increased fibrin(ogen)olysis (early phase)
• Suppression of fibrinolysis (late phase)
– Acute coagulopathy trauma-shock (ACOTS)
• Activated protein C-mediated suppression of coagulation
• Activated protein C-mediated increased fibrinolysis
• Exogenously induced minor pathologies that modify DIC and ACOTS
– Dilutional coagulopathy
– Hypothermia-induced coagulopathy
– Acidosis-induced coagulopathy
– Others

[a]Detailed description is given in the text

Trauma Induced Coagulopathy and DIC

The introduction of the concept of acute coagulopathy of trauma-shock (ACOTS) was proposed following a reacknowledgement that trauma itself and hemorrhagic shock can induce coagulopathy [138, 139]. Following the proposal of ACOTS, which is now a part of the more global term "trauma induced coagulopathy," there has been some confusion with the use of this terminology. Integration of this nomenclature is described in Table 13.3. Trauma induced coagulopathy consists of varying disease conditions that induce coagulopathy, each with a condition specific pathogenesis. Differences and similarities between DIC and the ACOTS have been extensively debated [14, 16, 25, 130, 140]. A detailed discussion on this point is beyond the scope of this chapter, but it is important to acknowledge that DIC has been the main pathophysiology of trauma induced coagulopathy [25, 140].

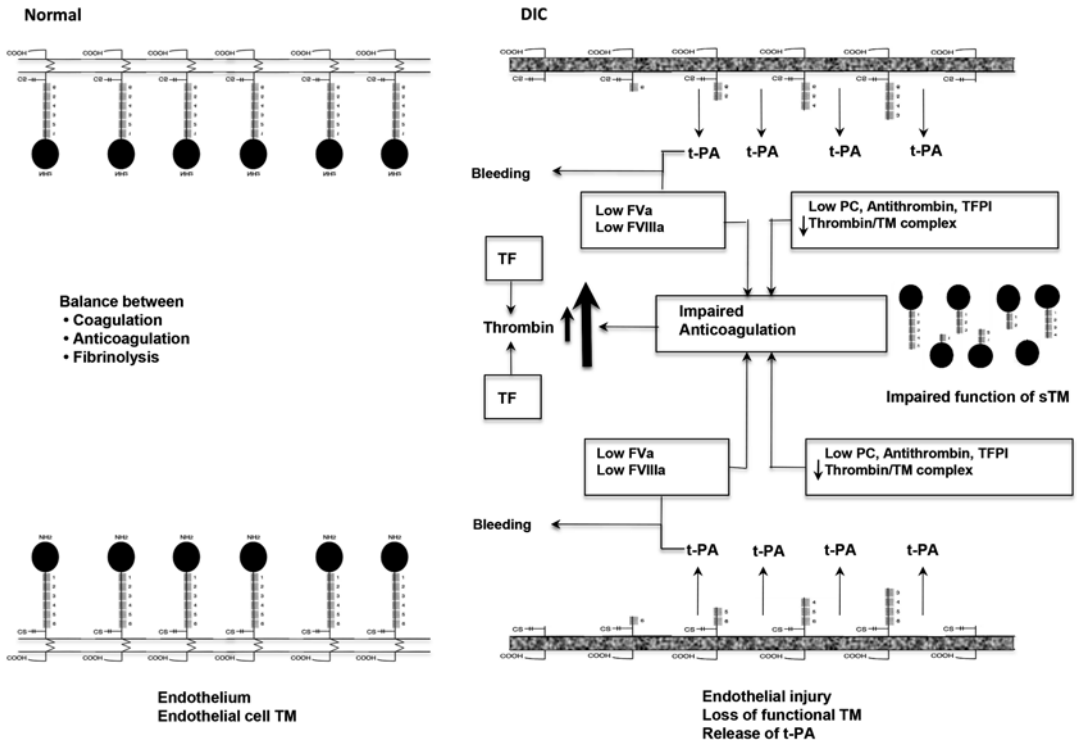

Fig. 13.10 Mechanisms of DIC with the fibrinolytic phenotype. *Left*, there is a balance between activation of coagulation, anticoagulation, and fibrinolysis during physiological hemostasis; *right*, DIC with the fibrinolytic phenotype. Consumption induced decreases in protein C, antithrombin, and TFPI and functional loss of both soluble and endothelial thrombomodulin severely impairs anticoagulation, enhancing systemic thrombin generation. *TM* thrombomodulin, *sTM* soluble TM, *TF* tissue factor, *PC* protein C, *TFPI* tissue factor pathway inhibitor. Modified with permission [16]

Conclusion

The main pathophysiological mechanism of trauma-induced coagulopathy is considered to be DIC. Disseminated intravascular coagulation in the early phase of trauma presents itself as a fibrinolytic phenotype, is associated with hyperfibrin(ogen)olysis and consumption coagulopathy, and contributes to massive hemorrhage (Fig. 13.10) [16]. This type of DIC progresses to DIC with the thrombotic phenotype during the late phase of trauma and drives multiple organ dysfunction. To understand the pathogenesis and appropriate management of DIC, deep insights are needed into the interplay between innate immunity, inflammation, and coagulation and fibrinolysis.

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Part III

Coagulation Assessment in Trauma Induced Coagulopathy

Prothrombin and Partial Thromboplastin Time

14

Ruchika Goel and Paul M. Ness

General Description and Clinical Use of Basic Coagulation Tests PT and PTT

Basic plasma coagulation function is readily assessed with a few simple in vitro laboratory tests: prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT), and quantitative fibrinogen determination. Traditionally, the PT and PTT assays were developed for diagnostic purposes unrelated to diagnosing or treating trauma patients. The PTT was originally used to identify subjects with hemophilia and subsequently to assay the level of antihemophilic factor VIII in hemophilia A patients [1] or factor IX in hemophilia B patients. The PT was initially used to assay the prothrombin level [2, 3] and its principal clinical utility now is in monitoring the adequacy of warfarin anticoagulation or providing an early diagnosis of patients with coagulopathy due to liver disease.

Figure 14.1 depicts the well-known "coagulation cascade" culminating eventually in thrombin generation. This model of the coagulation process was derived from the biochemical interactions of the coagulation proteins and describes very well the mechanisms of the PT and PTT assays.

While the PTT assesses the intrinsic limb of the coagulation system (prekallikrein, high-molecular-weight kininogen, factors XII, XI, IX, VIII), the PT assesses the extrinsic or tissue factor-dependent pathway that consists of tissue factor and factor VII. Both tests also evaluate the common coagulation pathway factors (prothrombin, V, X, and fibrinogen) involving all the reactions that occur after the activation of factor X (Fig. 14.1). Simultaneously drawn PT and PTT are needed to properly evaluate the coagulation system.

In PT, clotting is initiated by recalcifying citrated patient plasma in the presence of a thromboplastin (tissue factor) reagent. The PTT, on the other hand, is performed by recalcifying citrated plasma in the presence of a thromboplastic material that "does not" have tissue factor activity (hence the term partial thromboplastin) and a negatively charged substance (e.g., celite, kaolin, silica), which results in contact factor activation, thereby initiating coagulation via the intrinsic clotting pathway. Both PTT and activated partial

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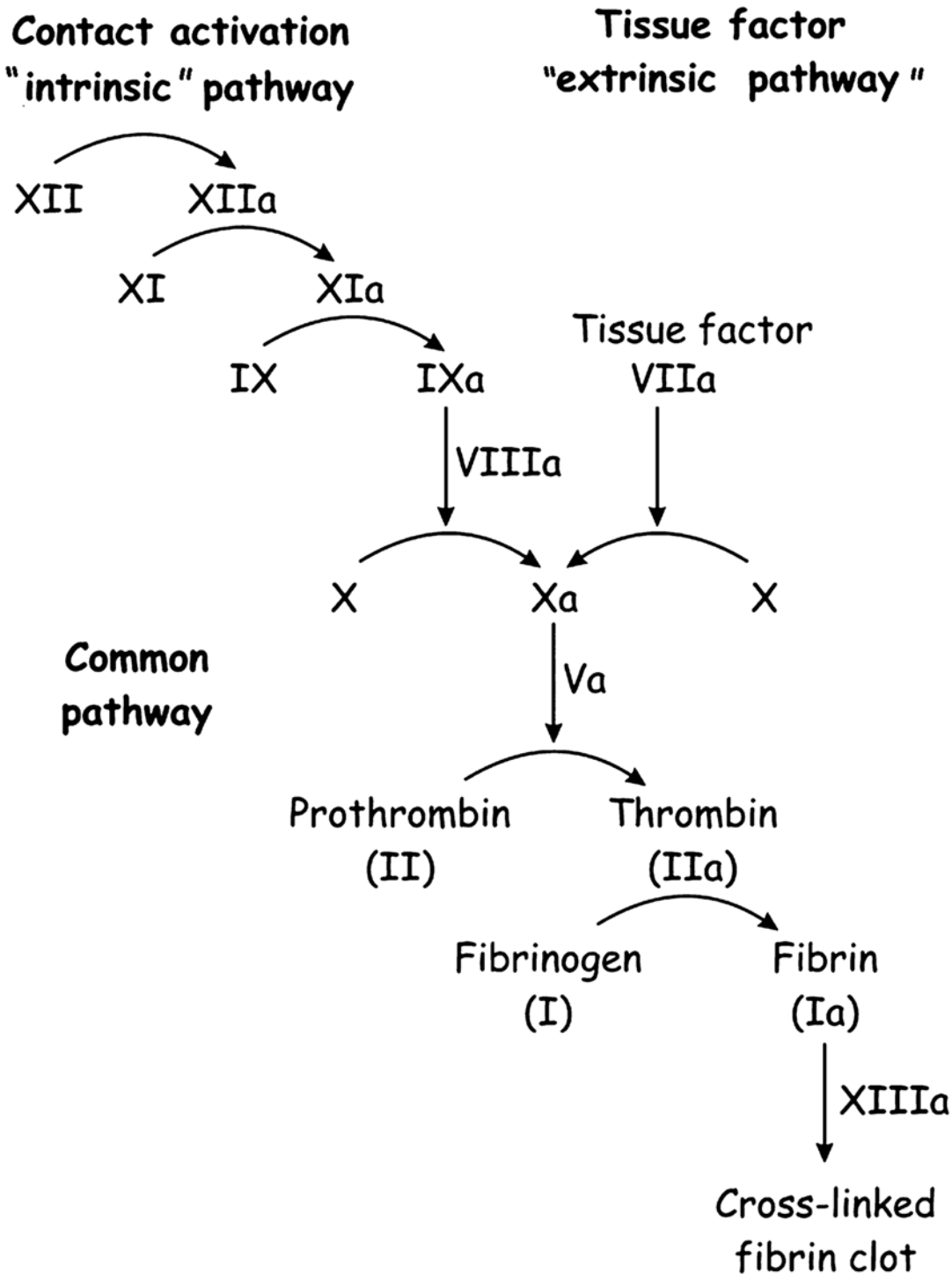

Fig. 14.1 The waterfall/coagulation cascade model of coagulation: the model is outlined as a Y-shaped scheme, with distinct "intrinsic" and "extrinsic" pathways initiated by FXII and FVIIa/Tissue Factor, respectively, with convergence of the two on a "common" pathway at the level of the FXa/FVa (prothrombinase) complex

thromboplastin time (aPTT) are used to test for the same functions; however, in aPTT, an activator is added that speeds up the clotting time and results in a narrower reference range. The aPTT is considered a more sensitive version of the PTT and is used to monitor the patient's response to heparin therapy. The reference range of the aPTT is 30–40 s. The reference range of the PTT is 60–70 s. The endpoint for the PT and PTT (and also thrombin time) is the time (in seconds) for the formation of a fibrin clot, which is detected by visual, optical, or electromechanical means.

A PT is expressed in seconds and/or as an international normalized ratio (INR). INR is the PT ratio that would result if the WHO reference thromboplastin had been used in performing the test. The PT/INR is most commonly used to measure the effect of warfarin and regulate its dosing as warfarin blocks the effect of vitamin K on hepatic production of extrinsic pathway factors. Utilizing the INR system of notation rather than the PT in seconds accounts for the variability of laboratory reagents used throughout the world and allows the effectiveness and dosing of warfarin therapy to be assessed by different laboratories with results that are consistent for patient care.

Prolongation of PT occurs with vitamin K deficiency (nutritional/antibiotic related), liver disease, DIC, deficiency of factors VII, II, X, fibrinogen, oral direct thrombin, or Xa inhibitors. Prolongation of the PTT can occur with a deficiency of, or an inhibitor to, any of the clotting factors except for factor VII. Medications that prolong the PTT include heparin, direct thrombin inhibitors, and direct factor Xa inhibitors. Notably, certain lupus anticoagulants, which are antibodies directed against plasma proteins bound to anionic phospholipids, cause PTT prolongation by interfering with the *in vitro* assembly of the prothrombinase complex. These *in vitro* events are paradoxically associated with an increased risk of venous and arterial thrombosis.

It is important to note that the cascade concept of coagulation as depicted above has often incorrectly been interpreted as an "actual *in vivo* model of coagulation" implying two parallel running systems. Although the cascade model remains useful to explain the mechanisms and utility of the PT and PTT, it does not explain the physiology of hemostasis or thrombosis very well. A 'cell-based' model of hemostasis as proposed by Hoffman et al. recognizes the major contribution of platelets to the clotting process, is more physiological, and shows that the two pathways have different functions and are both needed for adequate hemostasis. For details, please refer to Dr. Hoffman's description of the cell-based model of hemostasis [4, 5] in Chap. 1.

It is clear now that PTT and PT alone do not reflect the underlying physiology very well and furthermore they do not predict hemorrhagic or thrombotic predisposition. The absolute prolongation of the PT/PTT is not always clinically relevant. If the PT or PTT is abnormal, we can only determine the patient's risk of bleeding if we know the reason that the test is abnormal [6]. These screening tests are most useful in determining the cause of bleeding in a patient with hemorrhage, guiding the selection of other testing to look for specific factor deficiencies, and in guiding which blood components to transfuse to bleeding patients [6]. For example, a patient with an elevated PTT due to FVIII deficiency (hemophilia A) has a high risk of bleeding compared to a patient with an elevated PTT due to FXII deficiency who has no increased risk of bleeding and may actually be at risk for thrombosis. Likewise, a patient with a prolonged PTT due to the presence of a lupus anticoagulant usually does not have an increased risk of bleeding and may actually be at risk for thrombosis instead.

Role of PT/PTT in Coagulation Assessment in Trauma-Induced Coagulopathy

PT/INR and PTT are the most readily obtainable coagulation tests and so they remain the current standard for establishing a diagnosis of coagulopathy. While elevated PT is an integral part of the definition of early trauma induced coagulopathy (ETIC) itself, PT and PTT have been used largely as an early indication of severity in trauma.

Hess et al. reported high prevalence of "abnormal coagulation tests" in patients at the time of presentation to trauma centers, even in the absence of significant crystalloid resuscitation. In an analysis of records of all patients admitted to a large urban trauma center from 2000 to 2006, abnormal PT was increasingly frequent, with abnormal results occurring in 5–43 % of all trauma service admissions as the injury severity scores (ISSs) increased from 5 to more than 45. Similar trends were noted for PTT [7].

MacLeod et al. analyzed a trauma registry database of 7638 patients investigating PT, PTT, platelet count, age, ISS, presence of head injury, admission vital signs, and base deficit as predictors of mortality [8]. This study reported that in univariate analysis, abnormal PT had an odds ratio (OR) of 3.6 (95 % confidence interval [CI], 3.15–4.08; $p<0.0001$) for death with and an OR of 7.81 (95 % CI, 6.65–9.17; $p<0.001$) for deaths when combined with an abnormal PTT. In a multivariable regression model, PT and PTT remained independent predictors of mortality, whereas platelet count did not. The study concluded that a prolonged PT independently predicted a 35% increase in the likelihood of mortality and a substantially greater risk of mortality with a prolonged PTT as well [8].

Maegele et al. reported data from a large German Trauma registry database. They defined coagulopathy upon ER admission as abnormal prothrombin time test and/or platelets <100,000/μl. They detected evidence of ETIC in more than 1/3rd of patients (34.2%) upon presentation to ER. They noticed an increasing incidence of coagulopathy with increasing amounts of intravenous fluids administered prior to coagulation assessments. Coagulopathy was observed in >40% of patients with >2000 ml, in >50% with >3000 ml, and in >70% with >4000 ml of fluids administered [9].

Corroborating the data from German registry, Shaz et al. recently characterized the role of hemodilution in ETIC. They asserted that ETIC is associated with decreased factor activities without significant differences in thrombin and fibrin generation, suggesting that despite these perturbations in the coagulation cascade, patients displayed a balanced hemostatic response to injury. The "lower factor activities" are likely secondary to hemodilution and coagulation factor depletion. Thus, decreasing the amount of crystalloid infused in the early phases following trauma and early administration of coagulation factors through plasma resuscitation may prevent the development of severe ATC [10]. The data support the evolving picture that more severe injury, as demonstrated by increased ISS with resultant hypotension, can result in increased amounts of crystalloid administration in transit to the emergency department. This subsequent hemodilution results in a lower hematocrit and lower coagulation factor activities, most importantly factor VII, leading to the prolonged PT, which defines ETIC. The recent trends in trauma resuscitation with the avoidance of crystalloids and the early administration of plasma may mitigate these earlier observations.

Other groups have corroborated these findings using different trauma registries. In a study of 182 patients from sub-Saharan Africa, the prevalence of coagulopathy was 54 % (98/182) and mortality was significantly higher in the ATC group (29 deaths compared to 9 deaths in the non-ATC group). Notably, this study reported PTT as a strong independent predictor of mortality. They reported a significant difference in probability of survival between patients with elevated PTT and those with normal PTT ($p=0.001$). Most deaths related to elevated PTT occurred early in the hospital stay, with the probability of survival becoming parallel in the two groups as time went on [11]. Hess et al. have also reported abnormal PTT to be associated with excess mortality with PTT values that were abnormal but below the conventional PTT transfusion triggers [7].

Isolated traumatic brain injury (TBI)-associated coagulopathy defined as PTT >40 s and/or a PT/INR >1.2 and/or a platelet count <120,000/μl has also been shown to be strongly associated with an unfavorable outcome [12].

Utility and Limitations of PT/PTT Measurements

While there are considerable benefits to PT/PTT assessments in trauma patients, there are also major limitations. While a prolonged PT remains

a strong predictor of mortality and correlates with injury severity, it may not fully explain the mechanism of coagulopathy. It is very important to remember that these tests were originally designed to monitor oral anticoagulant therapy or diagnose hemophilia. The current testing procedures are performed on platelet-poor plasma at 37 °C and usually require 30–60 min to process by conventional methods [13], which may not be very helpful or timely in the management of acute trauma. Thus, for hemorrhaging trauma patients requiring acute interventions, these results may not be a contemporary and accurate reflection of coagulation function [5].

Nevertheless, currently available PT and PTT remain the most widely utilized and most cost-efficient first-line tests available for coagulopathy evaluation at most testing facilities especially so in resource-limited settings and smaller emergency room settings that are independent of major trauma centers [11]. They may also serve as an early warning about the severity of injury in a patient who presents with elevated PT but who is not actively bleeding.

Alternative Tests for Coagulopathy Evaluation in ETIC

Thromboelastography (TEG, Haemonetics, Braintree, MA) or rotational thromboelastometry (ROTEM, Tem Systems, Inc., Durham, NC) tests measure viscoelastic properties of the clot and have gained traction recently as testing methods that can be performed on whole blood. TEG and ROTEM are being increasingly performed as "point-of-care" assays in the emergency department or perioperative settings providing quick turnaround times compared to testing performed in a centralized clinical laboratory [14–16]. Although TEG has been used in clinical practice for many years, only recently has the equipment become rapid and stable enough for its use to be extended to the resuscitation room or surgical suite. A recent study on RoTEM measurements in ATC aimed to compare the utility of the RoTEM results to the standard tests (PT/PTT) of coagulopathy. While the study showed that TEG is feasible in early trauma, there are numerous programs using these systems under way which may confirm these , results and ultimately allow an assessment of their utility in trauma management [17]. These tests and their applicability in ETIC will be addressed in more extensive detail in the ensuing chapters.

Conclusions/Summary

PT/INR and PTT are the most readily obtainable coagulation tests and retain clinical utility to provide an early diagnosis of coagulopathy due to trauma. While both PT/PTT have been shown to be highly predictive of severity and mortality risk in trauma patients, they may not provide an accurate and contemporary description of the coagulation dysfunction in a trauma patient being resuscitated and may not be predictive of the bleeding or thrombosis risk *per se*. The results of PT/PTT testing typically have inadequate turnaround time to be useful to manage concurrent patients, but they retain some utility in assessing efficacy in treated patients as part of research protocols and ongoing databases.

Other testing systems such as TEG/ROTEM may provide more rapid and comprehensive assessments of the hemostatic system in trauma. As better testing algorithms for diagnosing and treating hemorrhage and/or thrombosis, clinicians should suspect and empirically treat acute coagulopathy in patients at risk. It is likely that ongoing development and validation of robust point-of-care tests of coagulation and thrombosis will ultimately permit better tailoring of resuscitation to the individualized needs of each trauma patient.

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Fibrinogen Assays

15

Christoph J. Schlimp and Herbert Schöchl

General

Fibrinogen plays a crucial role in haemostasis. It is not only the precursor of fibrin but has also a high affinity to glycoprotein IIb/IIIa receptors, which are expressed on activated platelets. Thus, fibrinogen contributes to both platelet aggregation and the formation of a stable fibrin network. Importantly, fibrinogen seems to be the most vulnerable coagulation factor, reaching critically low levels earlier than any other coagulation protein in the course of severe bleeding [1–4]. A variety of reasons, such as major blood loss, dilution, consumption, hyperfibrinolysis, hypothermia and acidosis, are responsible for the rapid deterioration of the plasma fibrinogen concentration [5–7]. Importantly, low fibrinogen levels are strongly related to the amount of blood loss, the extent of shock and the severity of injury [8]. Recent guidelines recommend maintaining the plasma fibrinogen concentration above 1.5–2 g/L in bleeding trauma patients [9, 10]. Moreover, these guidelines strongly recommend early and repeated assessment of fibrinogen in trauma patients with substantial bleeding.

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Fibrinogen, Principles of Measurement

More than 60 different methods for measuring plasma fibrinogen concentration have been described. The number depends on the type of activation method and read-out [11]. The four major fibrinogen assay groups are chronometric (Clauss), derived (prothrombin time), immunological, and clottable. Typical read-outs are mechanical or optical. Moreover numerous reagents may multiply the possibilities of measurements [11].

Recently, in contrast to plasma fibrinogen measurements, whole blood fibrinogen measurement methods have been introduced, potentially providing faster results than plasma-based techniques [12–15].

Chronometric Clauss Fibrinogen Measurement

Most commonly the Clauss method, which is a modification of thrombin time, is used to determine fibrinogen plasma concentration. As a note,

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consistency in the nomenclature of the Clauss method should be a goal as in the past it has been erroneously referred to as the "von Clauss" method [16]. In the original publication in German [17], "von" is not part of the name (i.e. a title of nobility), but simply means "written by" A. Clauss (Arnold Clauss). The Clauss method measures fibrinogen levels generated in a diluted, citrated platelet-poor plasma sample in the presence of an excess amount of thrombin (~100 U/mL). The time of the coagulation process is determined and extrapolated into a measurement of the functional fibrinogen concentration using calibration standards [18, 19]. The clotting time is inversely proportional to the amount of fibrinogen in the sample. Several methods have been developed to detect the fibrin clot endpoint generated by the Clauss assay. Automated coagulation analysers incorporating mechanical, electromechanical or photo-optical detection are commonly used. Mechanical devices detect the movement of a metal hook or the removal of a steel ball from a magnetic field upon clot formation. In contrast, electromechanical devices measure the effect of clot formation on the oscillation of a steel ball. The photo-optical techniques assess changes in light transmission [20]. Multiple commercially produced Clauss assays are available, with marked differences in thrombin concentration and source (e.g. bovine or human) and in the concentration of stabilisers, inhibitors and buffers [21]. In addition, a number of different reference plasmas are used as calibrators with substantial differences in composition between these products [20].

Derived Fibrinogen Measurement

Derived fibrinogen is based on the fact that the difference between baseline and maximum turbidity during prothrombin time (PT) measurement is proportional to the fibrinogen concentration between 0.5 and 16 g/L [22]. PT-derived fibrinogen measurement is established in some countries such as the UK, but it is not recommended by guidelines on fibrinogen assays because of the potential for discrepancies versus the Clauss method [21].

Whole Blood Fibrinogen Measurements

A new concept, which avoids prior centrifugation of the blood sample, is fibrinogen measurement in whole blood. Amukele et al. used an electromechanical device for the determination of whole blood fibrinogen, with acceptable results for the lower fibrinogen range but higher variations in fibrinogen levels above 4 g/L [12].

Ogawa et al. reported the "dry reagent method" (dry haematology), which assesses thrombin-induced clot formation in an oscillating magnetic field [14]. This technique has been introduced for whole blood PT, activated partial thromboplastin time and activated clotting time measurements. After adjusting for haematocrit an excellent correlation between whole blood fibrinogen measurements and the Clauss method has been observed.

This finding is in alliance with recent studies by Schlimp et al. in which fibrinogen concentration was measured in whole blood using a standard Clauss assay and a steel ball coagulometer [13]. The feasibility of this approach was tested with a Clauss assay using multiple porcine fresh blood samples obtained during in vivo bleeding, haemodilution and after treatment with haemostatic therapy. Two different Clauss assays were then tested using multiple human volunteers' blood samples diluted in vitro and supplemented with fibrinogen concentrate. After adjustment of the measured whole blood fibrinogen concentration with a quickly measureable haemoglobin-derived haematocrit, regression and Bland-Altman analyses of derived "plasma-equivalent" fibrinogen and measured plasma fibrinogen concentration were excellent in porcine and human blood samples, especially in the ranges relevant to traumatic or perioperative bleeding. Hayakawa et al. also just reported whole blood coagulation analyser-measured fibrinogen levels within 2 min in critical care settings [15].

Fast whole blood fibrinogen measurements could be considered as an alternative to plasma fibrinogen measurement for acute bleeding management in trauma and perioperative care settings. Further studies are needed to prove this concept and determine the turnaround times for its clinical application in emergency rooms and operating theatres.

Immunologic and Clottable Fibrinogen Measurement

Immunologic and clottable fibrinogen measurement are frequently applied for research purposes but hardly used in clinical routine. Compared to the Clauss method or PT-derived measurements, immunologic assays are expensive and/or inappropriate in terms of turnaround times for clinical routine.

Moreover, immunologic assays count the number of molecules to some extent independent of its functionality. Therefore in trauma and shock it must be taken into account that an immunological assay shows total fibrinogen but not the actual functional fibrinogen concentration. However, from an experimental point of view the difference between an immunological and functional assay could give a hint to fibrinogen degradation occurring due to shock [23].

Plasma Fibrinogen, Shortcomings

Unfortunately, the Clauss method is not standardised. Numerous variations in respect to the read-out method (photo-optical, mechanical or electromechanical), type of calibrators, analyser platforms and assay brands could deliver substantially different results for the same plasma sample. A variety of studies showed that the measurement of plasma fibrinogen concentration can be affected by the type of device [24, 25], reagents [25], assay methods [26] and calibrators [27, 28] used. It has been shown recently that significant differences exist in the performance of assays for measuring plasma fibrinogen concentration, particularly between different laboratories ([16], and Fig. 15.1).

All of these variations have the potential to influence the result, especially in bleeding patients where further clinical variables (e.g. haemodilution, hydroxyethyl starch or the presence of fibrin/fibrinogen degradation products or lipids) may amplify any differences between measurement methods. It has been reported that the presence of artificial colloids (e.g. dextran or hydroxyethyl starch) significantly raises measured fibrinogen concentration above that predicted by the dilutional effect [29]. Thus, when high volumes of synthetic colloids are infused in

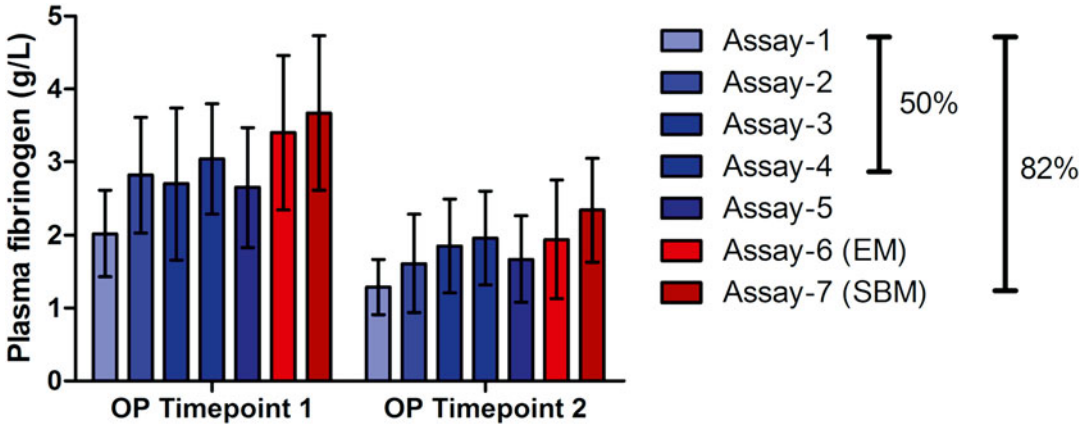

Fig. 15.1 Mean (standard deviation) values of fibrinogen concentration measurements obtained from the same set of plasma samples measure with seven different Clauss assays. Assays 1–5 represent assays with photo-optical read-out, assay 6 with an electromechanical (EM) read-out and assay 7 with a steel ball mechanical (SBM) read-out. Differences in the absolute fibrinogen concentration up to 82 % have been observed [16]

the course of massive transfusion, hypofibrinogenaemia may potentially be overlooked. Adam et al. reported that photo-optical methods significantly overestimate the fibrinogen concentration in blood diluted with hydroxyethyl starch [22, 30]. Fibrinogen concentration was overrated by >80 % and >110 % with 30 % and 50 % dilution, respectively. Similar observations have been made by Fenger-Eriksen et al. [24].

The most important shortcoming of plasma-based fibrinogen measurements is the unacceptable long turnaround time. Davenport and co-workers reported a median turnaround time for standard coagulation assessment of 78 min with an interquartile range of 62–103 min [31]. This is in line with a French survey, which reported a corresponding median time of 88 min with a range of 29–295 min [32]. This significantly limits the clinical value of PT-derived and Clauss measurements of fibrinogen. A much reduced turnaround time (14 min) has been reported and was achieved by rapid centrifugation, swift sample transport, optimal communications and an extended calibration range [33]. However, in most centres it is doubtful whether this could be implemented in clinical routine.

Fibrin-Based Viscoelastic Tests

Modern viscoelastic methods such as thromboelastometry (ROTEM®, TEM International GmbH, Munich, Germany) or thrombelastography (TEG®, Haemonetics Corp., Braintree, MA, USA) provide information on the speed of initiation of coagulation, the kinetics of clot growth, the clot strength and the potential breakdown of the clot [34]. Specific ROTEM and TEG assays (FIBTEM and functional fibrinogen [FF], respectively) are designed to assess the fibrin polymerisation in whole blood [35, 36]. This is achieved by assessing clot strength in the presence of a platelet inhibitor: cytochalasin D for the FIBTEM assay and abciximab for the FF assay [37]. It allows specific evaluation of the fibrin component of the clot (Fig. 15.2). Fibrin clot strength is primarily (though not exclusively) dependent on fibrinogen and activated FXIII. Thus, any functional

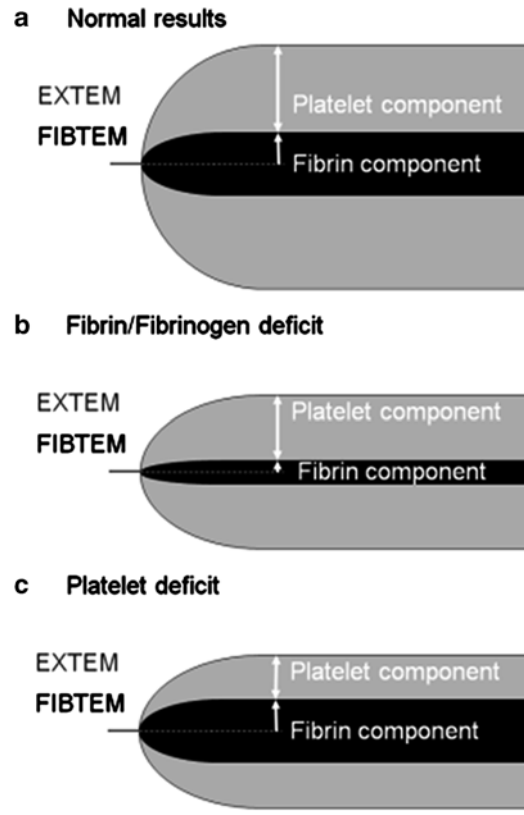

Fig. 15.2 EXTEM (extrinsically activated test) provides rapid information about the overall clot formation, whereas FIBTEM (extrinsically activated test plus cytochalasin D) focuses within the same time frame on the fibrin polymerisation of the clot after platelet inhibition (**a**). Fibrinogen and platelets both contribute to overall clot firmness. However, by only performing the EXTEM assay, fibrin(ogen) deficit (**b**) and platelet deficit (**c**) cannot be distinguished. With the supplementary platelet inhibition assay, the two coagulopathic conditions can be separated

impairment of fibrinogen (e.g. by colloids) will diminish the entire clot strength [38].

Recent data suggest that differences between FF and FIBTEM test results may be encountered, and therefore ROTEM and TEG results must not be used interchangeably [35, 37, 39, 40]. Recently a new assay (ROTEM assay: FIBTEM PLUS), which has been studied experimentally and clinically, provides higher effectiveness to inhibit the platelet contribution to clot strength (Fig. 15.3). Thus, a combination of cytochalasin D and a glycoprotein IIb/IIIa blocker allows a complete inhibition of platelet contribution and therefore

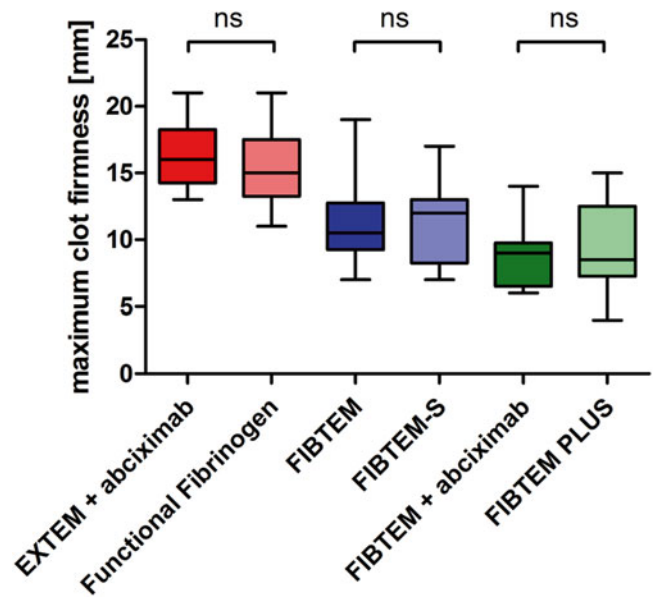

Fig. 15.3 Data from viscoelastic assays designed to assess the functional capacity of fibrinogen in whole blood. Graph shows the maximum clot firmness (median, interquartile range and range). All assays are activated extrinsically (tissue factor) and platelet function was inhibited either with a glycoprotein-IIb/IIIa receptor inhibitor (EXTEM + abciximab and functional fibrinogen), cytochalasin D (FIBTEM or FIBTEM-S) or a combination of both (FIBTEM + abciximab and FIBTEM PLUS). Unless stated "ns" (not significant), maximum clot firmness from all assays resulted in significant between-test differences [37]

more accurate information on the fibrin-based clot strength [37, 41].

Recent studies raised the question to which extent haematocrit influences whole blood viscoelastic test results [42–45]. It is important to keep in mind that the actual fibrin clot strength in whole blood only partially reflects plasma fibrinogen concentration, but gives a more comprehensive picture of fibrin polymerisation in whole blood. Moreover, it has been reported recently that fibrinogen interacts with erythrocytes [46, 47], and fibrinogen receptors on erythrocytes have been discovered [48, 49]. Binding of fibrinogen on these receptors might potentially interfere with clot strength. Although the amplitude of fibrin-based viscoelastic tests can be used to guide fibrinogen supplementation, we strongly discourage the reporting of functional fibrinogen in the way of a directly derived and calculated concentration (mg/dL) for the above-mentioned reasons [36]. However, when performed in platelet-free plasma an extrinsically activated thromboelastometric measurement correlates highly with the actual fibrinogen concentration [13].

Clinically, it has also been shown that the FIBTEM assay may have potential for early prediction of massive transfusion [50]. An important advantage of both the FIBTEM and FF assays is a short turnaround time. ROTEM and TEG analyses are performed in whole blood, thus avoiding the need for prior centrifugation of the blood sample. For ROTEM, a mean manipulation (set-up) time of 2 min 51 s has been reported for trained physicians [51]. Therefore, the first ROTEM test results allowing to estimate fibrin base clot strength, such as the clot amplitude at 5 min (A5), can be expected in around 8 min. Turnaround times for the FF assay have not been reported until now. Guidelines of the European Society of Anaesthesiology, and others, propose to use viscoelastic measurements in traumatic or perioperative bleeding patients [9, 10].

Plasma Fibrinogen Estimation by Surrogate Parameters

Rapid estimation of plasma fibrinogen in major trauma patients upon emergency room admission has been recently proposed, based on the results of blood gas analysis including the measurement of haemoglobin (Hb) and base excess (BE) as well as calculation of the Injury Severity Score [8] (Fig. 15.4). In a study of severely injured patients ($n = 675$) admitted with an Hb <12 g/dL, 74 % had low (<2 g/L) and 54 % had critical

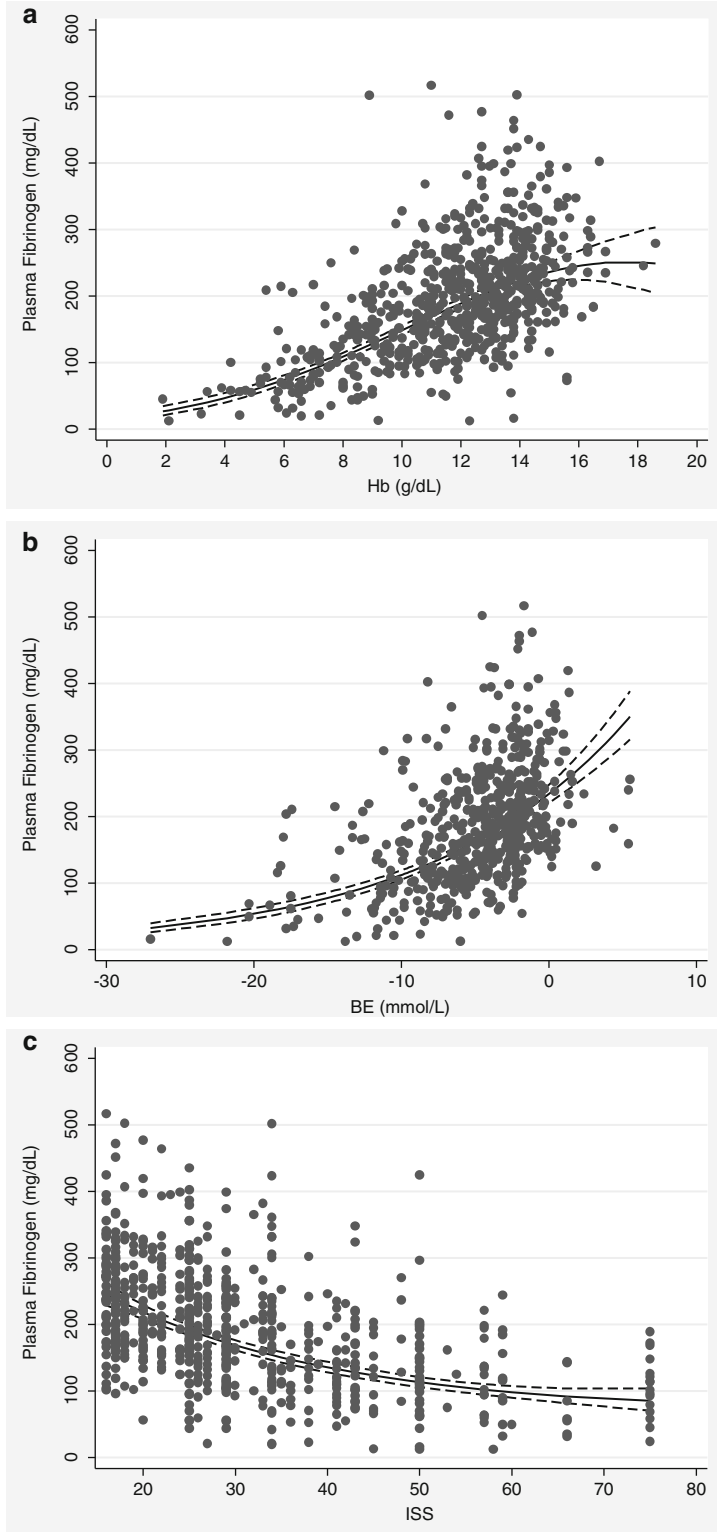

Fig. 15.4 Plasma fibrinogen concentration (mg/dL) versus (**a**) hemoglobin (Hb, g/dL), (**b**) base excess (BE, mmol/L) and (**c**) Injury Severity Score (ISS). The fitted regression line (solid) is shown along with a corresponding 95 % confidence interval (*dotted lines*) [8]

Table 15.1 Percentage of patients with adequate, low and critical plasma fibrinogen levels (FIB) when combining the parameters of Hb (g/dL) and BE (mmol/L) of severe trauma patients on admission [8]

Adequate FIB (≥200 mg/dL)	Hb ≥12.0	Hb 11.9–10.0	Hb 9.9–8.0	Hb <8.0
BE ≥−2	63 %	73 %	25 %	50 %
BE −2.1 to −6	57 %	30 %	24 %	17 %
BE −6.1 to −10	50 %	21 %	5 %	3 %
BE <−10	42 %	0 %	0 %	4 %

Low FIB (<200 mg/dL)	Hb ≥12.0	Hb 11.9–10.0	Hb 9.9–8.0	Hb <8.0
BE ≥−2	37%	27 %	75 %	50 %
BE −2.1 to −6	43 %	70 %	76 %	83 %
BE −6.1 to −10	50 %	79 %	95 %	97 %
BE <−10	58 %	100 %	100 %	96 %

Critical FIB (<150 mg/dL)	Hb ≥12.0	Hb 11.9–10.0	Hb 9.9–8.0	Hb <8.0
BE ≥−2	6 %	11 %	8 %	50 %
BE −2.1 to −6	18 %	36 %	56 %	75 %
BE −6.1 to −10	11 %	68 %	65 %	93 %
BE <−10	33 %	67 %	92 %	93 %

(<1.5 g/L) plasma fibrinogen levels. Of patients admitted with Hb <10 g/dL, 89 % had low and 73 % had critical plasma fibrinogen levels. These values increased to 93 % and 89 %, respectively, among patients with an admission Hb <8 g/dL. Sixty-six per cent of patients with only a weakly negative BE (<−2 mmol/L) showed low plasma fibrinogen levels. Of patients with BE <−6 mmol/L upon admission, 81 % had low and 63 % had critical plasma fibrinogen levels. The corresponding values for BE <−10 mmol/L were 89 % and 78 %, respectively (Table 15.1).

Conclusion

Many different plasma fibrinogen measurement methods are available. However, most commonly the Clauss method is used in order to measure fibrinogen plasma concentration. Several factors, including type of device and reagent, have been shown to affect the results of fibrinogen concentration measurements by the Clauss method and clinically significant differences exist between the performance of assays. Turnaround times for plasma fibrinogen in the clinical settings of a bleeding patient may be considered too long. Measurement of whole blood fibrinogen in perioperative or traumatic bleeding may be a future option to rapidly determine fibrinogen concentrations and can be easily calculated for plasma values as well. Plasma fibrinogen estimation in trauma patients on admission by surrogate parameters has been proposed. Viscoelastic tests estimating fibrin-based clot strength in whole blood are increasingly used to faster estimate the need for fibrinogen supplementation in the bleeding trauma patient.

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Platelet Aggregometry

16

Taizo Nakano and Jorge Di Paola

Introduction

After traumatic injury to tissue and vasculature, platelets facilitate the first line of defense against bleeding during primary hemostasis. Their complex role requires appropriate adherence to newly exposed subendothelial matrix composed mainly of collagen as well as interactions with plasma von Willebrand factor (VWF), adequate activation and secretion of stored granular content (adenosine diphosphate, adenosine triphosphate and serotonin) and robust aggregation in great number to tamponade bleeding. The formation of this platelet plug further serves as a scaffold to support the plasma factors responsible for fibrin clot formation (secondary hemostasis). Adequate hemostasis to prevent hemorrhage after extreme trauma depends on the body's ability to maintain this defense with adequate platelet number and function [1]. Although severe thrombocytopenia has been shown to impede primary hemostasis and correlate with early mortality, it is not present in all trauma patients [2, 3]. After rapidly assessing adequate platelet number, focus on the hematologic evaluation of traumatic injury includes the qualitative evaluation of platelet function. As platelet function assays become more standardized and more widely available, the ability to more rapidly assess platelet function to impact clinical decision making is growing. In this chapter, we review the field of platelet aggregometry as an in-vitro assay to measure platelet aggregation and its potential application in the assessment and intervention of acute trauma.

Platelet Aggregometry

Platelet aggregometry is a laboratory assay to qualitatively measure platelet activation and aggregation in response to known platelet agonists. Utilized in both the clinical and research setting, it represents the gold standard laboratory evaluation to assess platelet function in patients with suspected congenital or acquired platelet function disorders. The most commonly used assays to carry out platelet aggregometry include Light Transmittance Aggregometry (LTA) and Whole Blood Aggregometry (WBA).

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Light Transmittance Aggregometry

Light transmittance aggregometry was initially developed by G.V. Born in 1962 [4]. He designed an assay that measured light transmittance through a sample of platelets held in suspension. After the addition of a platelet agonist, he demonstrated an increase in light transmittance concurrent with progressive platelet aggregation. Over time, the utilization of different sample sources and various platelet agonists, each able to activate a unique functional aspect of platelet activation pathways, paved the way to a novel screening tool to identify disorders of platelet surface glycoproteins, signal transduction, and platelet granules. Born's LTA turbidimetric technique has endured and remains a widespread gold standard diagnostic tool that is now carried out in a more high throughput and standardized manner.

Despite ongoing efforts to standardize LTA technique, worldwide surveys continue to demonstrate significant variability in LTA practices [5]. In 2013, the Platelet Physiology Subcommittee of the International Society on Thrombosis and Haemostasis (ISTH) published methodological consensus guidelines to standardize LTA testing [5]. Current LTA assays require a 20–30 ml sample of whole blood. Centrifugation separates the heavier red and white blood cell pellet from the platelet rich plasma (PRP) supernatant. 270 μl of a patient's PRP is placed in a cuvette, maintained in suspension at a consistent temperature, and the baseline optic output is measured as the maximal optical density (Fig. 16.1) [5, 6]. After the addition of an agonist, platelets undergo an activated shape change from discoid to spiny spheres that increase their surface area, which is often associated with a brief increase in optical density readout.

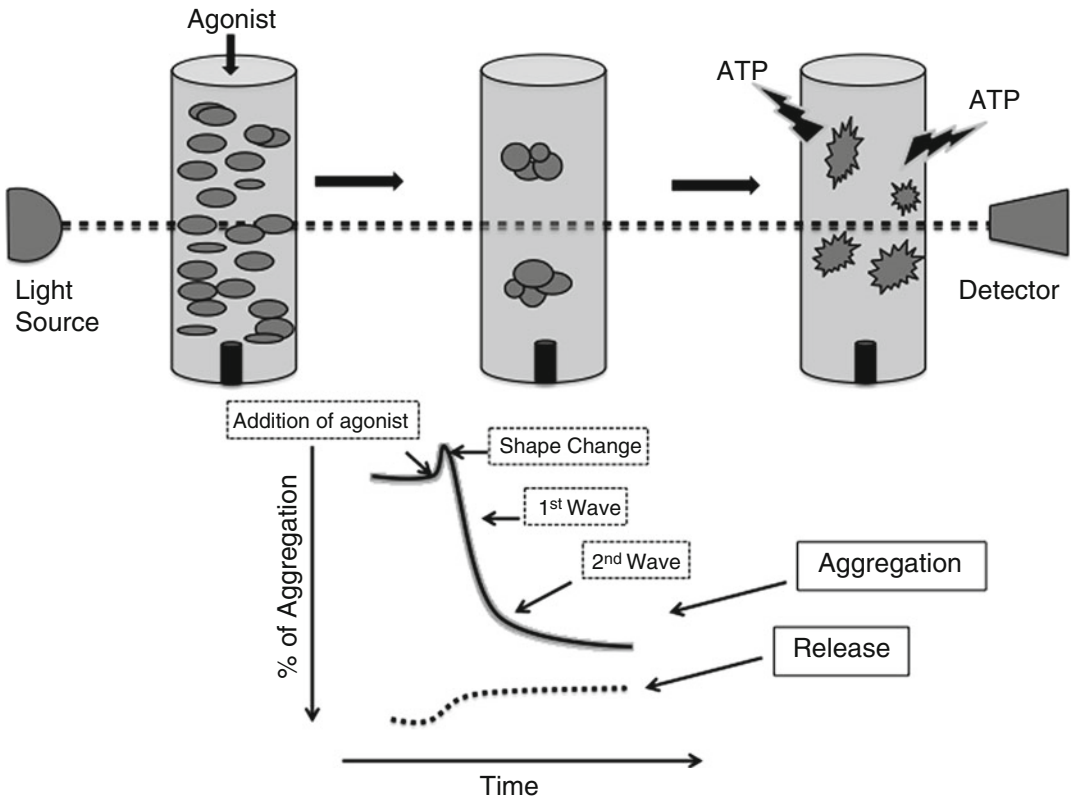

Fig. 16.1 Schematic representation of light transmittance aggregometry depicting initial turbid platelet rich plasma with the consequent aggregation due to the addition of a platelet agonist. This allows for the light to be transmitted and detected. The aggregation curve is represented by light transmittance as a function of time. When platelets get activated released ATP is measured. While the first wave of aggregation is dependent on exogenous agonist, the second wave is due to release of platelet granule contents and continued activation of surrounding platelets

As platelets begin to rapidly aggregate, more light is able to pass through the cuvette and the measurement of optical density decreases concurrently. Within minutes, the aggregation curve will reach a plateau at a point referred to as maximal aggregation. Depending on the specific choice and concentration of agonist used, the curve will either return to baseline (a process known as disaggregation) or maintain a state of irreversible platelet aggregation (Table 16.1). Individual platelet agonists, studied at various dilutions, are able to highlight unique defects in functional platelet pathways of activation, secretion and aggregation (Table 16.2) [7].

Epinephrine: A weak agonist that binds to α_2-adrenergic receptor on the platelet membrane leading to a primary wave of aggregation followed by ADP secretion and a second wave of sustained aggregation. Epinephrine will not demonstrate aggregation in storage pool defects or release defects. LTA stimulated with epinephrine will not induce an initial shape change. Epinephrine is used in the 5–10 μM range.

Adenosine Diphosphate (ADP): A weak agonist that binds to a specific ADP G-protein coupled platelet membrane receptor on the surface of platelets. Binding results in platelet shape change and a primary wave of aggregation. This is followed by release of dense granule stored ADP secretion and a second wave of aggregation, demonstrating a biphasic response. When studied at low doses, only primary aggregation is stimulated and disaggregation is observed. The concentrations of ADP typically used in the platelet aggregation assay ranges from 1 to 10 μM.

Collagen: A strong agonist that binds to the GPVI and GPIa/IIa receptors and directly induces granule release and thromboxane A_2 (TxA_2) generation without a primary wave of aggregation is usually used in the 1–5 μM range. Aggregation depends on intact collagen membrane receptors, membrane phospholipase pathway integrity, Arachidonic Acid production, normal cyclooxygenase and thromboxane pathway function.

Ristocetin: Not a true platelet agonist, ristocetin induces a passive process of platelet agglutination via von Willebrand Factor (VWF). At a concentration of 1.5 mg/ml ristocetin facilitates the interaction between platelet glycoprotein Ib (GPIb) and VWF. Platelets are not initially activated by the ristocetin; however, the close platelet-to-platelet contact during agglutination induces thromboxane A_2 (TxA_2) production, which in turn induces platelet shape change, release of stored granular content and formal aggregation.

Arachidonic Acid (AA): Arachidonic Acid is a precursor of TxA_2 in platelets. Upon platelet activation, AA is released from the membrane inner leaflet and converted to prostaglandins by cyclooxygenase. The prostaglandins are then converted to TxA_2, which induces platelet aggregation. AA, as an agonist, is used to assess the viability of the thromboxane pathway.

Thrombin receptor activating peptide (TRAP): Acting as a mimic of thrombin, TRAP is a strong agonist of the thrombin receptor PAR-1 that results in platelet aggregation. Used at a low dose, it can induce shape change, but no full aggregation. Used at a high dose, it can induce full aggregation.

Thromboxane A_2 analogue U46619: Acting as a mimic of endoperoxide prostaglandin PGH2, U46619 is a strong thromboxane A_2 receptor agonist. It induces shape change and aggregation of platelets.

LTA is highly prone to both preanalytical and analytical variables and, therefore, should be performed by specialized laboratory technicians equipped with standardized assay reagents. It is important to document a patient's recent exposure to medications (aspirin, nonsteroidal anti-inflammatory drugs, antiplatelet agents) and environmental factors (exercise, caffeine, smoking, high-fat meal prior to blood draw) to accurately interpret results. More specifically, ISTH consensus guidelines recommend patients abstain from caffeine for at least 2 h, refrain from smoking for at least 30 min, and allow a short rest

Table 16.1 Measurements of the platelet aggregation curve

Measurement	Definition
Maximal amplitude or percent aggregation	A ratio of the point of maximal percent aggregation after the addition of a platelet agonist to a control sample representing 100 % aggregation.
Second wave of aggregation	A more profound wave of aggregation dependent on secretion of ADP and thromboxane generation. Documented as either present or absent.
Disaggregation	After reaching maximal aggregation, there is possible immediate disaggregation to baseline. Documented as either present or absent.
Slope of aggregation	A slope tangent to the aggregation curve represents the rate of platelet aggregation, which can be interpreted as a ratio compared to a control sample that represents 100 % aggregation.
Shape change	Initial deflection in tracing secondary to platelet shape change that increases total platelet surface area when activated. Documented as either present or absent.
Lag phase	A brief flattening between primary aggregation and second wave of aggregation. Documented as a length of time between events.
Amplitude at the end of aggregation	A point representing the final extent of aggregation at the completion of the LTA assay.

period prior to collection of blood samples [5]. An effort should be made to draw blood using a high-caliber needle (at least 21 gauge), rapidly mix the sample in an anticoagulated and buffered Vacutainer tube (109 or 129 mM sodium citrate), stored at room temperature, and processed within 4 h of collection. After allowing the sample to rest for 15 min, it is then centrifuged at $200 \times g$ for 10 min at 21 °C. The PRP supernatant should be allowed to rest for an additional 15 min at room temperature prior to LTA testing. The LTA assay is then carried out on PRP from both the patient and a known healthy control subject. During testing, samples are stirred continuously at 1000 rpm to maintain a homogenous platelet suspension and temperature maintained at 37 °C.

Whole Blood Aggregometry

As LTA remains labor intensive and time consuming, there has been growing attention to the use of whole blood to assess platelet function. A notable benefit would be to maintain platelet interaction with red and white blood cells and retain any abnormal platelets that may have otherwise been lost during lengthy centrifugation steps. In 1980, Cardinal and Flower developed a technique to measure platelet aggregation of whole blood through the use of electrical impedance [8].

Whole blood aggregometry (WBA) is carried out on a 6–9 ml sample of whole blood diluted 1:1 with normal saline. 300 µl of the diluted sample is placed in a cuvette with two silver-coated cooper electrodes that have a current of 100 mV continuously passed between them. At baseline, the electrodes become coated with platelets and the investigators record a measure of initial electrical impedance in ohms. After a platelet agonist is added to the assay, platelets, WBC, and RBCs aggregate to progressively impede the current. The WBA output tracing resembles the LTA readout and measurements reflecting the stages of platelet activation and aggregation can be interpreted in a similar manner.

Industry has continued to develop high throughput, low cost and, importantly, more rapid WBA assays. Multi-well and multi-electrode high throughput plates have been developed that minimize the amount of patient sample required and greatly standardize assay conditions. Single-use test cells allows for a more standardized and sterile assay.

Lumiaggregometry

To better monitor platelet activation and the secretion of stored granular content, a technique was developed to measure biochemical luminescence

Table 16.2 Platelet agonists and their mechanism of action on platelet aggregation

Platelet agonist	Dose	Mechanism of action
Epinephrine	5, 10 μM	Weak agonist to induce platelet aggregation without secretion.
Adenosine diphosphate (ADP)	Low 1, 2.5, 5 μM High 10 μM	Weak agonist to induce platelet aggregation without secretion.
Collagen	1, 4 μg/μM	Strong agonist to induce/amplify platelet aggregation and secretion.
Arachidonic acid (AA)	500 μg/ml	Used to assess the viability of the thromboxane pathway.
Ristocetin	Low 0.5 mg/ml High 1.5, 5 mg/ml	Induces passive platelet agglutination through the interaction of GPIb and VWF.
(TRAP)	Low 50 nmol/L High 100 nmol/L	Thrombin mimic, strong agonist to induce/amplify platelet aggregation and secretion.
Thromboxane A_2 analogue U46619	1 μM	TxA_2 mimic, strong agonist to induce/amplify platelet aggregation and secretion.

as a surrogate marker of platelet adenosine triphosphate (ATP) secretion. The ability to monitor the ATP induced luciferase reaction has been applied to both LTA and WBA and is formally referred to as lumiaggregometry (Fig. 16.1) [1, 8].

Clinical Application

Screening for Inherited and Acquired Disorders

The evaluation of patients with abnormal bleeding symptoms begins by obtaining a thorough bleeding history, physical examination and is followed by appropriate laboratory screening. Disorders of platelet number and function should be screened for in all patients determined to have a pathologic etiology of bleeding given the prevalence of these conditions and the coexistence of them with other defects of coagulation [9]. Both light transmittance and whole blood aggregometry are utilized to assist the diagnostic evaluation of bleeding disorders due to inherited or acquired platelet dysfunction. LTA has been thoroughly studied in the clinical setting with validated reference intervals and remains the gold standard assay to detect platelet function abnormalities among individuals referred for bleeding problems [9]. An evaluation that utilizes a panel of agonists can distinguish between disorders of (1) platelet receptors required for adhesion, (2) platelet receptors for soluble agonists, (3) abnormalities of platelet granules, (4) abnormalities of signal transduction pathways, (5) abnormalities of procoagulant phospholipids, and (6) miscellaneous abnormalities of platelet function (Table 16.3).

The interpretation of LTA results remains challenging as variations in assay methodology between institutions makes it difficult to establish universal parameters for normal aggregation. Although discrepancies in assay reproducibility can be reduced by enforcing standardized LTA protocols, it remains a common factor that impedes interpretation of results. The determination of inadequate versus adequate aggregation is often made subjectively by experienced clinicians and/or laboratory technicians. A decision is made considering the documented lag phase, maximal amplitude, primary aggregation slope, and the presence or absence of shape change, secondary wave, and/or disaggregation for each agonist studied. The clinical impact of aggregometry results is best demonstrated when abnormalities follow that of a well-defined molecular defect that is associated with a well-characterized clinical disorder (Table 16.3). Therefore, the results of a patient's assay are

Table 16.3 Most common platelet functional disorders in which platelet aggregation is useful in the diagnosis

Disorder/defect	Platelet function abnormality/aggregation pattern (bold)
Bernard–Soulier Syndrome (BSS)	AR platelet adhesion disorder with giant platelets and defect of the platelet glycoprotein complex GPIb/IX/V unable to bind VWF or Thrombin**. BSS platelets will not agglutinate in response to ristocetin**.
Platelet-type (pseudo) von Willebrand disease	Phenotypic gain of function of platelet GPIbα that increases its avidity to VWF and results in the clearance of platelet/VWF complex from circulation. **These platelets will be hyperresponsive to ristocetin.**
Glanzmann thrombasthenia (GT)	AR bleeding disorder with normal size platelets and defect of the platelet glycoprotein complex GPIIB/IIIA (platelet membrane integrin αIIbβ3), inhibiting platelet-to-platelet aggregation and, in turn, platelet plug formation. **Aggregometry demonstrates a severely impaired response to all platelet agonists except ristocetin.**
Abnormal thromboxane A_2 receptor	**Platelets do not undergo platelet aggregation upon stimulation with arachidonic acid or thromboxane receptor analogue U46619.**
Abnormal α2-adrenergic receptor	**Platelets do not undergo platelet aggregation upon stimulation with epinephrine.**
Abnormal P2Y12 receptor	**Reversible aggregation and no secretion in response to weak agonists and impaired aggregation and secretion in response to strong agonists.**
δ-Storage pool deficiency (δ-SPD)	Deficiency of dense-granules (or δ-granules) in megakaryocytes and platelets**. A platelet secretion defect that demonstrates poor aggregation to weak agonists and lack of aggregation to collagen.** Seen as a component of both Hermansky–Pudlak or Chediak–Higashi syndromes.
Gray platelet syndrome (GPS)	AR deficiency of alpha-granules with notable large, agranular platelets in the peripheral blood smear. **These platelets demonstrate abnormal platelet secretion inducted by ADP and Collagen**.
Quebec platelet disorder	AD disorder characterized by increased storage of the fibrinolytic enzyme urokinase-type plasminogen activator (u-PA) in platelets. The u-PA can degrade proteins stored in platelet alpha-granules and, upon platelet activation, can be released accelerating clot lysis. **Platelets show poor response to epinephrine.**

AR autosomal recessive, *AD* autosomal dominant

often further characterized as a weak platelet agonist defect, an aspirin-like defect, an epinephrine-like defect, a pan-secretion platelet function defect, or a storage pool disorder.

Platelet Aggregometry in Trauma

A leading cause of preventable early death after trauma is hemorrhage [10]. In the setting of acute trauma with severe hemorrhage, resuscitation protocols focus on transfusion therapy to maintain an adequate platelet count (typically 50,000–100,000/μl) [11]. However, only a fraction of trauma patients initially present with severe thrombocytopenia, even if the present with significant bleeding [2]. Previous studies have demonstrated that platelet count upon admission is not predictive of mortality in acute trauma [3]. However, a patient's platelet count requires continuous monitoring throughout the resuscitation effort given the complications of ongoing consumption and vascular dilution from infused fluids and blood products. Less effort has been devoted to the study of platelet dysfunction in the setting of trauma, which may further contribute to coagulopathy, bleeding, and patient mortality. Although the mechanism of trauma induced platelet dysfunction remains unclear, our improved ability to study the dysfunction may uncover important opportunities for therapeutic intervention. However, thus far only a limited number of studies of platelet function in the setting of trauma induced coagulopathy have been published.

Platelet aggregometry, as a tool to quantify platelet dysfunction, remains logistically difficult

in the setting of acute trauma evaluation. LTA is the clinical gold standard screening assay of platelet function, but is labor intensive and not widely accessible to trauma centers [11]. Advances in multiple electrode impedance WBA now provide a high throughput option that is more readily available as a point-of-care monitoring device. These devices have recently been utilized in the field of cardiothoracic surgery to assist real-time decisions regarding platelet transfusion therapy and have proven sensitive to monitor inhibition of platelets by antiplatelet medication [11, 12].

The first study to utilize multiple electrode impedance WBA to asses platelet function in trauma patients was carried out by Solomon et al. in 2011 [11]. The authors carried out multiplate electrode aggregometry (MAE) using ADP, collagen, and TRAP agonists on whole blood obtained from 163 trauma patients at the time of admission to the emergency room. The study concluded that aggregometry values below the normal range for ADP and TRAP were significantly more frequent in non-survivors than in survivors ($p=0.0017$ and $p=0.0002$, respectively) and in patients with a greater injury severity. Although the results strongly support a relationship between platelet dysfunction and worse clinical outcome, the authors did not formally recommend any modifications to current trauma resuscitation protocols based on their MAE results.

Kutcher et al. prospectively studied platelet function in 101 blood samples from trauma patients obtained at admission and throughout their intensive care unit (ICU) course [13]. MAE assays were used to evaluate response to TRAP, AA, and collagen. Of clinical note, mean admission platelet count was $274 \times 10^3/\mu L$ with no admission count below $140 \times 10^3/\mu L$. Although 46 % of patients demonstrated platelet aggregation abnormalities at admission, 92 % were found to have platelet dysfunction at some point during their ICU course. These results reflect a gradual impairment of platelet function from the time of initial presentation throughout ICU stay. The authors additionally demonstrated an association between decreased platelet aggregation and low admission Glasgow Coma Scale score and a tenfold higher early mortality. As with the previous study, the authors provide no formal recommendations to modify current trauma resuscitation protocols based on MAE results.

More recently, in 2014, Windelov et al. completed a large multicenter prospective observational study of WBA in adult trauma patients entitled Activation of Coagulation and Inflammation in Trauma (ACIT) [14]. They used the MAE assay, triggered by TRAP and collagen agonists, to study blood samples obtained at admission to the emergency department. Samples were drawn at a median of 65 min after injury and stored at room temperature for 30–120 min prior to processing per their institutional protocol. The authors were unable to demonstrate a significant correlation between platelet aggregation and injury severity, shock, or severe brain injury. Furthermore, they demonstrated that an increased aggregation response (hyperaggregation) to TRAP was associated with death due to cerebral injuries. An initial association between decreased aggregation and use of a massive transfusion protocol was later determined to correspond more with low platelet counts.

Several studies have specifically correlated traumatic brain injury (TBI) to abnormalities of platelet function with resulting defects of aggregation in rodents and humans [15]. Even in the absence of hemorrhagic shock or multisystem injury, the coagulopathy of TBI typically involves the inhibition of platelet receptors to ADP and AA. In 2014, Briggs et al. carried out a prospective trial to determine if platelet transfusion impacts platelet aggregation in patients with traumatic intracranial hemorrhage (ICH) [16]. Twelve of their patients were on aspirin prior to injury and five of their patients were not. All patients demonstrated a trauma-induced platelet dysfunction. However, platelet transfusion only improved the aspirin induced dysfunction and had no impact on solely trauma induced platelet dysfunction.

Conclusion

Trauma induced coagulopathy has historically been defined by prolonged laboratory assays such as the international normalized ratio of prothrombin time and the partial thromboplastin time that place attention to dramatic dysfunctions of secondary hemostasis. However, platelets play a pivotal role in the initiation and propagation of hemostasis and their dysfunction after severe trauma represents a potential opportunity for clinical intervention. Although only a few studies have utilized platelet aggregometry to evaluate platelet dysfunction in trauma, they all present a similar hypothesis of rapid and diffuse platelet activation in response to severe tissue and vascular injury. This initial inappropriately robust activation of platelets may result in exhaustion of platelet secretion and the induction of a prolonged refractory state [10, 17]. Platelets then appear appropriate in number, but dysfunctional in their ability to further aggregate. Without a consistent platelet response, the foundation for secondary coagulation is impaired leading to exacerbation of hemorrhage. These studies report inconsistent clinical outcomes as a result of platelet dysfunction and, therefore, do not propose additions or amendments to current resuscitation protocols based on their results.

Platelet aggregometry continues to develop into a higher throughput and standardized assay. However, its clinical and research applications remain in the diagnosis of inherited and acquired platelet disorders. The use of LTA in the acute setting remains limited secondary to the length of time to carry out the assay, the necessity for subspecialty laboratory training, and being performed on platelet-rich-plasma [18]. The advent of multiplate electrode WBA provides the first opportunity for a point-of-care assay to evaluate platelet function. The additional use of lumiaggregometry allows one to monitor ATP release as a surrogate marker of platelet secretory activity and electron microscopy allows for evaluation of adequate stored content of platelet granules.

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Thrombelastography (TEG®)

17

Eduardo Gonzalez, Ernest E. Moore, and Hunter B. Moore

Introduction

Advances in our knowledge of clot formation and dissolution during pathologic states and physiologic extremis, as it occurs in trauma induced coagulopathy (TIC), has emphasized the importance utilizing coagulation assays that approximate hemostasis as it occurs in vivo. The viscoelastic assays thromboelastography (TEG; Haemonetics, Braintree, MA) and rotational thromboelastometry (ROTEM; TEM International, Munich, Germany) have emerged as the most comprehensive assays available to characterize clotting of whole blood. A significant increase in the utilization of viscoelastic assays in point-of-care settings that support care of complicated surgical procedures and trauma has been seen in recent years.

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Thromboelastography poses many theoretical advantages to standard available laboratory tests such as the international normalized ratio of prothrombin time (INR/PT), activated partial thromboplastin time (aPTT), fibrinogen level, platelet count, and d-dimer. First, it is a near-patient test that is easy to perform and can yield results within 30 min. By measuring various variables, it is able to yield information relating to the cumulative effect of several components of coagulation at a given time point. As a single test it provides data both on clot generation and clot dissolution (fibrinolysis) [1], aspects that are equally important in the care of the coagulopathic bleeding patient.

Hellmut Hartert conceived TEG in Germany, at the Heidelberg University School of Medicine in 1948 [2]. TEG was applied increasingly throughout Europe during the 1950s, validating its use for assessment of anticoagulant effect, effect of thrombocytopenia, fibrinolysis, and monitoring of coagulation during liver transplantation [3, 4]. The first report on the use of TEG outside of Europe came from Henry Swan at the University of Colorado, who in 1958 published on the use of TEG to characterize the effects of hypothermia and cardiopulmonary bypass during cardiac surgery [5]. Subsequently, Thomas Starzl utilized TEG to detect fibrinolysis during the anhepatic phase of liver transplantation [6, 7]. It gained further applicability for monitoring heparinization during cardiopulmonary bypass and was also found beneficial for predicting postoperative bleeding [8]. TEG gained additional

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acceptance as a tool for assessment of coagulation in the general surgical patient, permitting differentiation of coagulopathy from surgical bleeding [9].

Assay Methodology

The first descriptions on the methodology of this assay refer to TEG as "Hartert's instrument" [4]. The principles on which TEG is based remain the same since then. In the most current TEG system (Analyzer 5000; Haemonetics, Braintree, MA), a cylindrical plastic cup containing a 360 μl whole blood sample oscillates through 4°45′ (0.1 Hz) and a pin on a torsion wire is suspended in the blood (Fig. 17.1). Initially, movement of the cuvette does not affect the pin, but as the clot develops, resistance from the developing fibrin strands couples the pin to the motion of the cuvette. As the viscoelastic strength of the clot increases more rotation torque is transmitted to the torsion wire and detected by an electromagnetic transducer. The transducer signal is then interpreted by TEG software, which represents such changes as the characteristic TEG tracing. The first iteration of TEG used a light beam paired to the pin in motion; movement of the light beam was optically recorded through a visible scale on a roll film. The distance between the lines represents the degree of firmness with which the cup is bound to the pin. If the clot subsequently undergoes lysis, this is recorded as a decrease in amplitude, consequently decreasing the distance between the lines. These changes in amplitude are plotted as a function of time. The current instrument allows near real time data gathering with most results available between 10 and 30 min after blood draw.

At our institution, a dynamic TEG tracing is transmitted live to the emergency department trauma bay and operating room via computer, enabling prompt interpretation for goal-directed therapy.

Sample Collection

Whole blood for TEG analysis should be collected by standard laboratory practices recommended for assays of coagulation. These practices include minimizing tissue trauma during venipuncture, using a large bore needle (19 gauge or greater) in order to prevent hemolysis, discarding the first several milliliters obtained (at least two dead spaces), preferably not using a tourniquet when possible, filling the tube to indicated marking, avoiding sampling from heparinized lines, and using immediate gentle inversion (×5) for samples collected into a tube with anticoagulant. Samples should be collected into plastic or siliconized glass tubes. Glass surfaced tubes should be avoided as they cause contact factor activation.

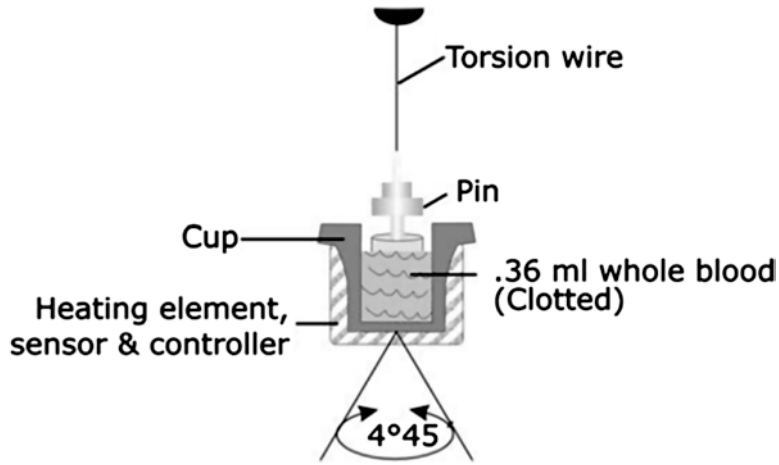

Fig.17.1 Thromboelastography, as first described by Hartert in 1948 [2]

TEG analysis can be performed on whole blood with no anticoagulant (native), whole blood collected into sodium citrate 3.2 % (0.109 mol/L) (9 volumes blood to 1 volume anticoagulant) and whole blood collected into heparin (>14.5 IU heparin/ml of blood sample).

There has been growing interest in the effects of citrate on thromboelastographic profiles, and whether citrate can introduce variation to interpretation of results. Studies on healthy volunteers performing serial TEG assays at different time points from collection show that citrated samples produce variable results when the assay is performed within 5 min from sample collection but stabilize thereafter for up to 2 h [10–13]. Regarding heparinized samples, primarily used to perform TEG platelet mapping (described below), it has been suggested that heparin can activate platelets. Heparinized samples showed platelet clumping and had procoagulant platelet microvesicles in levels double those collected in citrate [14].

Assay Execution

The first step in preparing a sample to execute a TEG assay is identifying the method of collection; i.e., whether the sample is anticoagulated or not. For samples collected with no anticoagulant, the assay must be performed, ideally, as soon as possible, and recommended within 4 min from sample collection. For samples collected in citrate, the assay must be performed between 5 min and 2 h from collection. Most centers adopt a 30 min time from collection for citrated samples, this standardizes practice and allows for logistics of transportation and quality control of instruments. However, data has demonstrated no changes in coagulation profiles when performed as early as 5 min from collection [10, 15], allowing for the use of citrated samples when TEG is performed at point-of-care in trauma settings. Citrated samples are re-calcified immediately prior to assay initiation. This is done by placing 20 µl of calcium chloride (0.2 M) into the plastic TEG cup followed by addition of the whole blood sample. The instrument can be adjusted to match the patient's core temperature, which is useful during cardiopulmonary bypass and intraoperative protective hypothermia. Some clinicians, in situations such as trauma, prefer to run the assay at the ideal temperature of 37°, in order to assess the hemostasis at the goal temperature to which the patient will be resuscitated.

Variables Measured by TEG

Based on time-resistance associations, the following TEG variables are obtained: *R*-time, activated clotting time (ACT), *K*-time, angle, maximum amplitude (MA), lysis at 30 min (LY30), lysis at 60 min (LY60), total thrombin generation (TTG), maximal rate of thrombin generation (MRTG), and time to maximal rate of thrombus generation (TMRTG). Of note, the TEG variables that have been clinically validated and are routinely used in clinical practice are: *R*-time, ACT, angle, MA and LY30; the other variables are mostly used for research purposes. A characteristic TEG tracing is shown in Fig. 17.2. A summary of the most commonly used TEG variables is shown in Table 17.1.

R-Time

R, the reaction time (or gelation time) represents the latent period from the beginning of the TEG assay to the establishment of a three-dimensional fibrin gel network with measurable rigidity of amplitude of 2 mm. This variable has also been referred to as the clot initiation time. *R*-time is measured in minutes and has been suggested to be analogous to PT/INR and aPTT, although results are not interchangeable. *R*-time reflects coagulation factor activity. Nielsen et al. [16] performed a study performing TEG on plasma with specific coagulation factor deficiencies (II, VII, X, XII). Of all TEG variables analyzed, *R*-time was the most affected by these deficiencies. In a study performed in severe hemophiliacs receiving recombinant factor VIII prophylaxis for bleeding, TEG and a thrombin generation assay were performed in order to characterize the

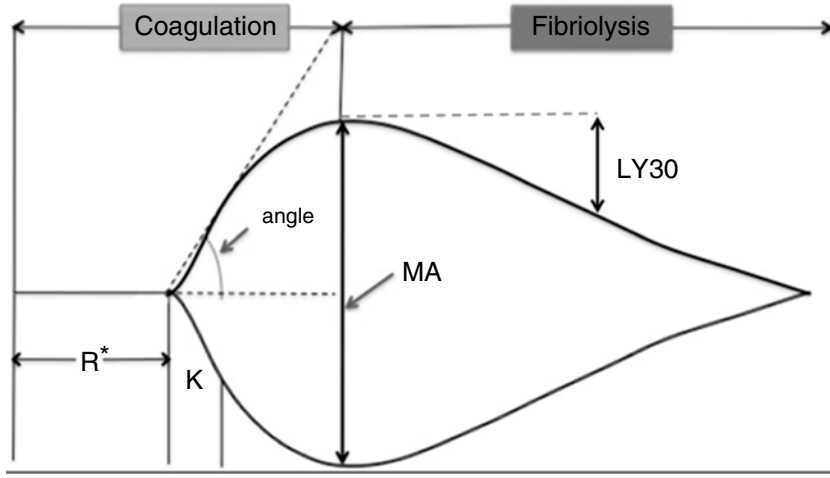

Fig. 17.2 Characteristic TEG tracing. *R* reaction time (*R*-time), *K* coagulation time (*K*-time), *MA* maximum amplitude, *LY30* percent reduction in amplitude 30 min after reaching MA. *For the Rapid-TEG assay, *R*-time is replaced by ACT

viscoelastic response of hemophiliac blood to factor VIII. Both TEG *R*-time and log thrombin generation velocity demonstrated a strong correlation with factor VIII activity; $R = -0.81$, $p = 0.001$, and $R = -0.90$, $p = 0.001$, respectively. *R*-time is currently used in clinical algorithms and protocols to trigger plasma transfusion [17].

ACT

ACT is a variables that is exclusive to the Rapid-TEG assay. Due to the rapidity of clotting in Rapid-TEG, the *R*-time (expressed in minutes) is extremely small, and the range is very narrow. Because of this, an ACT time is derived in seconds, providing a broader range that allows for defining normal and abnormal values [18]. ACT and *R*-time are thought to represent the same phase of hemostasis. Cotton et al. [19] performed a prospective observational study in 583 consecutive trauma patients (median injury severity score [ISS] = 14) using Rapid-TEG upon arrival. ACT had a significant correlation with INR and aPTT; $R = 0.71$ and 0.75 respectively. Linear regression demonstrated that ACT predicted red blood cell, plasma, and platelet transfusions within the first 2 h of arrival. Controlling for demographics and arrival vital signs, an ACT >128 s predicted massive transfusion (≥10 blood product units in the first 6 h); odds ratio: 5.1 (95 % CI, 1.3–19.4). In addition, an ACT <105 s predicted patients who did not receive any transfusions in the first 24 h; odds ratio: 2.80 (CI, 1.02–7.07). A recent study from our group has demonstrated that in trauma patients, an ACT >140 s can predict the angle and MA being abnormal [18]. Correspondingly, patients with an ACT >140 s required more cryoprecipitate and platelet transfusions. Thus, an ACT being available within 5 min allows for early delivery of cryoprecipitate and platelets units to the bedside based solely on ACT, before angle and MA are reported. This strategy has been adopted at our institution. ACT is currently used in clinical algorithms and protocols to trigger plasma transfusion.

K-Time

The *K* time is a measurement of the time interval from the *R*-time (2 mm tracing amplitude) to the point where fibrin cross-linking provides enough clot resistance to produce a 20-mm amplitude reading. It is thought to represent the propagation phase of enzymatic factors resulting in clot strengthening; which in this phase of clotting is

Table 17.1 TEG variables most commonly used in clinical practice

TEG variable	Significance	Unit of measure
R-time	Time elapsed from the initiation of the test until the point where the onset of clotting provides enough resistance to produce a 2 mm amplitude reading on the TEG tracing	minutes
ACT	Used as a surrogate of *R*-time in the Rapid-TEG assay, which uses tissue factor to obtain a quicker reading	seconds
Angle	Angle of a tangent line between the initial split point of the tracing and the growing curve. Reflects potentiation phase of enzymatic factors yielding clot strengthening mostly derived from fibrinogen cleavage and fibrin polymerization	degrees
MA	Point at which clot strength reaches its maximum measure in millimeters on the TEG tracing; reflects the end result of maximal platelet–fibrin interactions	millimeters
LY30	Percentage of clot strength amplitude lost 30 min after reaching maximal amplitude; reflects amount of fibrinolysis	percent

TEG thromboelastography, *R-time* reaction time, *ACT* activated clotting time, *MA* maximum amplitude, *LY-30* lysis at 30 min

mostly achieved by fibrinogen cleavage and fibrin polymerization. *K*-time occurs in the same time interval as angle (described below), which has replaced *K*-time in clinical practice. One of the reasons for this is that the calculation of *K*-time requires the TEG tracing to reach 20 mm of amplitude; in cases of severe coagulopathy this amplitude may not be reached and a *K*-time cannot be calculated. Consequently, most clinical algorithms and protocols do not use *K*-time to guide therapy.

Angle

This variable is the amount of degrees of the angle of a tangent line drawn between the initial split point of the tracing and the growing TEG curve. It is thought to represent the propagation phase of enzymatic factors resulting in clot strengthening; which in this phase of clotting is mostly achieved by fibrinogen cleavage and fibrin polymerization. A lower angle represents a decreased rate of clot strength growth, while a higher angle represents a greater rate of clot strength growth. Functional fibrinogen TEG (TEG-FF) (described later in this chapter) is thought to be the assay to best diagnose hemostatic alterations due to fibrinogen because this assay excludes platelets from contributing to clot strength. However, angle correlates well with functional fibrinogen concentrations (FLEV); $R=0.7$ ($p<0.0001$) [20]. Angle is currently used in clinical algorithms and protocols to trigger fibrinogen replacement in the way of cryoprecipitate or fibrinogen concentrate. For further details on fibrinogen replacement and cryoprecipitate transfusion refer to Chaps. 21 and 24.

MA

Maximal amplitude is the greatest strength achieved by the clot, which on the TEG tracing is represented as the width in millimeters of the widest gap in TEG tracing. MA assesses the combination of platelet count and function as well as fibrinogen activity (cleavage in to fibrin and polymerization). Kang et al. [6] studied patients undergoing liver transplantation and used linear regression to evaluate the relationship between TEG variables and PT, aPTT, platelet count, factors I, II, V, VII, VIII, IX, X, XI, and XII. In this study, *MA* correlated with platelet count ($r=0.59$, $p<0.001$) and fibrinogen level ($r=0.64$, $p<0.001$). The relationship of platelet count and MA was further investigated by measuring the MA and platelet count following infusion of platelets. Ten units of platelets led to

an increment of 40 (±31) × 10^9/L in platelet count and an increase in MA of 13.2 mm. Interestingly, another study reported that the association between MA and platelet count becomes linear when the platelet count was <100 × 10^9/L [21]. However, it is important to underscore that platelet count does not necessarily correlate with platelet function, particularly, in pathologic conditions such as hemorrhagic shock. As a measure of the maximal amplitude of strength reached by the clot, MA reflects all factors that contribute to clot strength, which is mostly represented by fibrin–platelet interactions. Functional fibrinogen TEG studies in healthy volunteers have demonstrated that approximately 20 % of the clot strength (MA) is determined by fibrin, with the remaining 80 % attributable to platelets. In trauma, studied upon admission, the contribution of fibrinogen to total clot strength is approximately 30 % [20, 22]. Thus, MA should be interpreted as a function of interactions between these two factors, and not a sole reflection of platelet function. If platelet dysfunction due to a pharmacologic inhibitor (i.e., aspirin of clopidogrel) is suspected, a TEG-PM assay should be performed (as reviewed later in this chapter). MA is currently used in clinical algorithms and protocols to trigger platelet transfusion.

Shear Elastic Modulus Strength (*G*)

Since MA reflects the modulus of clot strength, yet it is expressed as the amplitude of the tracing, a physical translation into an output of viscoelastic strength has been derived from MA. This variable is known as *G*, represents the shear elastic modulus strength, and is expressed in dynes per square centimeter. *G* is calculated as follows:

$$G = \left(5000 \times \mathrm{MA}\right) / \left(100 - \mathrm{MA}\right)$$

G is a parametric measure of clot strength. The relationship between MA and *G* is not linear but curvilinear—MA can vary from 0 to 100, while *G* varies from 0 to infinity. As it is expressed in units of strength, *G* facilitates interpreting clot strength as a viscoelastic variable. This relationship has lead investigators to believe that compared to *G*, MA may underestimate the changes in thrombus resistance characteristics. For example, when a clot with an MA of 35 mm is compared to another with amplitude of 70 mm, the difference in strength between the clots in terms of dyne/cm (*G*) is not twofold but rather fourfold. The *G* variable its used is mostly for research.

LY30

LY30 is the standard measure of fibrinolysis by TEG. LY30 is determined by calculating the percent reduction of clot strength (amplitude) 30 min after reaching MA. Per the manufacturer, an LY30 >7.5 % (i.e., 7.5 % clot strength reduction between MA and amplitude 30 min after reaching MA) is considered abnormal. Two recent studies have investigated the ideal threshold of LY30 in trauma patients. Our group prospectively studied 73 trauma patients meeting criteria for massive transfusion protocol activation with citrated Kaolin-TEG on admission. Those patients with an LY30 ≥3 % were more likely to require a massive transfusion (90.9 % vs. 30.5 %, $p = 0.0008$) and to die from hemorrhage (45.5 % vs. 4.8 %, $p = 0.0014$) compared to those with an LY30 <3 %. In a similar study performed using citrated Rapid-TEG, Cotton et al. [23] found that the LY30 value associated with a significant increase in mortality was ≥3 %. The odds of mortality increased tenfold when an LY30 ≥3 % was reached. These authors called for reconsideration of the LY30 threshold given that the normal reference range had been considered to be 0.0–7.5 %. These two studies have lead to the adoption of an LY30 ≥3 % as the trigger for administration of antifibrinolytic medication. It has been speculated that the use of a citrated sample and an activator (kaolin or tissue factor) can yield different LY30 sensitivities among TEG assays. Our group investigated this by performing a study on healthy control blood samples in which tissue plasminogen activator (tPA) was used to induce fibrinolysis in vitro and head to head citrated Kaolin, native Rapid and native TEG (non-citrated) were performed [24]. Citrated native TEG to detected

fibrinolysis by LY30 earlier and to a greater degree. This finding remains to be validated in coagulopathic trauma patients with fibrinolysis; however, a citrated native TEG should be considered the assay of choice for detecting fibrinolysis in experimental models and is the assay modality currently used in the newly developed tPA challenge TEG. Furthermore, our group has recently reported data demonstrating that in trauma patients, an LY30 approximating 0 is abnormal. We studied trauma patients with an ISS >15 by stratifying this cohort by the degree of fibrinolysis (LY30) evident upon arrival [25]. Sixty-four percent of patients had an LY30 <0.8 (referred to as fibrinolysis shutdown), which was associated with a greater mortality rate (26 %) compared to patients with an intermediate degree (LY30 0.8–3.0 %) of fibrinolysis (5 % mortality) ($p=0.001$). These data suggests that in trauma patients, the degree of normal fibrinolysis (i.e., physiologic fibrinolysis associated with the least mortality) is in the range of 0.8–3.0 % LY30, in contrast to the 0–7.5 % range reported by the manufacturer.

G-Derived Thrombus Generation Variables

The current analytic software of TEG has allows for three additional variables to be calculated based on changes in the *G* variable [26]. These are total thrombus generation (TTG, dynes/cm^2), maximum rate of thrombus generation (MRTG; dynes/cm^2 per second) and time to maximum rate of thrombus generation (TMRTG; seconds). MRTG represents the first derivative of the velocity of increase in clot strength as *G* begins to increase and ending after clot strength stabilizes (Fig. 17.3). TTG is the area under the velocity curve, representing the total change in elastic resistance until clot strength stabilization occurs. MRTG increases in an exponential fashion with angle, demonstrating a strong correlation ($R=0.88$) [27]. TMRTG is the time, from initiation of the TEG assay, to MRTG. TTG and MRTG are expressed using metric units of elastic resistance representing parametric measures of clot strength. These measurements are not currently used clinically; however, have proven to be useful in research.

Reference Ranges

While reference ranges based on multi-institutional studies have been published for TEG, recent proficiency data indicates very large inter-institutional variation in results; coefficients of variation of up to 80 % [28]. This is thought to be due to age related changes and variations in phenotypes of hemostasis seen across populations, as well as instrument operator variability. Thus generic reference ranges may not be applicable to individual centers and should be locally produced. Reference ranges for the most commonly used TEG assays, Rapid-TEG and Kaolin-TEG, are shown in Table 17.2 as reported by the manufacturer. Our group recently conducted a comprehensive analysis of TEG in 160 healthy volunteers in Denver in order to validate and standardize normal ranges of TEG. Results from this study are summarized in Table 17.3.

TEG Assay Modalities

TEG assays can be performed on a blood sample either with or without anticoagulant (citrate), except for the TEG platelet mapping (TEG-PM) assay which was designed to be performed on a sample collected in heparin. In clinical practice, the only assay that is routinely performed without an anticoagulant (citrate) is Rapid-TEG; this expedites results. It can be considered that the rest of the assays are usually performed on citrated blood samples and re-calcified prior to initiating the assay [12].

Native

Native-TEG allows for continuation of the clotting process in vitro as it is initiated in vivo given that no coagulation activator is added once the sample is obtained. However, due to the lack of activator, clotting is slower and TEG variables

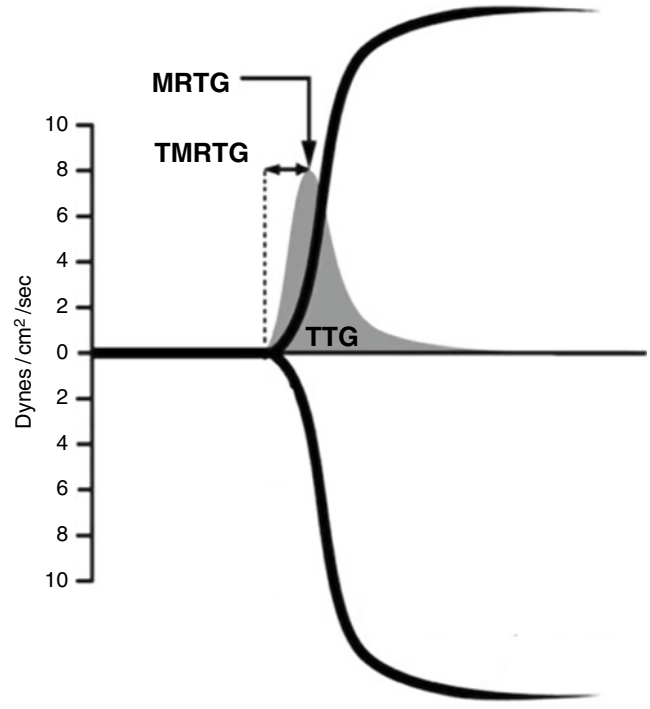

Fig. 17.3 Thrombus generation variables based on shear elastic modulus strength. *TTG* total thrombin generation, *MRTG* maximal rate of thrombin generation, *TMRTG* time to maximal rate of thrombus generation

Table 17.2 Reference ranges of TEG variables for the two most commonly used TEG assays in clinical practice: Rapid-TEG and Kaolin-TEG

	Sample collection	TEG variable				
TEG assay		ACT (s)	*R*-time (min)	Angle (°)	MA (mm)	LY30 (%)
Kaolin	Non-citrated	N/A	4–9	59–74	55–74	0–7.5
	Citrated		5–10	53–72	50–70	0–7.5
Rapid	Non-citrated	78–110	N/A	66–82	54–72	0–7.5
	Citrated	86–118		64–80	52–71	0–7.5

TEG thromboelastography, *R-time* reaction time, *MA* maximum amplitude, *LY30* percent lysis 30 min after reaching MA

Table 17.3 Range of TEG variables from healthy control study ($N = 160$) performed in Denver

	Sample collection	TEG parameter				
TEG assay		ACT (s)	*R*-time (min)	Angle (°)	MA (mm)	LY30 (%)
Native	Non-citrated	N/A	8–12	12–34	39–60	1.6–4
	Citrated		9–17	26–61	44–67	1.8–6
Kaolin	Non-citrated	N/A	4–11	53–75	56–75	0.7–5
	Citrated		6–12	49–69	54–71	0.9–4
Rapid	Non-citrated	78–146	N/A	66–81	56–74	0.2–5
	Citrated	91–140		62–81	53–71	0.1–5

Range determined by ±2 standard deviations from the mean
TEG thromboelastography, *R-time* reaction time, *MA* maximum amplitude, *LY30* percent lysis 30 min after reaching MA

take longer to be obtained compared to other TEG modalities (Table 17.3). Furthermore, there is a wide range of variation seen in Native-TEG *R*-time in a healthy reference population. Native-TEG has been used in research and experimental models given that some investigators consider this assay to be closest to in vivo physiology.

Rapid

Rapid-TEG is the most frequently used assay in trauma care [17–19, 29]. The sample is activated with the R-TEG reagent (8 % kaolin, human recombinant tissue factor, phospholipids, buffers, and stabilizer), which accelerates clotting by activating both intrinsic and extrinsic pathways and allows for rapid delivery of variables. The first Rapid-TEG variable (ACT) is available within 5 min, subsequent Rapid-TEG values (MA and angle) within 15 min [19].

Kaolin

Kaolin has been previously used as a coagulation activator in the kaolin clotting time, an assay similar to the activated partial thromboplastin time but without addition of phospholipids that is mostly used for the detection of lupus anticoagulant [30]. Kaolin acts as a negatively charged surface, activating contact coagulation factors [31, 32], as well as activating platelets and causing them to release platelet factor 3 [33]. Outside of the trauma setting, citrated kaolin-TEG is the most frequently used assay. The addition of kaolin as an activator standardizes the initiation of clotting, producing for less variation in TEG variables (Table 17.3). Consequently Kaolin-TEG is preferred to assess post-injury hypercoagulability.

Heparinase

One of the earlier clinical applications of TEG were in monitoring coagulation during cardiopulmonary bypass. A standardized dose of protamine was added to the sample to measure the degree of heparinization and used as a guide for heparinization reversal. Although protamine antagonizes the effect of heparin, it was subsequently found that protamine adversely affects coagulation if the dose of protamine is not matched to the amount of heparin in the sample. In this context, the heparinase TEG was developed in order to be used not only during cardiopulmonary bypass, but also during heparinization in other clinical scenarios. Heparinase catalyzes an eliminase reaction directly within the antithrombin binding site of heparin [34]. Heparinase has been shown to reverse the TEG effects of heparin [35]. A recent study suggests that heparinase may cause a slight degree of artificially increased fibrinolysis, however other TEG variables are not affected by heparinase [36, 37]. Heparinase-TEG is performed using a TEG cup that contains lyophilized heparinase (2 IU per cup). The amount of enzyme in each cup is sufficient to reverse 6.0 IU of heparin/ml of blood. The blood sample is placed directly into the heparinase cup. The TEG assay that has been standardized for its use with heparinase is the Kaolin-TEG; however, heparinase-TEG can also be performed on the native and Rapid-TEG assays. A Kaolin-TEG heparinase assay is run in tandem with a Kaolin-TEG (no heparinase) obtained from the same sample. This allows to obtain a TEG tracing and variables with the effects of heparinization and compare them to a sample after in vitro heparin reversal. The degree of heparinization will be proportional to the difference between the two TEG assays. An example of a heparinase TEG is shown in Fig. 17.4. Protocols and titration tables have been developed by individual cardiopulmonary bypass centers to indicate the amount of protamine reversal needed based on the difference achieved between the heparinase and non-heparinase TEG (*R*-time, angle, and MA are mostly used). However, such strategies are center specific and have not been validated in multicenter studies. Heparinase has been used to reverse the effect of high and low dose unfractionated heparin as well as low molecular weight heparin [38, 39]. The heparinase TEG assay correlates with the anti-factor Xa activity assay and has similar sensitivity in detecting heparinization [39]. Thus in trauma

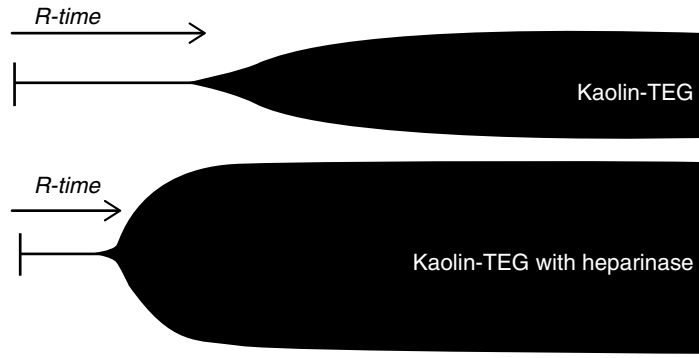

Fig. 17.4 Effect of heparinization revealed by a paired Kaolin and Kaolin with heparinase TEG

management the heparinase-TEG assay can be employed to monitor heparin anticoagulation during vascular reconstruction. Additionally heparinase-TEG has been used to monitor dosing of venous thromboembolism prophylaxis.

Functional Fibrinogen

This assay allows for estimation of the fibrinogen contribution to clot strength independent of platelets. The FF-TEG reagent creates a platelet-independent clot by using a platelet glycoprotein IIb/IIIa (GP IIb/IIIA) receptor antagonist. The assay excludes the contribution of platelets to clot strength and measures only fibrinogen's functional contribution to maximal clot strength (MA). The assay is performed in tandem with a Kaolin-TEG in order to compare the difference between fibrin-only clot strength (MA) and of fibrin and platelet clot strength; the difference between the two represents the fibrin's contribution to clot strength (Fig. 17.5). An estimated fibrinogen level (FLEV) (reported in mg/dL) is then obtained based on the FF-MA. A strong correlation between fibrinogen levels quantified FLEV and by the Clauss method has been demonstrated in a healthy control population ($R=0.92$) [40], as well as in liver transplant ($R=0.90$) [41] and trauma patients ($R=0.87$) [20]. The Clauss assay is considered the gold standard for measurement of fibrinogen. In the Clauss method, the patient's sample is paired with a calibrated fibrinogen standard; no such calibration is performed with FF-TEG and this is considered its main drawback. For further description of fibrinogen assays refer to Chap. 15.

Platelet Mapping

Platelet mapping was initially conceived to monitor antiplatelet therapy for cardiovascular disease but is now being used to assess post-injury platelet dysfunction. For further description of platelet dysfunction refer to Chap. 8. This assay isolates the contribution of fibrin and platelets to the clot's overall strength. Platelet aggregation is then stimulated by using either arachidonic acid (AA) or adenosine diphosphate (ADP). Platelet mapping is based on the MA variable and uses four channels (two analyzer machines) performed in tandem:

1. *Kaolin-TEG* (sample collected in citrate): represents thrombin dependent clot strength ($MA_{THROMBIN}$).
2. *Activator-F-TEG* (sample collected in heparin): heparin inhibits thrombin generation and platelet activation, while the addition the Activator-F reagent (reptilase and factor XIII) causes thrombin-independent fibrinogen cleavage and fibrin polymerization that generates a thrombin independent fibrin clot (MA_{FIBRIN}).
3. *Arachidonic acid* (AA) plus activator-F-TEG (sample collected in heparin): yields thrombin-independent platelet activation and

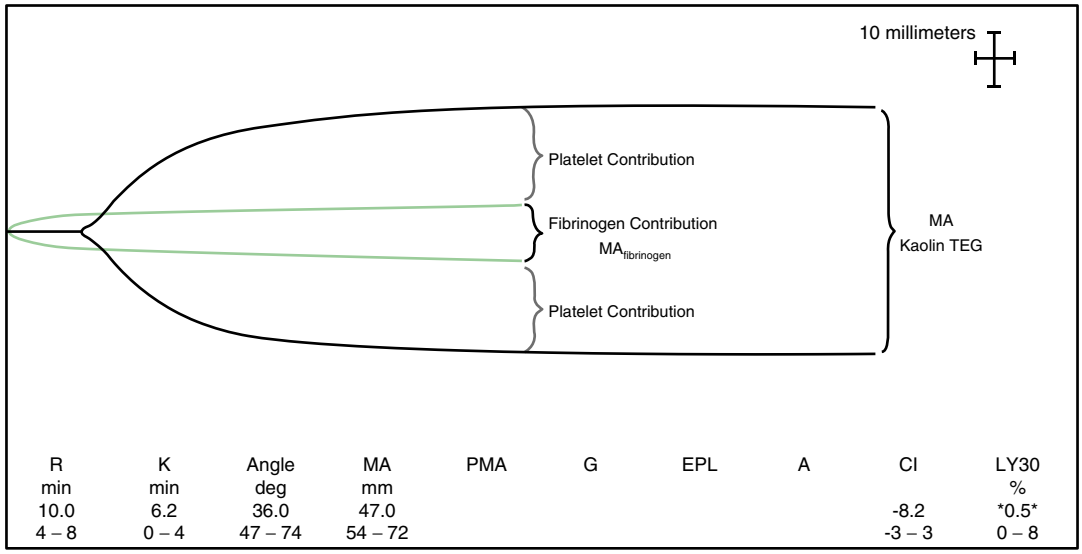

Fig. 17.5 Functional fibrinogen TEG

aggregation via the COX-1 (AA) pathway (MA_{AA}).

4. Adenosine diphosphate (ADP) plus activator-F-TEG (sample collected in heparin): yields thrombin-independent platelet activation and aggregation via the ADP pathway (MA_{ADP}).

The percentage of MA reduction is calculated using the following formula:

$$100 - \left[MA_{AA} \text{ or } MA_{ADP} - MA_{FIBRIN}\right] / \left[MA_{THROMBIN} - MA_{FIBRIN}\right] \times 100$$

Representative tracings are shown in Fig. 17.6. TEG platelet mapping has been shown to be useful in monitoring antiplatelet therapy [42–44]. Because of its rapid results it is currently being used to guide antiplatelet agent administration during percutaneous coronary interventions. There is good agreement between platelet aggregometry and TEG platelet mapping in determining the degree of platelet ADP inhibition in patients taking clopidogrel (Cohen's kappa coefficient of inter-assay agreement = 0.81) [43]. For further details on platelet aggregometry refer to Chap. 16. However, clinical applicability of platelet mapping has been limited due to the lack of prospective validation of thresholds of ADP and AA-MA inhibition to guide therapy following injury. Another limitation of TEG-PM is that the current assay does not assess platelet function through pathways initiated by collagen and thrombin. TEG-PM is used as a research tool in studies investigating platelet dysfunction in patients with TIC, particularly traumatic brain injury [45–47].

There is ongoing debate on whether heparin sample collection should be used for platelet mapping given that heparin directly activates platelets; citrate collection has been proposed [14].

tPA Challenge

Until recently, it was thought that TEG could not detect a decrease in fibrinolytic activity, known as hypofibrinolysis or fibrinolysis shutdown. The addition of a standardized tPA dose to the whole blood sample allows to assess the patient's ex vivo response to tPA. In other words patients who have fibrinolysis or are prone to it will have an exaggerated response to tPA, manifested by a greater LY30 compared to the response to the same dose of tPA in a healthy control population. In contrast, patients who have fibrinolysis shutdown will have

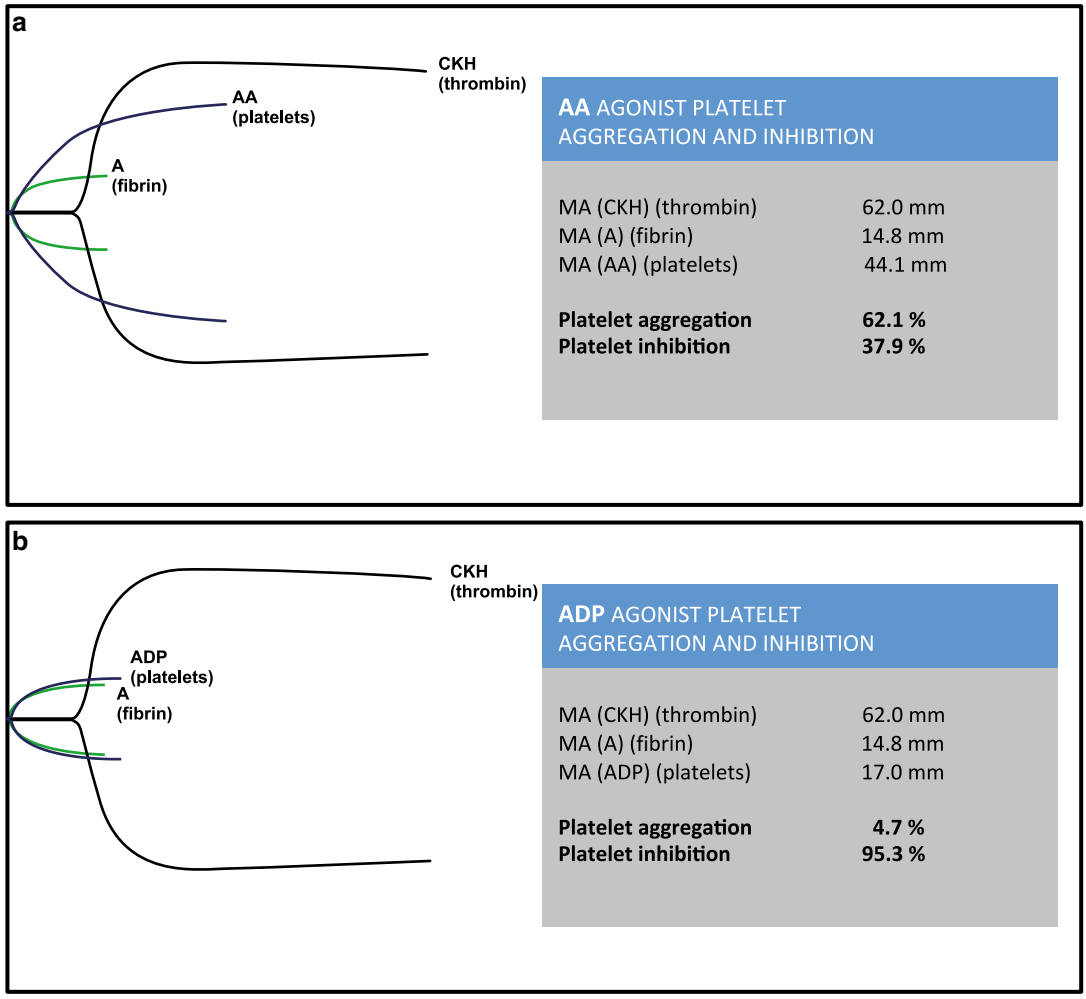

Fig. 17.6 TEG platelet mapping. (**a**) Aarachidonic assay stimulation (AA). (**b**) Adenosine diphosphate stimulation (ADP)

a decreased response to tPA, manifested by a lower LY30 compared to the response to the same dose of tPA in a healthy control population. A concentration of 75 ng/ml tPA is used (final concentration in the TEG cup with whole blood), as it represents the highest quartile tPA levels reported in trauma patients with TIC [48]. This assays is best performed on a citrated native TEG (no activator), as this assay modality yields the greatest degree of fibrinolysis compared to Rapid- and Kaolin-TEG [24]. Interestingly, the effects of streptokinase on whole blood were studied by TEG in the 1950s [4].

Differences Between TEG and ROTEM

In the TEG system, the sample cup oscillates [4] and a pin on a torsion wire is suspended in the blood sample. In the ROTEM system, the sample cup remains fixed while the pin [4] oscillates. The TEG cup is made of cryolite (acrylic polymer) while the ROTEM cup is made of polymethylmethacrylate. The ROTEM system is described in detail in Chap. 18.

ROTEM and TEG provide essentially the same information on clot formation kinetics and strength. Because of differences in operating characteristics,

the results are not interchangeable. Furthermore, the two tests use different nomenclature to describe the same variables. Although the sensitivity of TEG and ROTEM variables to detect coagulopathy is comparable between the two [63], the numeric output of each variable is not interchangeable. In a comparison study, it was noted that TEG and ROTEM values are not interchangeable [64]. European and American investigators studied a total of 184 trauma patients from three different countries prospectively by performing paired TEG and ROTEM analyses. Limits of agreement between most TEG and ROTEM variables exceeded the preset clinically acceptable deviation of 10 % [64]. This study concluded that interchangeability between TEG and ROTEM values is limited, and that development and validation of separate treatment algorithms for the two devices is required.

Limitations of Viscoelastic Assays

Understanding the limitations of viscoelastic assays is essential for adequate clinical implementation and therapy guidance. First, these assays do not reflect most interactions occurring between the fluid phase of coagulation and the endothelial cell surface; this dynamic is clearly present in the coagulopathic patient; however, it remains to be understood. Second, platelet inhibition/dysfunction may not be evident with the standard assays, unless thrombin is inhibited and platelet agonists are utilized in TEG-PM assay. Third, TEG and results are currently operator dependent and subject to sampling and/or processing errors, as well as inter-sampling variability. A newer generation of TEG analyzer may obviate these issues.

Lessons Learned from Clinical Applications Outside of Trauma

Liver Transplantation

The presence of intraoperative bleeding remains a major challenge in orthotopic liver transplantation (OLT). The underlying coagulopathy of cirrhosis, the intraoperative replacement of up to several blood volumes, and the changing metabolism of coagulant factors during the pre-hepatic, anhepatic, and neo-hepatic phases of liver transplantation create a complicated, dynamic hemostatic profile. The first comprehensive study that reported outcomes related to the use of TEG during liver transplantation was performed by Kang, Starzl et al. [6]. This study compared the use of TEG to guide blood transfusions during liver transplantation to a historic cohort that used conventional coagulation assays (PT/INR, aPTT, platelet count) and demonstrated that the use of TEG was associated with a decrease in red blood cell and plasma transfusion, while cryoprecipitate and platelet transfusions increased. This was the first report of a algorithm based transfusion strategy in OLT. A more recent randomized clinical trial in which patients were either monitored during OLT by TEG or conventional coagulation assays, demonstrated that significantly less plasma was transfused (mean [SD], 12.8 [7.0] units vs. 21.5 [12.7] units) in the TEG group compared to the conventional coagulation assay group [49].

Using TEG during OLT also allows for the detection of hyperfibrinolysis and guidance of antifibrinolytic medication administration. The anhepatic phase has been known since Thomas Starzl's first reports to be associated with hyperfibrinolysis [6]. Based on his findings monitoring OLT with TEG, Starzl abandoned the practice of empiric antifibrinolytic administration and reserved it for those in which hyper-fibrinolysis on TEG was not reversed during the neo-hepatic phase. As Starzl described, fibrinolysis often resolves spontaneously after graft reperfusion. In a retrospective review of over 600 liver transplants, 60 % developed hyperfibrinolysis, yet only 40 % of those patients required antifibrinolytic therapy based on viscoelastic variables [50]. When TEG monitoring is not performed, administering antifibrinolytics to patients in whom fibrinolysis has resolved, may expose them to a thromboembolism risk. With the availability of viscoelastic assays to monitor fibrinolysis intraoperatively, empiric antifibrinolytic administration during OLT is no longer recommended [51].

Cardiopulmonary Bypass

Cardiopulmonary bypass provokes a unique coagulopathy given that blood is circulated for a prolonged period of time via a plastic tubing system under non-physiological (continuous) flow, with areas of variable shear rate. This inevitably leads to bleeding which may be a challenge in managing cardiac surgery patients. Algorithms incorporating TEG analysis for diagnosis and treatment of bleeding have been studied in this setting. A study from 1999 randomized patients undergoing cardiac surgery to either a TEG guided transfusion algorithm or routine transfusion therapy [52]. Patients in the TEG group required less total blood products in both the perioperative and the postoperative phase. Other randomized clinical trials have demonstrated similar findings [53–56]. Collectively, while the overall burden of transfusion is reduced, the distribution of product administration tends to shift from a plasma predominant approach in favor to a more balanced distribution of platelet and cryoprecipitate. The typical transfusion algorithm uses the R-time as a trigger for transfusion of plasma, angle for transfusion of cryoprecipitate or fibrinogen concentrate (only available in Europe), MA for transfusion of platelets, and LY30 for administration of ant-fibrinolytic medication [57]. In addition, Heparinase-TEG is frequently used to titrate reversal of heparinization after cardiopulmonary bypass [57]. Studies in cardiac surgery have not yet been able to demonstrate a survival benefit from the use of TEG in transfusion practices, and its use is mostly supported by the decrease and optimization of blood product use.

Antiplatelet Therapy Monitoring

Antiplatelet therapy with a platelet COX-1 inhibitor (aspirin) and/or a $P2Y_{12}$ receptor blocker (clopidogrel) is the cornerstone of medical treatment to prevent recurrent ischemic events in patients with acute coronary syndromes (ACS) and in patients undergoing percutaneous coronary intervention (PCI). Recent studies that evaluated the pharmacodynamic effects of clopidogrel and aspirin revealed a wide variability in antiplatelet response to these medications and have brought attention to antiplatelet agent resistance [58–60]. The definition of resistance or nonresponsiveness to an antiplatelet agent is the failure of the agent to inhibit the target of its action. For example, the absence of a change in platelet response to ADP from baseline in comparison to after $P2Y_{12}$ receptor blocker administration is an indicator of $P2Y_{12}$ receptor blocker resistance, whereas the persistent presence of platelet COX-1 activity after treatment with aspirin is an indicator of aspirin resistance. Antiplatelet resistance has been independently associated with higher risk for thrombotic events, particularly stent thrombosis. Emerging data suggests that antiplatelet monitoring with assays such as TEG-PM can be used to personalize treatment [58].

Assays that use AA (a precursor of thromboxane) as an agonist have been recently employed to study aspirin responsiveness [61, 62]. There is wide variability in the reported occurrence of aspirin resistance (1–57 %). However, aspirin resistance decreases as the dose is increased. The definition of aspirin resistance also depends on what assay is used to characterize it. COX-1 pathway specific assays (e.g., AA TEG-PM) indicate the incidence is low (<5 %) at higher doses; however, COX-1 nonspecific assays (e.g., collagen stimulated Light Transmission Aggregometry [LTA] and PFA-100) report that aspirin resistance is in the range of 5–30 %, and is also dose dependent [62]. This suggests that even if aspirin is effective in inhibiting the COX-1 pathway, platelets can still be stimulated significantly via other pathways. Rare COX-1 and platelet glycoprotein polymorphisms, as well as increased platelet turnover particularly following surgery are also implicated in aspirin resistance [63]. In addition to these mechanisms, there are other factors that are known to augment platelet reactivity; e.g., smoking, diabetes, and hyperlipidemia [58]. Given that aspirin resistance is related to its dosing, attention has been placed on aspirin response monitoring assays. Aspirin

resistance by TEG-PM has been defined as <50 % AA inhibition (MA_{AA}) [59] while on compliant aspirin treatment, and has been used as a threshold for dose adjustment. However, there is no consensus as to whether aspirin treatment should be monitored routinely by COX-1 pathway assays (i.e., AA TEG-PM), and guidelines have not yet recommended this strategy.

Over a decade ago, clopidogrel response variability and resistance was first demonstrated using conventional platelet aggregometry and flow cytometry studies in patients undergoing PCI [64]. Up to 35 % exhibit either negligible or no antiplatelet response to clopidogrel [65, 66]. It has been demonstrated that high on-treatment platelet reactivity to ADP (HPR) during clopidogrel treatment is a strong and independent risk factor for post-PCI thrombotic events [65]. Moreover, recent data suggest that low on-treatment platelet reactivity to ADP (LPR) is associated with a higher risk of bleeding [67]. Thus, the concept of a therapeutic window has been proposed for $P2Y_{12}$ inhibitor therapy [68]. The ADAPT-DES (Assessment of Dual Anti-Platelet Therapy with Drug-Eluting Stents) study was a multinational, prospective registry involving >8500 patients. In this study, 43 % of patients receiving clopidogrel met the criteria of HPR, which was independently associated with a fourfold risk of stent thrombosis [67]. Studies utilizing ADP TEG-PM have determined that the clopidogrel on-treatment therapeutic window is an ADP TEG-MA between 31 and 47 mm [69]. This corresponds to the 85–208 PRU ($P2Y_{12}$ reaction units) therapeutic window of the VerifyNow platelet assay [60]. American College of Cardiology Foundation/American Heart Association guidelines recommend platelet function testing to determine the choice of $P2Y_{12}$ inhibitor and dose to be used, in selected, high-risk patients undergoing PCI [70]; although routine platelet function testing for patients on platelet inhibitors for other indications has not yet been recommended. Moreover, The Society of Thoracic Surgeons recommends platelet function testing to determine the timing of surgery in patients treated with clopidogrel [71].

TEG in Trauma Induced Coagulopathy

Until a decade ago, the diagnosis of TIC was made with conventional coagulation tests such as PT/INR. It is important to recognize that PT/INR and aPTT were initially developed for the screening of heritable coagulopathies such as hemophilia, and subsequently used to monitor anticoagulant therapy [72]. The end point for these tests is the time, in seconds, until the earliest formation of fibrin is detected. These assays do not assess the evolution of the clot beyond the formation of the first strands of fibrin. Furthermore, they have shown to correlate poorly with bleeding risk in elective general and vascular surgeries [73]. As classic coagulation tests have shortcomings when used to diagnose and monitor coagulopathy in the trauma patient, viscoelastic assays such as TEG and ROTEM have emerged in the last decade as a novel application of a well-established technology.

TEG was first used in trauma patients during the late 1990s as a research tool to characterize changes in the different stages of hemostasis during TIC [74]. It wasn't until a decade after that the first reports on standardized management strategies of TIC using TEG were published by our group and others [29, 75, 76].

The greatest clinical application of TEG in trauma is in the guidance of massive transfusion protocols (MTP). Such protocols are described in detail in Chap. 23. Until recently, the ideal strategy to guide MTP in injured patients remained elusive. In 2013 Tapia, Mattox et al. [77] published a study comparing their TEG-guided MTP to a historical cohort in which MTP was carried out using a fixed transfusion ratio of 1:1:1 red blood cell to plasma to platelet units. Patients studied were transfused 6 or more units of red blood cells in the first 24 h from arrival. The median ISS of patients ranged from 23 to 29 and was not significantly different between the groups compared. These authors found that patients with penetrating trauma that had an MTP guided by TEG had improved mortality compared to the fixed ratio MTP historic cohort. Our group

recently published a randomized clinical trial in which patients were randomized to either a MTP guided by TEG or conventional coagulation assays (CCA) (e.g., INR, aPTT, platelet count, fibrinogen, d-dimer) [78]. A total of 111 patients were enrolled with median ISS of 30 and 27 % had a penetrating injury mechanism. The TEG guided MTP group had a survival advantage over the CCA group; mortality of 19 % in the TEG group compared to 36 % in the CCA group ($p=0.04$); hazards ratio of mortality in the CCA group of 2.1 (95 % CI: 1.02–4.45). The difference in mortality was attributed to less early hemorrhagic deaths in the TEG group. More plasma and platelet units were transfused to the CCA group in the first 2 h from arrival, while more cryoprecipitate was transfused overall to the CCA group. There were no differences in the timing and amount of crystalloid and red blood cell units administered. This finding suggests that TEG allows for more appropriate hemostatic blood product use. Patients in the TEG group also had more ventilator-free and intensive care unit (ICU)-free days, which is potentially related to appropriateness of blood product administration. Our institution's goal-directed TEG-guided MTP is shown in Fig. 17.7.

TEG has also allowed for characterization of distinct phenotypes TIC, indicating that the pathophysiological mechanisms of TIC maybe different based on patient characteristics, comorbidities, and injury patterns. Our group performed a principal component analysis (PCA) on TEG variables of severely injured patients (median ISS of 31) [79]. PCA identified three principal components that together explained 93 % of the overall variance. The first phenotype reflected global coagulopathy with depletion of platelets and fibrinogen, a second group consisted of fibrinolysis as the main driver of TIC. Kutcher, Cohen et al. [80] from the San Francisco group performed a similar

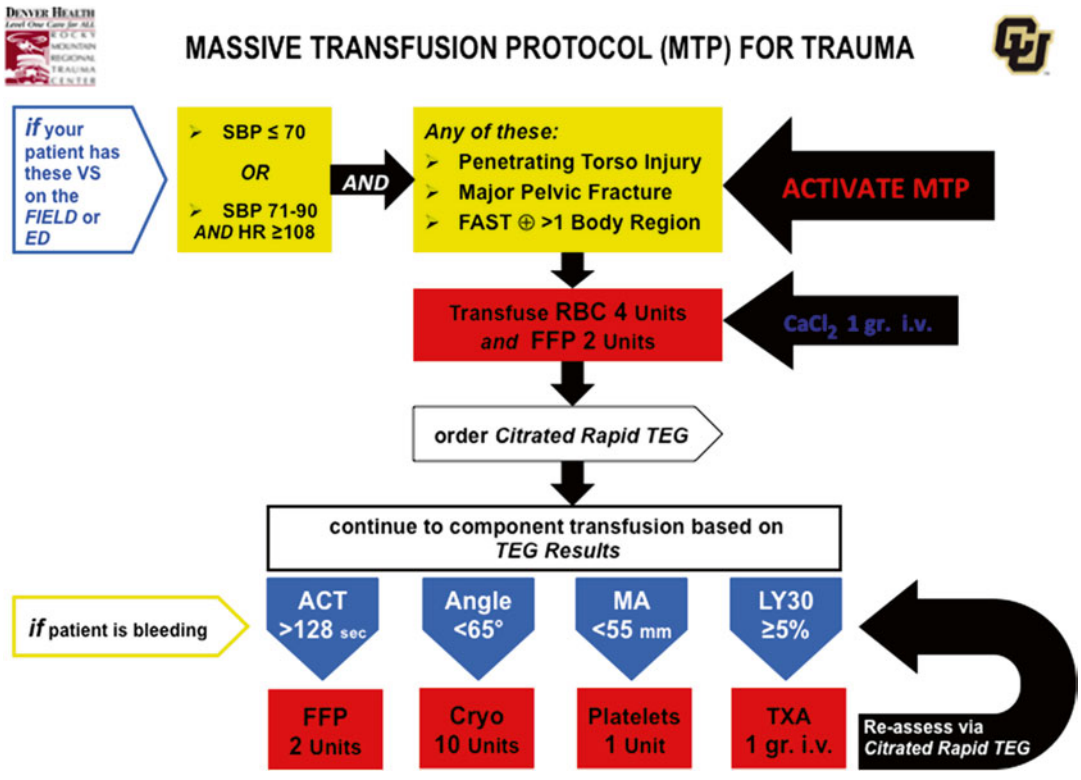

Fig. 17.7 Denver Health Medical Center Goal-directed massive transfusion protocol

study applying PCA to coagulation factor activity. These authors also reported two principal component groups; the first one consisting of global clotting factor depletion and a second one characterized by activation of protein C and hyperfibrinolysis. These data suggest that trauma patients present with different pathophysiological drivers of coagulopathy and underscore the importance of personalized resuscitation of TIC.

Clinical studies have identified that in those coagulopathic patients with the highest hemorrhage related mortality fibrinolysis is a conspicuous factor [25, 81, 82]. Thus, treating fibrinolysis with antifibrinolytic medication poses a potential strategy to improve outcomes of trauma patients. However, controversy exists regarding the threshold for administering this medication and potential adverse effects. Although the CRASH-2 trial reported a 1.5 % benefit in mortality when the antifibrinolytic tranexamic acid was administered empirically to all trauma patients [83], there has been significant criticism regarding the clinical applicability of this study's findings [84]. This trial did not perform any coagulation assays to either demonstrate the patients' degree of coagulopathy and fibrinolysis or to characterize the effect of the drug. Importantly, this study also found that administration of tranexamic acid >3 h after injury was associated with increased mortality; the study did not report any causality or mechanism to explain this finding. It seems intuitive that an antifibrinolytic medication (e.g., tranexamic acid) should only be administered to those who have demonstrable hyperfibrinolysis; however, advocates for its empiric administration to all trauma patients still exist. TEG is the only clinically available assay that can detect fibrinolysis accurately and in a point of care setting. Several studies have demonstrated an association of hyperfibrinolysis detected by TEG with increased mortality and need for massive transfusion [23, 25, 85]. Furthermore, with the recent characterization of fibrinolysis shutdown [25], present in 63 % of severely injured patients on arrival, administration of an antifibrinolytic to these patients should be avoided. Our institution's approach to hyperfibrinolysis is goal-directed [17], just as it is for transfusion of hemostatic blood products, administering tranexamic acid only to those patients who have an LY30 >3 %.

Conclusion

Viscoelastic assays such as TEG and ROTEM are the coagulation tests that better represent TIC and enable the clinician with data for critical decision making. The viscoelastic principals upon which TEG is based, are not different than when the assay was first described in 1948. Technology has allowed for expedited results and live transmission of a growing TEG curve in point-of-care clinical settings, such as the emergency department, the operating room and the ICU. TEG has been validated in monitoring patients undergoing liver transplantation and cardiopulmonary bypass and guiding blood product transfusion. TEG platelet mapping is utilized to identify resistance to aspirin and clopidogrel and used to guide dosing of antiplatelet agents in patients at high cardiovascular risk. In trauma, TEG's greatest application is perhaps in the guidance of massive transfusion protocols. A recent clinical trial has demonstrated improved survival when TEG is used to guide an MTP compared to conventional coagulation assays, while optimizing blood product use. In summary, the insights into TIC that TEG has provided, both from the clinical and biological mechanism standpoint, are substantial, and pose an opportunity for further investigations.

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Rotational Thromboelastometry (ROTEM®)

18

Klaus Görlinger, Daniel Dirkmann, and Alexander A. Hanke

Thromboelastometry Basics

The ROTEM® Device

Rotational thromboelastometry (ROTEM®, TEM International GmbH, Munich, Germany and TEM Systems, Inc., Durham, NC, USA) is a whole blood viscoelastic hemostasis analyzer, which evolved from the original thromboelastography (TEG) system, introduced by Helmut Hartert in 1948, and in the 90s by Andreas Calatzis to the ROTEG and later ROTEM system [1, 2]. Although the most recent TEG® (5000) and ROTEM® (*delta*) devices still share similarities, there are several distinct differences with regard to measurement technique, assays, and measurement variables (Fig. 18.1).

The present generation of the ROTEM® system (ROTEM® *delta*) consists of a compact measurement unit with four temperature-adjusted independent measurement channels, a prewarming plate, a reagent tray, and an integrated personal computer, allowing for remote viewing and LIS (laboratory information system) connection. An attached touchscreen and a software-assisted, automatic pipette are used to control the device and the specific ROTEM® software. This makes the device very user-friendly and reduces intra- and inter-operator variability of test results [3–5]. This allows for using the device in a multiuser environment, e.g., in the emergency room, operating room, or at the intensive care unit. Furthermore, the user is guided through the measurement process by the ROTEM® device with instructions and pictograms, displayed on the touchscreen, and a help menu can be activated if support in result interpretation is desired. Of course, this does not substitute for adequate education in hemostasis and decision-making by the attending physician.

Measurement Technique

The four independent viscoelastic measurement channels of the ROTEM® device allow for using a panel of specific assays. This improves the diagnostic performance of the device compared

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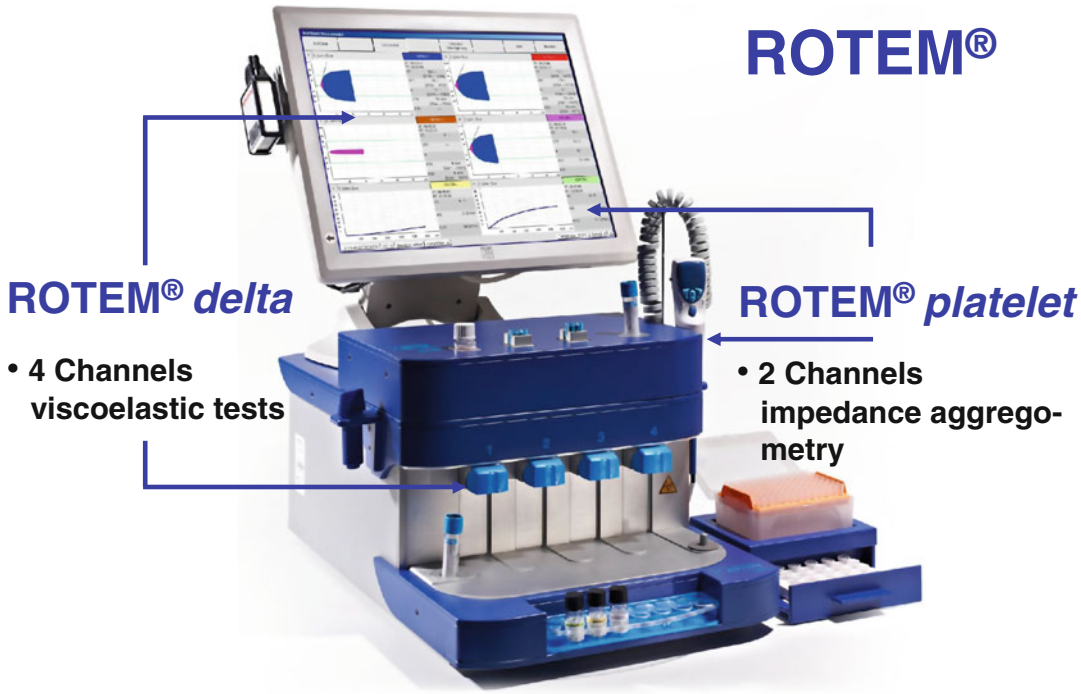


Fig. 18.1 ROTEM® *delta* device (thromboelastometry) plus ROTEM® *platelet* module (whole blood impedance aggregometry). Courtesy of Klaus Görlinger, Tem International

to a mono-assay system activated by kaolin [6]. Accordingly, the ROTEM® device is not only suitable to detect a coagulopathy, timely, but also to differentiate between different coagulopathies, e.g., between hypofibrinogenemia and thrombocytopenia, and is designed to guide hemostatic therapy in bleeding patients (Fig. 18.2). Each measurement channel consists of a disposable cuvette fixed in a temperature-adjusted metal cup-holder and a disposable pin attached to a moving axis, stabilized by a ball-bearing. The ROTEM® axis is alternatingly rotating forth and back by 4.75° twelve times per minute. After starting the test by recalcifying the citrated whole blood in the cup and adding an activator (tissue factor or ellagic acid) clot strands between pin and cup-wall are increasingly impairing the pin rotation. These changes in pin movement are detected by a LED light-mirror-light detector system, and the consequential signal is processed and transformed by the computer into a thromboelastometric curve (temogram), finally (Fig. 18.3). In addition, specific ROTEM® parameters are calculated by the computer and displayed on the touch screen in real-time. These technical modifications make the ROTEM® device on the one hand less susceptible to vibrations and movement artifacts, and on the other hand, allow for a continuous electronic quality control of the pin movement. Therefore, quality control using the reagents ROTROL® N and P is necessary once a week only, compared to daily QCs required for other viscoelastic test devices. This reduces costs and workload significantly. Furthermore, the device can be used in a mobile way at the bedside (e.g., in the emergency room, the operating room, the intensive care unit, or a satellite lab) and can even be moved around with the patient on a customized trolley providing uninterrupted power supply (Table 18.1). Accordingly, ROTEM® devices have successfully been used in military settings and other outdoor environments (e.g., mountaineering in the Himalaya and the Andes) [7–12].

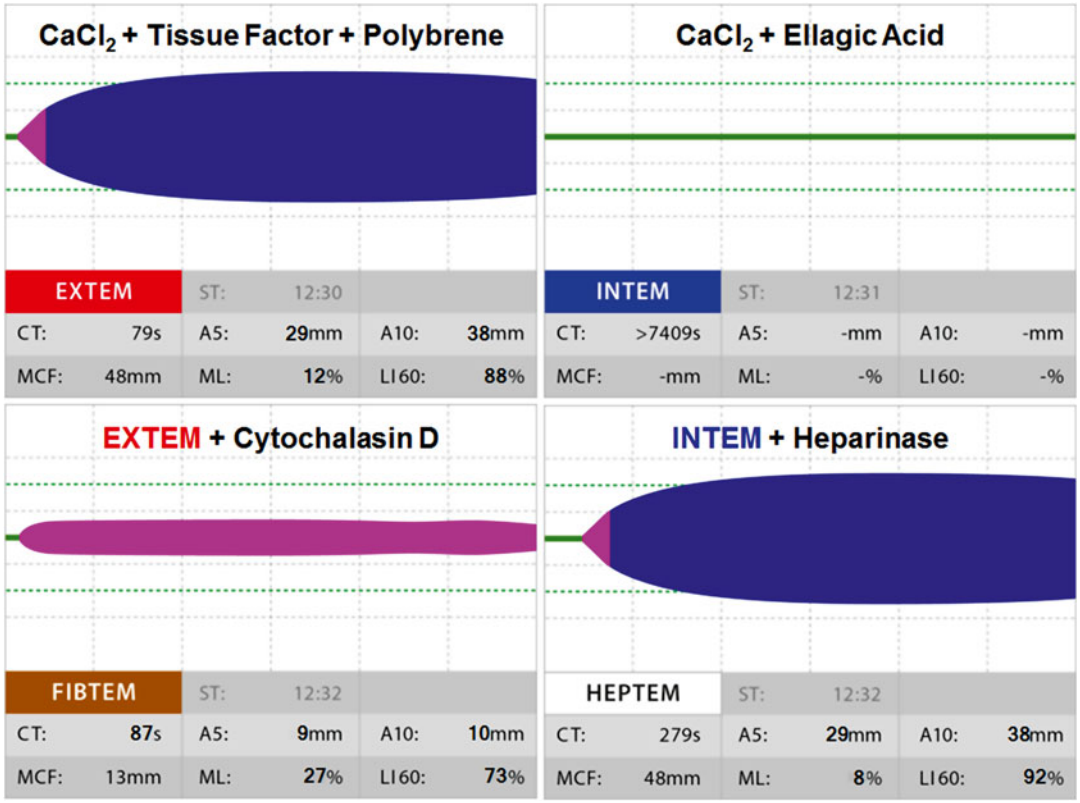


Fig. 18.2 ROTEM screenshot displaying four ROTEM® tests running simultaneously. Here, INTEM shows a flat-line since the patient is anticoagulated with heparin during cardiopulmonary bypass. EXTEM and FIBTEM contain polybrene and HEPTEM heparinase to eliminate a heparin effect. The test combination EXTEM and FIBTEM allows for discrimination between thrombocytopenia and hypofibrinogenemia, and the test combination INTEM and HEPTEM is used to detect a heparin effect. Courtesy of Klaus Görlinger, Tem International

ROTEM® Assays

Thromboelastometric assays use citrated whole blood (300 µL per assay), which is recalcified and activated by tissue factor (extrinsic pathway), ellagic acid (intrinsic pathway), or ecarin (direct prothrombin activation). Some assays contain further additives (Table 18.2). In contrast to the TEG system, all pipetting steps are guided by the ROTEM® software and performed using an automated pipette. This allows for improved multiuser handling with lower intra- and inter-operator variability of the results when compared to other devices [3–5]. Besides the standard liquid reagents, lyophilized single-potion or single-use reagents (SUR) are available in Europe [13]. Since SURs contain all reagents needed for one assay, lyophilized in one vial, pipetting is minimized to adding 300 µL of citrated whole blood to the reagent vial and transferring the activated blood 5 s later to the ROTEM® cup. SURs are labeled by the suffix S (e.g., ex-tem® S). Notably, extrinsically activated SURs do not contain and heparin inhibitor and, therefore, cannot be used in patients with therapeutic anticoagulation with unfractionated heparin, e.g., in cardiovascular surgery. This may have to be considered in patients with endogenous heparinization due to endothelial glycocalyx degradation, too [14]. The ROTEM® system provides various activated assays which in combination considerably improve the diagnostic performance of the device in comparison to a mono-assay system [6]. Here, extrinsically activated assays (EXTEM, FIBTEM, and APTEM)

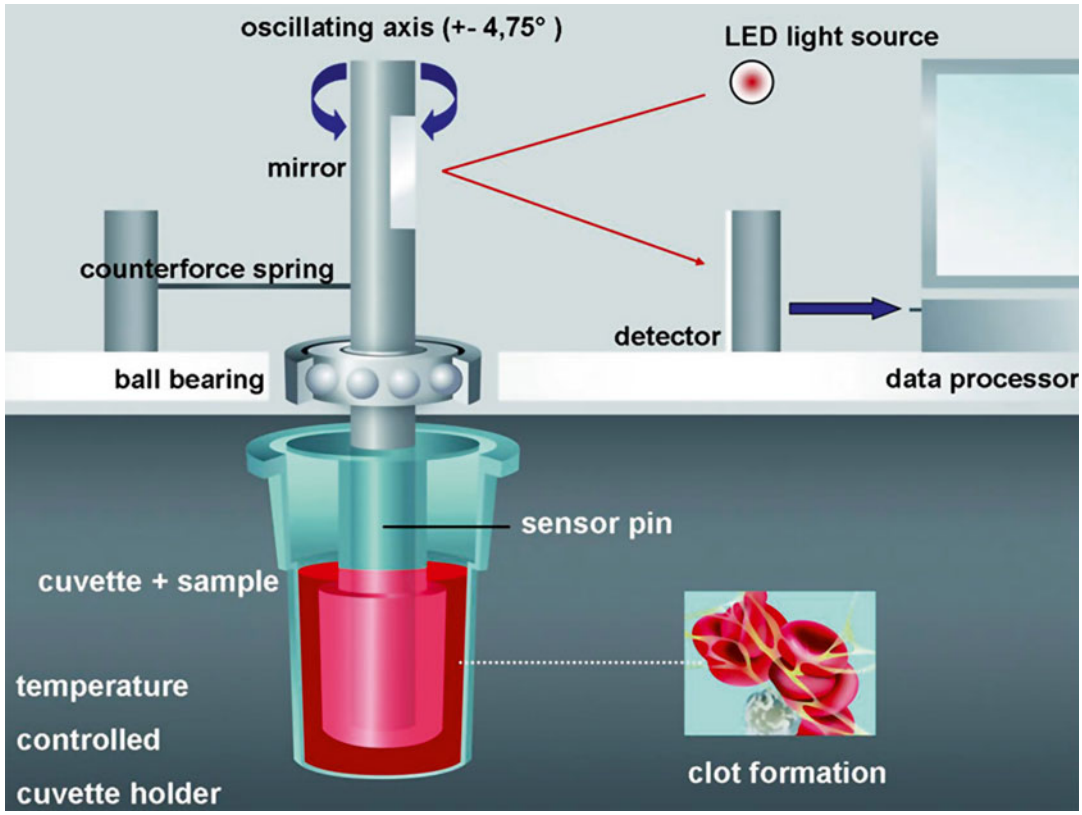


Fig. 18.3 Measuring principle of rotational thromboelastometry (ROTEM® *delta*). Courtesy of Klaus Görlinger, Tem International

intrinsically activated assays (INTEM and HEPTEM), an ecarin-activated assay (ECATEM), and a non-activated assay (NATEM) are available. Actually, ECATEM is available in Europe only.

Similar to the prothrombin time, **EXTEM** assays are activated by recalcification (star-tem® reagent, containing 0.2 mol/L calcium chloride) and addition of tissue thromboplastin (r ex-tem® reagents, i.e., recombinant tissue factor and phospholipids). Accordingly, since coagulation is initiated through the extrinsic pathway, initial thrombin generation and hence initial clotting mainly depends on the activity of the coagulation factors VII, X, V, II, and fibrinogen in EXTEM test.

The **FIBTEM** assay consists of a modified EXTEM assay with addition of a potent platelet inhibitor (cytochalasin D), which blocks platelet activation, shape change, and expression and activation of glycoprotein IIbIIIa (fibrinogen) receptors [15]. Thereby, platelet contribution to clot formation and clot strength is eliminated in this assay [16]. Accordingly, clot strength in FIBTEM is based on fibrinogen concentration and fibrin polymerization, solely, whereas clot strength in EXTEM depends on platelet count, platelet function, fibrinogen concentration, and fibrin polymerization. Therefore, the combination of EXTEM and FIBTEM allows for discrimination between thrombocytopenia and hypofibrinogenemia. The difference in clot strength between EXTEM and FIBTEM allows for estimation of the platelet part of clot firmness (referred as PLTEM by some authors) [17].

A third extrinsically activated assay—the **APTEM** test—includes an antifibrinolytic drug (in the past aprotinin and nowadays tranexamic acid (t ap-tem®)) allowing for in vitro assessment of an antifibrinolytic therapy.

Table 18.1 Technical improvement in thromboelastometry and its impact on device performance

Technical improvements in thromboelastometry (ROTEM®)	Device performance
Axis stabilized by a ball-bearing	Low sensitivity to agitation and movement artifacts enables mobile use (even in military settings)
Contactless detection of pin movements by a LED light-mirror-light detector system	
ROTEM® trolley (with optional power supply)	
Software-assisted automatic pipette results in low intra- and inter-operator variability	User-friendly, higher precision and reproducibility of results; enables multiuser environment (bedside testing in the ER, OR, and ICU)
System-integrated personal computer and touchscreen	User-friendly
Test procedure guided by instructions and pictograms on the touchscreen	
Single use reagents avoid the need for reagent pipetting (optional)	
Temogram overlays and help menu for support in result interpretation	
Continuous electronic quality control of pin movements (QC with control reagents only once a week necessary)	User-friendly; reduced workload and QC costs
Four channels for viscoelastic testing (ROTEM® *delta*)	Improved diagnostic performance; enables guided therapy with allogeneic blood products and coagulation factor concentrates ("theragnostic approach")
Two channels for whole blood impedance aggregometry (ROTEM® *platelet* module)	
Panel of 7 ROTEM® *delta* and 3 ROTEM® *platelet* assays	
Tissue factor activation (CT of 40–80 s compared to 4–8 min with kaolin activation)	Reduced turn-around-time (10–15 min) and short time-to-treat (in particular in combination with coagulation factor concentrates)
Early variables of clot firmness (A5, A10)	
Remote viewing and LIS (laboratory information system) connection	Real-time results at the bedside if device is placed in the lab

ER emergency room, *ICU* intensive care unit, *OR* operating room, *QC* = quality control. Courtesy of Klaus Görlinger, Tem International

All extrinsically activated liquid assays contain polybrene, a heparin inhibitor which allows for immediate elimination of heparin effects (up to 5 units heparin per mL). This enables the use of these tests even in heparin treated patients, e.g., during cardiopulmonary bypass [18].

INTEM assays are activated by recalcification and addition of ellagic acid and phospholipids. Due to the intrinsic activation, similar to the activated partial thromboplastin time, initial thrombin generation and clot formation in INTEM mainly depends on coagulation factors XII, XI, IX, VIII, X, V, II, and fibrinogen. As in EXTEM, clot firmness reflects both platelet and fibrin contribution to the clot. In contrast to all extrinsically activated assays, INTEM does not contain a heparin inhibitor. However, a modified INTEM assay, containing additional heparinase (**HEPTEM**) can be used in combination with INTEM in order to reveal (residual) heparinization or protamine overdose.

The **ECATEM** assay uses the viper venom ecarin as an activator. Ecarin directly converts prothrombin to meizothrombin which has already a low level of thrombin activity. Crucially, meizothrombin is inhibited by hirudin and other direct thrombin inhibitors (such as hirudin, argatroban, bivalirudin, and dabigatran), but not by heparin [19, 20]. Clotting time in ECATEM is also unaffected by other enzymatic coagulation factor deficiencies (except prothrombin deficiency), coumadin (warfarin), direct factor Xa inhibitors (such as rivaroxaban, apixaban, and edoxaban), and the presence of phospholipid-dependent anticoagulants (such as lupus anticoagulant). The eca-tem® reagent is actually approved in Europe only.

Finally, the **NATEM** assay is activated by recalcification (star-tem® reagent) only. Therefore, the test is very sensitive to any endogenous activator such as tissue factor-expression

Table 18.2 ROTEM® *delta* and ROTEM® *platelet* assays

Assay	Activators and additives	Clinical comments
ROTEM® delta assays		
EXTEM	$CaCl_2$ + recombinant tissue factor + polybrene	Deficiency of factors of the extrinsic pathway; VKAs (coumadin/warfarin); indication for PCC administration
FIBTEM	$CaCl_2$ + recombinant tissue factor + cytochalasin D + polybrene	Fibrin polymerization; dose calculation for fibrinogen concentrate or cryoprecipitate
APTEM	$CaCl_2$ + recombinant tissue factor + aprotinin/tranexamic acid + polybrene	Verifying the effect of antifibrinolytic drugs; differential diagnosis to clot retraction and FXIII deficiency (in combination with EXTEM)
INTEM	$CaCl_2$ + ellagic acid	Deficiency of factors of the intrinsic pathway; heparin and protamine effects (in combination with HEPTEM)
HEPTEM	$CaCl_2$ + ellagic acid + heparinase	Heparin and protamine effects (in combination with INTEM)
ECATEM	$CaCl_2$ + ecarin	Direct thrombin inhibitors (e.g., hirudin, argatroban, bivalirudin, dabigatran); not sensitive to heparin; actually only available in Europe
NATEM	$CaCl_2$	Tissue factor-expression on monocytes; other anticoagulants (e.g., LMWH)
ROTEM® platelet assays		
ARATEM	Arachidonic acid (AA)	COX-1 (e.g., aspirin) and GPIIbIIIa receptor inhibitor effects; effects of CPB, trauma and sepsis
ADPTEM	Adenosine di-phosphate (ADP)	ADP (P2Y12) (e.g., clopidogrel and prasugrel) and GPIIbIIIa inhibitor effects; effects of CPB, trauma and sepsis
TRAPTEM	Thrombin receptor-activating peptide-6 (TRAP-6)	Thrombin (PAR-1) (e.g., vorapaxar) and GPIIbIIIa inhibitor effects; effects of CPB, trauma, and sepsis

COX-1 cyclooxygenase-inhibitor 1, *CPB* cardiopulmonary bypass, *LMWH* low molecular weight heparin, *PAR-1* protease-activated receptor 1, *PCC* prothrombin complex concentrate, *VKA* vitamin K-antagonists. Courtesy of Klaus Görlinger, Tem International

on circulating monocytes in infection, sepsis, cirrhosis, and in patients treated with extracorporeal assist devices [21–24]. Therefore, this assay may be helpful to detect a pathophysiological change from trauma-induced coagulopathy (TIC) to disseminated intravascular coagulopathy (DIC).

ROTEM® Parameters

The ROTEM® test results are characterized by several ROTEM® parameters. Besides the standard ROTEM® parameters several other parameters are used for research only (Fig. 18.4, Table 18.3, and ROTEM® *delta* manual) [25–28]. ROTEM® reference ranges for the standard parameters are presented in Table 18.4 [29]. ROTEM® **reference ranges** can slightly vary from country to country (e.g., between Europe and the USA) and even from hospital to hospital (Table 18.4). Therefore, these reference ranges are for orientation only, and it is recommended to establish hospital-specific reference ranges. Here, the reference population, age, blood sampling vials and technique, sample transport, and other pre-analytic factors may affect the results. Notably, specific age-related reference ranges for **infants/children** and trimester-related reference ranges for **pregnant woman** have been published, too [30–34].

Coagulation Activation and Clot Polymerization Parameters

The thromboelastometric coagulation time (**CT**) in seconds corresponds to the reaction time (*r*) of TEG® assays. In ROTEM assays, CT is defined as the time from test start until a clot firmness amplitude of 2 mm is reached. In tissue factor activated tests the CT is usually achieved within about 1 min. The CT reflects the speed of throm-

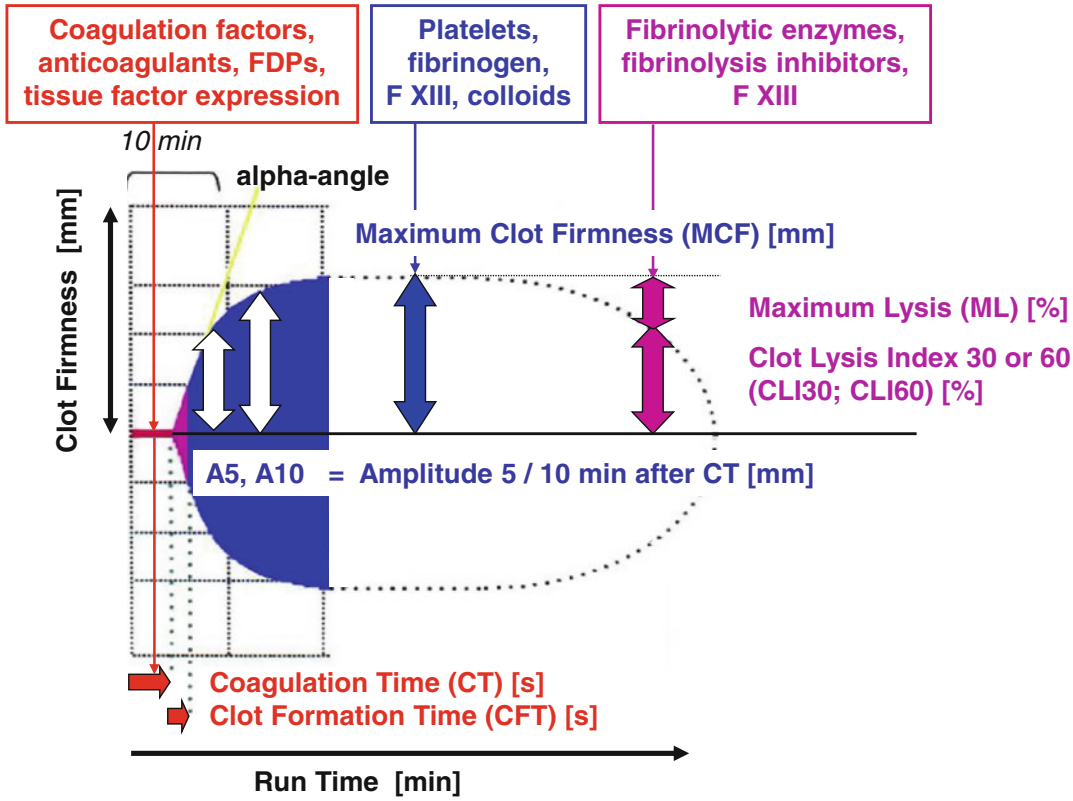


Fig. 18.4 ROTEM® trace ("temogram") displaying the clinically most important parameters and their informative value. FDPs = fibrin(ogen) split products. Courtesy of Klaus Görlinger, Tem International

bin generation and is mainly affected by the enzymatic activity of coagulation factors (extrinsic or intrinsic, depending on the assay used), the concentration of anticoagulants and fibrin split products, as well as tissue factor expression on circulating cells (e.g., monocytes or malignant cells) [21–24].

The clot formation time (**CFT**) in seconds indicates the time between 2 and 20 mm clot firmness amplitude is achieved. The CFT corresponds to the kinetic time (*k*) of TEG® assays and reflects the kinetic of clot formation. CFT mainly depends on thrombin generation, platelet count and platelet function, as well as fibrinogen concentration and fibrin polymerization.

The alpha angle (α) in degree (°) reflects the kinetics of clot formation, too, and is defined as the angle between the baseline and a tangent to the clotting curve through the 2 mm point.

Clot Firmness Parameters

One of the most important ROTEM® parameters is maximum clot firmness (MCF) in millimeters which corresponds to the maximum amplitude (MA) of TEG® assays. MCF is defined as the maximum amplitude of clot firmness reached during the test. The clot amplitude reflects the mechanically strength of the clot and mainly depends on platelet count and platelet function, fibrin concentration and fibrin polymerization, factor XIII activity, and colloids.

In order to speed up decision-making in severe bleeding, the amplitude of clot firmness 5 or 10 min after CT (**A5** or **A10**, respectively) is increasingly being used. Actually, A5 is not yet available in the USA. A20 is used during quality control measurements. A5 and A10 correlate very well with the MCF (Spearman's coefficient of 0.91–0.98) and allow for decision-making within

Table 18.3 ROTEM® *delta* parameters

Acronym	Parameter	Unit	Definition
Coagulation activation and clot polymerization parameters			
CT	Coagulation Time	s	Time from test start until a clot firmness amplitude of 2 mm is reached
CFT	Clot Formation Time	s	Time between 2 and 20 mm clot firmness amplitude is achieved
α	Alpha-angle	degree (°)	Angle between the baseline and a tangent to the clotting curve through the 2 mm point
Clot firmness parameters			
A5	Amplitude at 5 min	mm	Amplitude of clot firmness 5 min after CT
A10	Amplitude at 10 min	mm	Amplitude of clot firmness 5 min after CT
A20	Amplitude at 20 min	mm	Amplitude of clot firmness 5 min after CT
MCF	Maximum Clot Firmness	mm	Maximum amplitude of clot firmness reached during the run time
Clot lysis parameters			
ML	Maximum Lysis	%	Maximum lysis detected during the run time, described in % of MCF
LI30	Lysis Index at 30 min	%	Residual clot firmness at 30 min after CT, described in % of MCF
LI60	Lysis Index at 60 min	%	Residual clot firmness at 60 min after CT, described in % of MCF
LOT	Lysis Onset Time	s	Time from CT until clot firmness is decreased by 15 % as compared to the MCF
Research parameters			
MCE	Maximum Clot Elasticity	–	$MCE = 100 \times MCF/(100 - MCF)$
G	Shear Elastic Modulus Strength	–	$G = 5000 \times MCF/(100 - MCF)$
TPI	Thrombodynamic Potential Index	s^{-1}	TPI = MCE/CFT
LT	Lysis Time	s	Time from CT until the clot firmness is decreased to 10 % as compared to the MCF
CLR	Clot Lysis Rate	degree (°)	Angle between the baseline and the tangent to the declining clot firmness curve
Research parameters for the first derivative curve [25]			
maxV	Maximum Velocity	mm/min	Maximum of the first derivative of the curve
maxV-t	Time to maximum velocity	s	Time from test start until the maximum of the first derivative of the curve is reached
AUC	Area under the curve	mm × min	Area under the curve of the first derivative from test start until MCF is reached

Courtesy of Klaus Görlinger, Tem International

10–15 min after starting the test [4, 9, 17, 35–37]. EXTEM and INTEM A10 and A5 correlates with platelet count and fibrinogen concentration, FIBTEM A10 and A5 correlates well with plasma fibrinogen concentration and PLTEM A10 and A5 (EXTEM A10 (A5)—FIBTEM A10 (A5)) correlates well with platelet count [17, 37–39]. Furthermore, low clot firmness values have been demonstrated to be associated with an increased incidence of hyperfibrinolysis. Here, an EXTEM $A5 \leq 35$ mm can identify more than 90 % of patients developing hyperfibrinolysis, finally [40]. This is in line with the threshold of EXTEM $A5 \leq 35$ mm reported by Davenport et al. to identify trauma-induced coagulopathy on arrival in the emergency room [41].

Table 18.4 ROTEM® *delta* reference ranges for adults (non-US)

ROTEM® *delta* reference ranges for adults (non-US) (reference ranges 2012-02 Ver0007; Tem Innovations GmbH; 2012-02-07)								
Assay	CT (s)	CFT (s)	α angle (°)	A10 (mm)	A20 (mm)	MCF (mm)	LI30 (%)	ML (%) within 1 h
EXTEM	38–79	34–159	63–83	43–65	50–71	50–72	94–100	<15
FIBTEM	–	–	–	7–23	8–24	9–25	–	–
APTEM	Comparison with EXTEM. A better clot formation in APTEM as compared to EXTEM demonstrate the in vitro effect of an antifibrinolytic drug (e.g., aprotinin or tranexamic acid)							
INTEM	100–240	30–110	70–83	44–66	50–71	50–72	94–100	<15
HEPTEM	Comparison with INTEM. A better clot formation in HEPTEM as compared to INTEM indicates the presence of heparin or heparin-like substances in the blood sample							
Disclaimer	ROTEM® *delta* reference ranges can slightly vary from country to country (e.g., between Europe and the USA) and even from hospital to hospital. They are dependent on the respective reference population, age, the blood sampling vials and technique, sample transport, and other pre-analytic factors. Therefore, these reference ranges are for orientation only, and it is recommended to establish hospital-specific reference ranges							

Courtesy of Klaus Görlinger, Tem International

Clot Lysis Parameters

The clot lysis parameters maximum lysis (**ML**) and lysis indices 30 and 60 (**LI30** and **LI60**) provide information about the activity of fibrinolytic enzymes, fibrinolytic inhibitors, and factor XIII. ML detected during run is described as the difference between MCF and the lowest amplitude after MCF, displayed as % of MCF. LI30 and LI60 indicate the percentage of MCF still present 30 and 60 min after CT, respectively. In contrast, the TEG® lysis parameter LY30 and LY60 indicate the amount of lysis in percentage of MA, 30 and 60 min after MA is achieved. The ROTEM® lysis onset time (**LOT**) in seconds is characterized by the time period from CT until 15 % of clot lysis is achieved [42].

ROTEM® Limitations

A major limitation of standard viscoelastic testing is its insensitivity to the effects of antiplatelet drugs (e.g., cyclooxygenase-1 (COX-1) inhibitors, and ADP ($P2Y_{12}$)-receptor inhibitors) [26, 27]. This limitation is caused by the generation of high amounts of thrombin in viscoelastic test systems which mask the effects of antiplatelet drugs by stimulating the platelets via the thrombin-receptor pathway (protease-activated receptor (PAR) 1 and 4) [43]. Since thrombin is the strongest activator of platelets, the inhibition of other pathways (e.g., arachidonic acid- or ADP-pathway) does not affect viscoelastic test results in the presence of high amounts of thrombin.

ROTEM® Platelet Module

To overcome this limitation, **ROTEM® *delta*** can be combined with the **ROTEM® *platelet*** module, which is CE-marked in Europe since November 2013. It provides two channels for whole blood impedance aggregometry in addition to the four viscoelastic channels of ROTEM® *delta* (Figs. 18.1 and 18.5). Arachidonic acid (**ARATEM**), adenosine di-phosphate (**ADPTEM**), and thrombin receptor-activating peptide-6 (**TRAPTEM**) can be used as activators in ROTEM® *platelet*. The corresponding reagents are designed as user-friendly lyophilized single-use reagents.

Whole blood impedance aggregometry has been shown to detect the effect of COX-1 inhibitors and ADP-receptor inhibitors, effectively, and to predict stent thrombosis/ischemic events and bleeding/platelet transfusion in interventional cardiology and cardiac surgery, as well as mortality in severe trauma and sepsis [27, 44–58]. Furthermore, the effects of drugs, such as desmo-

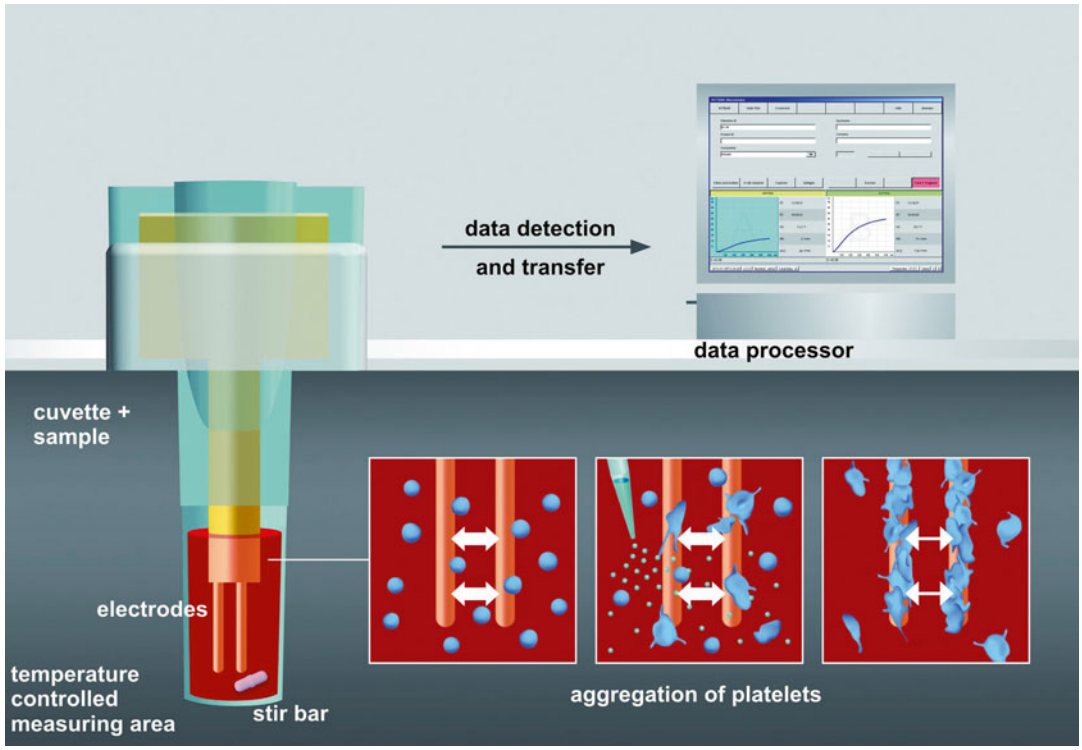


Fig. 18.5 Measuring principle of whole blood impedance aggregometry (ROTEM® *platelet*). Activated platelets are aggregating on the surface of the wires of the test cuvettes and, thereby, increasing the impedance between both wires. A magnetic stirrer prevents sedimentation of the blood cells during the 3 min incubation time. Arachidonic acid (ARATEM), adenosine-di-phosphate (ADPTEM), and thrombin receptor-activating peptide-6 (TRAPTEM) can be used as an activator. The measuring time is fixed to 6 min and the impedance curve is displayed on the screen. The main parameters are the area under the aggregation curve (AUC in Ω × min), amplitude at 6 min (A6 in Ω), and maximum slope of the aggregation curve (MS in Ω/min). Citrate, heparin, or hirudin can be used as an anticoagulant in the blood sampling vial. Different waiting times and reference ranges have to be considered dependent on the anticoagulant used. Courtesy of Klaus Görlinger, Tem International

pressin and tranexamic acid, on platelet function can be monitored by whole blood impedance aggregometry [59–61]. However, it is not clear whether platelet transfusion is beneficial or even harmful in patients with early platelet dysfunction in severe trauma and sepsis [62–67].

Diagnosis of Trauma-Induced Coagulopathy

Davenport et al. demonstrated in 2011 that TIC is functionally characterized by a reduction in clot strength. Here, thromboelastometry could identify TIC with a threshold of EXTEM A5 ≤ 35 mm on arrival in the emergency room [41]. Besides EXTEM A5 (A10), other thromboelastometric and aggregometric parameters, such as FIBTEM A5 (A10), EXTEM ML (LI60), EXTEM CT, INTEM/HEPTEM CT EXTEM, and APPTEM AUC can be helpful to characterize TIC and to guide hemostatic therapy (for details, see Fig. 18.6 and Section "Thromboelastometry-guided Hemostatic Therapy").

Prediction of (Massive) Transfusion

Since plasma transfusion may improve outcome only in patients requiring massive transfusion, whereas plasma transfusion in patients not requiring massive transfusion only show an

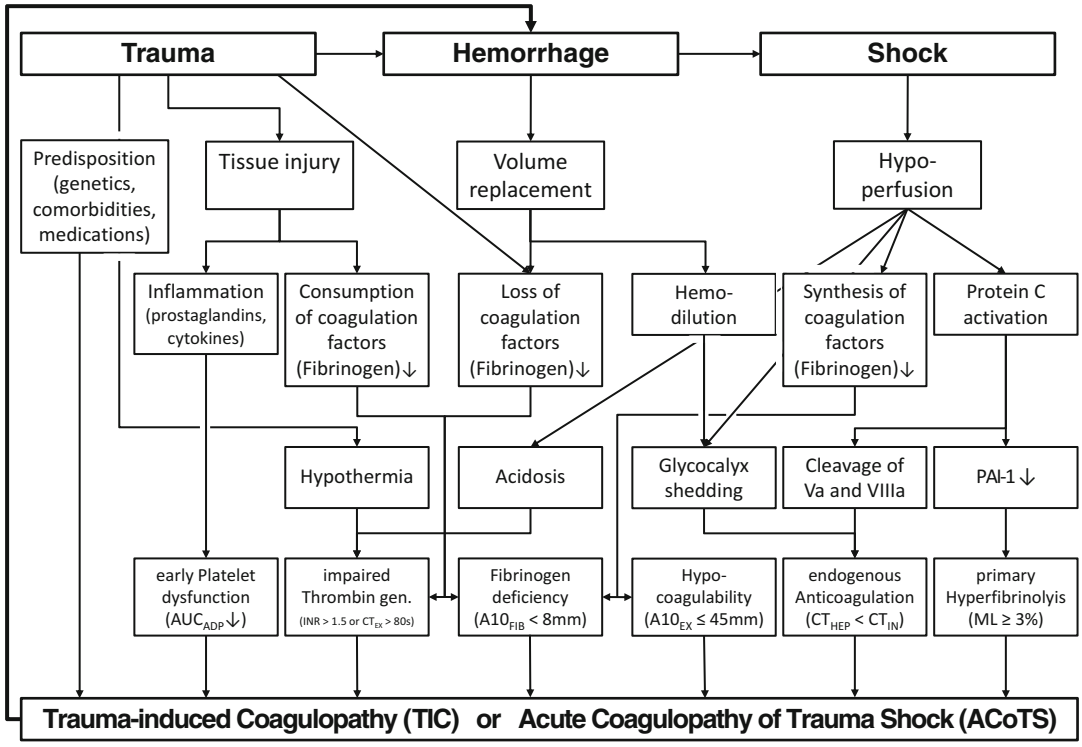


Fig. 18.6 Pathophysiology of trauma-induced coagulopathy (TIC) or acute coagulopathy of trauma and shock (ACoTS) and its detection by ROTEM® tests. $A10_{EX}$ = amplitude 10 min after CT in EXTEM, $A10_{FIB}$ = amplitude 10 min after CT in FIBTEM, AUC = area under the curve in impedance aggregometry (ROTEM® *platelet*), CT_{EX} = coagulation time in EXTEM, CT_{HEP} = coagulation time in HEPTEM, CT_{IN} = coagulation time in INTEM, INR = international normalized ratio, ML = maximum lysis (within 1 h run time), PAI-1 = plasminogen activator inhibitor-1. Courtesy of Klaus Görlinger, Tem International

increased complication rate, early prediction of massive transfusion is crucial for decision-making to start plasma transfusion in severe trauma [68–72]. On the one hand, the need for massive transfusion can be predicted based on clinical scoring systems, and, on the other hand, based on thromboelastometric results (INTEM, EXTEM, or FIBTEM A5, A10, or MCF) on arrival in the emergency room [41, 73–78].

In a retrospective analysis of 53 trauma patients, Leemann et al. reported that a decreased INTEM MCF < 50 mm (A10 < 44 mm) and a reduced hemoglobin level (≤10 g/dL) are independent risk factors for massive transfusion in severely injured trauma patients with an adjusted odds ratio (95 % CI) of 8.47 (1.19–62.50) and 18.18 (2.73–125.00), respectively [76].

Davenport et al. found in a prospective, observational, single center cohort study including 300 patients with full trauma team activation that a reduced EXTEM A5 (≤35 mm) is more accurate in predicting massive transfusion than INR (>1.2) [41].

In a retrospective study on 323 trauma patients published by Schöchl et al., both FIBTEM MCF and A10 were reasonable predictors of massive transfusion requirements. Here, patients admitted with a FIBTEM A10 ≤ 4 mm (MCF ≤ 7 mm), 84 % received a massive transfusion (≥10 units of RBCs). None of the patients with an FIBTEM A10 ≥ 12 mm on admission received a massive transfusion, finally [77].

These results have been confirmed by another prospective cohort study in 334 blunt trauma

patients. Here, Tauber et al. observed that a reduced FIBTEM MCF (<7 mm) on admission to the trauma center strongly correlated with the need for red blood cell transfusion [78].

Prediction of Thrombotic/ Thromboembolic Events

Three important mechanisms are involved in the pathophysiology of disseminated intravascular coagulation (DIC), microvascular thrombosis, and multiple organ failure: hypercoagulability, characterized by an increased clot firmness in EXTEM and INTEM, tissue factor (TF)-expression on circulating monocytes, characterized by a shorting of CT in NATEM despite prolonged PT and aPTT, and inhibition of fibrinolysis, characterized by less than 3 % lysis within 1 h [21–23]. This triad results in delocalization/dissemination of clot formation and microthrombosis and a simultaneous shutdown of the physiologic fibrinolytic cleaning system. Accordingly, it seems to be important to detect the time point when TIC shifts to DIC in trauma patients. This may also be one reason why tranexamic acid increased mortality in the CRASH-2 study when given later than 3 h after injury [79, 80].

Clot Firmness in EXTEM, INTEM, and FIBTEM

In a prospective observational study in 69 patients with cardiovascular diseases, Dimitrova-Karamfilova et al. assessed the ability of routine coagulation tests (PT, aPTT, fibrinogen, and platelet count) and ROTEM® tests to identify patients with hypercoagulability and thrombotic complications. No statistically significant difference could be found for routine coagulation tests. In contrast, significant difference in ROTEM® parameters could be observed in the 35 patients with thrombotic complications compared to the 34 healthy controls. In particular, EXTEM and INTEM CFT and MCF were able to identify patients with thrombotic complications using a MCF cutoff value of >68 mm with a sensitivity and specificity of 94 %. FIBTEM MCF, with a cutoff of >24 mm, achieved only a sensitivity and specificity of 77 % and 88 %, respectively. This suggests that an elevated fibrinogen level which compensates for a low platelet count seems not to increase the thrombotic risk. The EXTEM and INTEM thrombodynamic potential index (TPI = (100 × MCF/100 − MCF)/CFT), with a cutoff value of >3.5, provided even a sensitivity and specificity of 100 % and 92 %, respectively. In conclusion, ROTEM® analysis was definitively superior to routine coagulation tests in identifying patients with thrombotic complications [81].

These results could be confirmed by another recently published prospective observational study in 318 noncardiac surgery patients. Here, Hincker et al. evaluated preoperative routine coagulation tests (aPTT, INR, and platelet count) and ROTEM® tests to identify patients at increased risk for postoperative thromboembolic complications. 29 % of the included patient population has been recruited from the orthopedic and spine department. Again, none of the routine coagulation tests has been useful in predicting thromboembolic events, but preoperative EXTEM and INTEM CFT, alpha angle, A10, and MCF were predictive for thromboembolic complications. INTEM and EXTEM A10 were the best predictors with a cutoff value of 61.5 mm and a ROC area under the curve (AUC) of 0.75 and 0.72, respectively. None of the FIBTEM parameters predicted thromboembolic complications, confirming that elevated fibrinogen levels alone seem not to be an independent risk factor for thrombosis [82].

Tissue Factor-Expression on Monocytes

Stimulation with bacterial toxins, activation of purinergic (ADP) receptors ($P2X_7$), activated platelets, contact with surfaces of extracorporeal assist devices (e.g., cardiopulmonary bypass, ECMO, VADs, dialysis), as well as ischemia/reperfusion leads to TF-expression on circulating

monocytes [22, 23, 83–89]. This TF-expression in the intravascular space results in delocalization/dissemination of coagulation and is an early and important pathomechanism of DIC and thrombosis. TF-expression on circulating monocytes can be detected very sensitively (in picomolar concentrations) but not specifically by a reduction in CT in NATEM [21, 23, 84, 89, 90]. Since heparinoids (e.g., by glycocalyx degradation or therapeutic administration) can mask this effect, heparinase should be added to the blood sample or test system to eliminate a potential heparin effect [21].

Notably, TF-expressing monocytes inhibit fibrinolysis through a thrombin-activatable fibrinolytic inhibitor (TAFI)-mediated mechanism, which is the next step to microthrombosis and multiple organ failure [91].

Hypofibrinolysis

In contrast to TIC, physiologic fibrinolysis is shut down in the early phase of infection, sepsis, and thrombosis due to an upregulation of plasmin activator inhibitor type-1 (PAI-1) and activation of TAFI [24, 90–93]. Notably, whether the thrombin–thrombomodulin complex results in activation of protein C—with subsequent downregulation of PAI-1 and activation of fibrinolysis—or activation of TAFI—with subsequent shutdown of fibrinolysis—is regulated by platelet factor 4 (PF4) and dependent on the consumption of protein C as well as genetic polymorphisms [94–96].

Notably, Chapman et al. could demonstrate that not only increased fibrinolysis (>3 %) but also a shutdown of fibrinolysis (0 %) ad hospital admission is associated with increased mortality in trauma patients [97]. Accordingly, Adamzik et al. showed that the ROTEM® LI60 in NATEM can discriminate between intensive care patients suffering from severe sepsis (NATEM LI60 > 96 % corresponding to a ML < 4 % within one hour) and postoperative patients with systemic inflammatory response syndrome (SIRS) or healthy volunteers [21]. Furthermore, the LI60 (ROC AUC 0.901; $P < 0.001$) proved to be more accurate in detection of sepsis than classical laboratory parameters such as procalcitonin (ROC AUC 0.75; $P < 0.001$). Interleukin-6 and C-reactive protein were not able to differentiate between septic and postoperative patients. The same research group also found that ROTEM findings were a better predictor of 30-day survival in sepsis patients than established risk scores (SAPS II, SOFA) [98].

In conclusion, both hyperfibrinolysis and hypofibrinolysis seem to play an important role in the pathophysiology of TIC and DIC, and viscoelastic testing may be helpful in differentiating between both pathophysiologic entities and right decision-making regarding the appropriate use and timing of antifibrinolytic drugs.

Prediction of Mortality

Viscoelastic testing has been shown to be a good predictor of mortality in trauma in a recently published systematic review of the literature [99]. Levrat et al. included 87 trauma patients in a prospective observational trial. Here, patients with hyperfibrinolysis were more severely injured had greater coagulation abnormalities, and a higher mortality rate (100 % vs. 11 %) [100]. Schöchl et al. identified in his database 33 patients with hyperfibrinolysis at hospital admission, retrospectively. They found hyperfibrinolysis to be a strong predictor for mortality (88 %). Furthermore, it appeared that the earlier fibrinolysis could be detected by ROTEM®, the earlier the patient died, irrespective of appropriate treatment [101]. Theusinger et al. showed that in their patient population mortality in the trauma hyperfibrinolysis group (77 %), as diagnosed by ROTEM®, was significantly higher than in the non-trauma hyperfibrinolysis group (41 %) and the matched trauma non-hyperfibrinolytic group (33 %). Accordingly, hyperfibrinolysis was significantly ($P = 0.017$) associated with increased mortality in trauma [102].

In a prospective cohort study including 517 trauma patients, Rourke et al. found admission fibrinogen level to be an independent predictor of mortality at 24 h and 28 days.

Hypofibrinogenemia could be detected early by FIBTEM A5 (A10), and administration of cryoprecipitate or fibrinogen concentrate could correct coagulopathy and improved survival [38]. Similar results were shown in a prospective cohort study in 334 blunt trauma patients performed by Tauber et al. They identified cutoff values of FIBTEM MCF < 7 mm, and EXTEM MCF < 45 mm as predictors for increased mortality. EXTEM MCF was independently associated with early mortality and hyperfibrinolysis increased fatality rates, too [78].

Thromboelastometry-guided Hemostatic Therapy

Development of Thromboelastometry-guided Algorithms

Pathophysiology of trauma-induced coagulopathy is complex and cannot always be addressed adequately by hemostatic resuscitation (1:1:1 concept) only (Fig. 18.6) [103, 104]. In order to guide hemostatic therapy in bleeding patients, algorithms have been developed as a link between ROTEM diagnostics and hemostatic therapy ("theragnostic approach") [105–109]. These algorithms are based on the highest evidence, actually available. Implementation of such algorithms, have been shown to reduce transfusion requirements, complication rates, as well as morbidity and mortality in particular in cardiovascular surgery [110–112]. Several cohort studies reported similar results in trauma, but RCTs are still lacking in this field [38, 78, 99, 113–118]. A ROTEM®-guided algorithm for bleeding management in trauma is presented in Fig. 18.7. Since the ROTEM® parameter A5 is not yet FDA-approved, ROTEM®-algorithms for the USA have to use A10, whereas A5 is used as clot firmness parameter in the rest of the world in order to speed up decision-making. The difference between A10 and A5 for FIBTEM is 1 mm and for EXTEM, APTEM, INTEM, and HEPTEM 9–11 mm [35, 36]. Therefore, ROTEM® A5 and A10 algorithms can be transformed easily to each other. Characteristic thromboelastometric traces are presented in Fig. 18.8.

Clinical Assessment

Hemostatic interventions should be performed only in patients with diffuse bleeding and if blood transfusion is considered. Severity of trauma (ISS ≥ 25), clinical bleeding scores (e.g., TASH score ≥ 15), hypothermia (core temperature < 35 °C), and results of blood gas analysis (e.g., pH < 7.2, BE < −6 mmol/L, Hb < 10 g/dL, Ca^{++} < 1 mmol/L) should be considered, too, and may be associated with an increased risk of hyperfibrinolysis and hypofibrinogenemia [74, 119–121]. However, decision-making for hemostatic interventions should not be based on ROTEM® results, solely, in the absence of clinically relevant bleeding ("Don't treat numbers!").

Management of Fibrinolysis

Fibrinolysis greater than 3 % within 30 min or greater than 5 % within 60 min, respectively, is associated with increased mortality (64 % vs. 5 %) in severe trauma [97, 101, 122]. However, a fibrinolytic shutdown (0 % fibrinolysis within 30 min) is associated with increased mortality (26 % vs. 5 %) in severe trauma, too [97, 122]. This is in line with the data published by Adamzik et al. that fibrinolysis ≤3 % one hour after CT (LI60 ≥ 97 %) at ICU admission is associated with increased mortality in severe sepsis [21, 98]. Therefore, exogenous inhibition of the fibrinolysis system in severely injured patients requires careful selection, as it may have an adverse effect on survival, in particular if tranexamic acid is given later than 3 h after injury [79, 80, 122]. In order to enable quick decision-making, early thromboelastometric variables of clot firmness in EXTEM (A5 and A10) can be used to identify patients at risk for fibrinolysis. Here, an EXTEM A5 threshold of ≤35 mm (EXTEM A10 ≤ 45 mm) detects more than 90 % of patients with hyperfibrinolysis

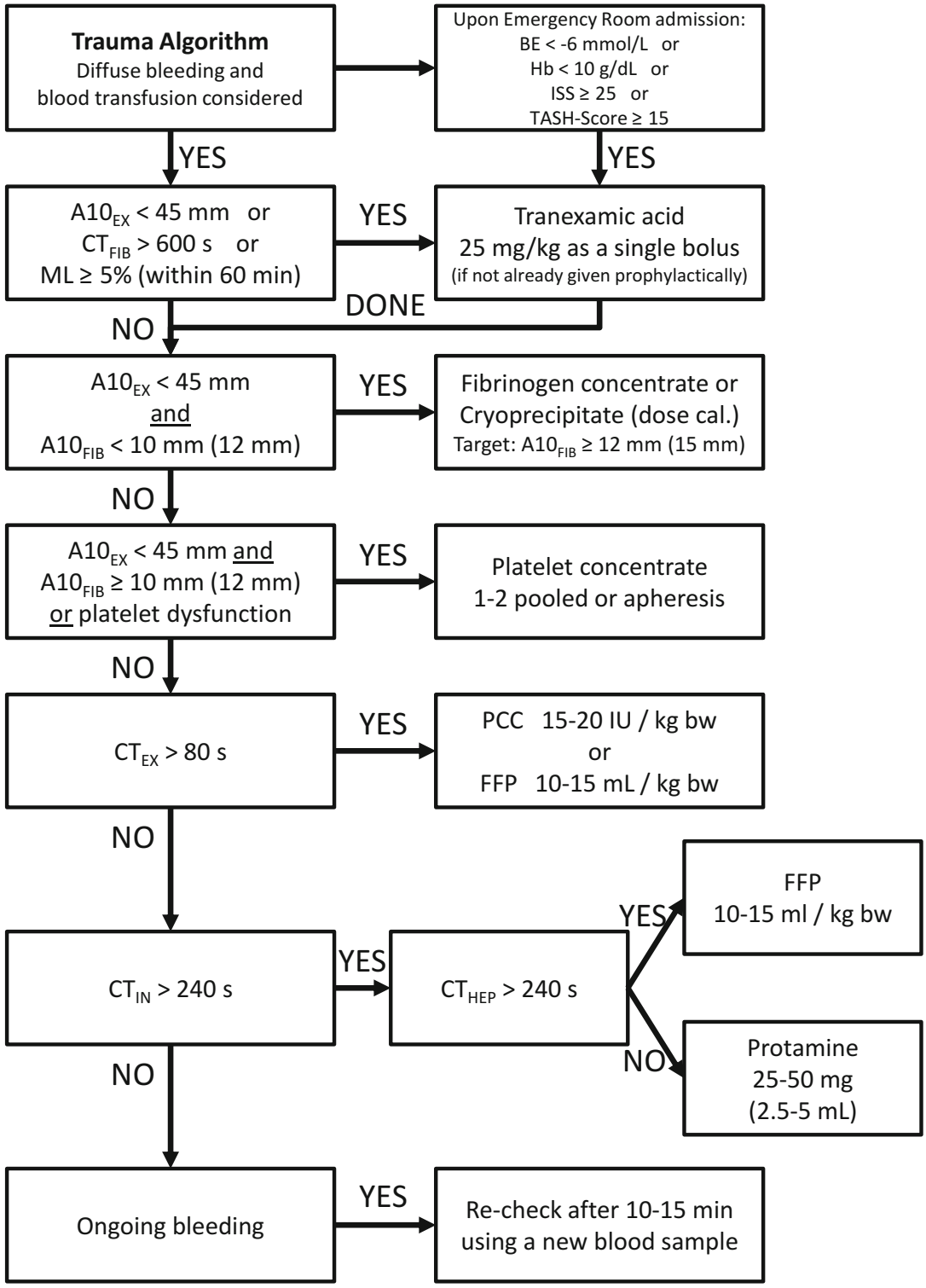
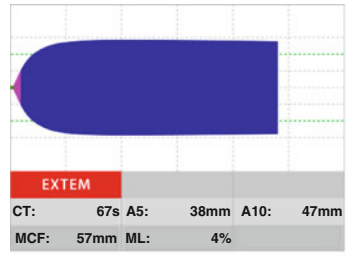
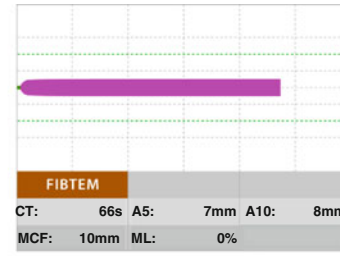


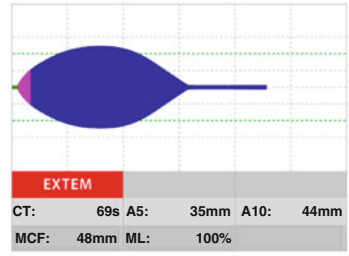
Fig. 18.7 Evidence-based ROTEM® A10 trauma algorithm. $A10_{EX}$ = amplitude 10 min after CT in EXTEM, $A10_{FIB}$ = amplitude 10 min after CT in FIBTEM, BE = base excess, CT_{EX} = coagulation time in EXTEM, CT_{FIB} = coagulation time in FIBTEM (CT_{FIB} > 600 s reflects a flat-line in FIBTEM), CT_{HEP} = coagulation time in HEPTEM, CT_{IN} = coagulation time in INTEM, FFP = fresh frozen plasma, Hb = hemoglobin concentration, ISS = injury severity score, ML = maximum lysis (within 1 h run time), PCC = prothrombin complex concentrate, TASH score = trauma-associated severe hemorrhage score. Courtesy of Klaus Görlinger, Tem International

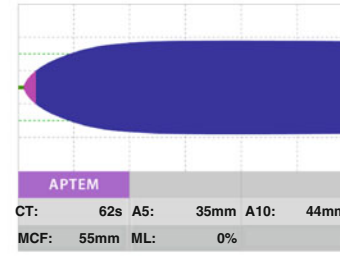
Normal clot:
EXTEM CT 43-82 s
EXTEM A10 40-60 mm
EXTEM MCF 52-70 mm
EXTEM ML < 15%
EXTEM LI60 > 85%
FIBTEM A10 6-21 mm
FIBTEM MCF 7-24 mm



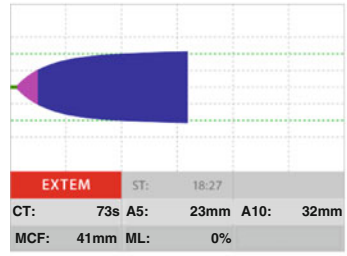


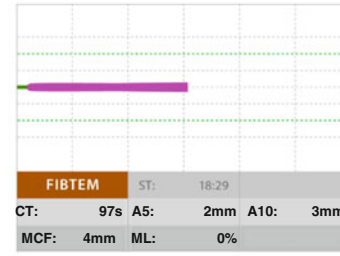
Hyperfibrinolysis:
EXTEM ML ≥ 15% within 60 min (LI60 ≤ 85%);
ML ≥ 5% may already be associated with increased mortality in severe trauma;
APTEM confirms TXA effect



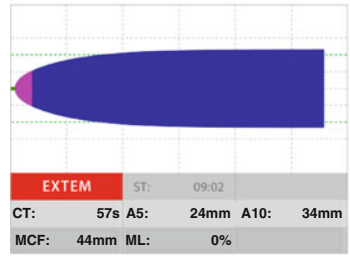


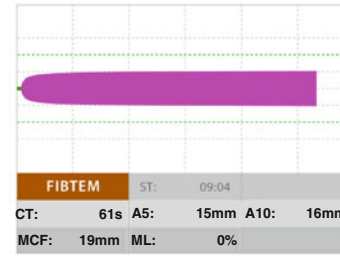
Fibrinogen deficiency:
EXTEM A10 < 40 mm
and
FIBTEM A10 < 10 mm



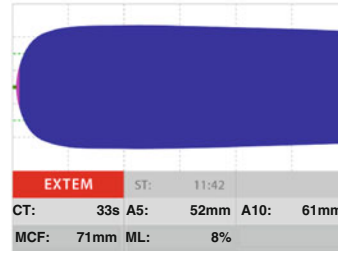


Thrombocytopenia:
EXTEM A10 < 40 mm
and
FIBTEM A10 ≥ 10 mm





Hypercoagulability (high thrombotic risk):
EXTEM CT < 40 s
EXTEM CFT < 50 s
EXTEM MCF > 68 mm
FIBTEM MCF > 24 mm
LI60 ≤ 3%



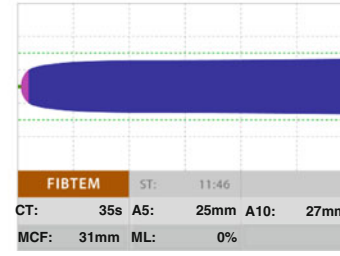
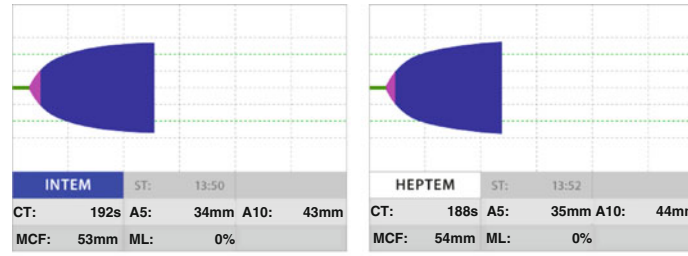
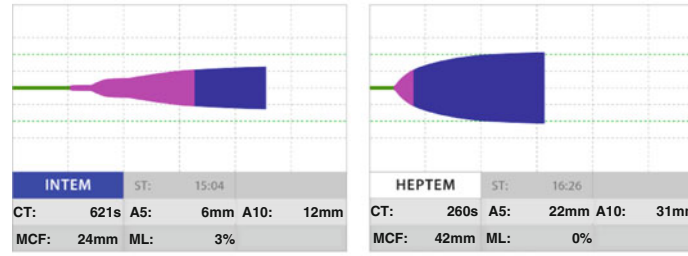


Fig. 18.8 Characteristic thromboelastometry (ROTEM® *delta*) traces. The diagnostic performance is increased by test combinations, e.g., EXTEM and FIBTEM, EXTEM and APTEM, or INTEM and HEPTEM. 4F-PCC = four factor prothrombin complex concentrate, CPB = cardiopulmonary bypass, OLT = orthotopic liver transplantation, TXA = tranexamic acid (or other antifibrinolytic drug) . Courtesy of Klaus Görlinger, Tem International

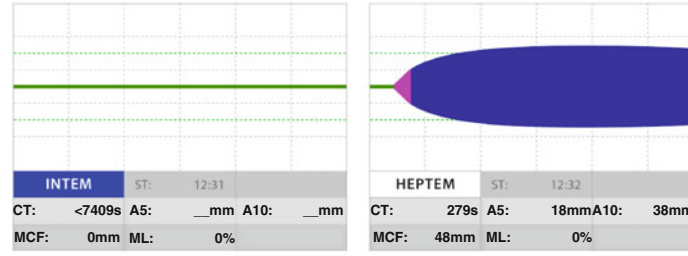
Normal clot (adequate heparin-reversal with protamine after CPB):
INTEM CT 122-208 s
INTEM A10 40-60 mm
INTEM MCF 51-72 mm
HEPTEM CT ≈ INTEM CT



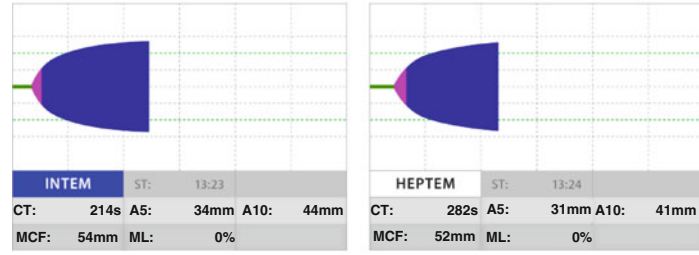
Heparin, low dose (endogenous anti-coagulation during OLT or severe trauma):
INTEM CT >> HEPTEM CT
(ΔCT > 20% HEPTEM CT)



Heparin, high dose (during CPB):
INTEM flat-line (CT > 1200s)
and
HEPTEM CT < 280 s



Protamine overdose (after heparin-reversal):
HEPTEM CT >> INTEM CT
(ΔCT > 20% HEPTEM CT)



Deficiency of vitamin K-dependent factors (warfarin therapy):
EXTEM CT > 80 s
(here, EXTEM CT decreased to 70 s after 4F-PCC administration; right graph)

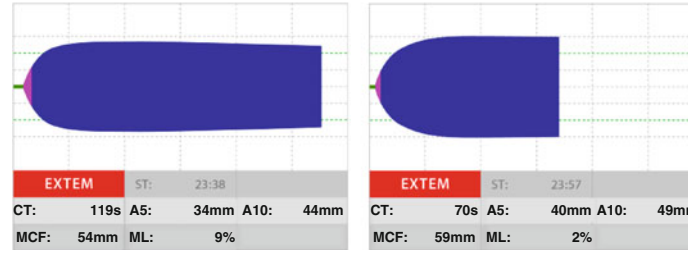
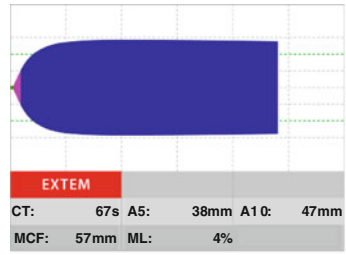
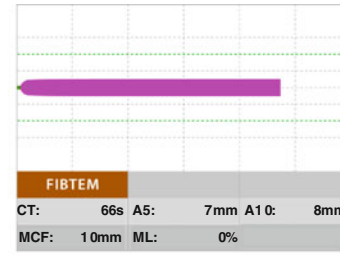


Fig. 18.8 (continued)

Normal clot:
EXTEM CT 43-82 s
EXTEM A10 40-60 mm
EXTEM MCF 52-70 mm
EXTEM ML < 15%
EXTEM LI60 > 85%
FIBTEM A10 6-21 mm
FIBTEM MCF 7-24 mm





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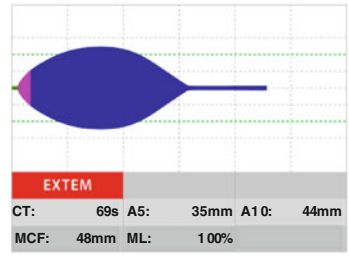

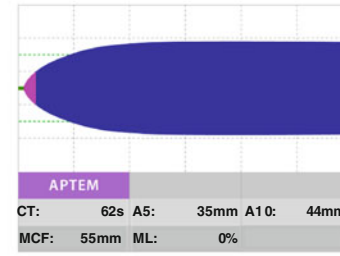

Fibrinogen deficiency:
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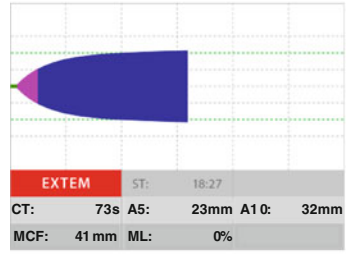

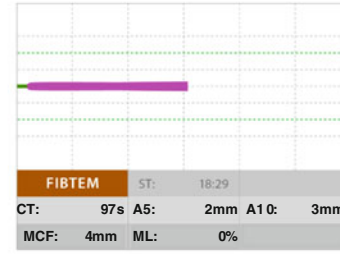

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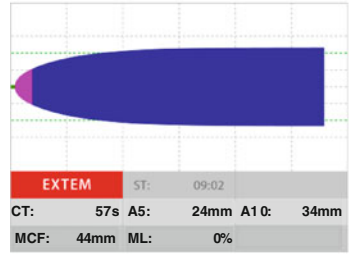

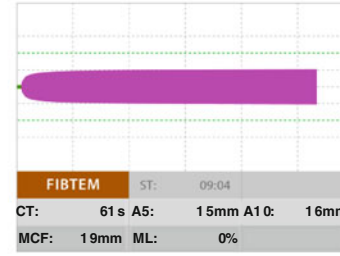

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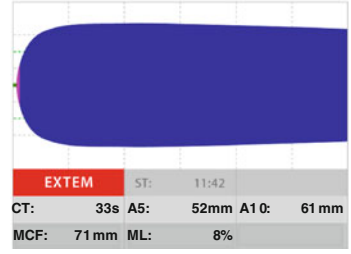

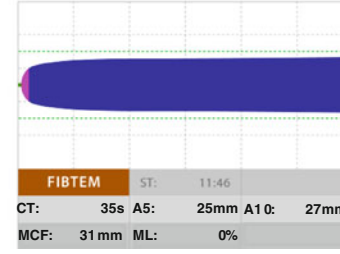

Fig. 18.8 (continued)

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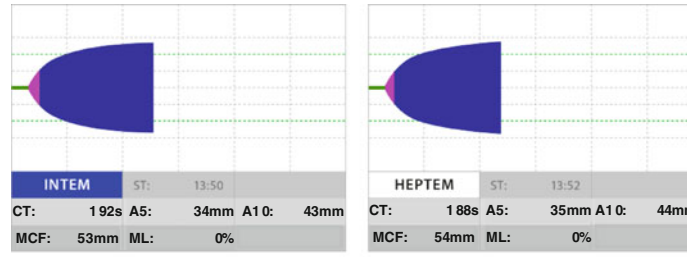

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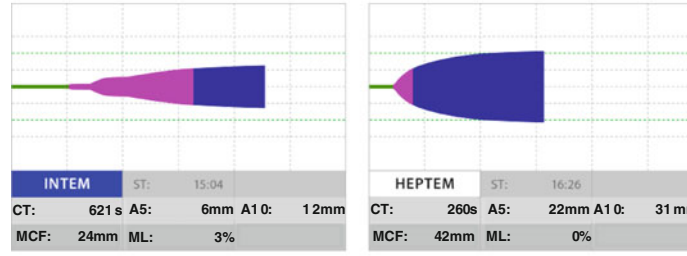

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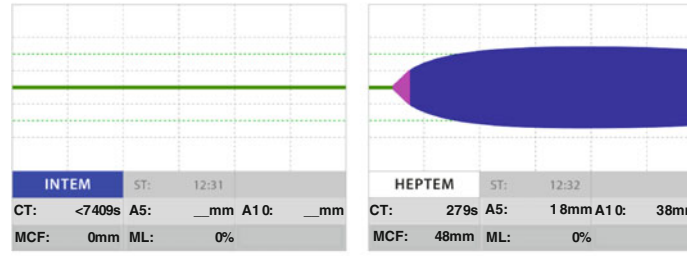

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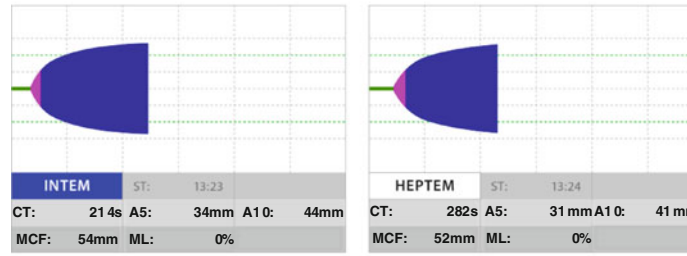

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EXTEM CT > 80 s
(here, EXTEM CT decreased to 70 s after 4F-PCC administration; right graph)

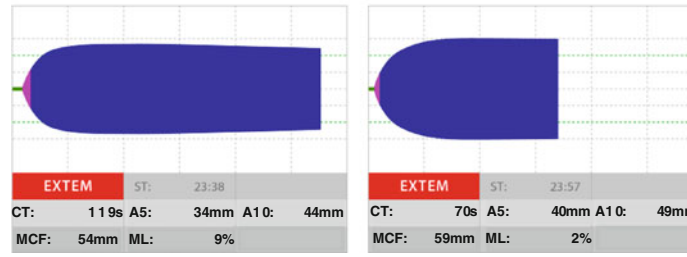

Fig. 18.8 (continued)

[40]. Colloid infusion (HES > gelatin) results in reduced resistance of polymerized fibrin to plasmin degradation [123]. In contrast, high factor XIII levels attenuate tissue plasminogen activator-induced hyperfibrinolysis in human whole blood [124]. Furthermore, a flat-line in FIBTEM characterized by a FIBTEM CT > 600 s seems to be associated with hyperfibrinolysis, too.

Management of Clot Firmness

Trauma-induced coagulopathy is functional characterized by a reduced clot firmness in EXTEM with an A5 < 35 mm or an A10 < 45 mm, respectively, and predicts the need for massive transfusion [41]. Here, reduced clot firmness can be based on hypofibrinogenemia, fibrin polymerization disorders (e.g., due to colloids), thrombocytopenia, and severe thrombocytopathy (reduced platelet aggregation due to activation of platelets' thrombin receptors).

FIBTEM A5 (A10) can be used for rapid and correct discrimination between hypofibrinogenemia and thrombocytopenia [17, 37–39, 77]. Here, a FIBTEM A10 < 8 mm is associated with an increased risk of massive transfusion and can be used as a trigger value for fibrinogen substitution [77]. However, some patients may even need a higher trigger value of 12 mm (with a targeted value of 16 mm)—in particular in patients with severe bleeding due to instable pelvic fractures, liver injury and traumatic brain injury [125, 126]. The fibrinogen dose can be calculated based on the targeted increase in FIBTEM A5 (A10):

Fibrinogen dose (g) = targeted increase in FIBTEM A5 (mm) × body weight (kg)/160

Here, the correction factor (140–160 mm kg g^{-1}) depends on the actual plasma volume [115, 119, 127–129]. Notably, the achieved increase may be lower than the calculated increase in severe bleeding. Fibrinogen substitution can be done by fibrinogen concentrate administration or cryoprecipitate transfusion, dependent on the local approval and availability. Table 18.5 provides a quick overview about the fibrinogen dose needed to achieve the targeted increase in FIBTEM A5 (A10).

If clot firmness in EXTEM is reduced (A5 < 35 mm or A10 < 45 mm) but FIBTEM clot firmness is above the trigger value (A5 ≥ 8 mm (or 12 mm)) platelet transfusion has to be considered in bleeding trauma patients. Notably, ROTEM® analysis has been shown to be superior to platelet count in prediction of bleeding in patients with severe thrombocytopenia [130]. The expected increase in EXTEM A5 (A10) per transfused pooled or apheresis platelet concentrate is 5–10 mm in adult patients [131, 132]. Therefore, the number of transfused platelet concentrates can be calculated based on the targeted increase in EXTEM A5 (A10).

Notably, standard viscoelastic assays are not sensitive to the effects of antiplatelet drugs such as COX-inhibitors (e.g., aspirin) and ADP-receptor antagonists (e.g., clopidogrel, prasugrel, and ticagrelor) since high amounts of

Table 18.5 FIBTEM-guided fibrinogen substitution

Targeted increase in FIBTEM A10 (A5) (mm)	Fibrinogen dose (mg/kg bw)	Fibrinogen concentrate (mL/kg bw)	Cryoprecipitate (mL/kg bw)
2	12.5	0.6 [1 g per 80 kg]	1 [5 U per 80 kg]
4	25	1.2 [2 g per 80 kg]	2 [10 U per 80 kg]
6	37.5	1.9 [3 g per 80 kg]	3 [15 U per 80 kg]
8	50	2.5 [4 g per 80 kg]	4 [20 U per 80 kg]
10	62.5	3.1 [5 g per 80 kg]	5 [25 U per 80 kg]
12	75	3.8 [6 g per 80 kg]	6 [30 U per 80 kg]

Here, fibrinogen dose calculation is based on the targeted increase in FIBTEM A10 (A5) in mm. In case of severe bleeding, the achieved increase in FIBTEM A10 (A5) may be lower than the calculated increase. Courtesy of Klaus Görlinger, Tem International

thrombin are generated in the test system which overcomes the effects of antiplatelet drugs. Therefore, platelet function analysis should be performed in patients with suspected platelet dysfunction. In the ROTEM system this is realized by the ROTEM® *platelet* module (CE-marked in Europe since November 2013), which provides two channels of whole blood impedance aggregometry in addition to the four viscoelastic channels of the ROTEM® *delta* device (Fig. 18.1). Whole blood impedance aggregometry provides rapid and reliable data about platelet function within 10 min (Fig. 18.9). Besides detection of the effects of antiplatelet drugs and other drugs with antiplatelet effects (e.g., analgetics, antidepressants, antibiotics, cardiovascular drugs), whole blood impedance aggregometry has been shown to detect early direct effects of trauma and sepsis on platelet function which is associated with increased mortality [27, 52, 55–58, 67, 133–135]. However, actually it is not yet clear whether early trauma-induced platelet dysfunction should be treated with platelet transfusion or not [64].

Management of Coagulation Time (Thrombin Generation)

Coagulation times (CT) can be prolonged due to a deficiency of enzymatic coagulation factors or the presence of an anticoagulant, e.g., warfarin, heparin, or new oral anticoagulants (NOACs). A CT prolongation in EXTEM, solely indicate a deficiency of vitamin K-dependent coagulation factors (factor VII, X, II; extrinsic pathway) due to a therapy with vitamin K-antagonists (warfarin) or hemodilution/consumption. Usually, the activity of the vitamin K-dependent coagulation factors are decreased below 30 % of their normal activity if CT in EXTEM exceeds 80s [115]. However, a severe fibrinogen deficiency can prolong CT in EXTEM, too. Therefore, EXTEM CT can be used for guiding therapy with prothrombin complex concentrate (PCC) or fresh frozen plasma (FFP) only in case of a normal A5 (A10) in FIBTEM. Accordingly, management of clot firmness precedes management of coagulation time in the ROTEM algorithm. Here, the use of three- or four-factor PCCs or FFP is dependent on the local approval and availability in the respective countries. Notably, four-factor PCCs (Beriplex® and Octaplex®) are approved in Europe for prophylaxis and therapy of bleeding in patients with hereditary and acquired deficiencies of vitamin K-dependent factors whereas four-factor PCC (Kcentra®) in the USA is FDA-approved for urgent reversal of vitamin-K antagonists only. Four-factor PCCs have the advantage of being a standardized product enabling a rapid and calculated increase in coagulation factor activity and avoiding transfusion-associated circulatory overload (TACO), transfusion-related lung injury (TRALI) and transfusion-related immunomodulation (TRIM) which are typical and serious complications of FFP transfusion [68, 113–115, 136–141]. Notably, direct thrombin inhibitors such as dabigatran can result in marked increase in EXTEM and INTEM CT as well as in ECATEM CT [142]. Here, the ecarin based ROTEM assay ECATEM is specific for direct thrombin inhibitors such as hirudin, argatroban, bivalirudin, and dabigatran [19, 20, 143].

Due to the high risk of arterial thromboembolic events, the off-label administration of rFVIIa should be restricted to bleeding not responding to comprehensive coagulation therapy [144, 145]. Activated PCCs (Factor Eight Inhibitor Bypassing Agent = FEIBA) are not indicated in trauma-induced coagulopathy, too. The implementation of thromboelastometry-guided bleeding management algorithms usually eliminates the need for rFVIIa administration as a rescue therapy [110, 111, 146, 147].

A prolongation of INTEM CT can be based on a heparin effect or a deficiency of coagulation factors of the intrinsic pathway. A heparin effect, e.g., due to endothelial glycocalyx degradation or re-transfusion of heparin by using a cell-saver in the emergency modus, can be confirmed by normalization of CT in HEPTEM [14, 148]. Here, protamine administration can be considered. In case of prolongation of CT in INTEM and HEPTEM, FFP transfusion can be considered in bleeding trauma patients.

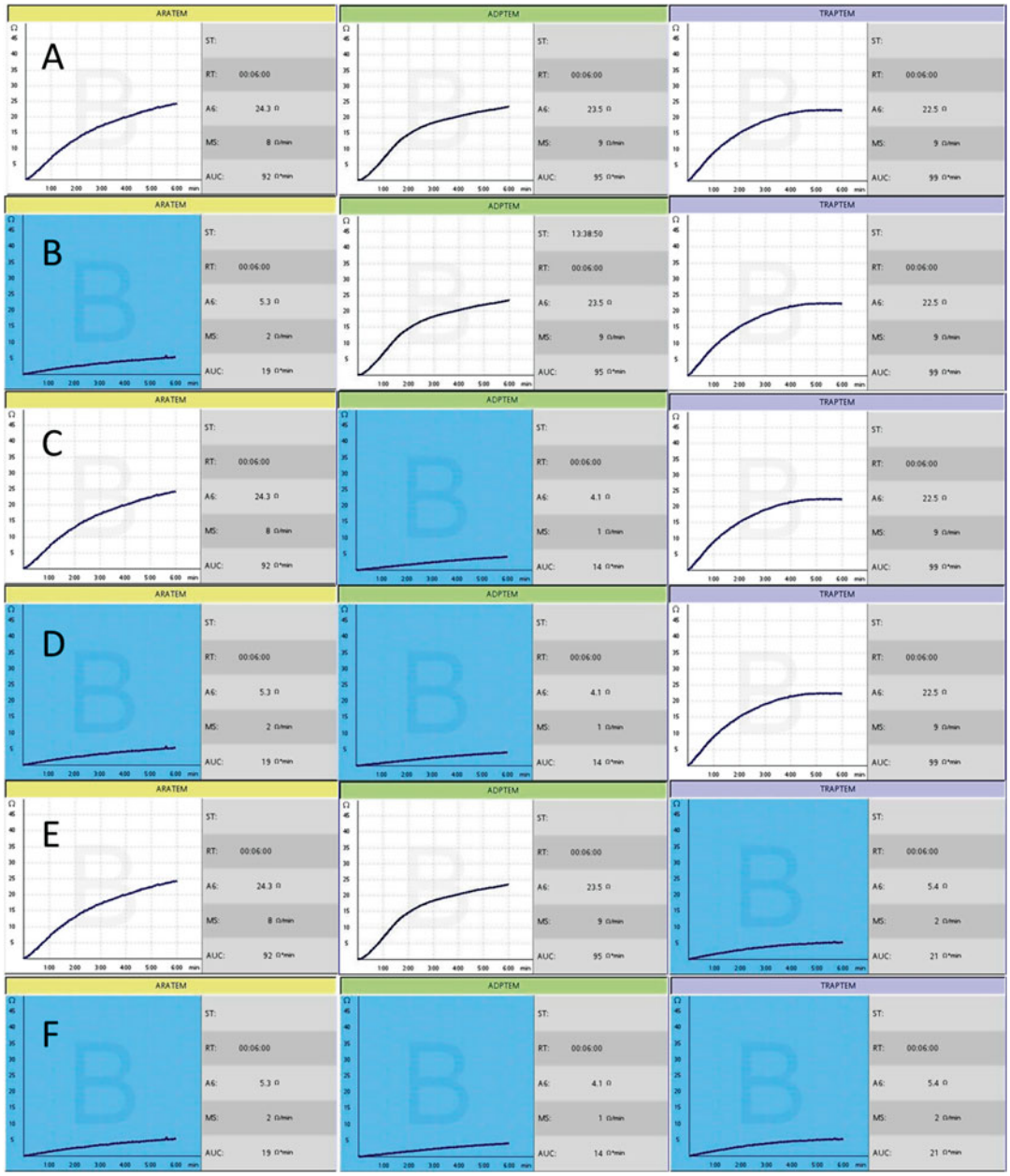

Fig. 18.9 Characteristic whole blood impedance aggregometry traces (ROTEM® *platelet*) achieved by activation with arachidonic acid (ARATEM; *left column*), ADP (ADPTEM; *middle column*), and TRAP-6 (TRAPTEM; *right column*). *Line A*: normal platelet function; *Line B*: selective inhibition of the arachidonic acid pathway (e.g., by aspirin); *Line C*: selective inhibition of the ADP-receptor pathway (e.g., by clopidogrel or prasugrel); *Line D*: inhibition of the arachidonic acid and ADP-receptor pathway (e.g., dual antiplatelet therapy with aspirin and clopidogrel); *Line E*: selective inhibition of the thrombin-receptor pathway (e.g., by vorapaxar); *Line F*: general platelet dysfunction due to triple antiplatelet therapy, GPIIbIIIa-receptor antagonists (e.g., abciximab, eptifibatide, or tirofiban), platelet receptor destruction (e.g., due to cardiopulmonary bypass, severe trauma, or sepsis), or severe thrombocytopenia. Courtesy of Klaus Görlinger, Tem International

Clinical and ROTEM® Reassessment

Finally, clinical bleeding has to be reassessed after running through the algorithm. In case of ongoing bleeding, ROTEM® has to be reassessed with a new blood sample and the algorithm has to be started again.

Thromboelastometry-guided Bleeding Management Algorithms—Impact on Outcome

Implementation of ROTEM®-guided bleeding management algorithms reduced bleeding and transfusion requirements in several clinical settings including severe trauma [99, 112, 118, 149].

Görlinger, Fries and Schöchl reported in their retrospective analysis that implementation of a ROTEM®-guided therapy with coagulation factor concentrates reduced transfusion requirements for FFP by 88–94 % in their institutions in different perioperative settings (trauma, visceral and transplant surgery and cardiovascular surgery). At the same time, the incidence of intraoperative massive transfusion (≥10 units of RBCs) could more than halved (1 % vs. 2.5 %; $P<0.001$), and RBC and platelet transfusion requirements could be reduced significantly by 8–62 % and 21–72 %, respectively [115].

Schöchl et al. analyzed transfusion incidence for RBCs and platelets in the emergency room and during primary surgery in a retrospective study including 80 trauma patients treated at the trauma hospital AUVA in Salzburg, Austria with a thromboelastometry-guided coagulation factor concentrate group versus 601 patients from the Trauma Register DGU, Germany with standard FFP-based therapy (≥2 units FFP; median 6 units FFP; range 2–51). Here, the transfusion incidence for RBCs (71 % vs. 97 %; $P<0.001$) and platelets (9 % vs. 44 %; $P<0.001$) was significantly lower in the thromboelastometry/factor concentrate group compared to the standard FFP group [114].

Nienaber et al. analyzed the incidence of multiple organ failure (MOF) in a matched-pair analysis including 18 severely injured and bleeding trauma patients from the Innsbruck Trauma Database (ITB), Austria treated according to a ROTEM®-guided coagulation factor concentrate protocol without FFP and 18 patients from the Trauma Register DGU, Germany treated with a FFP protocol without coagulation factor concentrates. Here, the incidence of MOF was significantly lower in the ROTEM®/factor concentrate group compared to the FFP group (16.7 % vs. 61.1 %; $P=0.015$) [150].

In a prospective cohort study including 517 trauma patients, Rourke et al. found that hypofibrinogenemia could be detected early by FIBTEM A5 (A10), and administration of cryoprecipitate or fibrinogen concentrate could correct coagulopathy and improved survival [38].

Schöchl et al. performed a retrospective analysis including 131 trauma patients who received ≥5 units of RBC concentrates within 24 h, and TIC was managed by ROTEM®-guided treatment with coagulation factor concentrates. After excluding 17 patients with traumatic brain injury, the mortality observed in this study was significantly lower (14 %) than predicted by the trauma injury severity score (TRISS; 27.8 %; $P=0.002$) and the revised injury severity classification score (RISC; 24.3; $P=0.014$) [113].

Lendemans et al. reported in a retrospective study before-and-after implementation of a ROTEM-guided algorithm in 172 trauma patients with an ISS > 16 a significant reduction in multiple organ failure (36.3 % vs. 66.7 %; $P=0.0001$) and in-hospital mortality (20.9 % vs. 38.3 %; $P=0.012$). In this study, performed in cooperation with the Trauma Register of the German Trauma Society (DGU) and supported by the German Interdisciplinary Association of Critical Care and Emergency Medicine (DIVI), 24 h mortality was reduced by 50 % (9.9 % vs. 19.8 %), too, but did not reach statistical significance [117].

Besides reduction in transfusion requirements, ROTEM®-guided bleeding management in particular in cardiovascular surgery has been shown to reduce complication rates as well as morbidity and mortality in cohort studies and RCTs [110–112, 115]. As shown above, several cohort studies reported similar results in trauma, but RCTs

are still lacking and urgently needed in this field [38, 78, 99, 113–118, 150].

Therapeutic Window Concept

The algorithm presented here, is based on the "therapeutic window concept". This concept has been developed for guiding antiplatelet therapy in patients undergoing percutaneous coronary interventions (PCIs) in order to minimize the risk of ischemia (stent thrombosis) and bleeding [151, 152]. Accordingly, bleeding management algorithms guided by thromboelastometry and whole blood impedance aggregometry are designed to minimize the risk of both bleeding and thrombosis by an individualized therapy. Here, the right therapeutic intervention, in the right dose and the right sequence is defining the framework of the therapeutic window, e.g.:

- EXTEM A10: 40–60 mm.
- FIBTEM A10: 10–20 mm.
- EXTEM CT: 40–80 s.
- ADPtem: 30–45 Ω min (in patients with drug-eluting stents).

Using this concept in cardiovascular surgery, it was possible to reduce both transfusion requirements and thrombotic/thromboembolic complications, significantly [110, 111, 153].

Lendemans et al. reported in their retrospective before-and-after trauma study a nonsignificant increase in thrombotic events (5.6 % vs. 2.2 %) in the POC group. However, this may be a result of the 50 % reduction in 24 h mortality in the POC group [117]. RCTs are urgently needed to assess if this concept is superior to other concepts for bleeding management in severe trauma.

Guidelines, Health Technology Assessments, Knowledge Translation, and Implementation

Based on the actually available evidence, the implementation of ROTEM®-guided algorithms is highly recommended (Grade 1C) by the guidelines for the management of severe perioperative bleeding from the European Society of Anesthesiology (ESA) and the updated European guideline for the management of bleeding and coagulopathy following major trauma [154, 155]. In particular, therapy with coagulation factor concentrates, such as fibrinogen concentrate and prothrombin complex concentrate (PCC), should be guided by thromboelastometry (Grade 1C). Furthermore, it is stated that the implementation of transfusion and coagulation management algorithms (based on ROTEM®/TEG®) can reduce transfusion-associated costs in trauma, cardiac surgery and liver transplantation (Grade B), and that targeted therapy with fibrinogen and/or PCC guided by ROTEM®/TEG® is not associated with an increased incidence of thromboembolic events (Grade C) [154].

The cost-effectiveness of ROTEM®-guided bleeding management has also been proven by several health technology assessments and pharmaco-economic analyses [156–159]. However, guidelines and heath technology assessments can only change practice an improve patients' outcomes in combination with knowledge translation and implementation [160–163]. Therefore, the 'STOP Bleeding Campaign' was set up in 2013 [164].

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Part IV

Management of Trauma Induced Coagulopathy

Red Blood Cell Transfusion

19

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Red Blood Cell: Manufacture, Storage, and Transfusion

Blood Collection and Manufacturing

Blood is classified as a drug and the collection and manufacturing processes of this industry are regulated by the US Food and Drug Administration (FDA) [1]. Throughout this chapter, references are made to the Circular of Information (COI), a joint publication of the AABB (formerly the American Association of Blood Banks), the American Red Cross, America's Blood Centers, and the Armed Services Blood Program, which is also recognized by the FDA as an extension of the blood label. This important document is a useful guide to the clinician and is available online [2]. The most recent National Blood Collection Utilization and Survey (NBCUS) reports that 13,775,000 million allogeneic units of whole blood and red cells (RBCs) were transfused in 2011, which is 8.2 % fewer units than in 2008 [3]. Over 20 million blood components were transfused in 2011 (there is a lag time between the receipt and publication of such data in this and similar reports). Blood centers collected 93.4 % of all components and the remaining amount was collected by hospitals [3]. The USA continues to transfuse red cells at a greater rate than many other countries, with 44 transfusions per 1000 population; this however, is a >10 % decrease from 48.8 transfusions per 1000 population in 2008 [3].

Blood transfusion is a common medical treatment and was listed in the most recent Healthcare Costs and Utilization (HCUP, a quality database supported by Health and Human Services) report as being in the top five procedures for patients aged 1–44 and was the most common procedure for patients aged 45–85 and beyond [4]. Moreover, allogeneic blood donation is a voluntary, unremunerated process. All donations are tested for blood type, specific antibodies and infectious diseases; platelets are also tested for bacterial contamination before they are released [3]. Units with positive or reactive results are destroyed, with appropriate deferral of the donor, per FDA. About 9 million people in the USA donate blood per year, although it is estimated that a much larger percentage could qualify for donation [3, 5].

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Whole blood is the unmanipulated product collected from a donor. Autologous blood donations are usually whole blood, though specific components may be drawn using automated collection devices. Allogeneic whole blood collections are separated into individual components soon after collection by centrifugation and manufactured into red blood cells (RBCs), plasma, or platelets destined to become pooled platelet products. Apheresis blood collection uses automated technology that allows for the targeted collection of red cells, single-donor platelets, plasma, and leukocytes (usually stem cells or granulocytes). Products may be collected singly or in multiples depending on donor qualification, the device, and FDA regulations. Plasma may be further manufactured into factor derivatives, albumin, and other medical products. Frozen plasma can be further manufactured into cryoprecipitate and cryoprecipitate-poor plasma. Blood products are a limited commodity with variable shelf-lives and the balance between supply and demand is dynamic, requiring cooperation between donors, blood centers and hospitals. Licensed blood products may be shipped across the USA to other areas in need.

Blood is collected into sterile, disposable, plastic bag sets which are manufactured to meet FDA specifications. Whole blood is collected through a large gauge needle (usually 16 or 17 gauge) by gravity flow from the donor's arm and into the primary bag, which contains a citrated solution to prevent immediate clotting. Additive solutions extend the RBC storage time. Apheresis collections use specialized bag sets and automated instruments that are designed to continuously draw and centrifuge small volumes of blood, remove the desired component(s) and return the remainder, with minimal impact to the donor's fluid balance.

ABO/Rh and Compatibility

The ABO blood group system was identified in 1900 by Landsteiner and colleagues and this is undoubtedly one of the most important medical discoveries, as prior to this time, there were myriad deaths due to transfusion incompatibility [6–8]. Variations or subtypes of the common blood types are occasionally seen and present patient blood typing challenges to the laboratory such that even routine blood orders require extra time to fulfill.

Codominant Mendelian inheritance of an A or B allele on chromosome 9q34 predicts blood type [9]. The A and the B allele each encodes for a glycosyltransferase which adds a sugar to the H antigen (FUT 1 gene, chromosome 19 q13.3) [10]. The H antigen resides on an oligosaccharide chain which extends beyond the RBC surface. Adding a specific sugar results in the formation of an A or B antigen [10]. Type O blood results from the homozygous inheritance of a nonfunctional allele (amorph) most commonly caused by a frameshift mutation, resulting in no glycosyltransferase being produced, and leaving the H antigen on the red cell unaltered [10]. The phenotypes of the ABO blood group system are based on expression of the A, B, and H antigens. A and B alleles are codominant. The O phenotype is recessive. In other words, if the genotype is AO the phenotype is A, and if the genotype is AB the phenotype is AB.

H carbohydrate is present on RBCs of almost every ethnic group worldwide and is the substrate upon which A or B antigens are formed [7]. Rarely, RBCs completely lack precursor H substance due to inheritance of two silent alleles of the H antigen, which is the *hh* genotype, also known as the "Bombay" phenotype [7]. Originally identified in Bombay, India in 1952 by Dr. Bhende, the complete lack of H antigen is seen in approximately 1 in 10,000 Asian Indians and 1 in 150,000 Japanese and is occasionally found in Caucasian populations at an incidence of approximately 1 in 1,000,000 [7].

A clinical example of this rare blood type is typified by a recent elderly patient who was hospitalized following a fall. She claimed that she had the Bombay phenotype, however, the staff did not deem the information to be important and

the blood bank was not notified. After several unsuccessful attempts to crossmatch blood for the patient, the sample was referred to the immunohematology reference laboratory (RL) for identification. After extensive workup the Bombay phenotype was identified, and these findings were confirmed with the patient. Fortunately she did not require transfusion, as the only compatible blood for a person with the Bombay phenotype is from a donor with this precise phenotype. The point is that if a patient communicates that they have a rare blood type, the blood bank should be informed, in order to investigate it, and to allow adequate time to locate compatible blood. Such patients may participate in autologous donation and their red cells may be frozen for future use. Patients with unusual blood needs may wear an emergency alert bracelet stating their condition or may verbally relay the information to the clinical staff.

Type A RBCs express A surface antigen, and naturally occurring anti-B antibody is found in the plasma [11]. Type B RBCs express B antigen and anti-A antibody is present in the plasma. Type O RBCs lack A or B antigen and have anti-A and anti-B antibody in the plasma. Finally, type AB blood expresses both A and B antigens on the red cell surface and lack naturally occurring plasma antibodies [11]. Naturally occurring blood group antibodies, also called isohemagglutinins, are not present in the newborn (apart from antibodies present due to passive maternal transmission) but develop around 4–6 months of age by a thymus-independent mechanism following exposure to carbohydrate epitopes on gut bacteria and food [11]. A and B antigens are found on cardiac, gut, and renal endothelium (and other organs) and additionally exist in a soluble form in secretions; for this reason they are also known as "histo-blood group antigens" and as such, are an important consideration in solid organ transplant [10] (Table 19.1).

ABO type compatibility is fundamental to avoiding a hemolytic transfusion reaction, which may occur within minutes of beginning the transfusion and with possibly fatal results. ABO antibodies are primarily of the IgM type, which fix complement well, and are capable of causing acute, intravascular hemolysis. ABO antibodies of the IgG subtype may cause a delayed, extravascular hemolysis. IgG subtypes may also cross the placenta from the maternal circulation to cause hemolytic disease of the fetus and newborn (HDFN) [12]. Antibodies from some blood group systems are more potent than those in other blood group systems in their ability to cause hemolysis [12]. Only one fatality resulting from ABO-mismatched RBCs was reported to FDA in 2013, accounting for 3 % of the total fatalities reported in that year [13]. Undoubtedly, many more ABO-mismatching events transpire without resulting in fatality. In 2011, a total of 42 such events were reported to the NBCUS, which amounted to 1 occurrence per 495,207 transfusions in the USA [3]. It is worth noting that not all hospitals participate in a program of voluntary transfusion reaction reporting, however fatalities due to transfusion must be reported to FDA [3].

Table 19.1 Frequency for ABO and Rh (D) type, red cell antigens and expected plasma antibodies [6, 7, 104]

Blood and D (Rh) type by ethnicity and approximate percentages					Red cell antigen	Plasma antibody
	Caucasian	African-American	Hispanic	Asian		
O	44	49	55	43	None	Anti-A and anti-B
A	43	27	28	27	A	Anti-B
B	9	20	13	25	B	Anti-A
AB	4	4	4	5	A and B	None
D (Rh) Pos.	83	93	93	98		
D (Rh) Neg.	17	7	7	2		

Table 19.2 Red cell type and compatibility

Patient blood type	Compatible red cells for transfusion
O	O only
A	A or O
B	B or O
AB	O or A or B or AB

Transfusion

Blood Type, Screen and Crossmatch

Persons with type O blood are often called the "universal donor" since their blood is compatible with all recipients. Type O blood is also the first choice of blood in emergency transfusion or trauma situations. Persons with A blood may receive type A or type O blood; persons with type B blood may receive type B or type O blood. Persons with type AB blood may be given any blood type and are sometimes referred to as being the "universal recipient" (Table 19.2).

The Rh blood group system is large and the D antigen is one of more than 50 Rh antigens found to date, but the presence or absence of the D antigen on RBCs is still commonly referred to as Rh-positive or Rh-positive, respectively. D antigen expression varies among ethnic groups (Table 19.1). With respect to transfusion, the D antigen is second in importance to the ABO blood group system. For routine transfusion every effort is made to match the ABO/Rh of the unit to the patient; for example, a patient with B-negative blood should ideally receive blood from a donor who is B-negative (type B, Rh-negative), but O-negative blood would be compatible. Anti-A and anti-B antibodies are naturally occurring and are present depending on blood type (Table 19.1). Non-ABO red cell antibodies are sometimes found in patient serum and are called "unexpected" alloantibodies. About 5 % of patients have unexpected alloantibodies. These antibodies have formed following exposure (usually from transfusion or pregnancy but possibly from other blood exposure) to red cells possessing antigens foreign to the recipient. Determination of RBC phenotype may be performed by serology and molecular testing and may help to predict what antibodies a sensitized person could make. Antibodies differ in their clinical significance, or in their ability to cause hemolysis and/or hemolytic disease of the fetus and newborn (HDFN). Extra time may be needed by the blood bank to locate blood for patients with rare or multiple alloantibodies.

If a "type and screen" is requested, the "type" is the determination of the patient's ABO and Rh type and the "screen" detects unexpected alloantibodies in the patient serum, such as anti-K (of the Kell blood group system), anti-Fy (of the Duffy blood group system) and so on. Multiple blood group systems are represented on screening and extended blood panels to improve the chances of detecting clinically significant alloantibodies. Once a person has formed an alloantibody, red cells negative for the offending antigen should be provided if possible, whether or not the alloantibody is detectable. If a "crossmatch" is requested, this is the pairing of patient serum against donor red cells and is the last check of compatibility prior to issue. If there is agglutination or hemolysis the unit is incompatible with the patient. Crossmatched units may be held in reserve for a time, depending on the institution's policies. Emergency-release blood is usually O-negative or O-positive and is uncrossmatched and requires a physician's signature to approve the product, either prior to, or in a specified time-frame following its release. Uncrossmatched blood is not necessarily incompatible.

RBC Transfusion

Patients require RBC transfusion in situations including hemorrhagic shock, other blood loss, and symptomatic anemia for myriad medical reasons including surgery, cancer treatment, and red cell exchange transfusion for sickle cell anemia. RBC and oxygen deficit in the patient is manifested by symptoms including fatigue, tachycardia, tachypnea, and hypotension. Informed consent must be provided and signed

prior to transfusion of any blood product, by the patient receiving the transfusion or by a parent, guardian or person with power of attorney to make medical decisions. A "time-out" should be performed by staff administering the transfusion, prior to the infusion of each unit, to make sure the right patient is being transfused the correct blood product; every hospital should have a clear policy for such a protocol that is easily accessible by all staff. Certain religious faiths will decline blood product transfusion and their refusal should be respectfully honored and carefully documented according to hospital policy and applicable laws.

Standard blood infusion filter sets must be used when administering blood products, including granulocytes. These filter sets have pore sizes ranging from 150 to 260 μm, which trap tiny clots and particulate aggregates, but still allow blood cells to pass through [2].

Routine transfusions should be administered slowly, especially in the first 15 min, in order to watch for the possibility of a transfusion reaction. Vital signs (temperature, blood pressure, heart rate, and respiratory rate) should be taken prior to the infusion and periodically during and then after the transfusion is complete. Oxygen saturation is useful if available. The remainder of the unit may be transfused at a slightly faster rate, with the goal of infusing a RBC unit over 2–4 h. Rapid infusion, unless medically necessary, should be avoided to mitigate the risk for transfusion-associated circulatory overload (TACO). Although many blood products will not require 4 h to infuse, the COI states that blood products must be infused within 4 h of entry into the bag [2]. If the amount of ordered product cannot be infused within 4 h, request that the blood bank prepare aliquots, or smaller amounts, to avoid product wastage. Pediatric blood products are often aliquoted into regular-size transfer bags, smaller bags designed for pediatric use, or into syringes.

All suspected transfusion reactions should be clinically managed and reported to the blood bank. The most common symptom of a transfusion reaction is fever; however, if a transfusion reaction is suspected for any reason, stop the transfusion and keep the line open with saline and manage the patient clinically.

The Rationale of Switching from O-Negative to O-Positive Blood

The first choice of blood product in a patient of unknown blood type is O, since blood type O possesses no A or B red cell surface antigens and is therefore more likely to survive in the plasma of any recipient. Once the blood type of the patient is determined, and this is usually determined soon after arrival, type-specific blood is given. Upon receiving an alert of an incoming trauma or for emergency blood needs, most hospitals automatically issue 2 or more O-negative RBC units at once, unless the patient's blood type is already known. As the supply of O-negative blood is limited by the small percentage of donors possessing this blood type (usually around 6 %), good stewardship is necessary. Hospitals often choose to add an O-negative switching protocol to aid clinicians in this endeavor.

In most protocols of this type, women of childbearing potential (or age) receive O-negative blood until their blood type is known. What constitutes childbearing age is determined by each institution but is generally accepted to be females under the age of 45 or 50. If a woman of childbearing age is indeed O-negative, this is the blood type she should continue to receive, to decrease her risk of forming a potentially deadly anti-D antibody that is capable of crossing the placenta and targeting the fetus' RBCs. Another group that may automatically receive O-negative blood is all patients under 18 years of age, male or female. For women beyond childbearing age and all males greater than 18 years, O-positive blood may be dispensed right away. This approach has been adopted in many large, urban institutions as the need for emergency or trauma blood could never be sustained if only O-negative units were used. Once the patient's blood type is known, type-specific or compatible blood should be given. An example of a switching protocol is illustrated in Fig. 19.1.

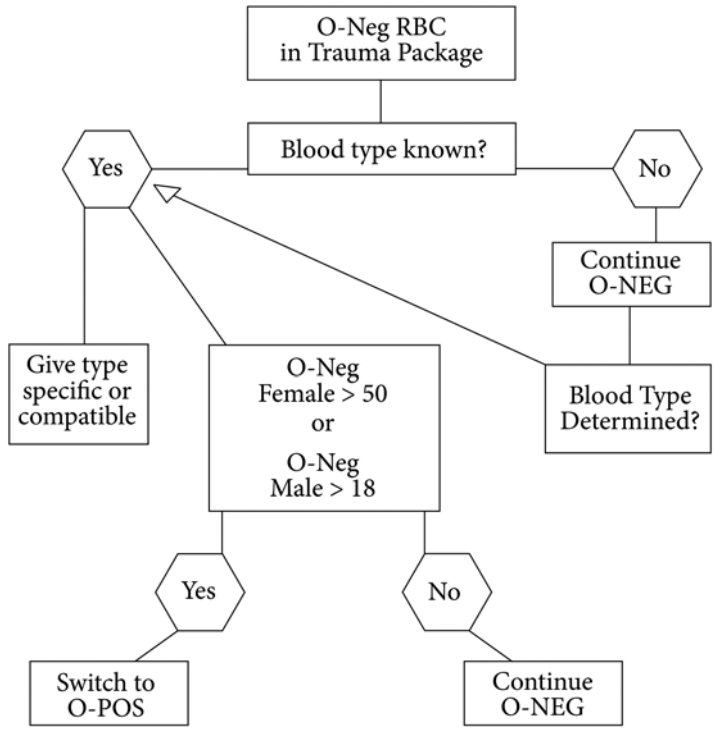

Fig. 19.1 Example of O-Negative Switching Protocol O-negative RBCs are released for trauma and emergency use in most hospitals. If the patient's blood type is known, type specific or compatible blood should be issued. If the blood type is not known, O-negative blood should be transfused until a blood sample has been sent to the blood bank for ABO/Rh determination and once blood type is known, type specific or compatible blood should be given. If the patient is a woman beyond childbearing years (usually accepted as being older than 45 or 50 years of age), or a male beyond the age of 18 years; some centers switch these patients to O-positive RBCs upon arrival. All women of childbearing capacity and all patients below 18 years of age (pediatric patients) should receive O-negative blood until their blood type is known, and then they should receive type-specific or compatible blood

In transfusion situations that are more serious than what can be managed by an MTP, some hospitals have instituted a catastrophic transfusion protocol (CTP) option which doubles or triples the amount of product that is offered in an MTP package. In these situations, in which very heavy bleeding occurs, it is permissible to temporarily switch a patient from O-negative to O-positive and then switch back to O-negative when the bleeding has stabilized. This approach also allows for better inventory management of a limited resource. Rh immune globulin should not be given to O-negative patients after the infusion of O-positive red cells, but may be considered if an O-negative person has received Rh-positive platelets; consultation with the blood bank or pharmacy may be helpful in such cases.

Intraoperative Blood Salvage

An autotransfusion service (ATS) may be available at medical institutions with robust surgical or trauma services. Program oversight is usually provided by the transfusion medicine, anesthesia or surgery department and procedures are ideally performed by a perfusionist or other specially trained personnel. The service may or may not be accredited by the AABB.

Specialized instruments, or "cell savers," may be used during planned or emergency surgery in which blood loss is excessive (equal to or greater than 20 % total blood volume loss), for patients with religious objections to receiving allogeneic blood and for patients with known multiple alloantibodies or rare blood types. ATS teams on-call

allow for a rapid response and instruments may be partially set-up ahead of time.

Various instruments on the market function in essentially the same manner: intraoperatively shed blood is gently suctioned to preserve red cell morphology and function, washed with an isotonic solution, filtered, and reinfused either intraoperatively or postoperatively. Sponges used intraoperatively may also be washed and rinsed and that fluid can be added into the circuit. Abdominal, thoracotomy, and drain blood from other surgical sites may be processed. Extracorporeal anticoagulation is achieved with heparin (up to 30,000 units per liter of normal saline) or citrate solutions (often ACD-A, which is anticoagulant citrate dextrose, solution A) or a combination of the two. The filters have fairly large pore sizes (40–120 μm) to remove debris such as bony spicules or cement and large cellular aggregates. Due to the washing phase, very little plasma or its solutes (free hemoglobin, interleukins, hemostatic factors, and so on) remains in the final product.

Depending on the processing, the final product may be kept at room temperature for up to 4 or 6 h, or at 1–6 ° C for up to 24 h in a monitored refrigerator [14, 15]. Since the whole-blood product is freshly obtained from the patient and washed, some transfusion reaction risks are reduced, but reactions may still occur, such as from fluid overload or bacterial contamination [16]. The final red cell product is of high quality, with a hematocrit between 40 % and 80 % depending on the device and method used, and is suspended in a small amount of isotonic solution with very little remaining anticoagulant. It has been shown that the red cell survival of cell saver units obtained during bypass surgery are comparable to circulating venous blood after 24 h time [17].

Literature is not abundant regarding the consistent use of the cell saver in the setting of trauma, specifically regarding the quality of chest blood as most current cell saver data are obtained from planned cardiothoracic surgeries. Direct reinfusion of unwashed shed blood does not appear to be well-supported in the setting of trauma, though more studies are needed in this area to clarify the risks and benefits. A recent prospective study of unwashed thorax (pleural) blood from 22 patients at a large trauma hospital was conducted to characterize the solutes present in shed chest blood [18].

An ATS may help the hospital significantly reduce allogeneic red cell costs and meet the aims of its blood management program. Receipt of fewer allogeneic units reduces donor exposure, possible alloimmunization, and some transfusion risks, but beware of the possible coagulopathy which may accompany its use in some patients. Collaboration between the service providing the blood and/or overseeing the cell-saver program and the end-user services, in accordance with AABB guidelines, is essential in order to establish evidence-based guidelines, and to cover topics such as what surgical situations might constitute an absolute (open bowel) or relative contraindication (malignancy) for use of the cell saver [15].

Composition, Shelf Life, and Storage

RBCs, whether separated from whole blood or obtained directly from apheresis collections, are manufactured in such a way that the residual plasma volume is minimal (5–10 mL) [19]. The volume of allogeneic whole blood collections ranges from 400 to 550 mL and the hematocrit of a whole blood unit is approximately that of the donor's [2]. Donors may not have a pre-donation hematocrit less than 38 % in accordance with FDA collection guidelines [2]. RBCs are collected into anticoagulant/nutritive solutions containing the following solute combinations: citrate–phosphate–dextrose (CPD), citrate–phosphate–dextrose–dextrose (CP2D), and citrate–phosphate–dextrose–adenine (CPDA-1) [2, 20]. Citrate chelates ionized calcium in the donor's blood to suspend the coagulation cascade and inhibit clotting. Phosphate and dextrose directly provide nutrients to the RBCs. Adenine is a nucleic building block that is added to some RBC solutions (CPDA-1) and additive solutions, allowing shelf life to exceed 3 weeks [2, 20]. RBCs collected in CPD and CP2D have a shelf life of 21 days, and those collected in CDPA-1 have a shelf life of 35 days. The volumes of RBC units vary between 225 to 350 mL with a hematocrit ranging from 65 % to 80 % (average 75 %) [2, 20].

Additive solutions currently available in the USA are: AS-1 (Adsol, Fenwal), AS-3 (Nutricel, Haemonetics), AS-5 (Optisol, TerumoBCT), and AS-7 (SOLX, Haemonetics) [2, 20]. These solutions add an additional 100 or 110 mL of fluid, post-collection, to the RBCs which are originally collected into the anticoagulant/nutrient solution [2, 20]. Additive solutions contain combinations of phosphate, adenine, mannitol, dextrose (glucose), and additional citrate. The solutes provide nutrients and help stabilize the RBC membranes, allowing for increased storage times [2, 20]. These units have a hematocrit of 55–60 %, a unit volume around 300–400 mL, and a shelf life of 42 days [2, 20].

One unit of RBCs is expected to raise the hemoglobin by 1 g/dL and the hematocrit by 3 % in an "average-sized adult"; however, this is a broad statement which cannot apply to every clinical setting. Pediatric patients should be transfused according to weight, usually at a dose of 10–15 mL/kg [21]. One milliliter (mL) of RBCs contains 1.08 mg (mg) of iron [22]. Thus if 200 mL RBCs are donated, approximately 200 mg of iron is lost by the donor and that same amount is ultimately transfused into the recipient [22]. Hemoglobin levels are not an accurate predictor of iron stores and this is currently an area of great interest regarding US blood donors [22]. The 2012 REDS-II donor iron status evaluation (RISE) study results reported absent iron stores (ferritin < 12 ng/mL) in as many as 27 % of frequent female donors and 16 % of frequent male donors [22]. Conversely, the issue of iron overload may exist in blood recipients. Clinically significant iron accumulation may be seen following the transfusion of 50 or more RBC units and possibly sooner in pediatric patients [23]. The total amount of iron transfused may be calculated using the following equation, where Kin is the total amount of iron infused:

$$\text{Kin} = \text{RBC transfused in mL} \times 1.08\ \text{mg Fe / mL}\ [24]$$

Iron is stored in the body as soluble ferritin or as insoluble hemosiderin [23, 24]. Frequently transfused patients have increased morbidity associated with tissue iron deposits in the heart, endocrine organs, and liver [23, 24]. Patients with hemoglobinopathies, hematological malignancies, inherited or acquired anemias, and myelodysplasias are most vulnerable. Depending on the clinical situation, chelation therapy and red cell exchange or erythrocytapheresis, rather than simple transfusion, may benefit some patients [23, 24].

Component Modification

Clinical indications for modified components vary and it may be advantageous to consult with the reference laboratory, blood bank, or a transfusion medicine pathologist prior to ordering these products. Not all products are readily available and considerable time may be required to manufacture or obtain some blood products.

Leukocyte Reduction (and Provision of CMV-Negative Blood)

Leukocyte reduction, or leukoreduction (LR), of RBCs or platelets is a widely performed component modification. Pre-storage LR is done during automated apheresis collections or after whole blood collection. Bedside LR, or post-storage LR, remains an alternate but infrequently chosen option. To qualify as a "Leukocyte Reduced" product, the residual white cell count must be less than 5×10^6 per unit per FDA criteria, which may be easily met by use of any of the current third and fourth generation LR filters [25]. LR of RBCs decreases the incidence of febrile non-hemolytic transfusion reactions, HLA and platelet alloimmunization, and the amount of biologic response modifiers (BRMs), which accumulate during product storage [26–28].

The equivalency of LR to CMV-negative products continues to be debated. Some physicians consider LR blood to be adequate and essentially equivalent to LR blood that is from CMV-seronegative donors. The rationale is that CMV resides within white cells, and with the efficiency of modern LR filters, any risk of CMV would be exceptionally low [29–31]. For CMV-seronegative patients, especially those who are peri- or post-transplant, or low birthweight infants, many clinicians request CMV-negative products and accept LR-only products if CMV-

negative and LR products are unavailable [29–31]. In addition, CMV-seronegative donors who test "negative for CMV" on their most recent donation carry a small but real risk of transmitting CMV to a recipient, if the donor happens to be newly infected and is in the window period (the time between infection and the time at which the infection can be detected by testing) [29–31]. The test performed on donor blood detects anti-CMV antibodies [29–31]. Nucleic acid testing (NAT) testing for CMV DNA is available but is not used for donor testing [29–31]. Frequent donors (such as platelet donors) have a theoretically higher risk of transmitting CMV since they are able donate several times during the 6–8 week window period, whereas a single red cell donor can only donate a single unit in the same period of time [29–31]. Indications for CMV-negative blood include the following populations: CMV-seronegative patients who are immunocompromised, fetuses, pregnant females, very-low and low-birthweight infants, transplant recipients of either hematopoietic stem cells or solid organs, and any severely immunocompromised patients including those with HIV.

Again, it must be stated that LR components may suffice in certain clinical situations and when CMV-negative blood is unavailable and the patient requires transfusion [2, 32]. LR should not be confused with the use of standard blood infusion filter sets used when administering blood products. These filters have pore sizes ranging from 150 to 260 μm, to trap large cell and particulate aggregates, but still allow blood cells to freely pass (for reference, RBCs are about 5 μm in diameter) [2, 33].

Washing

Washed RBCs (or platelets) are indicated in cases of very severe or progressively worsening allergic or anaphylactic transfusion reactions to cellular blood products and patients known to be IgA-deficient [34, 35]. Washed cellular products may also be requested for pediatric patients with renal impairment, elevated potassium and related issues, to remove excess potassium in the blood product [34, 35]. However, for the vast majority of patients, the amount of potassium in the plasma should not have any untoward effects on the recipient even if the RBCs are irradiated and near the outdate, if infusions are given slowly, over 2–4 h [34, 35].

Frozen RBCs)must also be washed prior to infusion, to remove the glycerol in which they are stored [20]. RBCs (and platelets) are washed by specialized, automated instruments that progressively wash a single unit in normal saline, and sometimes dextrose, to create a final product that is essentially devoid of plasma and which contains a minimal amount of saline [20]. The final product is usually left in the wash bag, which resembles a flat doughnut. A small percentage of product is normally lost during the washing process [20]. The hematocrit of the RBC product is usually around 75 % with a volume of 180 mL [20].

Up to two additional hours of time may be required to produce a single, washed product. It is important to communicate to the reference lab or blood bank, the time frame in which the product will be needed [2]. Washing changes the expiration date of RBCs to 24 h, stored at 1–6 °C (washed platelets expire in 4 h and are stored at 20–24 °C), or the original expiration date and time, whichever comes first [2]. Requests for washed plasma are not possible to fulfill for obvious reasons. Plasma for IgA-deficient patients should come from donors who are IgA-deficient. Such products are rare and may require nation-wide coordination with such entities as the American Rare Donor Program (ARDP) to identify possible donors and request that they come in for donation [36]. Altogether, several days' time may be needed to procure the donation, receive the donor's infectious disease testing results and to transport the product to the patient's location.

Irradiation

Cellular products (RBC, platelets) may be irradiated to preclude the development of transfusion-associated graft-versus-host disease (TA-GVHD), which is a donor T-cell mediated destruction against the recipient's immune system. Plasma

and cryoprecipitate are by definition, acellular and should not require irradiation. TA-GVHD is most-commonly caused by the infusion of competent donor T-lymphocytes into an immunocompromised recipient, though there have been cases involving immunocompetent recipients [37]. LR is inadequate in comparison to irradiation for preventing TA-GVHD, since even with the most recent LR filters residual leukocytes remain and there is no known minimal threshold of T-cells needed to trigger the reaction [38]. TA-GVHD is similar to post-transplant GVHD, affecting HLA-antigen-dense tissues such as the skin, gastrointestinal tract and liver [38]. There are, however, two findings seen with TA-GVHD: bone marrow aplasia and an earlier onset, usually between day 2 and day 50 following transfusion, which distinguishes it from post-transplant GVHD [38].

Irradiation of cellular blood products is accomplished by X-ray irradiators or gamma-ray irradiators specifically manufactured for blood establishments, or by linear accelerators used in the field of radiation oncology [39]. Linear accelerators are also used for blood irradiation in developing countries and other areas where dedicated blood bank irradiators unavailable [39]. Irradiation causes the damage to the red cell membrane and escape of intracellular potassium, which increases with the age of the red cell and over storage, but is not usually harmful to the recipient [34, 35, 40]. Hyperkalemia has been reported in pediatric patients when large volumes are given or blood is quickly infused, however washing irradiated red cells prior to infusion can remove the supernatant potassium if the clinician is concerned [34, 35, 40]. The use of X-ray compared to gamma rays have not been shown to be significantly different in their effects on red cell membranes [41].

The isotope decay of cobalt-60 or cesium-137 produces gamma-rays, providing the energy source for nuclear irradiators. These radioactive sources are sealed within the body of the instrument and require increased security controls [42]. X-ray irradiators and linear accelerators both emit X-rays and this form of energy is known as ionizing radiation [42]. Whatever energy source is used, irradiation renders residual allogeneic T-lymphocytes incapable of replication by rendering inactive the DNA of leukocytes to a level greater than 5 logs [42]. Mature red cells and platelets lack nuclei and are not affected in the same manner as white cells. Red cells outdate at 28 days from the date of irradiation, or keep the original expiration date, whichever comes first. Platelets retain their usual date of expiration, post-irradiation.

Per FDA criteria the dose to the center of the irradiation chamber must be 25 Gray (Gy) and the dose at any point in the periphery must be 15 Gy, never exceeding 50 Gy at any point in the chamber [43]. Irradiated cellular products may be routinely provided for the following patient needs: intrauterine transfusion, premature, very-low or low birth weight infants, infants needing exchange transfusion, any child suspected of or persons with known congenital immunodeficiency, pediatric extracorporeal membrane oxygenation (ECMO), patients with lymphomas and leukemias, any profoundly immunosuppressed patients with solid tumors, patients on nucleoside (purine) analogs or T-cell function altering drugs (examples: clofarabine, alemtuzumab, fludarabine).

Components that must be irradiated include donations from family members who are blood-related to the recipient, any human-leukocyte-antigen (HLA)-matched products, and granulocytes [33, 38, 44, 45]. It is not standard practice to irradiate blood for most patients undergoing whole organ transplantation or patients with HIV/AIDS as the *sole* diagnosis [33, 38, 44, 45].

Component irradiation is performed to prevent interactions from possible human leukocyte antigen (HLA) similarities between donor and recipient [43]. Shared HLA haplotypes are more commonly seen among blood-related family members (consanguinity) or in communities lacking HLA diversity [43]. If the recipient is transfused with T-cells that share an HLA haplotype with a donor who is homozygous for that haplotype, the recipient will not view the donor's cells as foreign and will allow them to enter the body without opposition. [43]. However, in time the donor's cells may come to view the recipient

as foreign and mount a response to destroy the recipient; this is known as TA-GVHD) [43].

Adverse Events Related to Transfusion

Transfusion complications, or transfusion reactions, may be broadly divided into infectious and noninfectious causes. Donor screening begins prior to the actual donation, with the reading of educational materials, a brief physical exam and completion of a donor history questionnaire; at each step the donor may self-defer or may be deferred. If all criteria are met, a donation may take place. Every time a donor is drawn for allogeneic transfusable products, blood samples from that donation undergo testing for blood ABO and Rh type, unexpected red cell antibodies and infectious disease testing. Tests for the following infectious diseases are also performed in accordance with FDA guidelines: human immunodeficiency virus (HIV) types 1 and 2, hepatitis B and C virus, human T-cell lymphotropic virus (HTLV) types I and II, *Treponema pallidum* (the organism that causes syphilis), and West Nile Virus (WNV). Donors must be negative for antibodies to the parasite *Trypanosoma cruzi*, which causes Chagas disease, once in their donation lifetime. In regards to other infectious diseases, the donor questionnaire is written in a way such that donors with the possible risk of transmitting diseases such as malaria or hepatitis A are deferred from donation that day. Testing for cytomegalovirus (CMV) and HLA antibodies may be additionally performed. Donors may be temporarily, indefinitely, or permanently deferred based on the criteria set by FDA or AABB [46]. Criteria for autologous donation are not as strict.

Much of the infectious disease testing is antibody-based and detects the donor's immune response to the offending agent. Adequate time, days to weeks, must pass until antibodies form; this also known as the window period [47–49]. Nucleic acid testing (NAT) detects viral nucleic acid particles and has greatly reduced the window period to just days [47–49]. NAT is used for detection of HIV-1 RNA, hepatitis B virus DNA, hepatitis C virus RNA, and WNV RNA [47–49]. For example, prior to NAT testing for HIV-1, the window period for detection of antibody formation, even with third generation tests was 21–24 days after infection; in contrast, NAT testing reduces the window period to less than 10 days [47–49] (Table 19.3).

Table 19.3 US blood donor screening and risk of recipient infection [51, 105–108]

Blood donor test	Risk of infection per number of transfused units
HBV	1:800,000 to 1:1,200,000
HCV	1:1,100,000
HIV	1:1,500,000
WNV	Very rare; two cases reported 2008–2014
HTVL	1:641,000
Chagas	Very low, no cases since screening has been implemented (FDA, 2010)
Syphilis	Very low, no cases in last 40 years
Bacterial contamination of platelets	1:3000

Blood donation is not a right and the FDA's primary responsibility is to ensure that the blood supply is safe for recipients. Despite thorough donor health screening and skin site prep, it has been estimated that approximately 1 in 2000 blood products are positive for bacterial contamination, though most are presumed to be normal skin flora which do not survive well at colder temperatures (RBC and plasma storage) [50, 51]. For this reason, the majority of implicated contaminated blood products are platelets, as they are stored at 20–24°C, which is fairly close to room temperature.

Following donation, a representative sample is taken from every platelet and is tested for bacterial growth prior to release. Since platelets are stored at room temperature, there are more reports of sepsis related to platelet products than any other blood product. RBCs are not prospectively tested for bacterial contamination, and are uncommonly implicated in septic reactions, but when they are, it usually involves an organism that thrives at colder temperatures, such as *Serratia* spp., since red cells are stored at 1–6 °C.

With the numerous current screening and testing measures set into place and the relatively low risk transfusion-transmitted infection, focus has shifted to the noninfectious complications of transfusion. Transfusion-related acute lung injury (TRALI); transfusion-associated circulatory overload (TACO); hemolytic reactions, both acute and delayed; transfusion-associated graft-versus-host disease (TA-GVHD); febrile non-hemolytic transfusion reaction (FNHTR); allergic/anaphylactic; and post-transfusion purpura (PTP) encompass the major noninfectious complications of transfusion (Table 19.4). Of these, only TRALI and TACO are discussed in detail.

Transfusion-Related Acute Lung Injury (TRALI)

TRALI continues to be a significant and underreported cause of transfusion-related morbidity and mortality in the USA. TRALI has previously been reported in medical literature as occurring as frequently as 1 in 3000 to 1 in 5000 transfusions but the true incidence of TRALI in the USA is unknown as all transfusion reaction reporting is currently voluntary [52]. Critically ill patients (ICU) have up to an 8 % incidence of TRALI; and has been reported in up to 15 % in patients with gastrointestinal bleeding and 30 % in patients with end-stage liver disease [53, 54]. The vast majority of patients with TRALI recover within 96 h, with aggressive, supportive treatment [52, 55]. Recent data from Medicare billing files have concluded that TRALI is much more prevalent than expected in the elderly, which makes sense because the incidence of ALI increases with age [56–58]. With respect to TRALI in pediatric patients, a recent large review performed by the Canadian Blood Services showed that the incidence of reported TRALI was not much different in children (5.58 per 100,000 transfusions) compared to adults (3.75 per 100,000 transfusions) [59].

The outcomes of these studies also reiterate that the incidence of TRALI is much greater in comparison to the data published by NBCUS, which is a voluntary data reporting program.

Table 19.4 Transfusion reactions [2, 36]

Entity	Etiology	Incidence
Immediate reactions		
Urticarial (allergic)	Patient response to donor Ag	1:100 to 1:300
Febrile non-hemolytic transfusion reaction (FNHTR)	Patient antibodies to donor WBC or cytokines in component	1:100 to 1:1000
Transfusion-related acute lung injury (TRALI)	Donor anti-WBC antibodies, pro-inflammatory molecules/biologic response modifiers	1:500 to 1:63,000
Anaphylactic/anaphylactoid	IgA-deficient pts with anti-IgA antibodies; antibodies to cytokines, haptoglobin or C4	1:20,000 to 1:50,000
Hemolytic transfusion reaction (HTR)	ABO-mismatch	1:76,000
Immunologic, delayed		
Hemolytic (non-ABO)	Patient anamnestic response to RBC antigens	1:2500 or rarer
Transfusion-associated graft-versus-host disease (TA-GVHD)	Donor destruction of patient immunity	Rare
Post-transfusion purpura (PTP)	Patient anti-HPA 1a Antibodies destroy donor platelets	Rare
Non-immunologic, immediate		
Sepsis	Bacterial contamination	Depends on component
Transfusion-associated circulatory overload (TACO)	Volume overload	Less than 1:100
Other Possible Events		
Air embolus, non-immune hemolysis, hypothermia, hypotension with ACE-inhibitors, citrate toxicity, and hypocalcemia		

There were 327 reports of TRALI submitted to the NBCUS in 2011 for an incidence of 1:63,940 transfused components [3]. The reporting of any death suspected to be related to transfusion, however, is mandatory per FDA [13]. During fiscal years 2009–2013, 38 % of all transfusion-related deaths reported to FDA were confirmed to be TRALI-related [13]. In fiscal year 2013, FDA reported that 37 % of all transfusion-related deaths were attributable to TRALI. Regarding the 14 TRALI-related deaths in 2013, 51 donors were implicated [13]. Gender data were available for 49 of the donors; 22 were male and 27 were female [13]. Thirty-eight of the donors were tested for the presence of HLA and HNA antibodies and ten of the 14 implicated donors reportedly had antibodies against cognate antigens in the recipient [13]. The majority of the deaths were among patients who received RBC transfusions alone or in patients who received multiple blood products [13].

Post-transfusion reactions consistent with what would now be described as TRALI were first reported in the medical literature in the 1950s; however, the coining of the term "TRALI" is attributed to Popovsky and Moore in the mid-1980s as the constellation of findings and symptoms temporally related to transfusion were unified under one diagnosis [60–62]. The diagnosis of TRALI begins with the recognition of acute lung injury (ALI) in the patient, defined by the American-European Consensus Conference as $SpO_2 < 90$ % or $PaO_2/FiO_2 > 300$ mmHg on room air or other demonstration of hypoxemia and bilateral pulmonary edema seen as lung infiltrates by frontal chest radiograph [63]. A combined definition of TRALI, as defined by the National Heart, Lung and Blood Institute Working Group and the Canadian Consensus Conference is an acute, non-cardiogenic lung injury occurring within 6 h of beginning a transfusion (though symptoms are often seen in the first 1–2 h) with respiratory symptoms of tachypnea, dyspnea, pulmonary edema which may be mild to severe and sometimes seen as acomplete "white-out" on frontal chest X-ray [64]. Frothy secretions are sometimes seen coming from the patient's mouth or endotracheal tube. If measured, the pulmonary artery wedge pressure should be less than 18 mmHg and there must not be any new, abnormal cardiac function [64]. Per this definition, TRALI may also be diagnosed in a patient with worsening preexisting pulmonary insufficiency (unique to the NHLBI definition), such as COPD or pulmonary fibrosis [64]. A diagnosis of possible TRALI may apply to patients with preexisting clinical risk factors for ALI, such as recent surgery, burn injury, coagulopathy, chronic alcoholism, sepsis, and carcinoma [64]. There may be hypotension (hypertension followed by hypotension may be seen), fever, chills, non-productive cough, and transient decreases in white cell counts, especially neutrophils [64–66].

TRALI is diagnosis of exclusion and septic transfusion reaction, volume overload, severe anaphylaxis or a newly manifesting medical problem are often in the differential. If TRALI is suspected during the infusion of multiple consecutive products, all products given to that point within a 6-h time frame are implicated.

All routinely transfused blood products (whole blood, RBCs, platelets, plasma, and cryoprecipitate) have been implicated in the diagnosis of TRALI [52]. Granulocyte and hematopoietic progenitor cell (HPC) products have also been associated with TRALI. Case reports have linked TRALI with the administration of immunoglobulin, first documented in 2001 [67–69].

The pathophysiology of TRALI is attributed to factors present in both the recipient and the transfused blood product. Up to 85 % of TRALI cases may be explained by the infusion of donor antibodies as demonstrated in an ex vivo animal model using isolated perfused rabbit lungs [70]. In these studies, antibodies against human leukocyte antigens (HLA) or human neutrophil antigens (HNA) had the ability to bind to neutrophils which expressed the cognate antigen and induce pulmonary edema [70]. However, the antibody, the cognate antigen on the leukocyte surface, and source of complement (plasma) had to be present in order for ALI to occur and if any of the components was omitted, lung damage was obviated [70]. This model has been refined using rats and demonstrated that a minimum number of antigen sites

needed to be present on the neutrophils such that antibody binding must reach a threshold before ALI would occur [71]. This work was also relevant in showing that priming with fMLF (N-formyl-methionine-leucine-phenylalanine,a component of bacterial cell walls) allowed anti-HNA antibodies to directly activate neutrophils in the absence of complement [71].

In vivo TRALI models have demonstrated that a specific monoclonal antibody could cause ALI at a concentration seemingly similar to relatively well patients who come in for a transfusion and develop TRALI [72]. However, in an animal model, when the mice were housed in a pathogen-free environment no TRALI was demonstrated indicating a likely two-event model [72, 73]. Such an in vivo two-event pathogenesis was confirmed in a rat model which also demonstrated that lipids and other biologic response modifiers (BRM) could cause TRALI in older, stored RBCs irrespective of leukoreduction [74]. Both antibodies to MHC class I ligands and the lipids from stored RBCs were capable of priming quiescent neutrophils (PMNs), activating primed PMNs (defined as superoxide anion production and release of granule constituents), and inducing ALI [74]. Specifically, rats that were infused with endotoxin (lipopolysaccharide, or LPS) alone did not develop ALI, however those rats who received LPS and then received a lipid extraction from 42-day-old RBCs did develop ALI [74]. Importantly extractions from fresh (day 1) RBCs or plasma did not result in ALI [74]. In addition, antibodies to common MHC class I or even class II antibodies caused ALI as the second event in this animal model of TRALI, indicating that TRALI, whether caused by antibodies to specific leukocyte antigens or due to BRMs that directly prime PMNs, appears to be the results of two distinct events [74]. The first event is the clinical condition of the patient, which predisposes him to TRALI and the second event is the infusion of the specific antibody or BRM into the patient which activates the sequestered PMNs inducing damage to the vascular endothelium, resulting in capillary leak and ALI [52, 74].

Antibodies implicated in TRALI include those against class I or II HLA and antibodies against HNA [52, 55, 75]. Antibodies to HLA class II antigens have a two-event pathophysiology that differs due to their recognition of these surface antigens on antigen-presenting cells and the in vivo modeling supports the threshold or two-event model [76]. Antibodies to HNA loci, especially HNA-3a, also are postulated to cause TRALI by a two-event pathogenesis as well as inducing TRALI by activating the pulmonary endothelium alone through the CTL-2 receptor [77, 78]. Such direct activation may occur without PMNs and could cause TRALI in neutropenic patients [77]. Other BRMs include soluble CD40 ligand (sCD40L) which has been shown to prime PMNs and are linked with clinical TRALI [79]. Whether due to antibodies or other BRMs, the majority of TRALI cases are the results of PMN activation inducing pulmonary endothelial damage [52]. Histologically, TRALI is indistinguishable from etiologies of diffuse alveolar damage, which include acute interstitial pneumonia, early acute respiratory distress syndrome (ARDS), and others [80, 81]. The two pathways by which TRALI may occur are not mutually exclusive and many patients have cognate antigens to transfused antibodies, yet do not develop TRALI, lending credence to a "threshold" or two-event model [52, 73, 75, 82].

Mitigation

TRALI mitigation strategies are vital to reducing morbidity and mortality related to this transfusion complication. During the 1990s the UK National Blood Service created a voluntary hemovigilance program, launching the Serious Hazards of Transfusion (SHOT) and were the first to collect plasma from male donors, after noting that TRALI or probable TRALI was seen almost seven times more frequently with plasma transfusion and about eight times more frequently with platelet infusion, (in comparison to TRALI occurrence in red cells) when these products were collected from female donors [83]. Their efforts were rewarded following the

adoption of this collection strategy for platelets and plasma, and reports of TRALI from plasma transfusion fell from 15.5 cases per million units transfused (1999–2004) to 3 per million (2005–2006) for plasma and from 14 cases per million to just under six cases per million for platelet products [83]. Densmore and colleagues also showed that HLA antibody formation increased with pregnancy, with about 8 % of never-pregnant females showing sensitization, increasing to 15 % of women after one pregnancy, and to about 26 % after three pregnancies [84]. Never-transfused men and never-pregnant women have a 1.7 % prevalence of HLA antibodies [85]. The American Red Cross (ARC) examined their own hemovigilance data and subsequently adopted a similar strategy of collecting plasma from predominantly male donors (95 %) and saw a significant (80 %) decrease in TRALI cases related to plasma infusion [86]. This similar and dramatic decrease in TRALI when female donors were avoided was also shown by Toy and colleagues, who designed a prospective study involving two major academic centers [87].

Effective April 1, 2014, the 29th edition of the AABB Standards outlined one facet of TRALI mitigation [33]. Standard 5.4.1.2 states that for the collection of plasma and whole blood for allogeneic donation, donations must be from males, never-pregnant females, or females with a history of pregnancy only if they have been tested for HLA antibodies since their last pregnancy and are found to be negative [33]. Many institutions have proactively extended this AABB Standard to the collection of platelets, switching their HLA-positive female donors to red cell collections or plasma collections bound for fractionation and some smaller donor centers, especially hospital-based, have applied the standard to all blood product collections.

Additional mitigation strategies include the use of licensed, pooled, solvent-detergent treated plasma products (Octaplas™ Pooled Plasma (Human) Solvent/Detergent Treated; OctaPharma, Lachen, Switzerland). Related to the use of these solvent-detergent plasma products in European hemovigilance systems have not yielded any TRALI cases after using these products for 10–20 years' time. This may be explained because the pooling of numerous units dilute out the concentration of antibody and by the presence of soluble HLA antigen which is able to bind free antibody. Octaplas may be used interchangeably for frozen plasma including in plasma exchange for patients with thrombotic thrombocytopenic purpura (TTP) but has its limitations, and besides being more expensive than frozen or thawed plasma products, it may not be used in patients with IgA deficiency or severe deficiency of Protein S.

Factor concentrates including Kcentra™ Prothrombin Complex Concentrate (Human) (CSL Behring, King of Prussia, PA) have also never been associated with TRALI, likely due to the same dilution theories.

Additional mitigation strategies include the avoidance of directed donations from blood-related donors, especially from mother to child as the mother has likely been sensitized to any paternally inherited antigens the child has inherited and therefore maternal transfusions have the potential to contain anti-HLA antibodies; this has been reported multiple times in the literature [88]. In addition, irradiation must be performed on donations from blood relatives, to mitigate TA-GVHD, but does not prevent possible TRALI from occurring.

In summary TRALI remains an important and underreported cause of transfusion-related morbidity and mortality. It is a clinical diagnosis for which there is no single, pathognomonic test and until the USA adopts a national, nonvoluntary reporting system or at least one entity able to receive data from multiple reporting systems, it is likely to continue to be underrecognized.

Transfusion-Associated Circulatory Overload (TACO)

TACO is an acute, hydrostatic pulmonary edema which occurs in the setting of transfusion. Especially vulnerable populations include the very young, older patients (over 70 years of age),

and those with compromised vascular systems or renal failure. TACO closely followed TRALI in the number of FDA-reported transfusion-related deaths, with 13 confirmed cases in fiscal year 2013, comprising 34 % of transfusion-related deaths, up from eight deaths the previous year [13]. TACO was reported by the 2011 NBCUS (which is the most recent as of 2014) to be voluntarily reported a total of 1512 times, amounting to 1 in 13,843 transfusions [3].

There are several distinguishing factors between TRALI and TACO (Table 19.5). Pulmonary wedge pressure is an invasive measurement to determine the back pressure from the heart, or filling ("wedge") pressure of the left atrium, by a catheter in the pulmonary artery, and unless the patient already has a Swan-Ganz catheter in place it is not likely to be performed. BNP is a 32 amino acid polypeptide secreted in response to stretched cardiac ventricles, to counteract the renin–angiotensin–aldosterone system. Baseline BNP is not likely to be measured unless the patient is already being monitored for heart failure and may not be useful unless the differences are marked. In general, two of the most helpful distinguishing features are that TACO responds quickly to diuresis, whereas this approach should be avoided in TRALI unless the patient is also fluid overloaded, and, though fever may or may not be seen with TRALI, it is never a feature of TACO. It may be helpful to sit the patient upright; oxygen should be given as needed. It is possible that TRALI and TACO may occur together.

Red Cell Storage

In adults, bone-marrow-derived hematopoietic stem cells may differentiate into myeloid or lymphoid lineages, and it is through the myeloid lineage with subsequent maturation through erythroid progenitors from which mature red cells arise. The kidneys are key regulators in red cell production and when the renal vasculature senses decreased oxygen delivery, renal cortical interstitial cells produce the hormone erythropoietin, which acts on cells in the bone marrow to stimulate red cell production. Oxygen-sensing bodies are also present in the lung and the carotid bodies (near the bifurcation point of the carotid arteries in the neck).

The primary function of red cells is to transport oxygen from the lung to the body's tissues. RBCs are biconcave disks approximately 5 μm at their widest diameter with a volume of approximately 90 fl [89]. The relatively thinner centers allow the cells great flexibility as they traverse the large capillary bed in the body, participating in gas exchange [89]. Prostaglandins and nitric oxide are two key regulators of the small vessels. Mature red cells lack a nucleus and mitochondria, contain only key organelles and rely on glycolysis for energy production [89]. Approximately 250 billion RBCs are in an average adult and the daily turnover is about 1 % of the circulating volume [89].

The life of a red cell is approximately 120 days and as the cell ages it undergoes changes in its shape, sheds lipids, proteins, and other particles, enduring metabolic and oxidative injury.

Table 19.5 Some distinguishing features between TRALI and TACO) [2, 36]

	TRALI	TACO
Pulmonary edema, bilateral	Yes	Yes
Fever	Possibly	No
Tachypnea, dyspnea	Yes	Yes
Leukopenia	Possibly	No
PWP (pulmonary wedge pressure)	Normal	Increased
BNP (B-type natriuretic peptide)	<200 pg/mL	Greatly elevated
BP	Usually hypotension	Usually hypertension
Increased vascular congestion/heart size	No	Yes
Diuresis	Hypoperfusion	Resolution of symptoms

The RBC storage lesion is a broad term describing these and other changes that occur to the red cell for the duration of its life outside of the body. The consequences of aging are reflected by what is accumulated in the storage plasma, preservative and additive solutions in which the RBCs are stored, and ultimately transfused into the patient.

When a blood donation is made RBCs of every age are collected and pH of the donor's blood likely ranges from 7.32 to 7.42 (venous blood). Blood banks primarily use a first in, first out (FIFO) model for RBC distribution to hospitals and transfusion services, though clinicians may request fresher blood for certain patient populations, such as transplant, neonatal, or extracorporeal membrane oxygenation (ECMO). This rotation model reduces waste and helps manage inventory.

During routine RBC storage, the shape of the smooth, biconcave disk (discocyte) is changed to an echinocyte, which retains the discoid shape but has small, rounded surface protrusions, then to a spheroechinocyte which resembles a round bumpy ball, and finally to a spheroctye [91, 92]. The longer the RBCs remain in storage, the more the shape reversibility diminishes [91, 92]. These less flexible shapes are unable to easily traverse the smallest vessels and shed lipids and other products and may occasionally occlude vessels [91, 92].

In the body heavily damaged cells are phagocytized or scavenged by the spleen, whereas in a storage bag, dead and dying cells and their by-products accumulate. The individual quality of each donation is donor-dependent. Glucose is metabolized (glycolysis) via the Embden–Meyerhof pathway yielding adenosine triphosphate (ATP) which is a high-energy compound needed for red cell function. By-products are lactate or pyruvate. The two important branch pathways from the primary glycolytic pathway are the 2,3-DPG shunt (Rapoport–Luebering shunt) which uses some of the ATP made in the primary pathway to produce 2,3-DPG and the pentose shunt (hexose monophosphate shunt) which is an important metabolic pathway to produce NADPH.

Hemoglobin is a tetramer of four subunits. Each subunit consists of a large protein chain with an oxygen-binding heme group. In the center of each heme group (or molecule) is an iron atom which binds oxygen in the "relaxed" or high-oxygen affinity state and releases it in the "tensed" or low-oxygen affinity state. The ratio of 2,3-DPG (DPG) to hemoglobin is approximately equal in the red cell. The presence of DPG is important as DPG binds to the subunit and favors the unloading of the oxygen into the tissues. As RBCs age, the ability to produce DPG falls and the oxygen-releasing capacity is reduced. Aucar and colleages concluded that aging red cells had the capacity to significantly and adversely affect the global coagulation status of trauma patients when the activated clotting time (ACT) (and controlled for all other factors) was compared at different RBC storage ages, but was especially prolonged between weeks 3 and 5 of RBC storage [93, 94].

With the application of mass spectroscopy to cells both the proteome and the metabolome of RBCs and stored RBC units have been studied [90, 95–98]. During routine storage RBC metabolomics demonstrates that vital functions begin to change at about 14 days D'Alessandro A, et al. Routine storage of red blood cell (RBC) units in additive solution-3: a comprehensive investigation of the RBC metabolome. Transfusion. 2015 Jun; 55(6):1155-68. PMID:25556331.). At this time intracellular proteins are released with loss of cellular integrity including peroxiredoxin-2 peroxiredoxin-6, glycolytic enzymes, e.g., α-enolase [99]. Some of these enzymes may have the ability to affect extracellular processes and may be masquerading mediators for they may be fully active [99]. Proteomic analysis has determined that AS-3 is the best additive solution because compared to AS-1, AS-5, and SGAM the intracellular proteins are retained longer [97]. Further work is required to determine better RBC storage as sell as the effects of released enzymes, proteins, and lipids on the transfused patient.

Transfusion and, in particular, stored RBCs have been shown to be independent predictors of post-injury multiple organ failure (MOF) in injured patients with moderate ISS ($15 < ISS < 30$) who received >6 units of RBCs in the first 12 h following injury [100, 101]. In further analysis of

these patients, the transfusion of stored RBCs (age ≥ 21 days) was an independent predictor of those patients who developed MOF [102]. Importantly, these studies were controlled for the amount of blood transfused; all patients with intermediate ISS received 6 units of PRBCs in the first 12 h and similar amounts of other blood products. In addition the mechanism of injury was not different. With new, stricter transfusion parameters using a hemoglobin of 7 g/dl as the trigger, only about 5 % of injured patients developed MOF [103]. There is still a significant amount of post-injury ALI and ARDS that appears to be transfusion related; however <5 % of these patients succumb to this complication.

Summary

There is currently a great emphasis on appropriate blood use in all clinical settings. Creation of blood utilization guidelines should be based on the clinical service's requirements and the usage tools, including transfusion triggers and algorithms, should be developed by each clinical service, supported by medical literature and rooted in patient needs, with involvement from the transfusion medicine pathologist and blood bank. Longer red cell storage times undoubtedly results in cellular alterations, diminished viability and accumulation of undesirable byproducts in the plasma in which cellular blood products are stored. This raises the question: despite the fact that blood may be stored for up to 42 days in additive solution, should it really be transfused up to its date of expiration?

Metabolomics is a burgeoning field and recent study in this area has helped to further distinguish the additive solutions and have promoted the development of new additive solutions such as SOLX, or, AS-7.

Previous retrospective studies, such as the 2008 Cleveland Clinic study led by Dr. Koch, showed that older blood resulted in increased postoperative complications and increased mortality. The concept that fresher red cells are better is supported by other clinical studies. Conclusions from prospective clinical studies including RECESS, ABCPICU, and other trials are anxiously awaited.

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Plasma Transfusion

20

Ryan A. Lawless and John B. Holcomb

Introduction

There have been many advances in the management of patients suffering from traumatic injuries. However, trauma remains the leading cause of death in the civilian population younger than 44 years of age [1]. Uncontrolled hemorrhage, including non-compressible hemorrhage, accounts for approximately 40 % of trauma-related deaths [2], in addition to 20–40 % of deaths following hospital admission [3]. As hemorrhage continues, patients develop hypovolemic shock in addition to tissue injuries that lead to the activation of multiple factors resulting in acute traumatic coagulopathy. Trauma-induced coagulopathy in and of itself has been associated with an increase in mortality in the trauma population [4–7]. Recently, Pati et al. described the endotheliopathy of injury and used plasma to reverse or prevent this systemic injury altogether [8]. In this chapter, we discuss the role of plasma in the resuscitation of the traumatically injured patient to combat acute traumatic coagulopathy, but more importantly to prevent it all together.

In 2012, the Department of Health and Human Services published data from a survey collected in 2011 describing the utilization of blood and blood products across the USA. 5,926,000 units of plasma were produced for transfusion in total. This included 2,802,000 units of plasma frozen within 24 h (FP24), 1,813,000 units of fresh frozen plasma (FFP), 560,000 units of liquid plasma (LQP), and 251,000 units from apheresis collection. The overwhelming majority of units were collected and produced by blood centers with less than 10 % derived from hospitals. Additionally, 8,195,000 units were produced for further manufacturing. In total, 3,882,000 units were transfused in 2011; a decrease of 13.4 % from 2008. The amount of FFP and FP24 transfused decreased over the 3-year period. However, the utilization of thawed plasma (TP) only became reportable in 2011. 1,181,000 units of TP were transfused, amounting to 30.4 % of all transfused plasma, and mostly being transfused at larger institutions. Finally, only 2000 units of LQP were transfused in 2011. The average cost of a unit of FFP and FP24 were $57.91 and $56.08 respectively [9].

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Plasma is the aqueous portion of blood that contains coagulation factors, fibrinolytic proteins, albumin, immunoglobulins, and up to 6000 individual proteins. It is derived from whole blood or apheresis collection. Once collected and produced it can be stored in the liquid state, frozen until needed for transfusion, or frozen then thawed and kept in the liquid state. The concentration of labile coagulation factors, namely factors V, VII, and VIII, are affected by the type of storage form plasma has undergone [10].

Types of Plasma

In order to achieve the more balanced transfusion goal set forth by multiple military and civilian studies an immediate and plentiful supply of plasma is necessary. This becomes difficult in centers where thawed plasma is unavailable and in austere environments where cold chain storage and transportation is a limiting factor. As a result, much research and development has gone into the production and distribution of plasma products suitable for human transfusion. In this section we discuss the different plasma products available for transfusion. The characteristics of the different plasma preparations available for transfusion are summarized in Table 20.1.

Fresh Frozen Plasma (FFP)

Fresh frozen plasma is prepared either by separating the red blood cells and platelets from whole blood with centrifugation or by apheresis. The plasma is then frozen at −18 °C and stored until needed for transfusion. To be called FFP the freezing process must occur within 8 h of donor phlebotomy. FFP contains high levels of all coagulation factors including the labile factors V and VIII. Whole blood yields approximately 200–250 mL of FFP, where apheresis collection yields 400–600 mL. The components are diluted approximately 8–20 % by the anticoagulant, a mixture of citrate, phosphate buffer, and dextrose [10–12]. Normal levels of factors V and VIII are found in FFP [10]. However, different blood groups yield different concentrations of coagulation factors confounding efforts to standardize therapy. The variations in expression can be as much as a 30 % difference between blood groups. This is exemplified by blood group O level of factor VIII and von Willebrand factor being 30 % lower than other blood groups [11, 12].

FFP must be thawed in a water bath between 30 and 37 °C prior to transfusion, taking anywhere from 20 to 40 min. Furthermore, breakage of bags occurs in the water bath in approximately 10 % of cases, further delaying delivery to the bedside.

Table 20.1 Characteristics of differing forms of plasma for transfusion

	Derivation	Yield	Storage	Preparation time
Fresh frozen plasma (FFP)	Centrifugation of whole blood or apheresis	Centrifugation—200–250 mL Apheresis—400–600 mL	Frozen at −18 °C within 8 h of phlebotomy	20–40 min water bath thaw
Frozen within 24 h (FP24)	Centrifugation of whole blood or apheresis	Centrifugation—200 to 250 mL Apheresis—400 to 600 mL	Frozen at -18 °C between 8 and 24 h of phlebotomy	20 to 40 min water bath thaw
Thawed plasma (TP)	FFP or FP24 thawed and not transfused within 24 h	Centrifugation—200–250 mL Apheresis—400–600 mL	1–6 °C for 4 days following thaw	Immediately available
Liquid plasma (LQP)	Centrifugation of whole blood	n/a	1–6 °C for up to 30 days	Immediately available
Dried plasma	Lyophilization or freeze drying	200 mL	Sterile container	10 min reconstitution

This can lead to a significant time delay to transfuse a dying patient. Once thawed, FFP should be transfused within 24 h or relabeled as thawed plasma [10, 12].

American Association of Blood Banks (AABB) indications for FFP transfusion include [10, 12, 13]:

1. Preoperative or bleeding patients with multiple coagulation factor deficiencies (e.g., liver disease, DIC).
2. Massive transfusion with clinically significant coagulopathy.
3. Reversal of warfarin effects in patients who are bleeding or undergoing a procedure without enough time for vitamin K reversal.
4. Transient reversal of warfarin in patients who are bleeding or undergoing an emergent procedure.
5. Thrombotic thrombocytopenic purpura transfusion or plasma exchange.
6. Management of rare specific plasma protein deficiencies when recombinant products are not available.

The AABB also recommends against the utilization of FFP when a coagulopathy can be corrected by a more specific therapy, such as vitamin K, prothrombin complex, or specific coagulation factors. FFP is not the optimal therapy when complete reversal of warfarin is desired and should not be used in the absence of severe bleeding [13].

Plasma Frozen Within 24 h (FP24)

Plasma collected via phlebotomy or apheresis and frozen between 8 and 24 h following collection becomes labelled as plasma frozen within 24 h (FP24). When derived from whole blood, a unit of FP24 yields a volume of 200–250 mL. However, an apheresis unit of FP24 yields 400–600 mL. FP24 contains high levels of stable coagulation factors and slightly diminished levels of the labile factors V, VIII, as well as Protein C due to the delayed in freezing from donor collection time [10]. FP24 undergoes the same thawing process as FFP and also must be transfused within 24 h of returning to the liquid state [14]. If not transfused by that time, it can then be relabeled as thawed plasma [10].

The indications and contraindications for FP24 utilization are identical to FFP (see section above). FP24 should not be used when the sole replacement of factors V and/or VIII is necessary [12].

Thawed Plasma (TP)

Thawed plasma (TP) is the liquid form of FFP or FP24 following a thaw at 30–37 °C. It is then stored at 1–6 °C to be used for up to 4 days following initial thaw. The levels of stable factors remain close to those of FFP and FP24 even at the 4-day point. However, there is a decline in the levels of labile factors, the most significant of which being factor VIII [10, 12].

AABB indications for transfusion of TP include [10, 12, 13]:

1. Preoperative or bleeding patients with multiple coagulation factor deficiencies (liver disease, DIC).
2. Massive transfusion with clinically significant coagulopathy.
3. Reversal of warfarin effects in patients who are bleeding or undergoing a procedure without enough time for vitamin K reversal.
4. Transient reversal of warfarin in patient who are bleeding or undergoing an emergent procedure.
5. Thrombotic thrombocytopenic purpura transfusion or plasma exchange.

Thawed plasma is not recommended for the management of specific coagulation factor deficiencies as the concentration of the factors in the product is variable [10].

Liquid Plasma (LQP)

Liquid plasma (LQP) is produced from whole blood no later than 5 days after expiration (21 days) and is never frozen [10–12, 14]. LQP is

kept at 1–6 °C and can be stored for up to 30 days. The primary indication for transfusion of LQP is the management of the acutely hemorrhaging patient requiring massive transfusion. Studies have shown that the initial and prolonged hemostatic profile of LQP, as determined by thromboelastography and calibrated thrombograms, is better than FFP or thawed plasma. Furthermore, the levels of coagulation factors remained ≥88 % of original levels out to 26 days, except for factors V and VIII [14, 15]. As discussed, the vitamin K dependent factors (factors II, VII, IX, and X) are relatively stable under approved LQP storage conditions. Therefore, LQP is currently indicated in patients on warfarin therapy who are suffering massive hemorrhage. The labile factors (V and VIII) deplete over time during storage, making LQP less effective in patients with these specific deficiencies [10–12].

The coagulation properties of LQP are superior to TP from FFP. Matijevic et al. analyzed the hemostatic potential of LQP as compared to TP derived from FFP. LQP had a superior capacity to form clot and generate thrombin. This phenomenon may be explained either by the presence of platelet microparticles in LQP not present in FFP, FP24, or TP, cold activation of coagulation proteins, the decline in protein S activity, or a combination thereof [14]. It is known that the freeze–thaw process required for FFP and FP24 degrades proteins; however, it is not understood why certain factors are less tolerant then others. By avoiding the freeze–thaw process the proteins remain functional longer to the point that 15-day-old LQP appears to function as well as 5-day-old TP [14].

Dried Plasma

Currently, the goal of transfusion in the patient requiring massive transfusion is a balanced resuscitation with a ratio of platelets–plasma–red blood cells (RBC) units of 1:1:1 [16–18]. Achieving this goal is often difficult in environments where a large and rapidly accessible supply of plasma is not available. As described previously, FFP and FP24 require frozen storage and re-warming under very controlled conditions. Furthermore, TP and LQP require storage under refrigerated (4 °C) conditions. This makes the rapid (within minutes of requirement) administration of plasma difficult at most civilian institutions and nearly impossible for military personnel. These factors, along with data indicating improved survival with balanced resuscitation, led to the recent redevelopment of dried human plasma. Lyophilization, a low-pressure, low-temperature, low-humidity process to convert plasma into powder, is not a new concept as it was first developed and introduced into practice during World War II. Lyophilized plasma (LP) was widely used, and became the primary resuscitative fluid on the battlefield by the end of WWII. Nevertheless, the concept was abandoned following unacceptable rates of viral transmission in the survivors, until modern screening methods improved transmission rates significantly [2]. LP possesses the same viscoelastic coagulation parameters as fresh plasma. In an animal model, Shuja et al. found an insignificant decrease in the activity of factors II, VII, and IX [19]. The French military has utilized dried plasma and has maintained a hemovigilance program since 1994. No adverse effects of infectious transmissions have been reported with the transfusion of more than 1100 units [20]. In 2011, Martinaud et al. described the utilization of freeze-dried plasma (FDP) in French intensive care units in Afghanistan, while caring for casualties from all the coalition forces [21]. The process of preparing the FDP involves the centrifugation of plasma. The supernatant is then removed and frozen by slow rotation in an ice slurry. The product is then freeze-dried at −8 °C for 48 h removing much of the CO_2, resulting in an alkaline product upon rehydration [19]. FDP was described as easy to use, reconstituting in medical water within 10 min to provide 210 mL of fluid with hemostatic and volume expansive properties. 236 units have been delivered and evaluated without a single adverse event reported [21]. Israeli Defense Forces (IDF) also utilizes FDP (from the German Red Cross) as the preferred resuscitation fluid in the military prehospital setting. Using these dried products facilitates

an environment of balanced transfusion from the point of initial resuscitation without playing "catch-up" while FFP thaws [22].

To date, dried plasma products are not approved for use in the civilian population within the USA [23, 24]. FDP from the French military has recently been approved by the Department of Defense, FDA and White House and is utilized by selected units of the US Special Forces. In addition to the French military and IDF, dried plasma products are utilized by the German and Norwegian militaries, Norwegian civilian emergency aeromedical services, and the US Army Special Operations Forces [22]. Further investigation for utilization in US civilian emergencies and ABO group universality are necessary as these products will allow plasma resuscitation to occur in environments not conducive to current plasma storage [23–25].

Current Practices in Civilian Trauma Centers

Spinella et al. recently performed a study on transfusion practices at Level I and II trauma centers using data from The American College of Surgeons (ACS) Trauma Quality Improvement Program (TQIP). These investigators report that he types of plasma used at these centers are as follows: 78 % thawed fresh frozen plasma/plasma frozen within 24 h, 16 % thawed fresh frozen plasma/plasma frozen within 24 h or liquid plasma, and 7 % (6 of 90) liquid plasma.

Why Do We Transfuse Plasma?

Patients in hemorrhagic shock suffer from a multitude of physiologic derangements. The early administration of blood products has been shown beneficial throughout the literature. However, the reasoning behind plasma transfusion is not as simple as previously thought. Trauma patients lose blood; therefore, giving blood back makes sense. However, reaching for packed RBCs first does not accomplish the goal which the trauma community is trying to achieve: restoration of circulating volume. The transfusion of plasma will restore circulating blood volume while delivering coagulation factors to bleeding patients actively consuming coagulation factors. Early administration of plasma, in the prehospital setting, as well as in the emergency center, has been shown to improve acid-base status upon admission, decrease the total transfusion requirement in the first 24 h following admission, and reducing mortality risk [25–27].

A decade ago, military and civilian physicians transfused plasma in order to correct coagulopathy. With increasing clinical experience on earlier and balanced use of plasma, the prevention of trauma-induced coagulopathy and endothelial dysfunction became the intent for transfusion based upon laboratory-based measures of coagulopathy. With increasing research and understanding as to the endothelial injury associated with massive trauma, treating the endotheliopathy of trauma has also become a goal for plasma transfusion [28]. This evolution of thought has been derived from a greater understanding of how hemorrhagic shock systemically affects physiology. Restoration of circulating coagulation factors is not the sole purpose for transfusion of plasma in patients with hemorrhagic shock.

The endotheliopathy of trauma (injury) refers to the breakdown of the endothelial glycocalyx on the endoluminal surface of blood vessels increasing permeability and decreasing their integrity. It has been hypothesized that injury to the endothelial glycocalyx leads to interstitial edema, inflammation, and tissue hypoxia [29, 30]. For further description of the endotheliopathy of trauma please refer to Chap. 7. At the Texas Trauma Institute we have anecdotally observed a decrease in edema, as well as a paucity of abdominal compartment syndrome secondary to resuscitation with the initiation of blood product based resuscitation. Animal data suggests a decrease in inflammation with transfusion of plasma when compared to infusion of albumin, artificial colloid, and crystalloid in a hemorrhage shock model [31–35].

Projecting from the endoluminal surface of blood vessels is a complex network of soluble components creating an endothelial glycocalyx.

Multiple proteoglycans and glycoprotein comprise this network providing surfaces for interactions with glycosaminoglycans, neutrophil-endothelial cell interactions that occur with injury to the glycocalyx, hemostasis, coagulation, and fibrinolysis. The glycocalyx allows the plasma component of blood to interact with the vessel wall while maintaining a barrier to erythrocytes and leukocytes [31–33]. Kozar et al. demonstrated that the endothelial glycocalyx is injured during hemorrhagic shock, manifested by shedding into plasma of syndecan-1, one of the endothelial glycocalyx's proteoglycans [36]. This had been previously demonstrated in models of ischemia and reperfusion. Furthermore, these authors demonstrated that the injured glycocalyx is partially repaired with the transfusion of plasma in comparison to the infusion of crystalloid solution [36–38]. In their study, significantly less volume was needed in the plasma group to maintain mean arterial pressure. The intact glycocalyx was ablated by hemorrhagic shock and signs of early restoration of the glycocalyx were present in the plasma group in comparison to the crystalloid group. Shown in Fig. 20.1 are electron microscopy images of mesenteric venules showing the effects of shock and resuscitation on the endothelial glycocalyx along with its thickness. Furthermore, the authors investigated lung injury with respect to hemorrhagic shock based on alveolar thickness, capillary congestion, and cellularity. They found an increase in all three parameters in the shock model compared to sham. However, the infusion of crystalloid increased all three parameters above the model, while the plasma transfusion group had improvement of all three parameters, suggesting attenuation of lung injury [36]. These findings were also demonstrated by Pati et al.; however, they also demonstrated that the age of

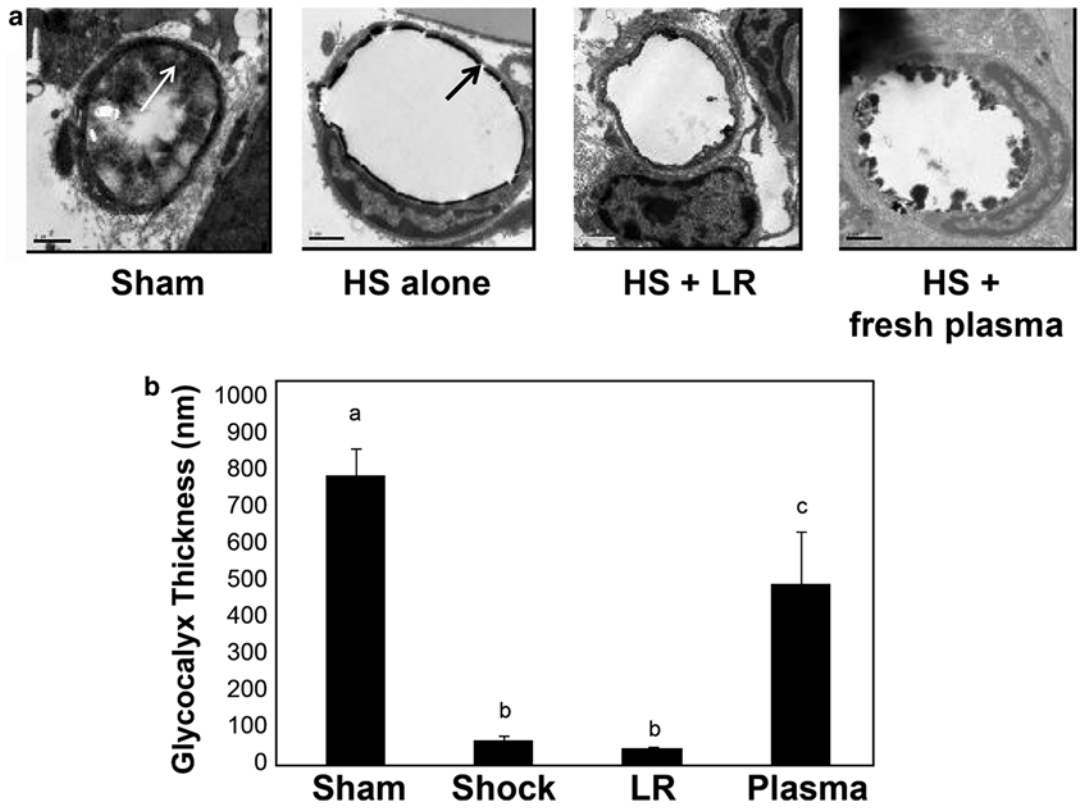

Fig. 20.1 Electron microscopy of mesenteric venules stained to reveal endothelial glycocalyx following hemorrhagic insult and resuscitation

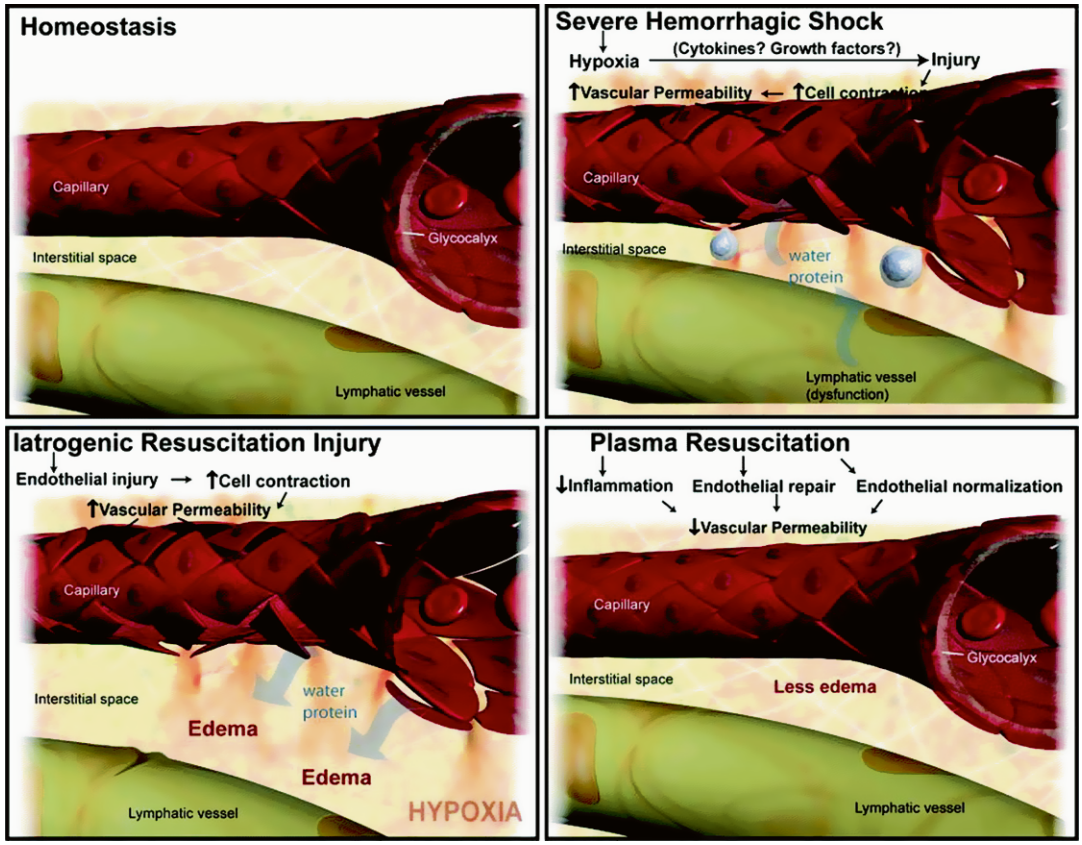

Fig. 20.2 Working biological model of the mechanism of action of fresh frozen plasma (FFP)

the plasma transfused also had an effect on this attenuation of lung injury (older plasma being less beneficial) [8]. Nevertheless, day 5 plasma stored at 4 °C remained superior to crystalloid infusion with respect to reparative capacity in the animal model [8]. This indicates that a component of plasma, in soluble form, interacts with the endothelial membrane to restore the endothelial glycocalyx. Figure 20.2 shows the vascular injury caused by hemorrhagic shock through hypoxia leading to cell contraction and decreased vascular permeability. Resuscitation with plasma decreases the inflammatory response, promotes endothelial repair, and leads to normalization of the endothelium. This leads to a decrease in vascular permeability; clinically seen as less edema.

In 2011, Haywood-Watson et al. found that patients arriving to a level 1 trauma center in hemorrhagic shock had an elevated level of the endothelial proteoglycan syndecan-1 [39]. The shedding of syndecan-1 was negatively correlated with pro-inflammatory cytokines INF-gamma, fractalkine, and IL-1beta, and positively correlated with IL-10, an anti-inflammatory cytokine [39]. This correlation suggests that syndecan-1 is not just a marker of endotheliopathy; it may be involved in the restoration of glycocalyx integrity as depicted in Fig. 20.3 [39]. Furthermore, Johansson et al. found an elevated admission syndecan-1 level in severely injured trauma patients to be associated with inflammation, coagulopathy, and increased mortality [40].

Protocols

The institution of exsanguination protocols at major trauma centers has been shown to improve survival [41, 42]. The original investigation did not specifically involve a balanced ratio resuscitation.

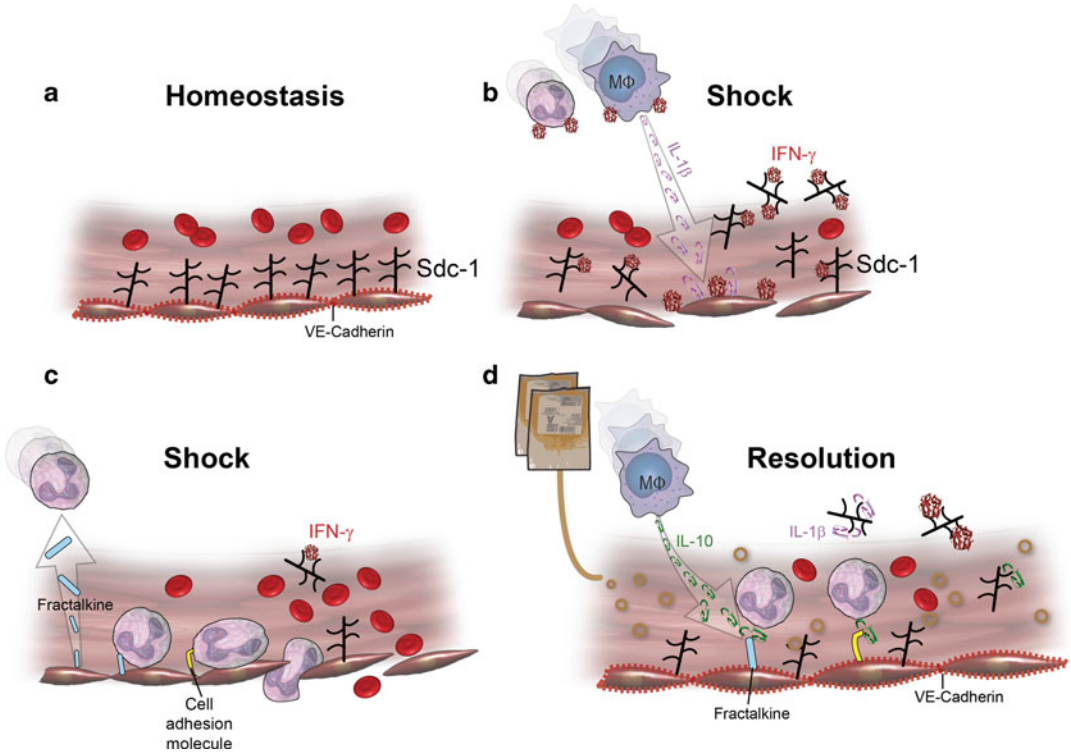

Fig. 20.3 Proposed model of syndecan-1 interaction with inflammatory cytokines after shock and resuscitation with fresh frozen plasma (FFP)

Simply by initiating a protocol for the delivery of blood products to the emergency center for an exsanguinating patient, mortality improved. The activation of a "massive trauma protocol" delivered blood component therapy to the emergency center without the request of specific components by the trauma team. This was also demonstrated in the military environment with improved survival in both Iraq and Afghanistan [43, 44]. Furthermore, a decrease in the total amount of blood products transfused was seen [41, 42, 45, 46].

The majority of trauma centers store uncrossmatched RBCs in the emergency center. However, plasma often remains in the blood bank awaiting the activation of the massive transfusion protocol or a direct order from a physician. The lack of readily available plasma makes it difficult to achieve a high plasma–RBC ratio in an expedited fashion, which has been shown in multiple studies to decrease mortality [47, 48]. By implementing an emergency center thawed plasma protocol, with 4 units of thawed AB plasma stored in the emergency center, the time to first plasma transfusion was improved by 46 min resulting in a decrease in 24-h transfusion of PRBCs, plasma, and platelets and a significant decrease in mortality [27]. Furthermore, a decrease in the rate of the activation of the massive transfusion protocol was seen following the implementation of the thawed plasma in the emergency center [27], as well as with attaining increased plasma–RBC ratios [49].

Recent Studies Involving Plasma

PROMMTT

The Prospective Observational Multicenter Major Trauma Transfusion (PROMMTT) study demonstrated that earlier transfusion of plasma, within minutes of identification of hemorrhagic shock, and achieving high early plasma–RBC ratios decreased 24-h and 30-day mortality.

This was evidenced by the three- to fourfold increased mortality risk associated with plasma–RBC ratios <1:2 [17, 28, 50]. Furthermore, gradual achievement of balanced transfusion ratios may not be as beneficial as early plasma transfusion [50]. Initiating plasma transfusion early also led to a decrease in the total amount of RBCs transfused during the initial 24 h following admission [50]. PROMMTT clearly showed that earlier plasma was associated with improved survival; however, few patients received consistent ratios. There was significant concern that higher ratios would lead to increased hypoxia, acute respiratory distress syndrome (ARDS) and transfusion related acute lung injury (TRALI). However, Robinson et al. showed that hypoxia was associated with excessive crystalloid infusion rather than plasma [51]. Lastly, it was clear from the PROMMTT data that the two ratios that clinicians were trying to transfuse were 1:1 and 1:2.

PROPPR

The PROMMTT study was followed by the Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) study which evaluated the effectiveness and safety of two transfusion strategies in patients with major trauma and severe bleeding. The PROPPR study was the first multicenter randomized trial comparing transfusion strategies with mortality as the primary endpoint. It provided evidence for the commonly held belief that balanced transfusion protocols were beneficial to severely injured patients with hemorrhagic shock. Although no significant differences in 24-h and 30-day mortality were found between the two transfusion ratios investigated (plasma–platelet–RBC ratio of 1:1:1 versus 1:1:2), the early availability of blood products transfused within minutes of arrival in a balanced (1:1:1) ratio achieved hemostasis more often and decreased hemorrhage-related deaths within the first 24 h following admission [18]. Furthermore, while the balanced transfusion group received significantly more plasma and platelets within the first 24 h, no difference was found in the rate of 23 prespecified complications including the systemic inflammatory response syndrome (SIRS), ARDS, transfusion associated circulatory overload (TACO), TRALI, and allergic reactions. Consistent with the biology of bleeding patients, the median time to hemorrhagic death in PROPPR was 2.3 h. In a post hoc analysis, at 3 h after admission there was a significant mortality difference between the two groups [18].

Texas Trauma Institute: Resuscitation

The concept used at the Texas Trauma Institute at Memorial Hermann Hospital in Houston has been derived from the experiences gained on the battlefield, and supported by military and civilian studies. In the prehospital setting the emphasis centers on the cessation of bleeding. This occurs in parallel with hypotensive resuscitation utilizing liquid plasma and packed red blood cells. LQP and packed RBCs are utilized by our hospital-based helicopter program, Life Flight. Patients are identified as requiring a prehospital transfusion based upon the assessment of blood consumption (ABC) score. Over a 20-month study period, 942 units (244 RBCs and 698 plasma units) were placed on the Life Flight helicopters with a 1.9 % waste rate [26]. Once a severely injured patient reaches the trauma center, a rapid thromboelastograph (r-TEG), venous blood gas, and hemoglobin levels are drawn and immediately analyzed. In patients that have an ABC score <2 (1 point each for penetrating mechanism, systolic blood pressure <90 mmHg, heart rate >120 beats/min, and a positive focused abdominal assessment with sonography for trauma) or without substantial bleeding (<3 units/h), the r-TEG is used to guide blood product resuscitation [52]. Patients that are in shock, are hypotensive, or have an ABC score ≥ 2 are started on the massive transfusion protocol and receive 1:1:1 ratio-driven resuscitation [53]. The patient then either proceeds to the operating room or interventional radiology. The response to resuscitation is closely monitored. Once clinical bleeding has slowed considerably or ceased the resuscitation is converted to goal-driven resuscitation utilizing r-TEG [53].

Adverse Effects/Events

Of all transfusable blood products, plasma, specifically FFP, is considered the most hazardous according to multiple studies into the mid-2000s. However, the overall risk remains low. The major risks include TRALI, TACO, infectious disease transmissions, acute transfusion reactions, and leukocyte-associated reactions. However, most of these studies only evaluated some of the blood products that are infused, frequently not evaluating the effects of RBCs, platelets or crystalloids, all which have well described deleterious effects.

TRALI

Transfusion related acute lung injury manifests as hypoxia, pulmonary edema, pulmonary infiltrates with radiographic changes, fevers, and possibly hypotension within 6 h of plasma transfusion. The presentation is similar to ARDS; however, >80 % of patients typically recover within a few days of symptom onset and treatment is mainly supportive [54–56].

The UK hemovigilance Serious Hazards of Transfusion (SHOT) reports a TRALI risk of 1 in 63,940 transfused units, a 28.8 % decrease since 2008 [9, 57]. It is the most common cause of death from transfusion and is the most frequent serious complication of FFP transfusion [58]. In 2013, the FDA reported that TRALI represented 37 % of all fatalities secondary to blood transfusions for that year and 38 % from 2009 to 2013. Plasma, specifically FP24 was implicated in two of the 17 cases and a plasma product may have been implicated in four other cases that received multiple transfusions. Interestingly, no units of FFP were implicated in 2013. The number of cases of TRALI has significantly decreased over the study period following voluntary measures taken by the transfusion community [59]. The absolute numbers of TRALI are very low, a testament to the rigorous testing performed by the blood banking community.

TRALI is significantly associated with leukocyte alloantibodies found in the donor plasma. These specific antibodies are found exclusively in post-partum female plasma and in male plasma from donors who have previous received a transfusion. Some authors believe TRALI to develop in two steps. A predisposing condition must be present that incites the release of cytokines leading to the attachment of neutrophils to the pulmonary capillary endothelium. The second step occurs with neutrophil priming, activation and pulmonary injury [13, 60]. In 2008, Eder et al. reported a reduction in the incidence of TRALI with the conversion to male-predominant plasma for transfusion [13, 61]. Unpublished data from the Texas Trauma Institute demonstrate that with the increase usage of plasma in resuscitating trauma patients the incidence of TRALI did not increase. There were nine incidences of TRALI related to plasma transfusion in over 180,000 units transfused. The incidence was approximately 1:20,000 units [62].

Further discussion amongst hemovigilance programs regarding standardization of the definition of TRALI versus TACO will improve the way health care workers identify and manage transfusion reactions [63, 64].

TACO

Transfusion associated circulatory overload occurs secondary to increased hydrostatic pressure resulting in pulmonary edema. This process can be indistinguishable from TRALI and the two entities may in fact co-exist [64]. In 2013, the FDA reported that TACO represented 34 % of transfusion related mortalities for that year. There has been an uptrend in mortality related to TACO over time. It is difficult to determine whether the uptrend is secondary to improved diagnostics or actual increase in incidence [59].

The incidence of TACO is not well described in the literature. Multiple retrospective reviews have reported an incidence of TACO from <1 % to 11 % [55, 64]. This range with multiple reports is secondary to the difficulty in determining the etiology of pulmonary edema as diagnostic evaluations for TACO are invasive and serologic tests for TRALI have yet to be validated. In Fig. 20.4, Gajic et al. designed an algorithm to determine the etiology of

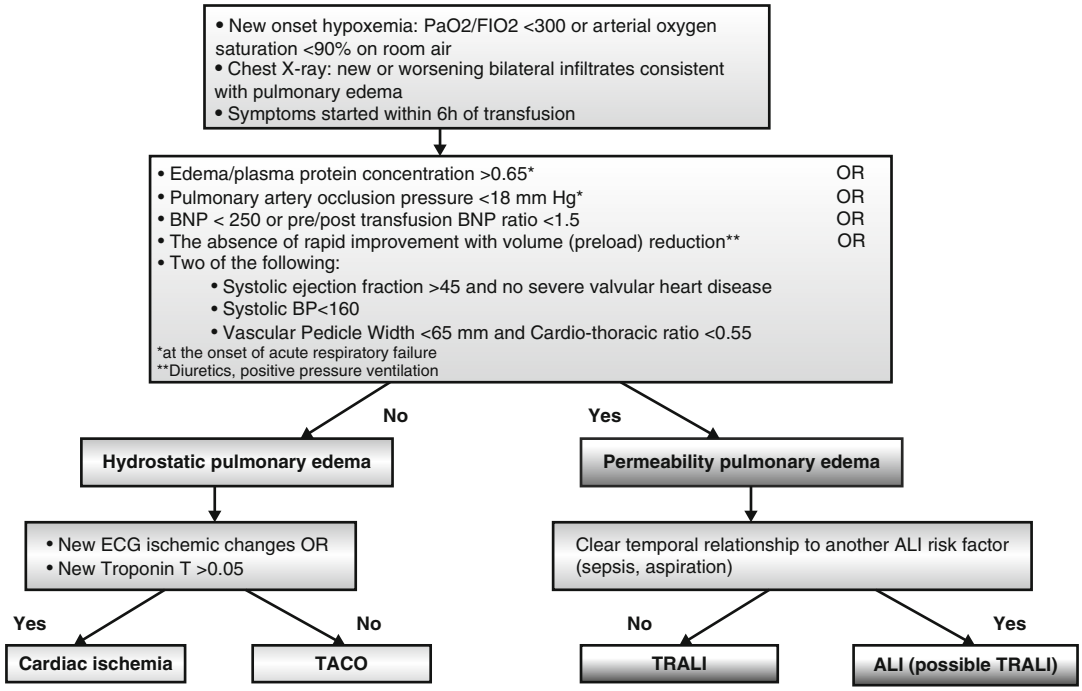

Fig. 20.4 Approach to post-transfusion pulmonary edema

post-transfusion acute pulmonary edema [64]. The algorithm is based on the European-American ARDS Consensus Conference definitions of TRALI and integrates specific laboratory values into the decision-making process. Frequently, TACO is a post-hoc diagnosis made once a patient responds to a specific therapy [55].

Infectious Disease Transmission

The transmission of infectious diseases has dramatically decreased with extensive donor screening and infectious disease testing. Nucleic acid testing sensitivity has improved leading to a decrease in transmission risk. This has led to an estimated risk of 1:1,467,000 for acquiring HIV, 1:1,149,000 for hepatitis C virus, and 1:280,000 donations for hepatitis B virus [58, 65, 66].

Outside the USA, many blood centers utilize donor retested plasma, pathogen inactivated plasma, and pathogen reduced plasma [67]. Donor retested plasma are units that are quarantined until the donor submits a subsequent donation which tests negative for infectious disease [67]. Pathogen inactivated and pathogen reduced plasma are prepared via the addition of chemicals (solvent/detergent, methylene blue, amotosalen, riboflavin, and UV light) to prevent the transmission of lipid-enveloped viruses [68, 69]. Two companies leading this technology are Terumo Medical Corp. (Somerset, NJ) and Cerus Europe B.V. (Amersfoort, NL). The Cerus process was FDA approved in early 2015.

The freezing process during preparation of plasma inactivates bacteria. Furthermore, bacterial contamination with the production of endotoxin prior to freezing is unlikely [58]. The 2013 FDA report describes a single bacterial infection transmitted through plasma transfusion [59]. The process of removing cellular components via filters also removes cell-associated bacteria, most protozoa, and cell-associated viruses including malaria, CMV, and HTLV. Freezing does not remove the free viruses including Hepatitis A, B, and C, HIV 1 and 2, and parvovirus B19 [13, 58].

Currently, no screening protocol exists for the detection of prion diseases. Furthermore, modern techniques of decreasing infectious transmission

are ineffective against prion diseases. In the UK, three possible cases of variant Creutzfeldt–Jakob disease (vCJD) have been reported. Each case involved transfusion of non-leukocyte reduced red blood cells [70]. There have been no reported cases of prion disease transmission following plasma transfusion; however, animal studies have shown that it is possible [71].

Acute Transfusion Reactions

In 2013, the 17th Annual SHOT Report described just over 3000 acute transfusion reactions in over 13,000 transfusions. Three hundred and twenty incidents occurred in 2012 including allergic, hypotensive, and severe febrile reactions. Thirty-one cases were associated with transfusion of FFP, which included 13 anaphylactic/severe allergic reactions, 12 moderate allergic reactions, four hypotensive reactions, one febrile reaction, and one mixed febrile/allergic reaction. No deaths were related to any type of transfusion reaction [57]. Moderate allergic reactions present with an urticarial rash, wheezing, and other symptoms not severe enough to be termed anaphylactic and occur in 1–3 % of transfusions. Anaphylaxis includes bronchospasm, angioedema, severe hypotension, and cardiovascular collapse and is a rare occurrence [13, 55, 57, 58, 68].

Other than human immunoglobulin A (IgA) and haptoglobin, the proteins involved in acute transfusion reactions are unknown and generally unpredictable. For patients known to have an IgA sensitivity, plasma obtained from patient with IgA deficiency is available for transfusion [13, 72].

Leukocyte-Associated Reactions

Leukocyte-associated reactions following plasma transfusion are rare. The freeze–thaw process yields a small percentage of viable leukocytes prior to transfusion [73, 74]. The presence of viable leukocytes leads to the rare occurrence of febrile non-hemolytic transfusion reactions (FNHTR), transfusion-associated graft versus host disease (TA-GVHD), and transmission of leukocyte viruses [68].

Following the freeze–thaw process nonviable leukocytes release mediators that may contribute to FNHTRs. These reactions are generally clinically insignificant and resolve quickly with supportive therapy [57, 68].

TA-GVHD requires viable leukocytes to be transfused and then engraft and proliferate in the host patient. This is a rare transfusion reaction amongst all blood components transfused and has yet to be reported with FFP transfusion. Therefore, irradiation of FFP is not currently recommended [68].

Future Endeavors in Plasma Resuscitation and Research

The utilization of plasma as the primary resuscitation fluid has been the subject of multiple retrospective studies, a large prospective observational study and a recent prospective randomized trial. The benefits of balanced transfusion with respect to hemorrhagic mortality in the trauma population have been reported. Current randomized studies are evaluating the utility of plasma in the prehospital area. Future investigations will likely include the utilization of dried plasma products in the US civilian population with the longer storage life and substantial logistic benefits.

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Cryoprecipitate Transfusion

21

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Product Details

Cryoprecipitate is prepared by thawing 1 unit of plasma at 1–6 °C, centrifuging to separate the cryoprecipitate from the cryosupernatant, removal of the supernatant, and then re-freezing the residual cryoprecipitate (see Fig. 21.1). The volume is approximately 10–15 mL per unit and has a shelf-life while frozen of 12 months. The starting plasma can be from either a whole blood donation or an apheresis collection. Each unit must have at least 150 mg of fibrinogen (although median levels are closer to 400–550 mg per bag) and 80 IU factor VIII content (although we never use cryoprecipitate exclusively for factor VIII replacement). There is considerable range per unit, ranging from approximately 200 to 1500 mg per bag due to donor variability, with variability reduced due to pooling from usually ten donors. The cryoprecipitate is either distributed to hospitals pre-pooled (usually in pools of 5–6 units) for immediate use after thawing, or as individual units, requiring reconstitution with saline and then pooling. The preparation time for the former product is 20–30 min for thawing and labeling. The latter product preparation time is much longer at approximately 30–45 min. The final volume of an adult dose of 10–12 units is approximately 200–250 mL. The product also contains factor VIII, von Willebrand factor, factor XIII, fibronectin, and platelet microparticles (see Table 21.1). There is also fibrinogen in plasma, platelet, and whole blood units, and therefore, patients will receive additional fibrinogen supplementation throughout resuscitation with these components as well. One unit of 250 mL of plasma contains approximately 400–900 mg of fibrinogen or 2.5–3.0 g per 1000 mL. Cryoprecipitate has been discontinued in some countries due to concerns regarding viral transmission and replaced with virally inactivated fibrinogen concentrates, which will be discussed in Chapter 24. Fibrinogen concentrates (RiaSTAP™) are lyophilized, human derived, virally inactivated concentrates. Each vial contains is 1 or 2 g of fibrinogen and the product is reconstituted immediately before use. In this chapter, given the variable dosing strategies used in the literature, the number of units transfused is reported, rather than the number of pools.

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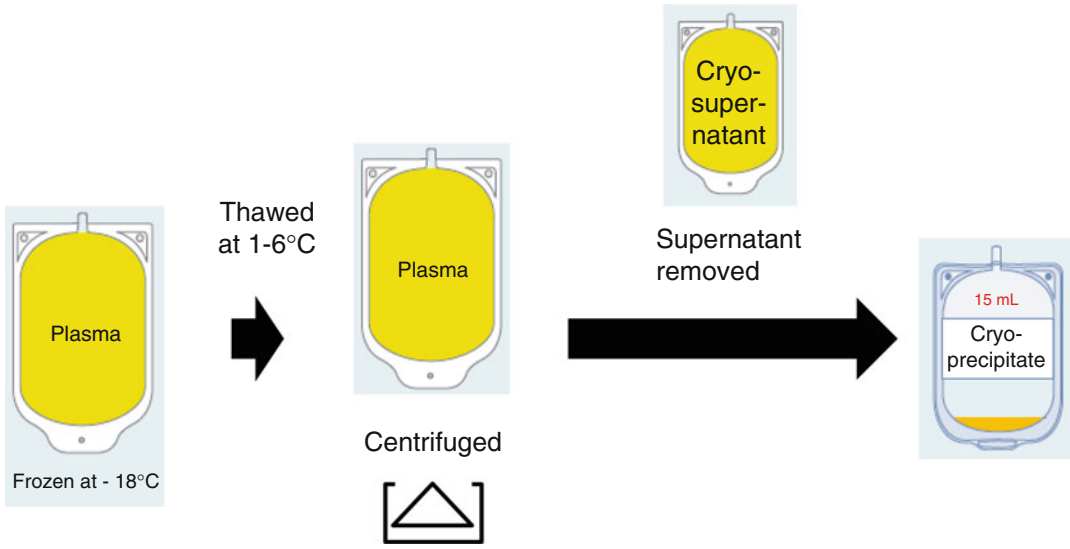

Fig. 21.1 Illustration of the production process for cryoprecipitate

Proportion of Cryoprecipitate Issued to Hospitals Used in Trauma

Several published audits on the use and appropriateness of use of cryoprecipitate have been published after national audits. Canada audited the use at 25 hospitals including 603 orders for 4370 units of cryoprecipitate [1]. Overall, 12.3 % were used for trauma resuscitation with a rise in fibrinogen from a median of 1.3–1.9 g/L. Similarly, an audit in 39 hospitals in the UK (423 episodes of cryoprecipitate infusion) found 12 % of adults and 6 % of pediatric orders were for trauma care. The median dose in adults was 10 units and the rise in fibrinogen observed in adults was 0.8 g/L and in children 0.9 g/L [2]. Lastly, an audit in Australia of 460 episodes of cryoprecipitate infusions at 23 hospitals found 9 % of all cryoprecipitate used was for trauma resuscitation [3]. A report from 2011, again from Australia, noted a rising rate of cryoprecipitate use at a single trauma center study [4]. The rate increased from 0.03 units per patient in 1998 to 1.3 units per patient in 2008 for patients undergoing a massive transfusion (10 units in 24 h), of which 26 % were trauma related hemorrhages. The reason for the rise in cryoprecipitate use was not determined by the authors, although they speculated that thromboelastography (TEG) guided hemostatic management may have been a contributing factor.

Clinical Use of Cryoprecipitate Transfusion in Trauma

The best study detailing the use of cryoprecipitate in trauma comes from the PROMMTT Study investigators [5]. The PRospective Observational Multicenter Major Trauma (PROMMTT) study group prospectively collected data on 1245 traumas patients in ten US level I trauma centers. To be enrolled in this trial, the patients had to survive at least 30 min after injury, get at least 1 red blood cell (RBC) unit and at least 3 units of hemostatic blood components within 24 h. Great variability in cryoprecipitate utilization was observed; one center used it for 7 % of patients and another center used for 82 % of patients. In general, the most common transfusion practice was to reserve this product for the very sickest of patients and was given late in resuscitation. The authors hypothesized that early and increased use of cryoprecipitate would save lives but unfortunately, they could not detect any signal to support their primary study objective. Overall, 359 patients (29 %) were administered cryopre-

Table 21.1 Content and roles of each of the components of cryoprecipitate

Substance	Specifics
Fibrinogen	This glycoprotein is synthesized by the hepatocytes and has a long half-life in the plasma of approximately 4 days. In contrast to factor VIII, fibrinogen activity remains stable with recovery of fibrinogen at 87 % after 24 h of liquid storage, when compared to original content at time of thawing
Factor VIII and von Willebrand factor (vWF)	Factor VIII and vWF represent approximately 5 % of the total protein in cryoprecipitate. vWF performs two major functions in primary hemostasis: it mediates the adhesion of platelets to exposed subendothelium and it stabilizes coagulation factor VIII in the plasma
Factor XIII	Factor XIII promotes clot stability by forming covalent bonds between fibrin monomers to enhance the mechanical strength of the fibrin clot and protect the clot from proteolytic degradation. Cryoprecipitate contains approximately 20–30 % of the original factor XIII of plasma
Fibronectin	Fibronectin is a dimeric α2-glycoprotein present at a concentration of 300 μg/mL in human plasma. It is thought to have opsonic activity assisting with the phagocytosis of particulate debris by the reticuloendothelial system. Cryoprecipitate is a rich potential source of fibronectin at 1500 μg/mL
Platelet microparticles	The process of preparing cryoprecipitate concentrates the platelet membrane microparticle content. The platelet membrane microparticle concentration of cryoprecipitate is 29-fold greater than the cryosupernatant plasma and 265-fold greater than the original plasma. Each clinical dose of cryoprecipitate (10 units) contains approximately 4×10^9 platelets in microparticle form. Microparticles can play active roles in thrombosis, inflammation, and vascular reactivity

cipitate in the first 24 h. The patients who were transfused cryoprecipitate were more severely injured and received more of every blood component type. Unfortunately, only the admission fibrinogen was recorded and it was below 1.5 g/L in only 30 % of cryoprecipitate transfused patients (non-transfused patients 17 % under 1.5 g/L) and pre-transfusion fibrinogen levels were not recorded. Predictors of cryoprecipitate use were admission fibrinogen under 1.0 g/L, lower hemoglobin level, and pelvic bleeding. The median number of units transfused was 10 units at a median time from arrival of 2.8 h (interquartile range 1.7–4.5 h). The median number of RBC units transfused before the first dose of cryoprecipitate was 8 (with a very wide interquartile range of 4–16 units). Only 1 % of patients were transfused cryoprecipitate before any plasma or platelets. It is unclear whether the long time to cryoprecipitate and the fact that no cryoprecipitate was given to many patients with hypofibrinogenemia was due to early death before infusion, an intentional clinician decision due to non-survivable injuries, or a long time delay in product preparation. In their analysis, cryoprecipitate use did not appear to increase, or decrease, survival; although perhaps used as a "last-ditch" as described at these trauma centers is not the best strategy. Unfortunately, they did not report mortality rates by site to give us insight into the differences in outcomes if you give 82 % of patients this component, as compared to only 7 %. What this study tells us is that there is highly variable practice across even major trauma centers and that we need prospective, randomized trials to allow us to make rational decisions when deciding to administer cryoprecipitate.

Impact of Cryoprecipitate on Outcomes in Trauma

No large, prospective randomized trials have been done comparing fibrinogen replacement with placebo in trauma patients to determine the impact of more aggressive fibrinogen replace-

ment on clinical outcomes. Five retrospective reports have attempted to determine the impact of more aggressive fibrinogen replacement on outcomes in pediatric and adult trauma and these studies will be described below. The first report was published in 2008 by Stinger et al. [6]. The authors analyzed data from the US Joint Trauma Registry which included patients from two combat hospitals in Iraq between 2004 and 2005. Only patients who received 10 or more RBC units or whole blood in the first 24 h were included ($n=252$ of 5586 injured patients). They calculated the fibrinogen to RBC ratio, considering fibrinogen content from all blood components, including cryoprecipitate; unfortunately, they used 2.5 g per 10 units of cryoprecipitate, when probably the true content was double this estimate. They divided patients into quartiles of fibrinogen replacement; patients who received less than 0.2 g per RBC had an increased risk of death. Patients who received even higher ratios of fibrinogen to RBC units did not have better survival (i.e., no dosage effect observed). The mortality rate was 52 % in the lowest quartile compared to 24 % in the highest quartile ($p<0.001$). This report was retrospective therefore the conclusions that can be drawn from this study are limited; with survival bias being the primary limitation of the report. Both plasma and cryoprecipitate require substantial time for preparation and therefore some patients may have died before having the opportunity to receive these frozen components.

The second report from Emory University [7], reviewed trauma patients who had received 10 or more RBC units in the first 24 h. They assessed patients in two time periods (pre- and post-massive hemorrhage protocol implementation). In the pre-MHP time period, cryoprecipitate was administered at the discretion of the clinical team. In the post-MHP period, their hemorrhage protocol provided plasma in a 1:1 ratio with RBC units, with cryoprecipitate (20 units) provided in pack 3 with RBC unit numbers 13–18 then 10 units of cryoprecipitate with every other pack of 6 units of RBC units. Interestingly, cryoprecipitate use overall was the same in both cohorts (pre 11 units vs. post 12 units). They found no difference in mortality at 24 h and 30 days when they compared the two time periods. They then pooled both time periods of patients and looked at the association between high cryoprecipitate ratio (≥1 unit per RBC unit), compared to a lower ratio, and mortality. A high ratio was associated with better 24 h (84 vs. 57 %) and 30 day (66 vs. 41 %) mortality, compared to a lower ratio. Obviously, we have to be concerned about survivorship bias as in the first report.

The third report by Rourke et al. [8] reviewed the fibrinogen levels of all trauma patients between 2008 and 2010 ($n=517$). The admission fibrinogen was greater than 1.5 in 78 %, less than 1.5 g/L in 14 %, less than 1.0 g/L in 5 %, and less than 0.5 g/L in 3 %. A low admission fibrinogen was associated with a higher Injury Severity Score (ISS) and a greater degree of shock. Cryoprecipitate was administered at a median of 103 min (interquartile range 78–134 min) from arrival in the 39 (7.5 %) patients who were administered cryoprecipitate. Fibrinogen levels were higher in survivors compared to non-survivors; but there was no difference in mortality for those given cryoprecipitate versus no cryoprecipitate at 24 h (22 % vs. 18 %) or 28 days (30 % vs. 34 %). In multivariate analysis, fibrinogen at baseline was an independent predictor of mortality at both 24 h and 28 days; the odds of death was reduced by 0.22 during the first 28 days for every 1 g/L rise in baseline fibrinogen. In an analysis of the fibrinogen to RBC unit ratio, the odds of death during the first 28 days (conditional on survival to 12 h) was decreased by a factor of 0.91 for every 1 g increase in ratio over the first 12 h, with the fibrinogen dose calculated for all components transfused (not just the cryoprecipitate).

In the sole pediatric study, Hendrickson et al. from Emory University [9] reviewed 102 trauma patients between 2006 and 2010 who were transfused with at least one blood component in first 24 h. Overall, 52 % of patients had an abnormal fibrinogen level; 21 % greater than 1.0 g/L, 20 % less than 1.0 g/L, and 11 % undetectable (48 % had normal levels). In total, 25 % of patients received cryoprecipitate. In multivariate analysis, fibrinogen level was not statistically significant

in predicting outcomes; only initial prothrombin time (PT), partial thromboplastin time (PTT), and platelet count remained significant in their model. The analysis was limited by the small sample size.

Lastly, the MATTERs II Study (Military Application of Tranexamic acid in Trauma Emergency Resuscitation) [10] reported data from the US and UK Joint Trauma registries for patients injured from 2006 to 2011 in Afghanistan. They included patients who had received at least 1 RBC unit in this retrospective analysis. The use of cryoprecipitate (and tranexamic acid (TXA)) was left to the judgment of the individual physicians. The dose administered was 10 units at a time. Overall, 1332 patients received at least 1 RBC unit, of whom 168 (12.6 %) received cryoprecipitate. Due to its retrospective design, numerous imbalances were observed between their four groups (no TXA or cryoprecipitate, only TXA, only cryoprecipitate, or both); the most severely injured group (ISS) was the cryoprecipitate only group and the cryoprecipitate/TXA group required four times the amount of RBC, plasma and platelet units. The median dose administered was 2.1 and 2.3 units per patient for cryoprecipitate only vs. cryoprecipitate/TXA (1 unit in this report was a 5 unit pool). Surprisingly, mortality was lowest in the cryoprecipitate/TXA group (11.6 %), compared to no cryoprecipitate/TXA (23.6 %). The administration of cryoprecipitate was associated with OR for death of 0.61 (95 % CI 0.42–0.89); with the cryoprecipitate/TXA combination being associated with a substantial reduction in the risk of death (OR 0.34, 95 % CI 0.20–0.58).

In summary, hypofibrinogenemia is common in the retrospective reports detailed above and is associated with worse survival. The administration of a high ratio of fibrinogen to each RBC unit is associated with improved outcomes; however, we need to be cautious that this may represent an effect of survivorship bias. The strongest evidence to support the use of cryoprecipitate, albeit non-randomized data, comes from the MATTERs II Study. Before adoption of early and aggressive use of fibrinogen replacement, we need to confirm these results through randomized controlled trials.

Guidelines

Despite the lack of evidence from randomized controlled trials, guidelines have recommended replacement during hemorrhage if the fibrinogen level falls below 1.0 g/L. This recommendation is based on expert opinion and a single non-randomized report of 36 patients [11]. This report detailed the bleeding outcomes of 36 patients transfused modified whole blood (RBCs in cryosupernatant) who had their fibrinogen levels measured for every 12 RBC units given. Four patients dropped their fibrinogen below 0.5 g/L and all four were classified as having "non-surgical" or coagulopathic bleeding. In addition, of the ten patients who had fibrinogen levels drop between 0.5 and 1.0 g/L, two had coagulopathic bleeding. Based on this very small report, numerous guidelines recommended maintaining the fibrinogen level in excess of 1.0 g/L. The British Committee for Standards in Haematology recommended fibrinogen replacement if the level drops below 1.0 g/L in their 2004 [12] and 2006 [13] guidelines. Similarly, the Association of Anaesthetists of Great Britain and Ireland recommended in 2010 [14] to replace if the fibrinogen fell below 1.0 g/L. Lastly, the US guidelines from American Society of Anesthesiologists (ASA) for perioperative blood transfusion 2006 [15] recommend a replacement threshold of 0.8–1.0 g/L.

More recently, the European Trauma Guidelines from 2013 [16] recommended initial and frequent measurements of fibrinogen levels and to replace if the fibrinogen level fell below 1.5–2.0 g/L if significant bleeding at a dose of 15–20 units of cryoprecipitate for a 70 kg patient (or 50 mg/kg of fibrinogen concentrate). The rationale listed in the text of this guideline cited data from postpartum hemorrhage [17] and two of the retrospective trauma studies listed above showing an association between higher ratios of fibrinogen to RBC units [6, 7]. It remains unclear what an appropriate replacement threshold should be in a bleeding trauma patient. There is also preliminary (again retrospective data) evidence that excessive blood loss dramatically increases after cardiac surgery if the fibrinogen level drops below 2.0 g/L [18].

Dosage and Infusion

ABO-compatibility is thought to be not necessary for cryoprecipitate due to its small volume. The cryoprecipitate is either distributed to hospitals pre-pooled (usually in pools of 5–6 units) for immediate use after thawing, or as individual units, requiring reconstitution with saline and then pooling. The preparation time for the former product is 20–30 min for thawing and labeling. The latter product preparation time is much longer at approximately 30–45 min. The final volume of an adult dose of 10–12 units is approximately 200–250 mL. Some hospital transfusion services do not pool the product but rather issue individual units for bedside reconstitution and immediate infusion. Once thawed, cryoprecipitate must be kept at room temperature and if not used, discarded at 4 h. The product is infused through a 170–260 μm filter, similar to all other blood components. The dose of cryoprecipitate is often listed at 1 unit per 5–10 kg of body weight, although no dosing studies have been performed to support this dosing recommendation. At our institution (Sunnybrook Health Science Centre) we evaluated the use of cryoprecipitate in 1004 patients [19] of whom 31 % were trauma patients. A median dose 8.7 units resulting in a fibrinogen rise of 0.55 g/L (0.83–1.38 g/L) for patients who received only cryoprecipitate between the two measurements ($n = 83$ events) or 0.07 g/L rise per unit of cryoprecipitate.

Adverse Reactions

In terms of the risk of viral transmission per unit transfused, each unit of cryoprecipitate has a similar risk to each RBC unit transfused, although there is a much larger donor exposure per pool (usually ten donors). The concern regarding the risk of viral and other infectious agents was one of the drivers prompting many European countries to switch from cryoprecipitate to fibrinogen concentrates. The adverse events reported from cryoprecipitate include very rare reports of acute hemolysis from anti-A/B, minor febrile and allergic reactions, respiratory distress, and thrombosis. Readers interested in reviewing these rare risks are directed to a review of cryoprecipitate [20]. Two retrospective studies, both in the setting of liver transplantation, have raised concerns about an association between cholestasis and exposure to cryoprecipitate in the peri-transplant period [21, 22].

Ongoing Trials

Numerous trials are being performed to try to improve our understanding of the use of fibrinogen factor replacement in the severely injured patient. We are performing a randomized pilot trial of 6 g of fibrinogen versus placebo in hypotensive, bleeding, trauma patients where RBC units have been either ordered for or administered to the patient within 30 min of arrival to hospital (NCT02203968). Fibrinogen concentrate (RiaSTAP™) is a human derived factor concentrate manufactured from plasma and is supplied as a lyophilized powder that is reconstituted in the blood bank or at the bedside. The goal of the study is to determine feasibility of performing a blinded trial with this design. We chose fibrinogen concentrate as our fibrinogen source due to our ability to blind with a saline infusion and its speed of preparation. Similarly, the Oxford transfusion medicine group (personal communication, Dr. Simon Stanworth) is performing a pilot randomized trial of early cryoprecipitate compared to no therapy in severely injured trauma patients to determine the feasibility of using cryoprecipitate up front in the setting of a large scale randomized trial. In Austria, a multicenter double-blind, placebo controlled trial (NCT01475344) is underway to assess the efficacy of pre-hospital administration of fibrinogen concentrate in trauma patients presumed to be bleeding. Lastly, although not a trauma study, a multicenter study from Copenhagen will randomize women with postpartum hemorrhage to 2 g of fibrinogen versus placebo (NCT01359878) to determine the impact on RBC transfusion

rates. It is unclear why such a small dose was chosen for this trial when a 10 pool of cryoprecipitate contains approximately 5 g of fibrinogen and this large dose only yields an increment of 0.55 g/L in the fibrinogen level.

Summary

Fibrinogen is a key part of the hemostatic system. Despite the availability of cryoprecipitate since 1964, its role in the management of the bleeding trauma patient remains unclear. Hypofibrinogenemia is common in adult (less than <1.5 g/L in 14 %, <1.0 g/L in 5 %, and <0.5 g/L in 3 %) and pediatric trauma patients (52 % <2.0 g/L, 20 % <1.0 g/L, and 11 % undetectable). A preserved fibrinogen level on arrival to hospital is associated with substantially better outcomes (odds of death reduced by 0.22 during the first 28 days for every 1 g/L rise in baseline fibrinogen). Worldwide, approximately 10 % of cryoprecipitate distributed to hospitals is transfused to trauma patients. Adult trauma patients in the USA on average receive a dose of approximately 10 units of cryoprecipitate at about 3 h after arrival to hospital (at about the eighth unit of red cells), yielding a rise in fibrinogen level of 0.5–0.9 g/L post-transfusion. Several retrospective studies in adult and pediatric, civilian and military, trauma settings suggest that more aggressive use of fibrinogen replacement may improve outcomes, although the data quality is poor due to the retrospective design in all trials. It is unclear what should be the appropriate dose, threshold for administration, or hemostatic target level. Studies from the settings of cardiac surgery and postpartum hemorrhage suggest that the target of 1.0 g/L in some guidelines may be inadequate for some patients and that a higher target may be necessary (>1.5–2.0 g/L). While we await the completion of several randomized trials, it is reasonable to administer cryoprecipitate if the fibrinogen is under 2 g/L and the patient has serious ongoing hemorrhage where immediate surgical control is not possible. A dose of 10 units or 5 g (or 50 mg/kg of fibrinogen in pediatrics) is a reasonable starting dose for adult trauma patients.

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Platelet Transfusion

22

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Objective of chapter:
To review the biology of platelets as a blood product, to provide the clinician with a practical guide for their optimal use, and to serve as a knowledge platform for those interested in platelet transfusion research.

The Evolving Role of Platelets in Trauma Care

Platelet (PLT) biology is deceptively complicated. From a morphological aspect, the elegant discoid shape of the resting platelet belies the intricate cytoskeleton and convoluted tubular systems that enable spectacular shape change upon activation. The evolution of platelet products and their use in transfusion is similarly convoluted and requires an immersion in history to understand. As described below, the current state of the art in platelet transfusion rests on surprisingly little robust clinical data, minimal efforts to tailor product characteristics to patient needs, and a regulatory framework that supports the inadequate status quo. Development of new platelet products and optimal transfusion strategies for bleeding patients represent opportunities for major advances in surgical care.

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The Origins and Evolution of Clinical Blood Banking and Platelet Component Use

The first description of platelets is generally credited to Alfred Donné, a nineteenth century French public health physician and microscopist, who published his drawings in 1842 [1, 2]. Prat published a primitive methodology for platelet counting in 1905, which was much improved upon by Wright in 1910–1911 [3, 4]. A more accurate manual counting method was not developed until the 1950s [5]. Duke, working with Wright at the Massachusetts General Hospital in 1910, published a seminal paper that described the spontaneous bleeding tendency resulting from thrombocytopenia and a method for testing platelet function that would become known as the Duke Bleeding Time (BT) [6]. In that paper, Duke showed that transfusion of fresh whole blood (WB) could raise a patient's platelet count and reduce both the BT and clinical bleeding, as evidenced by resolution of epistaxis,

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melena and other mucocutaneous bleeding. Based on the experience from the 30 cases presented in this paper and from experimental work on an unspecified number of dogs and rabbits, Duke concluded that a bleeding diathesis from thrombocytopenia was only likely when platelet counts dropped below 50,000/μL. Although his conclusions have not been thoroughly tested in the many clinical settings in which platelets are used, this transfusion threshold continues to inform guidelines to this day. Platelets were administered through whole blood transfusion until the development of platelet concentrates in the second half of the twentieth century, a practice that the military continues to use for the treatment of severe traumatic hemorrhage when platelet concentrates are unavailable [7].

As the light faded on the last days of World War II (WWII), a new era in medicine began. Building on the experience of pioneers of the field in WWI, US and British physicians in WWII conclusively established the vital importance of blood transfusion as a life-sustaining therapy for traumatic hemorrhage, providing not only oxygen delivery, but also hemostatic potential. Red cells, platelets, and plasma were delivered as fresh whole blood from a "walking blood bank," stored whole blood or freeze-dried plasma in WWII [8, 9]. Edwin Cohn pioneered the concept of blood fractionation in his work on plasma protein separation and the introduction of albumin as a resuscitative product in the 1940s [10, 11]. The therapeutic effects of donated blood would then be multiplied across the spectrum of human disease by the advances that led to modern blood banking and the adoption of component therapy, beginning in earnest in the 1950s with the early developments in blood apheresis. This revolution promised to meet the demands of "right product, right patient, right time" [12, 13]. Researchers shared a vision of an era in which whole blood would no longer be required to meet the needs of patients with a critical deficiency in a single blood cell line.

The major impetus for the development of blood banking in general, and platelet component transfusion in particular, was the harnessing of cytotoxic chemotherapy and radiation in the treatment of cancer. These advances were preceded by observations in the aftermath of the 1943 US Navy ship bombings in Bari, Italy, that sailors exposed to alkylating mustard agents exhibited myelosuppressive effects, including thrombocytopenia. By the early 1950s, civilian and military research had converged to build the foundations of clinical oncology, and patients were experiencing the infectious and hemorrhagic complications of increasingly effective cancer care [14].

From its beginning, platelet transfusion was focused on treating thrombocytopenia, mostly in the context of bone marrow suppression caused by chemotherapy. Early studies in this area identified the difficulties in isolating and storing platelets without activating them, in addressing the immunogenicity of platelets, and the potential for alloimmunization and platelet refractoriness in chronically transfused patients [7]. Early key improvements included the transition from open to closed and glass to plastic storage systems that decreased risks of bacterial contamination and platelet activation during storage, respectively [15]. Through all of this work, the sine qua non of success in platelet transfusion was defined by the ability to raise platelet counts and maintain these gains for as long as possible, thus decreasing the risk of alloimmunization and platelet refractoriness in frequently transfused chemotherapy patients [16]. Indeed, "platelet viability" was defined in these terms and platelet blood banking evolved along lines that optimized post-transfusion recovery and duration of platelet circulation, or survival over several days, in vivo [17–19]. An early observation was that refrigeration of platelets or whole blood caused a drop in platelet count in the stored product and more rapid clearance of the stored platelets from circulation over 2 days compared to fresh platelets, and as a result, the obvious benefits of refrigeration such as reduced metabolic stress and risk of bacterial growth were discounted [17, 20]. Room temperature (RT) storage was adopted after studies showed increased in vivo recovery and survival ("viability") compared with refrigerated storage (4 °C) [20]. Generations of physicians have thus been misled by the confusion caused by this use of the term "viability" and the underlying assumption that because refrigerated platelets do not circulate as long as fresh platelets, they are therefore dead or nonfunctional [21, 22]. "Viability" when defined in

terms of recovery and survival of stored platelets does not correlate with hemostatic efficacy or measures of metabolic function and there have been very few studies that have rigorously studied platelet dose-response [23–25]. In fact, a more clinically relevant understanding of platelet function, encompassing hemostatic, trophic, and immune functions, might place greater weight on measures of platelet function that correspond to desired transfusion outcomes such as acute hemostasis [26].

An example of how the focus on "viability," or in vivo survival time, has obscured clinically relevant metrics of platelet product function can be found in the Korean War experience. Wounded soldiers in this conflict, as in WWII, were transfused cold-stored whole blood, which was widely assumed to contain nonviable platelets. Detailed studies of hemostasis in Korean War casualties; however, describe normal postoperative platelet counts despite massive transfusion (>20 pints) with cold-stored whole blood, and only rare coagulopathic bleeding [27]. Such results belie the notion that cold-stored platelets are truly nonviable. These platelets were clearly able to support acute hemostasis during surgery and in the immediate postoperative period despite relatively accelerated clearance. The few cases of coagulopathic bleeding can be understood if one takes into consideration that at this time, as in WWII, blood was transfused cold, at its storage temperature (4 °C) to simplify administration and avoid the perceived risk of hemolysis during rewarming [28]. Massive transfusion patients of this era thus faced a major risk of hypothermia with resulting coagulopathic bleeding and indeed researchers at the time did not attribute occasional bleeding tendencies to a defect in stored platelets [27]. It was not until the mid-1960s that warming blood from refrigerator temperature to body temperature was identified as a critically important step in transfusion practice [29]. From WWII to the Vietnam conflict, blood transfusions were comprised of mostly stored, but also fresh, whole blood. During this time, the Korean War experience seemed to confirm Duke's assertion that a platelet count of 50,000 was the critical level below which disorders of hemostasis would result, because the whole blood contained effective platelets able to participate in acute hemostasis, and platelet counts rarely fell below that threshold [27, 30]. During the Vietnam experience, the transfusion of 8- to 20 day-old acid citrate dextrose (ACD) preserved whole blood was thought to cause a dilutional thrombocytopenia due to nonviable platelets in the blood with ensuing coagulopathy after 20 units and a platelet count of 50,000. In reality, a close examination of the data reveals that post-transfusion platelet counts exceeded predicted values by a significant margin, and platelet counts dropped to critical levels only after transfusion of about 30 units with simultaneous large-volume crystalloid resuscitation [31]. It is not surprising that coagulation disorders encountered under such circumstances were corrected with warm fresh whole blood, but it does explain why the surgical community was lulled into thinking that platelets mattered only when they were severely depleted—the condition was rarely encountered in the age of whole blood use [32]. As a result, the importance of platelets in the treatment of surgical bleeding and trauma went unrecognized and research in this field was stifled until brought to the fore by the difficulty in supplying platelet components during the wars in Iraq and Afghanistan in the twenty-first century.

Clinical Evaluation of In Vivo Platelets and Platelet Components

The evolution of assays to assess the clinical efficacy of platelet transfusion is a metaphor for this inability to appreciate its vital role in reestablishing hemostatic function in trauma patients with hemorrhagic shock. Between 1910 and the late 1980s, simple platelet counts and Duke's BT method were the only assays routinely used. The latter, which was modified from an earlobe incision to one on the underside of the forearm while inflating a sphygmomanometer to 40 mmHg (the Ivy BT), was frequently unreliable as a platelet function test, as results were dependent on skin structure, blood vessel structure and integrity, hematocrit, temperature, fibrinogen, Factor VIII (FVIII) and von Willebrand Factor (vWF) levels,

as well as platelet count, size, and function [33–35]. Over the last 30 years, the BT has been abandoned due to its imprecision, poor reproducibility, and discomfort for the patient. Platelet aggregometry, which measures platelet aggregation response to physiologic agonists like thrombin, collagen and adenosine diphosphate (ADP), was developed in the 1960s and eventually became a "gold standard" but was never widely adopted in clinical practice, particularly in surgical settings, because the relative technical complexity of performing and interpreting the assay was never addressed by manufacturers due to the lack of emphasis on platelet function [33, 36]. Similarly, flow cytometry can be used to characterize platelet function, but this method, like aggregometry, has mostly been applied in research settings [37]. Unlike in the field of trauma, cardiologists and cardiovascular surgeons were much more aware of platelet effects in acute coronary syndromes, thus modifications of aggregometry, viscoelastic testing (thromboelastography—TEG; rotational thromboelastometry—ROTEM) and newer point-of-care tests have been developed to support management of platelet inhibition therapy [38]. These technologies predict bleeding risk and platelet transfusion in surgery, but, in keeping with the general disregard of platelet efficacy, so far are not required by regulatory guidelines in the evaluation of platelet products for treatment of hemorrhage [39, 40]. In short, reliance on BT and failure to adopt other standards suggests that, until recently, measurement of platelet function after transfusion has not been a priority for trauma surgeons and critical care physicians, platelet transfusion researchers or regulatory agencies. In summary, the focus of post-transfusion evaluation has been on raising platelet counts in chemotherapy patients, with the underlying assumption that platelets that circulate must be functional [41].

The relative lack of emphasis on platelet transfusion for treating surgical hemorrhage and trauma should be surprising, given the Vietnam War-era evidence demonstrating that platelet dysfunction plays a role in trauma-induced coagulopathy, but awareness of the data's import was not evident in the writing of the time [42]. As noted, cardiothoracic surgery has since become the exception, but even there, interest is narrowly focused on correcting bypass circuit-induced thrombocytopenia. Prior to the twenty-first century, the relatively few studies that examined platelets as part of trauma care and massive transfusion did record the development of thrombocytopenia and evidence of platelet dysfunction [43–45]. Unfortunately, these observations were dismissed as irrelevant due to the infrequency of clinically apparent "medical bleeding"—oozing, petechiae, purpura, as commonly observed in severe hypoproliferative thrombocytopenia or consumptive conditions like disseminated intravascular coagulation (DIC) [43, 44]. In contrast, Counts et al. noted that non-platelet-containing products such as red blood cell (RBC) concentrates resulted in hemodilution during massive transfusion, and provided one of the few studies to suggest a role for platelets in preventing dangerous thrombocytopenia [46]. Nevertheless, even Counts and colleagues narrowly advocated platelet transfusion for treatment of an established dilutional thrombocytopenia and platelet dysfunction. Their critical role in primary hemostasis, known for much of the twentieth century, of plugging holes in the vasculature, catalyzing the generation of thrombin to form the clot-stabilizing fibrin mesh, and then retracting the clot in concert with blood vessel vasoconstriction was little considered. Investigators did not appear to see the need to support these functions by proactively replacing the platelets lost to severely traumatic or surgical hemorrhage. The link between thrombocytopenia and microvascular bleeding in oncology patients was much discussed, while the larger role platelets play in overall hemostasis and the response to injury, whether surgical or traumatic, was largely ignored.

Beginning in the late 1960s, platelet-containing stored and fresh whole blood products gradually disappeared from most hospitals as component therapy became widely available and the economics of blood banking favored fractionation of whole blood [46]. The growing threat of transfusion-transmitted disease virtually eliminated walking blood banks by the early 1980s. Surgeons in this era were using modified whole

blood (generally meaning that platelets and cryoprecipitate had been removed), RBC concentrates and saline, with limited amounts of plasma and platelets [31]. When massive hemorrhage from penetrating injuries became more common during the drug wars of the 1980s and early 1990s, interest in trauma-induced coagulopathy resurfaced, and with it an appreciation that surgical bleeding outcomes were adversely affected because, with whole blood largely unavailable, RBC-focused resuscitation created iatrogenic deficits in plasma and platelets [47–49].

Transfusion Products for Trauma Care

It is important to recognize that the current state of platelet transfusion science is inadequate to guide optimal resuscitation therapy, even if data driven guidelines covering timing and quantity were available. The regulatory framework currently in place throughout the world evolved with development of platelet products optimized for recovery and survival, and does not adequately address the hemostatic, trophic, or immune functions of platelets. While the US Food and Drug Administration (FDA) issued a draft "guidance to industry" document on platelet function testing in 1999, it does not appear to have been formally adopted or enforced and thus current standards assume functional integrity based on morphological appearance and responses [41]. Perhaps the most important innovation in platelet transfusion was the adoption of apheresis over preparation of platelet concentrates from whole blood donations (pooled platelets) [50]. Use of apheresis units reduces donor exposure; simplifies leukoreduction and thus prevents complications such as nonhemolytic febrile transfusion reactions; and allows human leucocyte antigen (HLA) type- and cytomegalovirus (CMV) seropositivity-matching. While these are genuine advantages in the setting of hematopoietic stem cell transplantation, use of apheresis platelets confers no advantage with respect to hemostatic function. The practice of RT storage (22–25 °C) of platelets thus persisted despite profound functional deficits and the increased risk of bacterial growth compared to refrigerated or frozen blood products [26]. As noted previously, the recognition that refrigeration leads to rapid clearance of stored (over 2 days) compared to fresh (over 7 days) platelets is well established. While circulation time is critical for some; however, trauma surgery patients typically have normal megakaryocyte function, and thus require functional platelets to support hemostasis during active bleeding, after which they are able to replace depleted stores. Refrigerated platelets may be the ideal product for this population, because they have enhanced ability to participate in coagulation and thus may control bleeding more quickly [51]. In both platelet concentrate and whole blood studies, cold platelets perform better with regard to platelet aggregation (collagen, ADP), viscoelastic testing (TEG and ROTEM), and other functional measures compared to those stored at RT [21, 22, 26, 51–54]. The RT platelet storage lesion results in increased levels of soluble CD40 ligand (sCD40L), a potent immune stimulatory molecule, which reflects platelet degranulation is thus considered a marker of platelet functional loss. Stored RT platelets accumulate more sCD40L over time compared to those stored at 4 °C [21, 55], providing indirect evidence that cold-stored platelets may enhance hemostasis due to improved function. Life-threatening bleeding is typically controlled within 6 h of onset, during which time refrigerated platelets (4 °C-PLT) circulate at high concentrations that are comparable to those stored at room temperature (RT-PLT). On the other hand, RT-PLT rapidly accumulate functional defects during storage, losing their ability to aggregate in response to arachidonic acid, collagen, and ADP [21, 56–59]. Furthermore, Dr. Becker and colleagues and, in a separate study, Dr. Valeri showed that 4 °C-PLT control bleeding more effectively in aspirin-treated volunteers compared to RT platelets [51, 60, 61]. Becker further demonstrated that 4 °C-PLT are effective in treating bleeding thrombocytopenic patients. Conversely, RT platelets may be ineffective in reversing the increased risk of surgical bleeding in patients taking aspirin and/or clopidogrel unless large doses (2 apheresis units) are given

preoperatively [62–64]. Unfortunately, 4 °C platelets were never compared to RT-PLT for the treatment of surgical or traumatic hemorrhage, and indeed no randomized controlled trials of platelet- versus non platelet-containing transfusion strategies in trauma or massive surgical bleeding are published. Platelet refrigeration was abandoned by a transfusion medicine community focused on the needs of oncology patients who were thought to benefit from prophylactic transfusions of platelets with longer circulation times. Other approaches to platelet preservation, motivated mainly by the need to expand available inventories were attempted. For example, cryopreserved platelets (CPP) frozen in the cryoprotectant, dimethyl sulfoxide (DMSO), were developed by Valeri as early as the 1970s, and Crowe pioneered the use of trehalose in platelet lyophilization [65, 66]. Since neither of these approaches yielded products that improved platelet counts in a sustained manner, their development stalled. Valeri conducted a small randomized controlled trial (RCT) comparing RT platelets to CPP in cardiothoracic surgery bypass-induced thrombocytopenia and showed that CPP were more efficacious than RT platelets and likely safe [67]. The available data thus indicate that 4 °C and CPP are superior to RT platelets in treating bleeding patients in some settings; nevertheless, comprehensive RCT evaluations of different platelet containing products, namely fresh whole blood (FWB), stored whole blood (SWB), RT-PLT, 4 °C-PLT, and CPP, are lacking. Recently, investigators in Bergen, Norway have begun a trial of 4 °C platelets stored for up to 7 days in platelet additive solution (PAS) versus standard of care platelets in plasma.

The result is that, despite over 6 decades of research on platelet transfusion, the only available platelet products for trauma and surgical care are not optimized for these populations, suffer major functional deficiencies, and may pose unnecessary risk of bacterial contamination. The neglect of platelet function and insistence on RT storage to maximize circulation time restricts shelf life to 5 days in order to reduce risks of bacterial growth. The resulting shortages in platelet inventories ensure that platelet transfusion is largely reserved to treat the most severe thrombocytopenias, primarily in oncology patients. Ironically, three large, multicenter RCTs in North America and Europe have recently demonstrated that prophylactic platelet transfusion in the oncology setting is of questionable efficacy, and no dose of RT platelets was identified as effective in reducing blood loss [24, 68, 69]. The notable exception to this finding is prophylactic platelet transfusion for patients undergoing induction chemotherapy or allogeneic stem cell transplantation for acute myelogenous leukemia (AML). These patients suffer a profound and long-lasting thrombocytopenia, as well as treatment-related toxicity. Prophylactic platelet transfusion in this cohort reduces intracranial hemorrhage and mortality and should be continued, but many oncology patients currently receiving prophylactic transfusions do not fall into this category. As these data are incorporated into current guidelines, the proportion of transfusions for prophylactically maintaining an arbitrary platelet count is likely to decrease, and therapeutic transfusion for severe, acute hemorrhage may become the most common indication for platelet use. Maintaining a single inventory of RT-stored platelets at the expense of function, safety, and availability may become an untenable practice in the future.

Evidence from the Gulf Wars and Beyond: A New Direction for Platelet Transfusion Medicine?

The relative unavailability of platelets and the iatrogenic deficits in plasma and platelets noted above essentially describe the state of affairs when the USA embarked on the recent conflicts in Iraq and Afghanistan, beginning in 2001. Civil unrest and insurgent activity heightened the conflict in 2004–2005 and the casualty rate rose, giving military physicians sufficient experience to realize that resuscitation with RBC concentrates and crystalloid was ineffective in managing combat trauma-related massive hemorrhage. Platelets and plasma were needed, but platelet concentrates were largely unavailable because, after being shipped from US blood banks, most were at or beyond the 5-day storage limit. The shortages

prompted emergency whole blood collections as a platelet-containing alternative, and in dramatic fashion, a new generation of physicians rediscovered the importance of providing whole blood functionality to the shocked, hemorrhaging patient [70, 71]. The military transfusion medicine community resisted this return to whole blood due to concerns regarding infectious disease risk and streamlined transport systems to provide timely delivery of plasma, platelets, and cryoprecipitate which were reconstituted into a whole blood-like cocktail [72, 73]. Retrospective analyses of this experience revealed that fresh whole blood was at least as effective if not more so than component therapy and that the effectiveness of component therapy was dramatically enhanced when plasma and platelets were included early to reapproximate whole blood function at the outset of resuscitation [70, 74–76]. These observations gave rise to the "1:1:1" ratio-based massive transfusion protocol, which was subsequently validated in large retrospective civilian trauma studies. Recently, the Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial, a large prospective randomized trial comparing a 1:1:1 ratio versus 1:1:2 in which the latter group received fewer platelets given later in resuscitation [77]. The study was limited by the similarity of the treatment arms due to ethical considerations, which may be the reason that there was no difference in the primary outcome of all-cause mortality. Further analysis; however, demonstrated a difference in secondary outcomes, including superior early hemostasis in the 1:1:1 group and fewer deaths due to exsanguination by 24 h. These results provided higher quality evidence to support equal ratio component use, but importantly, the PROPPR trial could not differentiate between platelet and plasma effects and was not designed to evaluate platelet transfusion efficacy. The intriguing finding of Hess and colleagues that trauma mortality outcomes are directly proportional to admission platelet count, including counts well above the 50,000 platelet per microliter accepted minimum threshold [78] does suggest that some of the 1:1:1 benefit is likely due to platelet effects and is corroborated by retrospective data from the recent Gulf conflicts [74–76, 79]. Hess and colleagues noted that as admission platelet count increased across the entire normal range, outcomes improved. The link between platelets and outcome has been further reinforced by others who described onset of a trauma-induced platelet dysfunction occurring prior to iatrogenic hemodilution and other interventions and illustrated that surgical hemostasis is a function of platelet activity as well as count [43, 44, 80, 81]. After 60-plus years of platelet transfusion history, this poorly understood anucleate blood cell has attained recognition as a critical component of transfusion therapy in trauma and surgical resuscitation.

Transfusion-Related Platelet Biology

Platelet Storage Lesion

The platelet storage lesion is defined as deleterious alterations in platelet quality from the time of collection until transfusion. Numerous complex factors contribute to the storage lesion and lead to changes in platelet structure, biochemical processes, and metabolic parameters due to collection technique, preparation, and storage conditions [82]. Factors that influence platelet quality include time, temperature, pH, lactate levels, suspension medias such as platelet additive solutions, the presence or absence of leukocytes, storage bag materials, and shear forces [56, 57, 82, 83]. These conditions affect the biology of stored platelets and modify their functional responses and circulating times. Understanding the metabolic, structural, biochemical, and functional changes will likely permit future improvements in the storage and transfusion of platelet products.

Metabolic Indicators

Proper oxygenation of stored platelet concentrates (PC) is important as hypoxia induces a switch to anaerobic glycolysis leading to lactic acid accumulation, reduced pH, and platelet impairment [56]. Studies demonstrating that PCs

have an oxygen consumption rate of approximately 1.10 nmol/min/10^9 following 24 h at room temperature, led to recognition of the importance of oxygen exchange during storage and to the development of novel bag materials and plasticizers to optimize gas exchange [84]. In the presence of adequate oxygen, PC bicarbonate levels drop and glucose consumption to lactate production occurs at a rate of approximately 1:2 moles respectively [84].

Identification of the changes in platelet metabolic parameters during storage thus led to strategies for preserving and supporting metabolic rate, such as refrigeration or the formulation of platelet additive solutions [57, 85]. As refrigeration slows metabolic rate, glucose consumption and lactate production decrease, whereas changes in bicarbonate levels and pH are minimized compared to 22 °C controls [21]. While the benefits associated with reduced metabolic demand are considerable, these come at the cost of a progressive drop in platelet count over time, suggesting aggregate formation, adhesion, or cell death might be occurring in PC stored at 4 °C [21]. Furthermore, cold-stored platelets have poor circulation times which are not ideal for prophylactic platelet transfusion to treat bone marrow failure [20, 86].

The decision to store platelets at 22 °C for clinical use led to the development of platelet additive solutions (PAS) to address adverse effects on energy metabolism and to minimize platelet activation during storage [85]. All PAS have some basic components in common: Sodium chloride (NaCl); a fuel source such as Na acetate or glucose; and Na citrate as an anticoagulant. Proprietary versions differ in composition with components such as sodium bicarbonate ($NaHCO_3$) or phosphate (PO_4^{3-}) to aid in buffering, and/or magnesium chloride (MgCl) and potassium chloride (KCl) to limit platelet activation [87]. Despite continued efforts to optimize the storage environment, studies have yet to demonstrate that PAS confer sufficiently substantial improvements in maintaining platelet viability that would justify the added expense compared to plasma storage [88, 89].

Structural Changes

Platelets circulate in an inactive discoid shape, and upon stimulation, undergo responses including shape change, filopodia and lamellipodia formation, granule secretion, and aggregation. Platelets in the resting state have a macroscopic swirling appearance when a manual "swirling test" is performed, which, in room temperature-stored samples, correlates well with preservation of the discoid shape and in vivo circulation after transfusion [90]. Studies examining the cytoskeletal properties of platelets following chilling or activation identified the importance of the circumferential band of microtubules in supporting and maintaining the discoid shape [91, 92]. Once platelets are chilled below 15 °C, they undergo cold-induced shape change, which is characterized by depolymerization and collapse of the microtubule ring, increased cytosolic calcium levels, and actin assembly [91, 93–96]. As the temperature drops, platelets lose their swirling phenotype and, upon transfusion, are cleared from circulation within 2 days, compared to 4–5 days if stored at room temperature [20]. Reports indicate that brief periods of rewarming prevent or reverse the cytoskeletal changes if the exposure to the cold is less than 18 h [97]. Thus temperature cycling improves microtubule reassembly, preserves platelet shape, improves aggregation responses, and increases circulation times compared to platelets continuously maintained in the cold [97, 98].

Activation Markers and Granule Contents

Platelet granule secretion is important for amplification of aggregation, recruitment of leukocytes, and vascular remodeling. P-selectin, an adhesion molecule found in the platelet α-granules, is rapidly expressed on the plasma membrane following activation [99]. Platelet storage studies indicate that P-selectin expression increases with time at room temperature and to an even greater degree when stored at 4 °C [21, 100, 101]. Additionally, α-granule constituents

such as β-thromboglobulin and platelet factor 4 increase over time in the storage media, indicating that partial degranulation has occurred [102–104]. While P-selectin is a sensitive marker for platelet activation, it is unlikely to have a significant role in clearance and may be an unreliable predictor of post-transfusional platelet function [103, 105–108].

Microparticles/Phosphatidylserine Exposure

Phosphatidylserine (PS) is an anionic phospholipid expressed on the inner leaflet of the plasma membrane and is externalized after necrotic or apoptotic pathways are triggered in platelets [109]. The exposure of PS on the platelet surface helps support primary hemostasis by promoting thrombin generation through the assembly of the coagulation factors activated factor five (FVa) and activated factor ten (FXa) [110]. The constant metabolic demand during platelet storage is associated with loss of mitochondrial membrane potential and PS exposure [111, 112]. Many of the necrotic and apoptotic features such as membrane fragmentation, cytoskeletal disruption, caspase activation, PS exposure, and microparticle (MP) formation have been observed following platelet storage [57, 109]. The clinical relevance of transfusing products containing MP and PS warrants further investigation. While platelet-derived MP may aid in the hemostatic process by accumulating at site of injury and supporting fibrin formation, transfusion of procoagulant MP may enhance or promote systemic coagulation in susceptible patient populations [113–115], but the clinical significance of these functions remain to be fully defined.

Surface Receptors

Glycoprotein Ib-IX-V (GPIb-IX-V) mediates primary hemostasis through its interaction with activated von Willebrand factor, and plays an essential role in clearing platelets from circulation [116]. Storage studies demonstrated that a GPIb-IX-V subunit, GPIbα, is shed from the platelet surface in response to the ADAM metalloproteinase domain 17 (ADAM17), also known as TNF-α-converting enzyme (TACE) [117, 118]. Inhibition of ADAM17 activity during room temperature storage improves platelet survival and recovery, illustrating the importance of this receptor in platelet clearance [117, 119]. Other studies investigating the mechanism of cold stored platelet clearance have identified two distinct modifications of GPIbα which mediate their removal from circulation [120]. Short term chilling of platelets leads to the clustering of exposed β-N-acetylglucosamine (bGlcNAc) on GPIbα which is recognized by hepatic macrophage αM-lectin receptors [121]. The fact that bGlcNAc glycosylation with UDP galactose, a method to prevent hepatic recognition, fails to improve platelet clearance after prolonged storage at 4 °C suggests the existence of other platelet clearance mechanisms. Follow-up studies demonstrated that refrigeration causes the release of platelet sialidases, resulting in desialylation of GPIbα and thus platelets with reduced α2,3-linked sialic acid are removed from circulation by hepatic lectins, Ashwell-Morell receptors, through the binding of exposed βGal and GalNAc [122–124]. Furthermore, a recent finding suggests that clearance of desialylated platelets by Ashwell-Morell receptors upregulates compensatory new platelet production by stimulating the expression of thrombopoietin [125].

Translating Platelet Research Findings into Artificial Platelet Products

As the preceding sections of this chapter make clear, platelet transfusion has had a complicated history, and currently available platelet products leave much to be desired. As platelet research moves forward in earnest to fully describe structure, function, and diverse roles, creation of artificial platelet substitutes may become possible and would reduce the hazards of infectious disease transmission, alloimmunization, short shelf life, and complicated storage conditions. It is possible

to envision that a standardized product could even allow for reliable dose-response hemostatic properties delivered with predictable pharmacokinetics, unlike current platelet concentrates whose properties are as individual as the donors themselves. Enterprising scientists have sought since the 1950s to develop artificial platelet products along two major lines of effort: platelet-derived hemostatic agents (PDHAs) and artificial hemostatic platelet substitutes [126]. The immune and trophic aspects have been less emphasized as these functions remain poorly understood, and because the challenges of merely delivering hemostatic function have been overwhelming. Nevertheless, applications of PDHAs in promotion of wound healing are already being explored [127].

PDHAs are made by simple lyophilization; platelet fixation and lyophilization; by using trehalose, a sugar made by desiccation-resistant organisms to stabilize platelet membranes prior to lyophilization; or by various attempts at generating thrombogenic platelet fragments including membrane preparations [128–130]. To varying degrees, these development efforts have succeeded in addressing the logistical challenges of providing platelet hemostatic activity to patients, mainly by increasing shelf life and simplifying storage. The most promising development projects to date have been Cellphire's Thrombosomes® (trehalose-loaded lyophilized platelets) and Entegrion's Stasix® products (glutaraldehyde-fixed, lyophilized platelets), funded by the US Department of Defense and the Department of Health and Human Services. The distinguishing in vivo features of products made by these approaches are rapid clearance, ability to support thrombin generation, variable adhesion to collagen, and reduced aggregation performance [131–133]. Animal model studies suggest safety and some ability to support hemostasis and reduce blood loss in thrombocytopenic models [134, 135]. More extensive testing in animal hemorrhage and trauma models will inform human clinical trial design. While success in fielding PDHAs has been elusive for over half a century, well-designed clinical studies may identify useful roles for these products [136].

Artificial hemostatic platelet substitutes have proven more difficult to develop. Numerous fibrinogen- and vWF-binding platforms have been evaluated, including: modified RBCs, albumin microparticles, liposomes, and hydrogels [137–139]. While the in vitro and animal model results of some of these technologies are promising, advanced preclinical or clinical development has been extremely limited. The limited shelf life of currently approved platelet concentrates continues to create unmet needs and generate opportunities for further development of these technologies.

Clinical Platelet Transfusion Medicine

Transfusion Volume and Anticipated Change in Platelet Count

The measurement of platelet transfusion efficacy is complex due to the multiplicity of variables that can affect platelet participation in clot formation. Traditionally, simple platelet counts were used; however, this approach does not account for volume of distribution, degree of thrombocytopenia, or the effect of chronic disease, among other factors. Platelet viability, pharmacologic agents, and medical conditions such as hypersplenism, hepatomegaly, and bone marrow transplantation can dramatically alter recovery [140–142].

The established research tool for determining platelet transfusion effect, a recovery and survival study, starts with the infusion of a radiolabeled platelet dose, followed by serial blood sampling to determine the percent of the original dose available for measurement (recovery), as well as the length of time, typically measured in days, over which the radiolabeled platelets are detectable (survival, see Fig. 22.1) [143]. Although higher values have been reported, approximately 66 ± 8 % of the platelet dose is recovered on average immediately after transfusion in healthy subjects, a number that is slightly lower (approximately 60 ± 15 %) in the setting of thrombocytopenia [144]. As investigators recognized that simply reporting percentages ignores the influence of multiple patient and donor factors, methods such as corrected count increment (CCI) [145] and percent platelet recovery (PPR) [140, 144] were developed, followed by platelet count increment regression analysis to reduce result variability (Fig. 22.2) [140, 142].

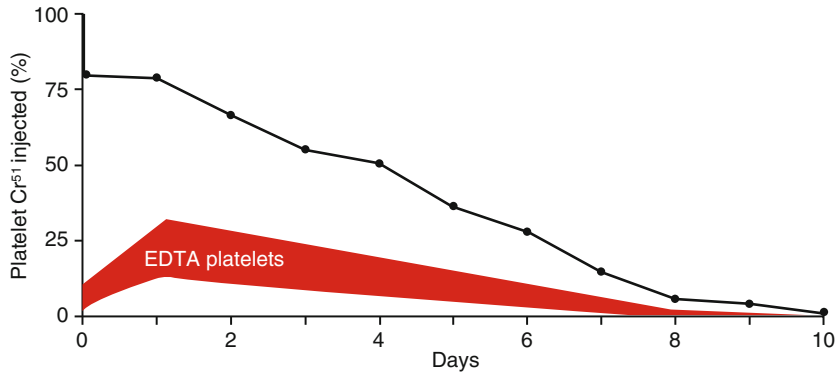

Fig. 22.1 Survival of autologous "citrate platelets" after transfusion to a normal subject demonstrates that approximately 75 % of labeled platelets were recovered in the circulation immediately after being transfused. The *red area* denotes the range of blood platelet radioactivity after the injection of Cr51-labeled "EDTA platelets" on ten occasions in seven normal subjects, demonstrating that the maximum recovery was <40 % with lower survival, due to EDTA-induced structural changes leading to more rapid clearance in the liver [143]

Fig. 22.2 Formulas for calculating platelet recovery, corrected count increment (CCI), and platelet survival post-transfusion [140]

PLT recovery, CCI, and survival

Recovery in percentage was calculated from the formula[11]

$$\frac{\text{PLT increment (after transfusion – before transfusion) } [10^9/\text{L}]\text{x blood volume[L]x100\%}}{\text{Transfused PLTS } [10^9].}$$

Blood volume was estimated by the formulas[12]

Women: $(0.3561 \times \text{height } [\text{m}]^3)+(0.03308\text{x weight[kg]})+0.1833=\text{blood volume[L]}$

Men: $(0.3669\text{x height } [\text{m}]^3)+(0.3219\text{x weight[kg]})+0.6041=\text{blood volume[L]}.$

CCI was calculated from the formula[11]

$$\frac{\text{PLT increment (after transfusion-before transfusion) } [10^9/\text{L}] \text{ x body surface area}[\text{m}^2]}{\text{Transfused PLTs}[10^{11}].}$$

Body surface area (BSA) was estimated by the formula[13]

$$\text{BSA}[\text{m}^2]=0.007184\text{x weight[kg]}^{0.425}\text{x height[cm]}^{0.725}.$$

Survival was defined as flow cytometric detection of a population of transfused PLTs of more than 1% in the patients' circulation.

Platelet refractory response is defined by two measurements in which 1-h CCI and PPR values are less than 7500 platelets/μL/m^2 and 30 %, and 20-h values are less than 4500 platelet/μL/m^2 and 20 % respectively [142]. Although platelet counts are higher after transfusion of apheresis platelets compared to pooled preparations, efficacy of transfusion as measured by CCI and PPR appears to be equal regardless of the method used to obtain platelets [142]. Expected count increases in an average sized adult, as estimated by one group of investigators, are in the range of 30,000–60,000/μL for each apheresis component containing approximately 3×10^{11} platelets, and 5000–10,000/μL for random donor components with an average of 7×10^{10} platelets [142]. While a "standard" platelet dose has not been defined, a reasonable estimate is the transfusion of one platelet concentrate per 10 kg of body weight, and can be expected to cause a 40,000/l elevation in platelet count [142].

Average In-Vivo Life-Span of Transfused Platelets

In-vivo platelet circulation is determined by the rate of utilization, as well as by random and senescent mechanisms of clearance [17, 146–155]. Radioisotope-labeled platelet deposition is primarily in the spleen and liver [155], with splenic uptake occurring early, consistent with sequestration in the splenic platelet pool, whereas liver uptake occurs late and increases over time [146]. Survival studies demonstrate that platelets circulate for approximately 5–7 days and their numbers decline over time, with only about 5 % remaining in circulation by day 8 [146, 156–158].

Overview of Platelet Transfusion Refractoriness

Thrombocytopenic patients can develop a clinical syndrome characterized by rapid post-transfusion platelet clearance, posing a considerable challenge if hemorrhage develops and requires treatment. While the etiology is diverse, the most common cause is alloimmunization due to frequent platelet transfusions. Platelet transfusion refractoriness is defined as inappropriate post-transfusion rise in 1-h CCI and/or PPR (two or more consecutive CCIs of $<5–10\times10^9$/l and/or two or more consecutive PPRs less than 20 %, when measured at 1 h after transfusion of compatible platelet concentrates that are within 3 days of storage) [159–161]. A large prospective platelet transfusion study titled the Trial to Reduce Alloimmunization to Platelets, or the TRAP trial, was analyzed for factors implicated in platelet refractoriness. The authors found that the clinically important variables included lymphocytotoxic antibody positivity; females with at least two pregnancies; heparin administration; bleeding; palpable spleen; fever; amphotericin; and disseminated intravascular coagulation (DIC) [160–162].

Immune causes of refractoriness can be broadly described as those related to human leucocyte antigens (HLA) and those acquired against platelets, involving the human platelet antigen (HPA) system [159]. HPA-related platelet refractoriness is less common, less severe, and typically transient, but also less preventable with leucoreduction [162]. Nonimmune causes include prior antigen exposure in multiparous females, enlarged spleen, DIC, medications [163], bleeding, fever, sepsis, venoocclusive disease (VOD), and transfusion-associated graft-versus-host disease (TA-GVHD) [160, 164]. Component-related factors that can influence refractoriness include gamma irradiation, storage duration, and ABO compatibility [160].

Management strategies to address refractoriness start with antibody tests to differentiate between the possible immune-mediated causes (Table 22.1) [162]. If immune causes are found, HLA-matched, crossmatched, or antibody specific platelet components are indicated. HLA-matches are categorized as A, BU, BX, C, or D, and efficacy is assessed by establishing the degree of improvement in post-transfusion CCI [165]. Cross matching improves platelet survival [166], and possibly, post-transfusion increments. Provision of platelets lacking the specific HLA antigens determined to cause platelet refractoriness is another method that improves post-transfusion platelet recovery and can be used if HLA-matched or crossmatched donors are not available [167]. Management for patients with nonimmune etiologies is aimed at treating the underlying causes, if possible; however, splenectomy has not proven useful when splenic enlargement is present.

Table 22.1 Laboratory tests commonly used to detect anti-HLA or platelet-specific antibodies [162]

Lymphocytotoxicity test (LCT)
Platelet immunofluorescence test (PIFT)
Lymphocyte immunofluorescence test (LIFT)
Enzyme-linked immunosorbent assay (ELISA)
Antigen capture ELISA (ACE)
Monoclonal antibody-specific immobilization of platelet antigens (MAIPA)
Solid-phase red cell agglutination test (SPRCA)
Multiplex flow cytometric bead assays

ABO/D Compatibility for Platelet Transfusions

Accidental transfusion of ABO-incompatible red blood cells results in a severe hemolytic reaction, often leading to shock and death. The inadvisability of transfusing ABO-incompatible platelets is less clear; however, unmatched platelets are often used in clinical practice. While adverse consequences such as low post-transfusion platelet counts, acute hemolysis, fever, inflammatory changes, the development of refractoriness, and even death [168–177] can occur, particularly with major incompatibility, maintaining a strict policy of requiring cross-matched platelets can also lead to waste, platelet shortages, and in emergency cases, could also contribute to death [169]. Given the lack of clarity and the competing risks, the American Association of Blood Banks (AABB) and other regulatory bodies require blood banks and hospitals to have written policies regarding incompatible transfusion, but do not prohibit the practice [168, 169, 178]. A recent study [168] examined the data from the platelet-dose trial (PLADO) by Slichter et al. with regard to transfusion of ABO incompatible platelets and found that, while major ABO incompatibility resulted in lower post-transfusion increments, it did not have a measurable effect on clinically significant bleeding (Fig. 22.3) [173].

Alloimmunization of Rh(D) negative patients after D+ platelet transfusion varies from 19 % to, most recently, 1.4 % [179, 180]. While D– platelets or Rh immune globulin (RhIG) are recommended for D– patients of child-bearing age [181, 182], the recommendations are not based on high quality data [180, 183]. A recent study followed D alloimmunization after D+ platelet transfusion in D– patients and reported a low percentage of study subjects who developed

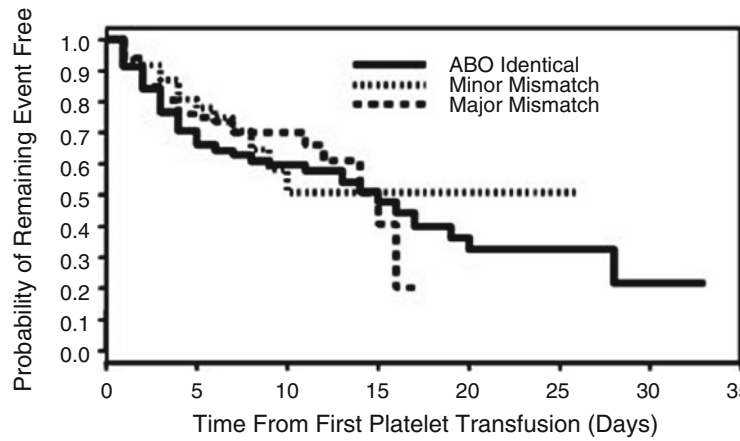

Fig. 22.3 Kaplan–Meier plots of time from platelet transfusion to first > grade 2 bleeding for time from first platelet transfusion to first grade 2 or higher bleeding by ABO matching status. Time to bleeding was censored at the first date that any of the following occurred: transfusion of a platelet dose with a different ABO matching status from the patient's initial platelet transfusion or missing data on ABO matching status; missing data on whether grade 2 bleeding occurred; or end of study. Divergence of the curves after 15 days is probably the result of the small number of patients still at risk by that time. ABO matching status was not a significant predictor of time to grade 2 bleeding ($p=0.33$) [173]

anti-D antibodies. The authors concluded that this should be considered when determining whether to give RhIG [180].

Practices in Leukoreduction for Platelet Units

Leukoreduction (LK) is currently the standard of care in many blood donation centers, blood banks, and medical centers due to proven and hypothesized clinical benefits (Table 22.2) [184, 185]. LK of platelet products can be accomplished many ways, including pre-storage platelet leukocyte filters, pre-transfusion platelet leukocyte filters, platelet-sparing whole blood leukoreduction, or direct leukoreduction during apheresis [186–188]. The efficacy of leukoreduction was compared to gamma irradiation in a large, prospective, randomized trial and found to be equivalent in preventing alloantibody-mediated refractoriness [161]; however, LK effectiveness in preventing TA-GVHD, a clinical entity associated with high morbidity and mortality, is not proven [184]. An alternative method of neutralizing white blood cell (WBC) transfusion effects, pathogen reduction, has not been fully evaluated and is not yet widely available in the USA, but preliminary data suggests promise in preventing both platelet refractoriness and TA-GVHD [189–195].

Table 22.2 Putative clinical benefits of leukocyte reduction

(A) Proven relevant clinically:
1. Reduced frequency and severity of NHFTRs
2. Reduced risk of CMV transmission
3. Reduced risk of HLA-alloimmunization and platelet refractoriness
(B) Likely clinically relevant:
4. Reduced infectious risk associated with immunomodulation (TRIM)
5. Reduced organ-dysfunction and mortality
6. Reduced direct risk of transfusion-transmission bacteria
(C) Unproven clinically:
7. Avoidance of vCJD transmission
8. Avoidance of HTLV I/II, EBV etc.
9. Reduced risk of GVHD
10. Reduced risk of TRALI

Putative clinical benefits of leukocyte reduction, subdivided as to whether each benefit has been proven by evidence-based guidelines to be: Relevant Clinically, Likely Relevant Clinically, or are Unproven to be Clinically Relevant [184]

NHFTRs non-hemolytic febrile transfusion reactions, *CMV* cytomegalovirus, *vCJD* variant Creutzfeld–Jacob disease, *GVHD* graft-versus-host disease, *TRALI* transfusion-associated acute lung injury

Adverse Effects of Platelet Transfusion

Bacterial contamination is a recognized risk of platelet transfusion due to room temperature storage [196–199]. Pooled platelet components were ten times more likely to be contaminated compared to refrigerated RBC components [200], and recognition of increased adverse events led the FDA to reverse their decision to extend storage time beyond 5 days [201]. Within the last decade, blood banks and hospitals have implemented numerous advances in how platelets are collected, processed, and tested during storage, and these changes have resulted in reduced morbidity and mortality [198, 199, 202, 203]. Changes include improved methods of skin preparation, addition of diversion pouches to transfusion kits that reduce exposure to the potentially contaminated initial blood sample, and routine bacterial testing [204], a requirement adopted by the AABB in 2004. Adoption by many centers of single-donor apheresis platelets, which are collected in a more sterile manner, and reduced use of multiple-donor pooled platelets, has also had a measurable effect on septic platelet transfusion reactions. After one center increased its use of apheresis-derived platelet concentrates from 51.7 % to 99.4 %, the incidence decreased by a factor of 3 [205], although a randomized controlled trial failed to document higher bacterial contamination in pooled buffy coat platelets [206]. Pooled platelet contamination risk is approximately 1 in 400, thus the risk to a trauma patient receiving massive transfusion can surpass 1 in 100 [207]. In response to concerns about bacterial contamination, the use of apheresis platelets continues to grow ($p<0.05$) and now accounts for 67 % of platelet concentrates administered to

patients according to the 2011 National Blood Collection and Utilization Survey Report [208]. Similarly, apheresis platelets are now commonly transfused in US military field hospitals, although this is primarily due to logistic considerations [72]. Despite advances in platelet collection, bacterial contamination remains a significant problem [196–198, 209, 210], resulting in related cases of bacterial sepsis and death [127, 198, 199, 211], which continue to be more common than human immunodeficiency virus (HIV), hepatitis B virus (HBV), or hepatitis C virus (HCV) transmission [212].

Due to rigorous screening and testing of blood product donors, blood product contamination with these viruses is very low, but now attention has turned to newly discovered threats to blood safety, with up to 5.3 previously unrecognized viruses emerging per year [213]. Examples from recent decades that have captured global attention include the variant Creutzfeldt-Jakob prion [214], West Nile virus [215], dengue viruses [216], Hepatitis E [217], and Ebola viruses [218], as well as nonviral agents such as *Babesia microti* [219], *Trypanosoma cruzi* [220], and *Leishmania donovani* [221]. In response, nonspecific pathogen reduction systems were developed to address the increasing risks and costs associated with emerging pathogens, and are already in use outside the USA [222]. These technologies promise to increase blood safety and reasonably preserve platelet function both in treated whole blood [22] and platelet concentrates [192].

As mentioned above, despite advances in the prevention of transfusion-related viral transmission, concerns over contamination of platelet products with infectious bacterial agents remain due to room temperature storage, and increasingly, the noninfectious sequelae of blood product transfusion are also recognized as important and commonly occurring entities. These are categorized as acute (within 24 h) and delayed (after 24 h). The most common acute transfusion reactions include febrile and allergic non-hemolytic transfusion reactions (NHTR) [223–228], and hemolytic transfusion reactions, which are the serious, life-threatening consequence of blood type misidentification. Febrile NHTR occur in response to anti-leukocyte antibodies, are typically self-limiting, can be minimized with leukoreduction, and occur most commonly in response to platelet transfusion [185, 229–231]. Allergic NHTR are distinguished by the absence of fever and presence of urticarial symptoms, and can be minimized by pretreating the transfusion recipient with antihistamines [232]. Although generally mild, anaphylaxis is possible, thus treatment often begins with halting the transfusion and monitoring the patient for development of more serious signs [233]. That said, the evidence for this strategy is not definitive [234], thus in the absence of a randomized controlled trial, pretreatment is relegated to physician discretion. The highest cause of death is attributed to transfusion-related acute lung injury (TRALI, Fig. 22.4) [235], defined by acute onset ($\leq$6 h), $PaO_2/FiO_2 < 300$ mmHg, bilateral pulmonary infiltrates, and absence of pulmonary edema from other causes [236]. Risk of TRALI is associated with transfusion quantity [237–239], transfusion of plasma or whole blood (particularly from female donors) [238–240], and high anti-HLA or other potentially causative antibody titers in the transfusion product [241, 242]. Platelet transfusion is not independently associated with TRALI according to several recent studies [238, 240, 241] and older reports of an association with pooled platelets may be due to the presence of plasma from several donors [243], particularly if leukocytes and/or high antibody titers are present. The etiology is predominantly due to a combination of activated pulmonary endothelium and recruitment of polymorphonuclear leucocytes (PMNs) due to trauma or critical illness, followed by transfusion of leucocyte-containing blood products [244]. Up to 40 % of patients with TRALI have only been exposed to leukoreduced blood products, and thus other etiologies occur that have yet to be fully described.

Late complications of transfusions include post-transfusion purpura (PTP), and transfusion-associated GVHD. PTP is a rare, but serious, entity typically due to anti-HPA-1a (an antibody against a platelet-specific antigen) [245], is most common in parous women who have been sensitized to platelets during pregnancy, and is treated

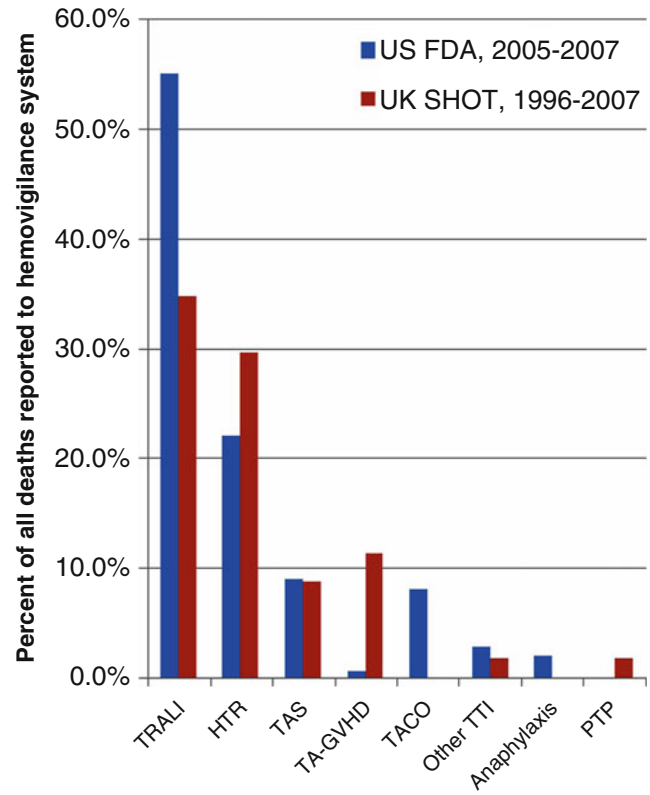

Fig. 22.4 Causes of allogeneic blood transfusion-related deaths as a percentage of all deaths reported to the SHOT (1996–2007) or the FDA (2005–2007) [235]

with high dose intravenous immunoglobulin. TA-GVHD is characterized by rash, fever, pancytopenia, and liver dysfunction [246]. Multiple etiologies for TA-GVHD, which is associated with greater than 90 % mortality, have been described, involving immunocompromised and immunocompetent patients. Risk factors associated with this life-threatening syndrome include immunocompromise, transfusion of HLA homozygous blood into heterozygous patients, intrauterine transfusion, advanced recipient age, and short RBC (<11–14 days) storage times, thus greater probability of containing active lymphocytes [246, 247]. When immunocompetent patients develop TA-GVHD, reactions are more likely to involve an incomplete mismatch that allows persistence of donor lymphocytes [247, 248]. Common symptoms are fever, erythema, and leukocytopenia [247]. Therapeutic intervention is primarily focused on prevention, which is primarily accomplished by gamma irradiation of transfused products to inactivate T lymphocytes, due to the difficulty of reversing the disorder once bone marrow is compromised and to the high mortality associated with this syndrome [248, 249]. Incidence markedly declined once widespread irradiation of transfused components was widely adopted for patients at risk [247].

Platelet Transfusion for Trauma-Induced Coagulopathy

Indications and Dosing for Platelet Transfusion in Injured Patients

Although physicians agree that platelet transfusion is life-saving for profoundly thrombocytopenic bleeding trauma patients, there is no high quality data to guide resuscitation, nor consensus regarding the timing of transfusion for non-thrombocytopenic patients with massive hemorrhage. Evidence-based guidelines are needed to establish transfusion thresholds and dosing strategies. Research

gaps are due to the ethical issues which proscribe studies comparing platelet unit transfusion to placebo, as well as to the technically challenging nature of prospective platelet dosing studies. Platelet functional deficits are recognized, but the degree of dysfunction is highly variable and in vivo methods of measurement are not validated in trauma patients. As mentioned above, commonly accepted indications for platelet unit transfusions include the treatment of active bleeding with thrombocytopenia (<50,000/μL or <100,000 in the presence of DIC or central nervous system bleeding), in preparation for an invasive procedure in a thrombocytopenic patient, and for prevention of spontaneous bleeding in high-risk patients with thrombocytopenia [250]. Although there are theoretical benefits, these thresholds are not based on evidence of efficacy in improving outcomes or reducing blood loss or blood product utilization. Until recently, actively bleeding trauma patients received platelet transfusions only after thrombocytopenia was documented, but this strategy is currently being challenged by physicians who have reason to believe that severe traumatic life-threatening bleeding could rapidly lead to death in this vulnerable population. As noted above, recent studies suggest that platelet function can be compromised in response to trauma despite platelet counts above classic target thresholds for transfusion [251], and that early empiric use of platelets are associated with improved outcomes [76, 79, 252, 253]. For all these reasons, empiric platelet transfusions are becoming standard practice in addressing traumatic hemorrhage [77, 254].

As previously discussed, military clinicians treating casualties of the recent conflicts in Iraq and Afghanistan were faced with a degree of injury and number of cases rarely if ever equaled in civilian trauma centers. In response to their experience with mass casualty activations, they noted that outcomes improved when patients with traumatic hemorrhage received platelet replacement during the initial resuscitation. Perkins et al. reported in a cohort of 464 patients with combat related trauma that the use of platelets was independently associated with improved 30 day survival [76]. Spinella et al., published that increased platelet to RBC unit ratios were associated with survival in patients with and without severe traumatic brain injury in a civilian cohort of patients [252]. An increased ratio of platelet to RBC units transfused was associated with increased survival in a 10-year review of patients with combat-related injuries by Pidcoke et al. and in civilian cohorts by Holcomb et al. [79, 253]. One small prospective RCT of 33 massively bleeding trauma patients compared transfusion of 6 units of random donor unit platelets to 2 units of fresh frozen plasma (FFP) administered with every 12 units of modified whole blood [255]. In this study, there was no difference in the incidence of microvascular bleeding between the two study groups, but results from the PROPPR trial demonstrated that hemostasis was achieved in more patients treated with the higher ratio of platelets and fewer expired due to exsanguination [77].

The use of platelet units for patients without life-threatening bleeding is not well studied; however, a retrospective analysis of 1788 transfused trauma patients treated with resuscitations that did not meet massive transfusion criteria (<10 units of RBCs in 24 h) reported that higher platelet (or plasma) to RBC unit ratios were not associated with increased survival but rather with increased morbidity represented by reduced intensive care unit (ICU) free and mechanical ventilator free days [256]. The degree to which these observations are attributable to specific platelet effects versus overall transfusion volume are difficult to ascertain and suggest the need for further exploration in randomized clinical trials.

The optimal dose of platelets to treat patients with life-threatening traumatic injury is unknown. It is unlikely that a single dose can be identified since the degree of thrombocytopenia and reduced platelet function is highly variable due to the heterogeneity of traumatic injuries and wide variation in time from injury to presentation at a treatment facility with platelet units available. Platelet transfusion is based upon clinical response and typically given at a ratio of 1:1 to 1:2 platelets to RBC units. A recent survey of 132 US trauma centers indicated that 79 % of centers target a platelet to RBC unit ratio greater than or equal to 1:2 for patients that require massive transfusion protocol activation [254].

In opposition to those who advocate empiric platelet transfusion, a growing body of literature supports so-called "goal-directed" hemostatic resuscitation based upon measures of platelet function, which can be measured by viscoelastic tests or platelet aggregometry [257]. Multiple platelet transfusion algorithms in patients with traumatic injury have been published. Some are applicable to patients with traumatic injury, but thresholds and therapeutic targets have not been formally validated [258]. While the use of functional platelet measures to drive goal-directed hemostatic resuscitation based on therapeutic targets appears more rational than merely targeting an arbitrary number of platelets, particularly in the light of recent data describing early platelet inhibition after severe traumatic injury, the evidence supporting this strategy remains inconclusive.

Whole Blood as a Source of Platelets

Whole blood, a product licensed by the FDA for up to 21 days of storage at 4 °C in citrate phosphate dextrose (CPD) or 35 days in citrate phosphate dextrose-adenine 1 (CPDA-1), is another, potentially more optimal, source of platelets for patients with traumatic hemorrhagic shock. A whole blood unit is less dilute compared to a comparable unit of reconstituted whole blood made from individual components of RBCs, plasma, and platelets, and it has the advantage of being simple to administer and rapidly available while allowing for the simultaneous treatment of both coagulopathy and shock [259]. Due to 4 °C storage, platelets in whole blood are more hemostatically active compared to standard of care (22 °C) platelet components. While whole blood is used at some children's hospitals [260], it is not routinely available for adults due to the wide-spread belief, in the absence of convincing data, that blood component therapy is not only adequate, but for some reason preferable to whole blood.

The only available prospective evidence in trauma patients that utilizes whole blood comes from a single small RCT of 107 patients requiring massive transfusion [261]. One arm received leukoreduced whole blood (resulting in platelet removal during filtration) supplemented with platelets, and the other was treated exclusively with components (RBCs, plasma, platelet units). While the primary outcome of 24-h transfusion volume was equivalent between groups, a secondary analysis excluding patients with severe traumatic brain injury demonstrated reduced 24-h blood use for the modified whole blood group. It is difficult to know if the addition of platelets to whole blood sufficiently changed the results to limit generalizability, thus the study was insufficient to definitively establish the efficacy of whole blood; however, published reports in a non-trauma patient population may provide further clues. A prospective RCT in children requiring cardiac surgery that examined blood use in both bypass circuits and in the ICU reported reduced blood loss and improved platelet function with whole blood at 4 °C compared to reconstituted blood from components, including platelets stored at 22 °C [262]. Another RCT in children compared whole blood to components in the operating room for bypass, after which subjects only received components in the ICU. The authors reported that ICU morbidity and mortality was no different between the two groups [263]. Retrospective data in adults, including an analysis of US casualties with 100 % follow up which reported an independent association between survival and the addition of whole blood as an adjunct to resuscitation, further support the use of whole blood compared to exclusive use of components [70]. Additionally, a study of US and non-US casualties reported no difference in outcomes for those transfused whole blood as part of their resuscitation compared to those who only received blood components; however, results should be viewed with caution as approximately 33 % of patients were lost to follow-up [75]. These two studies of combat casualties are unique in that the whole blood transfused in combat was given immediately after collection or was stored very briefly (<24 h) at 22 °C. They also differ from civilian data in that whole blood accounted for only a portion of the total blood used for resuscitation during the initial 24 h of admission. In the study that found an association with survival, 30 % of the 24-h

blood volume transfused was whole blood, whereas in the study that demonstrated equivalence, that figure was only 20 %.

The clinical availability of whole blood has been limited since the 1970s due to three main concerns. Whole blood must be ABO-specific, and maintaining sufficient inventory to ensure a ready supply of every ABO type would result in significant cost and waste. The second reason stems from the misperception that whole blood cannot be leukocyte-reduced, a process that typically removed platelets with the leukocytes and thus cannot be considered a platelet-containing product. The FDA recently approved a WB leukoreduction filter that is platelet-sparing, paving the way for leukoreduced WB as a platelet-containing product. A final concern is that the platelets in whole blood stored at 4 °C are not functional or viable, once again calling into question whether WB is truly a platelet-containing product. As reviewed earlier in this chapter, this misconception stems from the spurious assumption that spherical platelets are nonfunctional. Cold-stored platelets, despite irreversible shape change, aggregate better, are able to form a thrombus, and are associated with stronger clot formation when compared to the current standard of RT-stored platelet concentrates [21, 22, 53].

Type-Specific Versus O-Negative Low Titer Whole Blood

Transfusion of type O low titer whole blood (O LTWB) is an alternative solution to type specific whole blood (TSWB), but despite being the standard of care for treating hemorrhagic shock up to and during the Vietnam War [264], it is currently not permitted by AABB standards [265]. The rationale for this prohibition is difficult to understand. Hundreds of thousands of units of type O whole blood, with low anti-A and anti-B titers to reduce the risk of plasma incompatible ABO reactions, were used during both World Wars, the Korean War, and the war in Vietnam [264, 266, 267]. The threshold for low titer set by the US military in the Korean War was <1:256 [268]. A report by Nessen et al. indicated that, at US military forward surgical bases, the use of type O whole blood was associated with improved outcomes when compared to RBCs and plasma alone [269]. In addition to decades of use with few reports of adverse complications, there are multiple reasons to suppose that O LTWB is safer than TSWB. While a small risk of severe hemolytic reaction to O LTWB does exist, it is similar to that of transfusing type O RBCs, which are currently available for emergency release at major trauma centers. It is the plasma contained in the blood that causes the greatest concern for ABO incompatibility, but the associated hemolytic reaction is typically mild to moderate and the incidence, only 1:120,000 transfusions in the UK's Serious Hazards of Transfusion (UK SHOT) database, is low [270]. In contrast to this nonfatal risk, type-specific WB transfusion carries a higher risk (1:80,000) of severe hemolytic reaction, mainly due to human error [270]. Given the prevalence of type O donors, O LTWB is a viable alternative to type-specific WB, but to date it continues to be largely unavailable. Implementation of Type O LTWB would require evaluation of current methods for determining titers, since multiple assays are available, but evidence-based standards are lacking. In addition, a commonly accepted, data-driven threshold that defines "low titer" anti-A and anti-B Immunoglobulin M (IgM) and IgG is needed to replace the arbitrary values used in previous military conflicts. In the report by Nessen et al. described above, the authors also found that use of untitered type O whole blood was not associated with increased adverse events [269], but, outside of emergent cases or very austere environments, few would advocate this strategy given the small sample size and the relatively low cost of establishing titers.

In considering the advantages and disadvantages of the whole blood products discussed, it is clear that type-specific WB, chosen by regulatory bodies for its apparent safety, in actuality carries the highest risk because of the incidence of human error, and is the least cost effective of the three. The challenges associated with maintaining a large inventory of every blood type, and the inevitable waste, relegate this option to a minor role at

best for the treatment of hemorrhagic shock. Similarly, continued use of blood components for severely bleeding trauma patients should undergo reevaluation due to the possibly unnecessary financial burden of fractionation, the poor quality of platelet function, the added encumbrance of bacterial testing required for platelets stored at 22 °C, and the cost of frozen plasma wasted due to packaging fractures during the thawing process. In contrast, the cost of whole blood expiring before it can be used to treat hemorrhagic shock may compare favorably, even after factoring in the cost of establishing titers. Despite the possible advantages of using O LTWB to treat hemorrhagic shock, such a change in practice cannot occur until, as noted above, AABB standards for the use of whole blood are updated. Some centers, including the Mayo Clinic, have obtained permission to use O LTWB in the prehospital setting under an AABB exception, possibly opening the door for more widespread use.

According to a recent survey of 132 trauma centers, prehospital use of blood products, while increasing, is still only used for resuscitation by 34 % of first responders [254]. A smaller minority is carrying both RBCs and plasma, and very few, if any, ever carry platelets, although multiple studies demonstrate that they are important for hemostasis. The use of blood products to resuscitate patients in the prehospital phase has the potential to reduce the large risk of death from hemorrhagic shock prior to hospital admission. Military data indicates that 90 % of potentially survivable deaths occur from hemorrhagic shock [271]. Improved control of bleeding and early blood product use are required to reduce this toll. The logistical constraints of transporting RBCs, plasma, and platelets prehospital are considerable. In addition to the extra weight and complexity of transfusing product from multiple bags, intravenous or intraosseous catheters have a limited number of access ports. The use of whole blood at 4 °C stored for less than 10–14 days would dramatically reduce the logistical burden compared to the current approach, which too often provides the oldest component products in the inventory. Storage lesions can include fragile RBCs prone to hemolysis, thawed plasma with attenuated thrombin generation potential, and RT platelets with the deficits more fully described above. O LTWB, in addition to being safer, lighter, and logistically supportable, may also be more efficacious in providing hemostatic function.

In summary, for patients with traumatic injury and hemorrhagic shock, the current component products may not be optimal, but until further research leads to changes in regulations and guidelines, preemptive transfusion of RT-stored platelets in massively bleeding patients, in advance of thrombocytopenia, is associated with better outcomes. Refrigerated platelets may improve safety, and stored whole blood is an underutilized potential source of platelets, plasma, and RBCs that may be more logistically feasible both in the hospital and, recognizing the increased use of blood products during transport, in the prehospital setting. Due to safety, logistical, and cost considerations, type-specific WB is

Table 22.3 Future research needed for platelet transfusion efficacy and safety for traumatic hemorrhagic shock

Studies that determine most accurate measure of platelet function that is practical to use to indicate the need for platelet unit transfusion and goal directed therapy for platelet function
Studies that compare the efficacy and safety of platelet unit and whole blood storage solutions and additive solutions
Studies that compare the efficacy and safety of platelet unit and whole blood storage temperature
Studies that compare the efficacy and safety of ABO compatible versus noncompatible platelet units
Studies that compare the efficacy and safety of leukoreduced versus non-leukoreduced whole blood
Studies that compare the efficacy and safety of ABO compatible versus ABO noncompatible platelet or whole blood units
Studies that compare the efficacy and safety of using component therapy with platelet units compared to whole blood for patients with life-threatening hemorrhagic shock
Studies that define the appropriate method and threshold to measure and define “low titer” for Anti A and B antibodies
Studies that examine the non-hemostatic effect of platelet or whole blood transfusions on immune and endothelial function
Studies that compare the efficacy and safety of frozen or lyophilized platelets compared to liquid platelet units

not a feasible option for widespread deployment, thus adoption of O LTWB as the primary resuscitative fluid for massive hemorrhage would require a change in AABB guidelines. Future research needs are depicted in Table 22.3.

Future Directions in Platelet Transfusion Research and Areas of Opportunity for Investigators

Platelets came to occupy their functional niche through complex and poorly understood evolutionary pressures. Unlike invertebrates, birds, and fish, only mammals possess anucleate platelets derived from polypoid megakaryocytes that are confined to the bone marrow [272, 273]. Megakaryocytes and platelets evolved from ancestral diploid cells that performed both phagocytic and hemostatic functions [274]. Most likely due to enhanced activation signaling through ADP receptors and increased expression of the fibrinogen receptor, Glycoprotein IIb/IIIa (GPIIb/IIIa), mammalian platelets are capable of forming occlusive, shear-resistant thrombi with an ability to change shape that is unhindered by a nucleus. This enhanced hemostatic capacity may have provided mammals with an evolutionary advantage by improving survival after trauma. On the other hand, these evolutionary changes also set the stage for the mammalian scourge of thrombotic cardiovascular disease [272]. The role of platelets in the response to injury is complex and includes hemostatic, immune, and trophic aspects [275–277]. The megakaryocyte response to injury is not confined to increasing platelet production, but details regarding additional functions remain relatively obscure. Interactions between these cells and the endothelium, other immune effector cells including the hepato-splenic and other components of the reticuloendothelial system, and stem cells are potentially fruitful areas of research. In addition to probing the role of platelets at the nexus of the hemostatic and immune systems, platelet product development, whether addressing challenges in platelet preservation, development of synthetic hemostatic platelet substitutes, or even exploitation of platelets as drug delivery vehicles, remains an underexplored scientific frontier. As to clinical applications that require further elucidation, much work remains to be done to optimize platelet products and transfusion strategies for both medical and surgical patients, but several themes are being clarified. In treating severe, acute hemorrhage, platelet consumption and loss must be addressed early and aggressively to achieve the best possible outcome. Functional platelet counts matter and an "adequate" platelet count does not equate with adequate platelet function. The dogma that platelets should be stored at RT and transfused prophylactically in the setting of hypoproliferative thrombocytopenia has been challenged, and the assumption that platelet counts above 50,000 are adequate for trauma patients is refuted by the PROPPR study. In short, the field of platelet transfusion is at a crossroads and both regulators and scientists are called to critically reexamine current standards and recognize the importance of updating our understanding of the platelet.

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Massive Transfusion Protocols

23

Alexis M. Moren, Samantha J. Underwood, and Martin A. Schreiber

Introduction

Historical Background

Historically, knowledge regarding massive transfusion and resuscitation comes from military experiences. Blood transfusion services began during World War I (WWI), using whole blood for traumatic hemorrhage. During World War II (WWII), success in this area by the British Army has been partially attributed to the ability to provide accessible whole blood products to trauma patients [1]. In WWII, fractionation techniques were popularized creating packed red blood cells (PRBC), fresh frozen plasma (FFP), platelet (PLT) concentrates, and cryoprecipitate from whole blood [2, 3]. The vast majority of blood donated during the early part of WWII was utilized to create lyophilized plasma [4]. Both whole and component blood therapy were used until the 1970s, when blood component therapy surpassed whole blood in popularity as a means to improve resource utilization and to reduce transmission of infectious diseases [5–8].

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Management of coagulopathy is time-sensitive as hemorrhage-related mortality occurs within the first 2 h after injury. Attention to the lethal triad of coagulopathy, acidosis and hypothermia must be addressed. Traditionally, administration of component therapy was guided by laboratory values. Deficits identified by laboratory tests were corrected with specific component therapy. In the 1980s, component therapy largely replaced whole blood and transfusion guidelines were yet to be initiated. Many institutions used arbitrary ratios such as 1 unit of FFP for every 4–10 units of PRBC [9, 10]. Additionally, computer simulation models were made to evaluate circulatory changes; one model looked at and popularized 2 units of FFP to every 3 units PRBC. This administration was used to avoid compromising a patients' ability to clot and avoid plasma dilution [9]. Advance Trauma Life Support (ATLS) recommended FFP only after coagulopathy was recognized by laboratory values [11].

Platelets transfusions remain highly individualized. Many guidelines do not include recommendations for transfusion of platelets until patients are thrombocytopenic. Regardless of an intervention, function of the platelet often goes unknown unless a thromboelastogram (TEG) or rotational thromboelastography (ROTEM) is sent. Ketchum et al. was able to show a survival benefit with 1 unit of platelets (6 pack) for every 7.5 units of PRBC [9, 12]. Zink et al. also demonstrated that mortality decreased when patients received higher early

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ratios of FFP and platelets to PRBC especially within the first 6 h of admission [13].

Lucas and Ledgerwood published a study showing decreased transfusion of plasma during massive resuscitation exacerbated coagulopathy in trauma patients [14]. Expanding on the work of Lucas and Ledgerwood, numerous studies of trauma patients in the late 1990s reported that mortality improved in patients receiving higher ratios of FFP relative to PRBC [15–17]. The conflicts in Iraq and Afghanistan have provided a substantial amount of transfusion data in patients with severe injuries. The ability to study the effects of balanced versus component transfusion on survival, has been the military's focus as military physicians are seeing patients who require massive transfusion at four to five times the frequency seen in civilian practice. Balanced transfusion, mimicking whole blood, has been shown to be beneficial in both military and civilian settings [18–21].. Civilian centers first proposed the use of PRBC and FFP to provide goal directed therapy, damage control resuscitation and a survival benefit in 1982 [22]. Based on these studies many trauma centers have implemented a massive transfusion protocol (MTP) to reflect this resuscitation strategy.

Although the military reported positive experiences for use of whole blood (WB) resuscitation, the civilian studies continue to favor component therapy as it is thought to be better for resource utilization, reduce infections associated with transfusion, and reduce transfusion volume and 24 h- and 30-day mortality [23]. Studies using WB resulted in reduced transfusion volumes in non-brain injured patients [24, 25]. A modern MTP aims to approximate delivery of a 1:1:1 ratio of PRBC–FFP–PLT [26]. By addressing the early coagulopathy of trauma, MTPs have been associated with improved mortality in patients with multiple injuries [5, 27].

To more closely mimic whole blood transfusions while conserving blood component resources, varying transfusion ratios of PRBC, FFP, and PLT have been examined (Table 23.1). Hemostatic resuscitation provides transfusions with PRBC, FFP, and platelets (PLT) in a fixed ratio in an attempt to better approximate whole blood. This concept has been adopted in institutions worldwide as part of the transfusion protocol for hemorrhaging patients [28]. Due to the massive resources required to execute an MT, early recognition of patients at risk is critical [29]. MT is applied in a very small percentage of patients, whose mortality rates range between 40 % and 60 %; these individuals consume large amounts of blood and blood products [30]. Early and aggressive transfusion with balanced ratios of blood products has become the modern approach and has been shown to correct the three elements of the lethal triad [5, 28, 31].

Redefining Massive Transfusion

Hemorrhage remains a major cause of potentially preventable death. Trauma and MT are associated with coagulopathy secondary to tissue injury, hypoperfusion, dilution, and consumption of clotting factors and platelets [28, 32]. Massive transfusions are utilized in approximately 8 % of military casualties and 3 % of civilian trauma patients [29, 33, 34]. The current definition of massive transfusion is ten PRBC in 24 h; however, this definition remains under scrutiny and continues to evolve. This historic definition of MT fails to take into account the intensity of ongoing resuscitation in the hemorrhaging patient. It has been further criticized as being ineffective in capturing the most critically ill patients as they often expire prior to receiving 10 units [35–38]. Therefore, with respect to analyzing massive transfusion during acute resuscitation, the traditional definition not only "dilutes" the study sample with a stable group of patients who do not require intensive transfusion acutely, but also excludes a group of patients who die early and are potentially the group that is most likely to benefit from any change in resuscitation practice. Alternative definitions have been proposed that utilize rates of transfusion as opposed to total amounts but due to lack of supporting evidence, they have failed to be widely accepted. Regardless, all of the definitions proposed to date have failed to account for changes in transfusion patterns over

Table 23.1 Components of whole blood and an example of a guideline for usage during adult massive transfusions

Product	Composition	Usual transfusion trigger	Indication	Usual adult dosage	Adjustments for massive hemorrhage coagulopathy
Red cells (PRBC)	RBC, WBC	Symptomatic anemia and low HCT < 28	Increase RBC mass and O_2 carrying capacity	2 units PRBC	4–6 units of HCT < 18
Frozen plasma (FFP)	Fibrinogen with clotting factors V and VII (frozen)	Bleeding, INR > 1.5	Hemorrhage, INR > 1.5, PTT > 60	2 units FFP	4 units FFP if INR 2–2.5 6 units FFP if INR > 2.5
Cryoprecipitate	Factor VIII, vWF, Factor XIII, Fibrinogen (frozen)	Bleeding, Fibrinogen < 100	Hemophilias	10 units Cryo (2 pools)	20 units (4 pools) if Fibrinogen < 50
Platelets (PLT)	Platelets, Plasma	Bleeding, Platelets < 100	Control or prevention of bleeding	1 unit PLT (1 unit = 6 pack)	2 units PLT if <50

time, which may represent inherently different underlying pathophysiologies [33].

Isolating a threshold for activation of MTP has identified issues with missing salvageable hemorrhaging patients who may expire prior to reaching the predetermined transfusion threshold. This "missed patient population" results in survivor bias in the traditional MT patient cohort. The issue of survivor bias has been discussed in previous research, primarily regarding transfusion ratios. Most patients who die of hemorrhage do so within the first 6 h of hospitalization, while the higher transfusion ratios (approaching 1 unit of PRBC to 1 unit of FFP) may not occur until later [39, 40]. In many institutions, patients must live longer to achieve a high ratio and thus only survivors have a high ratio. Patients that fail to meet the definition of MT, may not survive to trigger transfusion protocols, and are not included in the compilation of research focused on improving patient salvage. Most definitions of MT poorly reflect intensity of transfusion, suffer from survivor bias, and dilute the patient cohort with inappropriate patients. Therefore, Kashuk et al. proposed that the definition be changed to 10 units of PRBC per 6 h based on data that 80 % of transfusion requirements were completed within the first 6 h after emergency department admission [41].

Savage and Rhabar have been at the forefront of redefining MT. Savage identified a critical administration threshold (CAT) which was defined as 3 units per hour of PRBC and compared this to the current MT definition. CAT was found to be advantageous as it has the potential to rapidly identify patients requiring damage-control resuscitation, leading to earlier use of plasma and platelets. CAT may also serve as a platform for future research in this patient population. Prospective studies are underway to validate CAT [42]. Utilizing data from the PROMMTT (PRospective Observational Multicenter Major Trauma Transfusion) study found that patients who received four or more units of any resuscitative fluid had a much higher 6-h mortality rate [43].

MT definitions should be optimized to benefit patient outcome. Multiple studies have shown differences in mortality based on the volume of blood transfused. Brohi et al. showed that patients receiving 6–9 units of PRBC had nearly 2.5 times the mortality of patients receiving 0–5 units [44]. One of the difficulties of identifying an appropriate definition is the lack of prospective data or trials that would allow for a real-time calculation, while minimizing the survival bias. Multiple prediction models for MT have been proposed; however, many of these are retrospective. Current

definitions of massive transfusion are not supported by clinical outcomes and are not useful for guiding management. We do know that mortality increases with each PRBC unit required, although not linearly [45]. The minute-to-minute prediction of MT from standard admission parameters remains difficult. New approaches and prospective studies are required for the early diagnosis of patients with acute traumatic coagulopathy who will require transfusions [45]. Redefining MT in a manner that accounts for both the intensity of the ongoing resuscitation and survivor bias will lead to a more accurate method with which to identify potentially salvageable patients at highest risk of death from hemorrhagic shock.

Scoring Systems to Predict Massive Transfusions

Blood loss of 20–40 % of the circulating blood volume often causes a change in vital signs; blood loss of over 40 % leads to hemorrhagic shock including organ failure and cardiac arrest if it is not identified and corrected rapidly [46]. However, identifying patients who require massive transfusion remains a challenge.

Multiple scoring systems have been suggested with the intent to identify patients in need of MT. Scoring systems and predictive models rapidly identify patients at risk for MT. The issue remains that these are often retrospective and incorporate laboratory data, which take time to obtain delaying the care of critically injured patients. These scores were put in place to attempt to identify patients early, and avoid under treatment thus allowing the benefit from a balanced resuscitation [38].

Many of the scores contain binary variables as shown in Table 23.2. One of the first was the assessment of blood consumption (ABC) model which uses four parameters: Penetrating mechanism, positive FAST exam, arrival SBP <90 mmHg, and arrival pulse >120 beats per minute. A score of 2 was used as "positive" to predict MT. Sensitivity and specificity of these scores have been extensively studied [38]. The ABC score has been deemed one of the easiest to apply as each of the predictors is equally weighted and requires no calculation; the usability is translated to the trauma bay [47].

Other systems have been point based. The Trauma Associated Severe Hemorrhage (TASH score), utilizes seven independent variables

Table 23.2 Examples of multiple binary scoring systems

	TASH	Rainer	Vandromme	ABC	Schreiber	Larson
Variables						
Gender	×					
Pelvic fracture AIS ≥ 5	×	×				
Femur fracture AIS ≥ 3	×					
FAST+	×	×		×		
Heart rate	×	×	×	×		×
SBP	×	×	×	×	×	×
Hg	×					
Base excess/deficit	×	×				×
Mechanism of injury				×	×	
INR			×		×	
GCS ≤ 8		×				
Lactate ≥ 5			×			

Each system has a designated cutoff for individual variables which are included in the final scoring system as predictors of morbidity and/or mortality

AIS abbreviated injury scale, *FAST* focused assessment with sonography for trauma, *SBP* systolic blood pressure, *Hg* hemoglobin, *INR* international normalized ratio, *GCS* Glasgow coma scale

(heart rate (HR), SBP <90 mmHg, Glasgow coma scale, displaced pelvic/long bone fracture, CT scan or positive FAST for abdominal fluid, base deficit, and Hg) to determine an individual's risk of life-threatening hemorrhage and need for MT after severe trauma. The TASH-score is based on a point system [48, 49]. A score of ≥15 was associated with an MT prediction of 40 % and was found to improve time to transfusion and survival [50].

An analysis validated six existing scoring systems which included TASH, Prince of Wales Hospital/Rainer (PWH), Vandromme, ABC, Schreiber, and Larson scores [51]. Each score is determined retrospectively and has been found to be sensitive and specific for MT. The Vandromme score includes blood lactate, HR, INR, Hgb, and SBP. ABC uses only non-laboratory and non-weighted parameters that are available during the first minutes after admission of a trauma patient using the parameters listed above. The McLaughlin score consists of HR, SBP, and HCT. The Schreiber and Larson scores were developed in the military setting based on patients who suffered a higher incidence of blast injury and penetrating trauma. The Schreiber score predictors for MT are: Hgb, INR, and a penetrating mechanism of injury. Larson score includes: HR, systolic blood pressure, Hgb, and base deficit. Brokamp et al. validated these scores by assessing them in one large civilian trauma database and concluded that the TASH score was superior [51].

Consideration of Trauma Induced Coagulopathy in Massive Transfusion

Seven key initiators of trauma induced coagulopathy (TIC) have been identified; these include tissue trauma, shock, hemodilution, hypothermia, acidemia, inflammation, and consumption of coagulation factors. The mechanisms of non-transfusion related coagulopathy have been previously described in this publication. Hemodilution from transfusion of fluids (crystalloid or colloid) during resuscitation is associated with a dose dependent increase in coagulopathy [11, 52, 53]. Crystalloid transfusions also promote hypothermia and acidosis, which further exacerbate coagulopathy. Hypothermia causes inhibition of coagulation and platelet function while acidosis, a consequence of the low-flow shock state and chloride administration, impairs patients' response to fluids [54–56].

Prior to arrival to the hospital, the massively bleeding patient has numerous reasons to have abnormalities in coagulation. Rapid resuscitation to correct tissue ischemia, hypothermia, and coagulopathy is a priority. With this in consideration, hemostatic resuscitation is essential. For the majority of these patients there is no time to wait for laboratory analyses to guide initial product administration. Therefore, the blood bank and the trauma service must be familiar with the institutional MTP to efficiently manage these high mortality patients. The early phase of a massive transfusion and attenuation of TIC is referred to as "damage control resuscitation" (DCR).

Damage Control Resuscitation

Hemorrhage control utilizing damage control techniques such as packing and the Pringle maneuver was first described for liver surgery in the early twentieth century by Pringle [57]. The benefits of rapid resuscitation were described by Shaftan in 1963 who showed an association with decreased mortality in patients with liver injury [58]. DCR differs from conventional resuscitation in that it attempts an earlier and more aggressive correction of coagulopathy and metabolic derangements [59, 60]. Key components include permissive hypotension, preferential use of blood products over isotonic fluids, and early correction of coagulopathy with goal-directed component therapy [33, 61]. This early administration of blood products allows for prevention and treatment of coagulopathy and for the ability to temporize ongoing hemorrhage in order to restore blood volume and hemodynamic stability [62].

The term DCR was coined in 1976 by Lucas and Ledgerwood regarding treatment of military casualties in the field [63, 64]. A clinical practice

guideline for DCR was first developed by the United States Army Institute of Surgical Research in 2004 which established the basic principles [65]. Patients in need of DCR constitute those with severe metabolic compromise. These patients continue to experience the sequelae of shock that manifest as persistent hypothermia, persistent metabolic acidosis and nonsurgical bleeding. Excluding head injured patients, DCR patients are resuscitated in the ED by keeping the SBP around 90 mmHg which prevents recurrent bleeding from recently clotted vessels while restoring intravascular volume in a 1:1 or 1:2 ratio with FFP and PRBC [9, 10, 33, 62, 66, 67].

The overarching goal of DCR combines minimizing fluid resuscitation with crystalloid, hemorrhage control via surgery while allowing permissive hypotension to address the lethal triad. Stone et al. published one of the first retrospective studies to report on traditional fluid resuscitation versus DCR. They showed that 14 % of patients managed with fluid resuscitation prior to surgery survived versus 93 % of those who underwent immediate packing resulting in rapid hemorrhage control [68]. Furthering the concept of rapid resuscitation, the technique of truncating laparotomies was adopted. This included surgical exploration of hemorrhage with intra-abdominal pack tamponade followed by a definitive surgical repair once the patient was stabilized [68]. Truncation laparotomy proved beneficial and was associated with decreased mortality for previously reported "nonsalvageable" cases. Many other institutions showed success with the concept of abdominal packing, finding up to a 70 % salvage rate of critically ill patients [63, 69–71].

The damage control resuscitation philosophy also dictates minimization of crystalloid to avoid dilutional coagulopathy and this practice has been associated with increased transfusion of plasma [72]. Prior to MTP, administration of platelets and FFP target laboratory values when the PT and PTT are <1.5 times normal and platelets are $<50 \times 10^9$/L. An observational study showed that patients who received FFP and continued to bleed (37 %), had lower fibrinogen levels suggesting that FFP did not contain sufficient levels of fibrinogen to achieve hemostasis [73]. Finally, lyophilized plasma is being developed that can be reconstituted quickly at the time and site of use and is already available and used in select European countries [74].

Why Have a Massive Transfusion Protocol?

The importance of the MTP is to optimize communication and coordination of care of the massively bleeding patient [75]. The US military was the first to show the success of MT guidelines in 777 patients; improvement in temperature on arrival to the ICU and reduction in resuscitation fluid [76, 77]. Early prediction of the need for MT is very difficult to establish, but could contribute to improving the development of MTPs. Scoring systems are particularly useful for identifying subjects at low risk of requiring MT, and helping to be objective in applying resuscitative measures [49, 78].

Massive Transfusion Protocols

Damage control resuscitation was most likely the catalyst for the development of modern MTPs [18, 33, 79]. The concept of hemostatic resuscitation through the implementation of MTPs, has been developed to prevent death from hemorrhage for patients with life-threatening bleeding from traumatic injury [33]. The importance of early identification of those needing MT using physiologic and hemodynamic transfusion triggers dates back to the 1990s. Ability to identify critically injured patients on initial evaluation has led to MTPs based on collaborations with trauma surgery, blood bank, and anesthesiology [16, 27, 80].

Commonly proposed triggers that were correlated with the need for MT include systolic blood pressure (SBP) <90 mmHg, hemoglobin (Hgb) <11 g/dL, temperature <35.5 °C, international normalized ratio (INR) >1.5, and base deficit >6. When three or more of these triggers were present, the likelihood of MT was increased [18–21, 52, 79]. Spearheading the way in 2005, Cotton

et al. implemented an MTP in which predefined blood product ratios were transfused at their institution [76]. Retrospectively, they found early protocol activation and achievement of predefined plasma and platelet ratios were independent predictors of survival, while emergency department activation and direct blood bank notification by the trauma attending were associated with a reduction in blood product waste [76].

Hyperfibrinolysis contributes to the coagulopathy of trauma. Antifibrinolytic agents are known to reduce blood loss by preventing plasminogen from binding to fibrin and by preventing plasmin degradation of platelet glycoprotein Ib receptors. Tranexamic acid (TXA) is a lysine analog which inhibits plasmin fibrinolysis, therefore stabilizing the clot. It has shown to provide a significant survival benefit in the CRASH-2 trial (clinical randomization of TXA in hemorrhage 2) where randomization of 20,000 patients with significant bleeding to placebo or TXA occurred. The group treated with TXA had a significant survival benefit within the first 3 h after injury [81]. TXA is often incorporated into MTP to assist with coagulation.

Implementation and Characteristics of Centers with MTPs

Modern MTPs are developed by multidisciplinary teams and blood bank services in which preset packages of various blood components in a predetermined sequence are delivered until hemorrhage slows and laboratory tests can guide further transfusions (Fig. 23.1). This standardization helps to

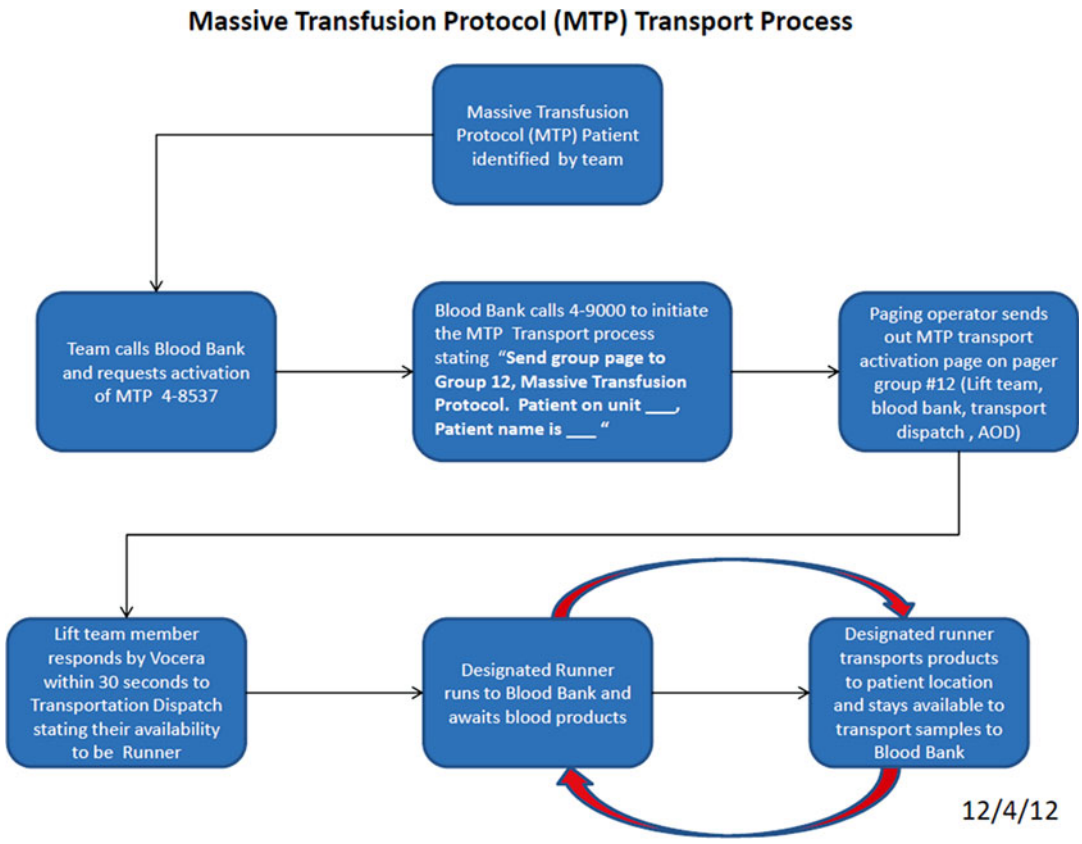

Fig. 23.1 Oregon Health & Science University (OHSU) MTP transport and initiation process. Each institution designs their site specific protocol involving multiple services each designated with a precise task

provide earlier administration of blood products during the resuscitation phase, improved overall efficiency, and decreased total blood product use during a patient's hospital stay resulting in substantial economic savings [82]. Additionally, the amount of crystalloid infused is decreased, thus reducing dilutional coagulopathy [62]. Once hemorrhage is controlled, the MTP is discontinued and further transfusions guided by clinical and laboratory assessment. Each MTP activation and execution is reviewed by a transfusion medicine service physician within 24 h, at which time feedback is shared with participants involved in the MTP. The hospital transfusion committee also provides oversight related to the MTP, including product waste and noncompliance.

A typical MTP requires activation by staff. In response to the activation, the blood bank immediately places 6 units of group O or type-specific PRBC and 6 units of group AB FFP in a cooler as the "initiation package" or "box of blood" (Table 23.3). For the purpose of MT, the blood bank maintains an inventory of thawed plasma products for immediate release. Once the thawed plasma is distributed additional FFP is thawed. During the period of hemorrhage, the blood bank prepares packages with a ratio of PRBC–FFP–PLT of 1:1:1 as needed. Laboratory testing is performed to gauge the efficacy of the MTP and to guide it once bleeding slows [27].

With the most severe injuries, the anecdotal experience and clinical series from the military setting suggest that fresh whole blood results in the most effective correction of coagulopathy. Component therapy remains the standard which limits effectiveness as there is a limited capacity to achieve 100 % of normal coagulation activity [7]. The combination of plasma, platelets, and PRBC in a 1:1:1 ratio is estimated to result in a hematocrit of 25 %, coagulation factor activity of 62 %, and platelet concentration of ~50×10^9/L and a fibrinogen concentration of 750 mg/dl. In comparison, a unit of fresh whole blood has a hematocrit around 45, 100 % coagulation factor activity, a platelet concentration of 200×10^9/L, and a fibrinogen concentration of 1500 mg/dl [7, 83].

Schuster et al. performed a survey of trauma centers in which 186 responses were questioned in 2010. Level 1 trauma centers were the first to adopt MTPs; however, only 85 % of centers had a protocol in place, many of which had been recently implemented within the previous year. The blood product components of each protocol differed; 23 % had one set of components, 25 % had two or three, 41 % had more than three, and 11 % did not release components in sets. When FFP was part of the first set, it was present in a ratio of 1:1 in 81 %. In the second set, FFP and PLT increased. Most second sets (98 %) contained FFP and 67 % contained PLT. In all cases, the third sets had FFP, 90 % of which were in a ratio of 1:1, and 92 % had PLT. Calcium was a component in 20 % of MTPs. The MTP was activated by trauma surgeons, anesthesiologists, other surgeons, blood bank physicians, and automatic activation. It was found that trauma surgeons most commonly activated the protocol

Table 23.3 Constituents of an MTP when ordered comes as a "Box of Blood"

Product	Box of blood	MTP	Super MTP (SMTP)
RBC	Issue 6 units	Issue 6 units at start of MTP Stay ahead 6 units for next 24 h Issue additional units as directed	Issue 6 units at start of SMTP and 10 units every 15 min or as directed
PPH	Issue as directed	Issue as directed	Issue 1 unit at start and 1 unit every 15 min or as directed
Thawed FFP	Issue as directed	Issue 6 units of A if type unknown (or type compatible) at start of MTP Stay ahead 6 units while on MTP	Issue 8 units A plasma, if type unknown ASAP and then 8 units type compatible every 15 min thereafter
Cryo	Issue as directed	Thaw and issue units as directed	Thaw and issue units as directed

Table 23.4 Roles of team members when an MTP is initiated

Policy:	
Responsible	Action
Trauma Team	1. Calls blood bank at x48537 and requests activation of MTP
Blood Bank	2. Calls x49000 to initiate the MTP Transport Process stating "send group page to Group 12, Massive Transfusion Protocol. Patient on unit _____, Patient name is _____"
Paging Operator	3. Send out MTP transport activation page group 12 (lift team, blood bank, transport dispatch, AOD)
Transportation Aide (lift team member)	4. Responds by Vocera within 30 s to Transportation Dispatch stating their availability to be Runner 5. Runs to Blood Bank and awaits products 6. Transports products to patient location and remains available to transport samples to Blood Bank 7. Repeat steps 5 and 6 above until massive transfusion complete
ICU Nurse	8. Notify the Transportation Aide when the Massive Transfusion/Box of Blood Protocol is over

(94 %) followed by cardiothoracic surgery (2 %). The frequency of MTP activation was also calculated. Eight percent of centers activate the MTP once per week or more often, 53 % activate less than once per week but more than once per month, and 37 % activate less than once per month but more than once every 6 months. In 81 % of institutions, MTPs are reviewed by a committee. The important trends identified by Schuster concluded most MTPs had been in place 5 years or less and MTPs were more common in Level 1 and Level 2 centers [84]. Prospective randomized studies are needed to confirm current retrospective data that will allow trauma centers to develop evidence-based MTPs.

At Oregon Health & Science University (OHSU), MTP is initiated when the clinical team or a pathologist directs the Transfusion Service to activate the MTP. Care providers including surgeons, anesthesia, emergency physicians, nursing, pathology, blood bank, transfusion services and ancillary staff work closely in conjunction to facilitate an MTP as outlined in Table 23.4. Prior to the initiation of an MTP it is the responsibility of the blood bank to have a minimum of 4 units of FFP thawed and available for immediate transfusion. The clinical team initiates the need when they feel it necessary and provides the laboratory with the patient identifying information. An MT or a super MTP may be declared. If the latter true, it indicates that the consumption of blood products is ongoing and the blood bank should have 60 units of PRBC prepared. It is important not to delay the release of blood products. Uncrossmatched blood (type O) is used until the patients' blood type is confirmed. Women under the age of 45 receive O negative PRBC, all other patients receive O positive PRBC. The pathology department is notified when a second box of blood (1 box PRBC = 6 units) is requested or 10 or more units of PRBC are issued within 2 h to ensure that coagulopathy panels are collected in a timely manner (Table 23.5). Pathologists ensure that the patients' coagulopathy is monitored every 30–60 min until they are deemed stable.

Table 23.5 The differences between a box of blood and an MTP

Product	Box of blood	MTP
RBC	Issue 6 units	Issue 6 units at start of MTP Stay ahead 6 units for next 24 h Issue additional units as directed
PPH	Issue as directed	Issue as directed
Thawed FFP	Issue as directed	Thaw 6 units at start of MTP Stay ahead 4 units while on MTP Issue units only when directed
Cryo	Issue as directed	Thaw and issue units as directed

Discontinuing an MTP is the role of the surgeon and occurs when the patient has stabilized and surgical bleeding is controlled. The products that have been transfused are documented and transfusion services are notified to halt further

delivery of products. It is important to communicate any anticipated future transfusion needs with the blood bank. Lastly, all unused blood products should be returned to the blood bank.

Laboratory Assessment of Coagulopathy During MT

In massively bleeding patients, often there is not time to await laboratory analyses to guide product administration and once the tests are available the coagulation milieu has changed. Thus, novel massive transfusion protocols were developed to treat severely bleeding patients empirically. Commonly, laboratory tests such as PT/PTT, INR, fibrinogen, and platelets are used to guide resuscitation. TEG is a laboratory test that examines whole blood clot formation and lysis in real time. It has been shown that coagulation tests are suboptimal in the management of massive hemorrhage, as they are laborious and time consuming. TEG rapidly assesses the coagulation cascade from the initial platelet–fibrin interaction to clot lysis, allows rapid point-of-care testing of coagulation and is useful in assessing fibrinolysis. Routine use of TEG to guide transfusion may optimize the utilization of blood components as it provides real-time interactive data which is promptly available in which to further guide resuscitation of the patient.

TEG is a simple, fast test that can broadly determine coagulation abnormalities and give information about fibrinolytic activity and platelet function. It has been extensively used in hepatic and cardiac surgery to guide resuscitation and has been shown in blunt trauma to be associated with specific transfusion requirements [85]. Tapia et al. compared TEG directed resuscitation with MTP resuscitation in patients receiving massive transfusions as they are coagulopathic and even with aggressive resuscitation only 65 % of coagulation factor activity is delivered [86]. TEG directed therapy was found to be superior to MTP in both blunt and penetrating injury. This study suggested that TEG in conjunction with MTP would provide further assistance in resuscitation of patients and avoid unnecessary transfusions.

The parameters analyzed on a TEG produce a comprehensive tracing of the clotting cascade more-so than laboratory values. It is known which blood components are responsible for phases of clot formation, any irregularity in the TEG will help guide product administration. A normal TEG in the presence of abnormal vital signs may indicate surgical bleeding and the need for exploration. This could, theoretically, reduce transfusion requirements of patients arriving in emergency departments, as it has for patients undergoing cardiopulmonary bypass [59].

Both FFP and platelet use have been diminished with the use of TEG, with no adverse effects on blood loss or on rates of reoperations for bleeding [87, 88]. Schochl has published the use of TEG to guide infusion of fibrinogen concentrate and PCC as primary therapies in coagulopathic trauma patients. The use of fibrinogen concentrate and PCC in these studies has resulted in minimal use of plasma and platelets and better than predicted outcomes. Routine use of TEG to guide transfusion may optimize the utilization of blood components [89, 90]. This was shown in a study out Ben Taub looking at TEGs before and after initiation of an MTP using 1:1:1. The findings showed that TEG-directed resuscitation is equivalent to standardized MTP for patients receiving 6 units or more PRBC [86].

Complications of MT

Numerous adverse effects of blood transfusions can cause early and late complications [91]. Concerns regarding massive transfusion include 1:1 resuscitation resulting in excessive plasma administration, which may result in fluid overload, TRALI, and DVT/PE, and increased risk of organ failure. Prior to implementation of MTPs, concern for infection, infectious complications and lung injury due to amount and type of resuscitative fluids administered, were of great concern. Two year post-injury complications were examined and revealed reductions in pneumonia, pulmonary failure, open abdomens, and abdominal compartment syndrome after MTP imple-

mentation with early initiated ratio based regimens. Regarding in-hospital complications, sepsis and multiorgan failure were also lower, and there were more ventilator-free days in the protocol patients [92–95].

Complications can be caused by the process of blood and product preservation. Adverse reactions occur in at least 20 % of all transfusion [96]. MT patients receive large amounts of blood from multiple different donors; studies have shown that trauma patients who received 25 units PRBC were had the potential to be exposed to 80 different donors [91]. Biologic or physiologic effects of blood preservation and storage that can cause complications include increased acidity, elevated potassium, and decrease in 2,3 diphosphoglycerate (both affect oxygen transport), the quality of erythrocytes, and platelet function.

Transfusion reactions can be immediate and life threatening or insidious. Acute hemolytic transfusion reaction is caused by an antigen–antibody reaction to ABO incompatibility, usually due to errors in administration of incompatible blood. Symptoms of fever and hypotension can occur with a minimal amount of infusion. Delayed hemolytic reactions can occur but also can go unnoticed. These reactions typically occur 2–14 days after transfusion with signs of fever, jaundice, and anemia as a result of clearance of the antibody coated erythrocytes.

Anaphylactic reactions are rare. Signs can include swelling, hypertension, fevers, chills, flushing, and gastrointestinal distress. Febrile non-hemolytic reactions are associated with fever within 2 h of transfusion and are managed medically.

Transfusion related acute lung injury (TRALI) occurs secondary to antibodies in the donor blood against recipient leukocytes. Symptoms include bronchospasm, hypoxia, fever, and diffuse bilateral pulmonary infiltrates which occur within 6 h of transfusion and may require mechanical ventilation. TRALI is defined as ALI PAO_2/FIO_2 (ratio <300 mmHg) within 6 h of transfusion. TRALI is mainly associated with plasma from women due to a cross reaction with antibodies to the fetus and the incidence has significantly decreased since removal of female plasma from blood banks, but it remains a significant concern in patients requiring MT. Although the incidence is lower than other complications, it is life threatening and mortality is up to 10 % [97]. Transfusion-associated circulatory overload (TACO) and transfusion-related immunomodulation (TRIM), must also be recognized as potential complications. TRIM is associated with impaired wound healing, ALI, multiple organ failure, and increased cancer risk [97].

Citrate, an additive to stored blood, is normally excreted by the liver, but when a patient receives more than 10 units of PRBC the capacity of the liver to metabolize it is impaired. Elevated blood citrate levels are associated with hypocalcemia which leads to decreased ventricular contractility and decreased peripheral vascular resistance causing hypotension.

Hyperkalemia is a common complication as potassium leaks out of erythrocytes during storage [91]. Lastly, acid base disturbances occur during MT. Stored blood and other resuscitative products such as crystalloid are acidotic. To add to the acidity, stored blood becomes increasingly acidic when it is older than 3 weeks [91].

MTP Outcomes

With MTPs in place, institutions began prospectively collecting data on all protocol activations, monitoring in a "real-time" fashion for the first year and evaluated quarterly. A retrospective cohort study of all MT protocol activations (69 patients) from one center was compared with a pre-MT protocol cohort of trauma patients who received standard MT (70 patients) [19, 92]. Given a relatively similar population, there was a 74 % reduction in the odds of mortality in MT patients with the implementation of the protocol, a decrease in PRBC, plasma, platelet use, and the release time for products was dramatically reduced to <10 min for the first "cooler"; the time between the first and second "coolers" was reduced from 42 to 18 min [19, 92]. This decrease in blood product use was calculated to result in a cost-savings of $2270 per patient or an annual savings of $200,000 [82, 95].

Further evaluation of the MTP in four separate studies concluded that implementation of the MTP was associated with better outcomes; improved mortality, faster time to first cross-matched blood product transfused, no change in the FFP–PRBC ratio, reduction of 30-day mortality and length of stay, fewer overall blood products, and fewer complications. The use of MTPs appeared to be associated with a reduction in mortality among trauma patients requiring MT without a clinically significant increase in the number of PRBC transfused, and a potential reduction in the amount of plasma transfused [27, 82, 98].

Prospective Studies in Massive Transfusion

Prospective Observational MT Trial

With the development of MT protocols at many trauma centers, a concern has been raised as to how to prospectively identify patients who are at a high risk for MT. The importance of properly identifying high risk patients is not only related to conservation of limited resources in the hospital and blood bank, but more importantly toward the identification of the resuscitation strategy that will most benefit this population.

The PROMMTT study provides support for the early application of plasma in a high ratio. In this multicenter study, data were collected from 1245 trauma patients across ten level I trauma centers in the United States, all of whom received blood products within 6 h of admission. Real time recording of administration time and volumes of blood products was performed. In a subset of patients who received a minimum of 3 units of blood, median time to death from hemorrhage was 2.6 h, and a survival advantage was seen in those who received higher ratios of plasma to RBCs. The survival advantage provided by higher ratios of plasma was greatest when it was achieved in the first 6 h of treatment, as compared to patients who received more plasma later in their resuscitation [61]. From the PROMMTT database, it has been shown that early resuscitation greater than 4 units of any fluid was associated with sicker patients and higher mortality which proves to be a good initial indicator for need for fluid resuscitation and possibly MT. Additionally, patients who required greater than 4 units PRBC within 3 h were likely to require MT. MT protocols and higher ratios of blood components appear to be associated with improved survival in patients with exsanguinating hemorrhage.

PROMMTT also showed that hemorrhagic deaths occur rapidly within a median time of 2.7 h of injury. PROMMTT is unique in that it begins to define MT by rate. By quantifying severe hemorrhage, the overall MT definition was improved [27, 43, 78, 99]. Better quality evidence involving the optimal ratio of plasma to RBC transfusion in severe trauma has been collected as part of the PROPPR (Pragmatic Randomized Optimal Platelet and Plasma) trial. In this multicenter trial, patients were randomized to a PRBC–FFP–PLT ratio of either 1:1:2 or 1:1:1. The results of PROPPR, which has reached its enrollment goal, will provide level one data regarding the utility of increased plasma and platelet ratios in the setting of MT. This trial should further help define the optimal PRBC–FFP ratio in the trauma population as well as examine the utility of adjunctive agents.

The ideal ratio of PRBC, FFP, and platelet transfusion remains under investigation. Recently published reports and current clinical guidelines recommend a 1:1:1 ratio (each unit defined as the amount in 1 unit of whole blood) for component therapy in an attempt to recreate whole blood [9, 100, 101]. This theory is based on the findings above; however, the ratio has been challenged by studies which have found the optimal ratio of plasma to be in the 1:2–1:3 range [67, 102]. To date, much has been published on the need for prospectively randomized trials regarding ratios. Most conclude that a fixed ratio is necessary. It was determined that the administration of a fixed ratio of 1:1:1 was feasible to implement in a small prospective trial of 69 patients, 37 in the fixed-ratio and 32 in the control group (laboratory guided transfusion) where 51 % of fixed ratio patients (21/37) compared to 6 % (2/32) of the control group had 1:1:1 transfusion achieved

[103]. Although the 28-day mortality in this study showed no statistical difference between the two groups, it proved that balanced transfusions can be achieved in a civilian setting. This study laid the groundwork for the Pragmatic, Randomized Optimal Platelets and Plasma Ratio (PROPPR) study.

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Fibrinogen and Clotting Factor Replacement

24

Massimo Franchini

Introduction

Severe trauma is a major cause of mortality, with more than 5 million deaths annually worldwide [1]. Uncontrolled post-traumatic bleeding is the leading cause of death among these patients and is a major challenge for trauma care providers [2]. Blood loss that leads to an endogenous derangement of haemostasis, as well as dilution and consumption coagulopathy confounds achieving haemorrhage control in these patients. It has been estimated that about one third of all bleeding trauma patients present with a coagulopathy upon hospital admission [3–5]. This early trauma-induced coagulopathy (TIC), which significantly worsens the patients' prognosis [6], is nowadays a well recognised multifactorial condition resulting from a combination of bleeding-induced shock, tissue injury-related thrombin–thrombomodulin complex generation and the activation of anticoagulant and fibrinolytic pathways [7, 8]. In this context, a prompt recognition of this underlying acquired coagulation disorder is mandatory to start immediately the most appropriate treatment. This chapter is focused on the role of fibrinogen and prothrombin complex concentrates (PCC) in the management of trauma-induced coagulopathy.

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Fibrinogen Replacement Therapy

The rationale for fibrinogen supplementation in severely injured trauma patients is based on the fact that fibrinogen seems to be the most vulnerable coagulation factor in this clinical setting, reaching critical levels earlier than any other coagulation protein [9–13]. Experimental data from animal studies and in vitro studies have shown that the application of exogenous fibrinogen, at a dose of 70–250 mg, increases clot firmness and reduces blood loss after blunt liver injury [14–16]. Clinical data from surgical patients have shown that post-operative haemorrhagic tendency is increased when fibrinogen levels are below 150–200 mg/dL [17, 18]. Current guidelines now recommend maintaining a fibrinogen level above this threshold in bleeding trauma patients [1, 19]. Evidence supporting the administration of fibrinogen to injured patients is derived from a retrospective study including 252 seriously injured soldiers and civilians who received a massive transfusion (defined as >10 U of red blood cells [RBC] in 24 h). The analysis of these data showed that the strategy of increasing the fibrinogen/RBC ratio (low [<0.2 g fibrinogen for each RBC Unit given] or high [≥0.2 g fibrinogen for each RBC Unit given]) was independently associated with

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improved survival to hospital discharge, primarily by decreasing death from haemorrhage [20]. An observational prospective study of 517 trauma patients demonstrated that low admission fibrinogen (<150 mg/dL) was associated with increased Injury Severity Score (ISS), shock and pre-hospital fluid volume requirements, and was an independent predictor of mortality at 24 h and 28 days [21]. Similarly, a recently published study of 260 critically ill patients who required massive transfusion demonstrated that patients with fibrinogen level <100 mg/dL had significantly increased in-hospital mortality (51.9 % versus 18.5 %) compared to patients with normal fibrinogen levels and that the critically low fibrinogen level was the most important predictor of mortality [22].

Fibrinogen supplementation can be provided by transfusion of fresh-frozen plasma (FFP), cryoprecipitate and fibrinogen concentrate [23, 24]. Chambers and colleagues [25] found that fibrinogen deficiency was almost always the initial haemostatic abnormality in patients with severe trauma, and that a change in haemostatic therapy toward a high ratio of FFP to RBC was not sufficient to overcome fibrinogen depletion within the first 4 h of treatment. Given the low concentration of fibrinogen (approx. 400 mg in 1 U of FFP) contained in each unit of FFP, correction of fibrinogen with FFP would require a large volume of this blood product. Thus, the risk of transfusion related complications (i.e. transfusion-related acute lung injury [TRALI], transfusion-associated circulatory overload [TACO] and viral transmission]), FFP is not a suitable haemostatic therapy to restore fibrinogen levels in this clinical setting [9].

Although cryoprecipitate contains a higher concentration of fibrinogen than FFP, the fibrinogen concentration is not standardised and ABO blood group matching is needed prior to transfusion. Time is also required to thaw cryoprecipitate, and this aspect represents a clear disadvantage in the emergency setting of massive haemorrhage. Furthermore, it carries a risk of viral transmission similar to that of FFP [9]. For these reasons cryoprecipitate has disappeared from blood banks in most European countries [24]; however, it remains the most common source of fibrinogen replacement in the USA. Thus, depending on your global location, the most suitable source of fibrinogen may be provided by fibrinogen concentrates.

Fibrinogen Concentrate

Fibrinogen concentrate is produced from pooled human plasma using the Cohn/Oncley cryoprecipitation procedure [26]. The concentration of fibrinogen is standardised; the product is stored as a lyophilised powder at room temperature and can be reconstituted quickly with sterile water and infusion volumes are low, allowing for rapid administration without delays for thawing or cross-matching [27]. In contrast to FFP and cryoprecipitate, viral inactivation steps by solvent/detergent exposure or pasteurisation are routinely included in the manufacturing process for fibrinogen concentrate, thus minimising the risk of viral transmission [28]. Four fibrinogen concentrates are currently available, as reported in Table 24.1. However, the most widely used is Haemocomplettan (CSL Behring, Marburg, Germany), a human pasteurised, highly purified, plasma-derived fibrinogen concentrate [29, 30], and a number of studies have evaluated the effects of fibrinogen supplementation with this agent in patients suffering from various forms of congenital or acquired hypofibrinogenaemic conditions [31, 32].

The main studies on the use of fibrinogen concentrate in trauma patients are summarised in Table 24.2. In a recent published study including 128 bleeding trauma patients, goal-directed coagulation management using thromboelastometry-guided administration of fibrinogen concentrate together with prothrombin complex concentrate was evaluated retrospectively. Observed mortality rate was lower in these patients compared with the mortality predicted by the Trauma Injury Severity Score (TRISS) and the Revised Injury Severity Classification score (RISC) [33]. In a subsequent retrospective study conducted by the same group, comparing blood product requirements between trauma patients treated with fibrinogen concentrate (median dose: 6 g) and/or prothrombin

Table 24.1 Main characteristics of the currently registered fibrinogen concentrates (see Ref. 46)

Brand	Company (site of manufacture)	Purification	Viral inactivation	Comments
Clottagen	LFB (France)	Adsorption on aluminium hydroxide gel; ion exchange chromatography and affinity chromatography	TNBP/polysorbate 80	–
Fibrinogen HT	Benesis (Osaka, Japan)	Ethanol fractionation; glycine precipitation	TNBP/polysorbate 80; dry heat, 60 °C, 72 h; 35 nm nanofiltration	No albumin added
FibroRAAS	Shanghai RAAS (Shanghai, China)	Multiple fractionation	TNBP/polysorbate 80	–
Haemocomplettan	CSL Behring (Marburg, Germany)	Multiple precipitation	Pasteurisation at 60 °C, 20 h	Albumin added

Abbreviations: *TNBP* tri-n-butyl phosphate

complex concentrate, but no FFP, and patients receiving only FFP; those patients receiving fibrinogen concentrate and/or PCC only required significantly less RBC and platelet transfusions than those treated with FFP only [34]. Innerhofer and colleagues [35] compared patients who received fibrinogen concentrate without FFP ($n=66$) versus fibrinogen concentrate with FFP ($n=78$), reporting a lower volume of blood products transfused, including RBC and platelets, in patients receiving only fibrinogen concentrate. There was no difference in clinical outcomes. This retrospective study increased the robustness of its findings by adjusting for patient severity using propensity scores. This propensity score–matched analysis conducted in 28 patient pairs confirmed the results. A retrospective study of 294 trauma patients further evaluated whether administration of fibrinogen concentrate is associated with improved outcomes [36]. Although 6-h mortality was significantly reduced in the fibrinogen concentrate group, overall mortality was not significantly different between groups. In contrast to other studies, RBC requirement was not reduced in the fibrinogen concentrate group. The only published prospective observational study in this clinical setting is that by Weiss and colleagues [37]. A total of 223 patients were included, of whom 62 (28 %) were trauma patients and received fibrinogen concentrate in association with FFP. After a median dose of 12 g of fibrinogen, plasma fibrinogen rose to 2.19 g/L at the end of surgery, corresponding to a median increment of 0.045 g/L per gram of fibrinogen administered. Three percent of patients sustained thromboembolic complications perioperatively. Interestingly, the authors found that plasma fibrinogen at the end of surgery and 24 h after administration of fibrinogen concentrate was significantly higher in the survivors compared with the non-survivors. A systematic review on the use of fibrinogen concentrate in trauma patients has been recently published [38]. From the analysis of the 12 articles identified, it emerged that fibrinogen concentrate administration was safe and associated with reduced blood product requirement. Two randomised trials have assessed the role of fibrinogen concentrate in cardiac surgery [39, 40]. In the first trial, involving 20 patients undergoing elective coronary artery bypass graft, the infusion of 2 g of fibrinogen concentrate significantly reduced post-operative blood loss [39]. In the second more recent study, thromboelastometric-guided intraoperative haemostatic therapy with fibrinogen concentrate was more effective than placebo in controlling bleeding during major aortic replacement surgery [40].

Current European guidelines on the management of bleeding and coagulopathy following major trauma recommend treatment with fibrinogen concentrate if significant bleeding is accompanied by signs of a functional fibrinogen deficit

Table 24.2 Main clinical studies evaluating fibrinogen concentrate in trauma patients

First author, year [reference]	Study design	Population (*n*)	Trigger for administering fibrinogen concentrate	Fibrinogen dose	Outcomes	Main results
Schöchl, 2010 [33]	Retrospective	128	ROTEM MCF <10 mm	Median 7 g	Comparison of the observed mortality with the predicted mortality by TRISS and RISC	The observed mortality was 24.4 %, lower than the TRISS mortality of 33.7 % ($P=0.032$) and the RISC mortality of 28.7 % ($P>0.05$)
Schöchl, 2011 [34]	Retrospective	681 (80 FC ± PCC vs 601 FFP)	ROTEM MCF < 10 mm	Median 6 g	PLTS and RBC units requirement; mortality	RBC: 71 % of patients in the FC group vs 97 % in the FFP group ($P<0.001$). PLTS: 9 % of patients in the FC group vs 44 % in the FFP group ($P<0.001$). No mortality difference
Innerhofer, 2013 [35]	Retrospective	144 (66 FC vs 78 FC + FFP)	ROTEM MCF < 7 mm; fibrinogen level < 1.5–2 g/L	2 g FC only; 4 g FC + FFP	Coagulation parameters before and after treatment; blood products for the first 24 h; clinical outcomes	Less blood products transfused in the FC group (RBC: 2 U vs 7 U, $P<0.001$; PLTS: 0 U vs 1 U, $P<0.001$). No difference in clinical outcomes
Wafaisade, 2013 [36]	Retrospective	588 (294 with FC and 294 without FC)	NA	NA	6 h, 24 h, 30 days and hospital mortality; MOF incidence	6 h mortality: 10.5 % (FC^+) vs 16.7 % (P 0.03). No difference in 24 h, 30 days, and hospital mortality. MOF 61.2 % in the FC^+ group vs 49 % ($P=0.003$)
Weiss, 2011 [37]	Prospective observational	62	Fibrinogen level <1.45 g/L, blood loss 2.0 L	4 g	Hospital mortality	Significant correlation between plasma fibrinogen level at the end of surgery and at 24 h post-FC

Abbreviations: *ROTEM* thromboelastometry, *MCF* maximum clot firmness, *TRISS* trauma injury severity score, *RISC* revised injury severity classification, *FC* fibrinogen concentrate, *FFP* fresh frozen plasma, *PCC* prothrombin complex concentrate, *MOF* multiple organ failure

at viscoelastic tests (i.e. rotational thromboelastometry [ROTEM, TEM Innovation GmbH, Munich, Germany] and thromboelastography [TEG, Haemonetics Corp, Niles, IL, USA]) (Grade 1C). An initial fibrinogen concentrate dose of 3–4 g is suggested, with successive doses guided by viscoelastic monitoring and laboratory assessment of fibrinogen levels (Grade "C") [1]. In the USA, fibrinogen is typically replaced by cryoprecipitate administration (each unit of cryoprecipitate contains 150–250 mg of fibrinogen): 1 U/10 kg increases plasma fibrinogen by 50–70 mg/dL [41]. Differing from Europe, in the USA the fibrinogen concentrate is not approved for patients with acquired bleeding and thus its use in the trauma setting is limited. As regards the costs, although fibrinogen concentrate may be perceived as being more expensive than allogeneic blood products, a recent cost-effective analysis showed that the overall costs (including compatibility testing, thawing and administration) of cryoprecipitate and fibrinogen concentrate are quite similar [42].

Clotting Factor Replacement Therapy

Prothrombin Complex Concentrates

To increase impaired thrombin generation, PCC have been preferentially used as coagulation therapy during trauma related bleeding [43]. Prothrombin complex concentrates, which were originally developed as a source of factor IX for the treatment of patients with haemophilia B [44, 45], are produced by ion-exchange chromatography from the cryoprecipitate supernatant of large plasma pools after removal of antithrombin and factor XI [46]. Different processing techniques involving ion exchangers permit the production of either three- (i.e. factors II, IX and X) or four-factor (i.e., factors II, VII, IX and X) concentrates with a final overall clotting factor concentration approximately 25 times higher than in normal plasma (see Fig. 24.1 for the mechanisms of action of PCC) [46, 47]. Four-factor PCCs are currently not available in some countries (e.g. the USA). To prevent activation of these factors, most PCC contain heparin. Prothrombin complex concentrates may also contain the natural coagulation inhibitors protein C and protein S. PCCs are standardised according to their factor IX content. All PCCs undergo at least one step of viral reduction or elimination (solvent detergent treatment, nanofiltration, etc.). Data on PCC pharmacokinetics are scant. The half-lives of the four clotting factors differ widely. The half-life of prothrombin is much longer (60–72 h) than that of the other factors (6–24 h). Factor VII has the shortest half-life (approximately 6 h) [48]. Importantly, the long half-life of prothrombin needs to be taken into account when considering the potential accumulation of prothrombin after multiple dosing. PCC are lyophilised, requiring reconstitution in a small volume and can be administered rapidly (e.g. over 10 min). Table 24.3 summarises the main characteristics of the currently available PCC.

Limited data are currently available on the use of PCC in trauma patients [49]. A part the two above mentioned study by Schöchl and colleagues [33, 34], robust data arising form prospective studies are still needed. In trauma patients who are on anticoagulation with warfarin prior to their injury, retrospective analyses showed that the use of PCC resulted in a more rapid time to reversal of the International Normalized Ratio (INR) [50–53]. According to the recent European guidelines, thromboelastometry appears to be a useful tool to guide PCC therapy in patients with traumatic coagulopathy [1].

Although some studies in animal models of liver and spleen injury [54–59] have demonstrated that PCC use reduced blood loss, shortened bleeding time and enhanced thrombin generation, two of them documented an increased thromboembolic risk [58, 59]. Indeed, particular concerns regard the risk of both venous and arterial thrombosis carried by treatment with PCC [43]. Although commercially available PCC (Table 24.3) have nowadays an improved safety versus products used in the 1970s and 1980s due to the exclusion of activated coagulation factors as well as the inclusion of coagulation inhibitors, their thrombotic risk should be carefully weighted

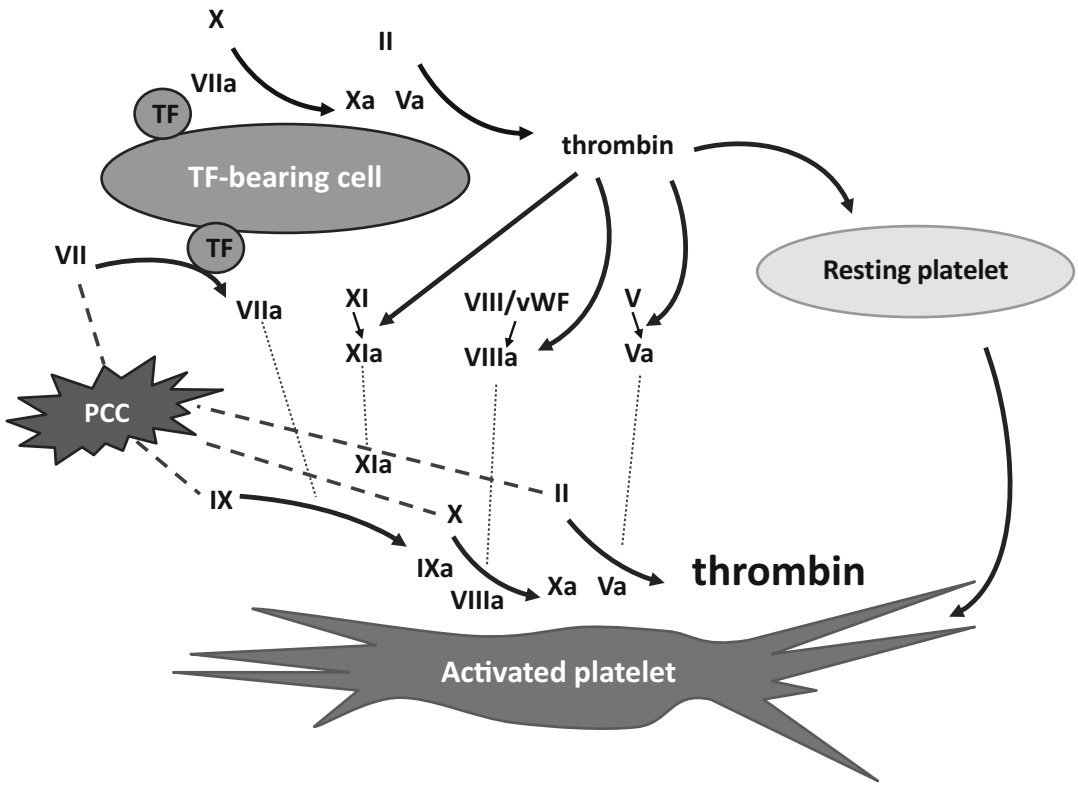

Fig. 24.1 Mechanisms of action of prothrombin complex concentrate (PCC)

against the need for rapid and effective correction of coagulopathy when initiation of PCC treatment is taken into account [45]. However, thromboprophylaxis as early as possible after control of bleeding has been achieved is recommended in trauma patients under PCC treatment [1]. The advantages of PCC over FFP include decreased volume, lack of ABO blood type matching, and lower risk of viral transmission along with the ability of transfusing without thawing. However, the US Food and Drug Administration (FDA) considers the use of PCC in the management of traumatic bleeding off-label [8].

In patients with life-threatening haemorrhages, recombinant activated factor VII (NovoSeven, Novo Nordisk A/S, Bagsværd, Denmark), which is able to generate a thrombin burst through both tissue factor-dependent and -independent mechanisms, has been used to control bleeding [60]. However, due to the limited clinical evidences from literature and the thrombotic risk, its use should be restricted to cases of massive bleeding where standard interventions failed [61]. The same considerations apply also for the activated PCC FEIBA (Baxter, Deerfield, IL, USA) which is, however, currently recommended for severe bleeding associated with target-specific oral anticoagulants [62].

Conclusions

Trauma-associated coagulopathy is a life-threatening condition with a high morbidity and mortality. This chapter focuses on the role of fibrinogen concentrate and PCC, among the newer therapeutic approaches. The majority of clinical data to date suggest that fibrinogen concentrate plays a key role as a primary haemostatic agent in trauma patients. Its early administration can enable successful treatment of trauma-related bleeding with reduced or even no requirement for

Table 24.3 Main characteristics of the currently registered prothrombin complex concentrates (see Ref. 40)

Brand	Company (site of manufacture)	Purification	Viral inactivation	Comments
Bebulin VH	Baxter BioScience (Vienna, Austria)	Ion exchange adsorption	Vapour heat 60 °C for 10 h, then 80 °C for 1 h	Heparin added
Beriplex P/N Confidex	CSL Behring (Marburg, Germany)	Multiple precipitation and adsorption steps, DEAE-sephadex	Pasteurisation 60 °C for 10 h; nanofiltration	Four factor PCC; PC, PS, AT, heparin and albumin added
Cofact	Sanquin (Amsterdam, Netherlands) Biotest (Vienna, Austria)	DEAE ion exchange chromatography	TNBP/polysorbate 80; nanofiltration	AT added; no albumin added
Facnyne	Greencross Corp (Seoul, Korea)	Ion exchange chromatography	TNBP/polysorbate 80	No albumin added
Haemosolvex Factor IX	National Bioproducts (Pinetown, South Africa)	DEAE-sephadex	TNBP/polysorbate 80	No albumin added; heparin added
HT DEFIX	SNBTS (Edinburgh, Scotland)	Ion exchange chromatography	Dry heat 80 °C for 72 h	AT added
KASKADIL	LFB (France)	Ion exchange chromatography	TNBP/polysorbate 80	No albumin or AT added; heparin added
Octaplex Pronativ	Octapharma (Vienna, Austria)	Ion exchange chromatography	TNBP/polysorbate 80; nanofiltration	Four factor PCC; heparin added; no AT or albumin added
Profilnine SD	Grifols (Los Angeles, CA, USA)	Double DEAE cellulose chromatography	Solvent/detergent	No albumin, heparin or AT added
Prothrombinex-VF Prothrombinex-HT	CSL Biotherapies (Melbourne, Australia)	DEAE cellulose adsorption	Dry heat 80 °C for 72 h	No albumin added; AT and heparin added
Prothromplex-T	Baxter BioScience (Vienna, Austria)	Ion exchange adsorption	Vapour heat 60 °C for 10 h, then 80 °C for 1 h; nanofiltration	AT and heparin added
Prothroraas	Shanghai RAAS (Shanghai, China)	PEG precipitation, DEAE- sephadex	Solvent detergent; nanofiltration	–
PPSB-human SD/Nano 300/600	German Red Cross NSTOB (Springe, Germany)	DEAE-sephadex; ion exchange chromatography	TNBP/polysorbate 80; nanofiltration	AT and heparin added; no albumin added
Uman Complex D.I.	Kedrion (Barga, Italy)	Anion exchange; DEAE-sephadex/sepharose chromatography	TNBP/polysorbate 80; Dry heat 100 °C for 30 min	AT and heparin added; no albumin added; FII and FX titration

Abbreviations: *PEG* polyethylene glycol, *DEAE* diethylaminoethanol, *PC* protein C, *PS* protein S, *AT* antithrombin, *TNBP* tri-n-butyl phosphate

allogeneic blood products. At least three prospective, randomised ongoing trials are currently investigating the use of fibrinogen concentrate in trauma and their results are greatly awaited to definitely assess their effectiveness and safety in this clinical setting. Less uncertainty exists regarding the use of PCC therapy in patients with traumatic coagulopathy, especially considering the lack of robust data from prospective studies and their increased thrombotic risk. Randomised controlled trials are needed to elucidate the PCC role in trauma-related coagulopathy.

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Anti-fibrinolytics

25

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Abbreviations

aPC	Activated protein C
ATC	Acute traumatic coagulopathy
CSF	Cerebrospinal fluid
EACA	ε-Aminocaproic acid
ICH	Intracranial haemorrhage
ISS	Injury severity score
LY30	Percent clot lysis 30 min after maximum amplitude of the clot has been reached assessed with thromboelastography
PAI-1	Plasmin activator inhibitor 1
PAI-2	Plasmin activator inhibitor 2
TAFI	Thrombin activatable fibrinolysis inhibitor
TBI	Traumatic brain injury
TEG	Thromboelastography
tPA	Tissue-type plasminogen activator
TXA	Tranexamic acid
uPA	Urokinase-type plasminogen activator

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The Fibrinolytic System

The fibrinolytic system consists of serine proteases, protease inhibitors and cell surface receptors that orchestrate the spatial proteolytic activation of the zymogen plasminogen to plasmin [1]. Plasmin in turn can act upon various substrates, but the most recognised being fibrin, the structural scaffold of blood clots. Hence, the fibrinolytic system is most recognised for its role in fibrin removal and the subsequent dissolution of blood clots.

Under physiological conditions plasmin generation is facilitated by tissue-type plasminogen activator (tPA) and to a lesser extent by urokinase-type plasminogen activator (uPA). In contrast to uPA, the activity of tPA is critically dependent on the presence of fibrin. Indeed, this fibrin dependency increases the capacity of tPA to generate plasmin by more than two orders of magnitude [1]. Both plasminogen and tPA bind to exposed lysine residues on fibrin (Fig. 25.1, panel a) that subsequently promotes plasmin formation on the fibrin surface. When associated with fibrin, plasmin is protected from its major natural inhibitor alpha$_2$ antiplasmin (α_2-antiplasmin). This strict regulation of fibrinolytic activity is not only facilitated by lysine specific binding and circulating α_2-antiplasmin, but also by inhibition at the level of the plasminogen activators. Plasmin activator inhibitor (PAI)-1 and in lower concentrations PAI-2, are potent circulating inhibitors of both tPA and uPA. Finally, an important

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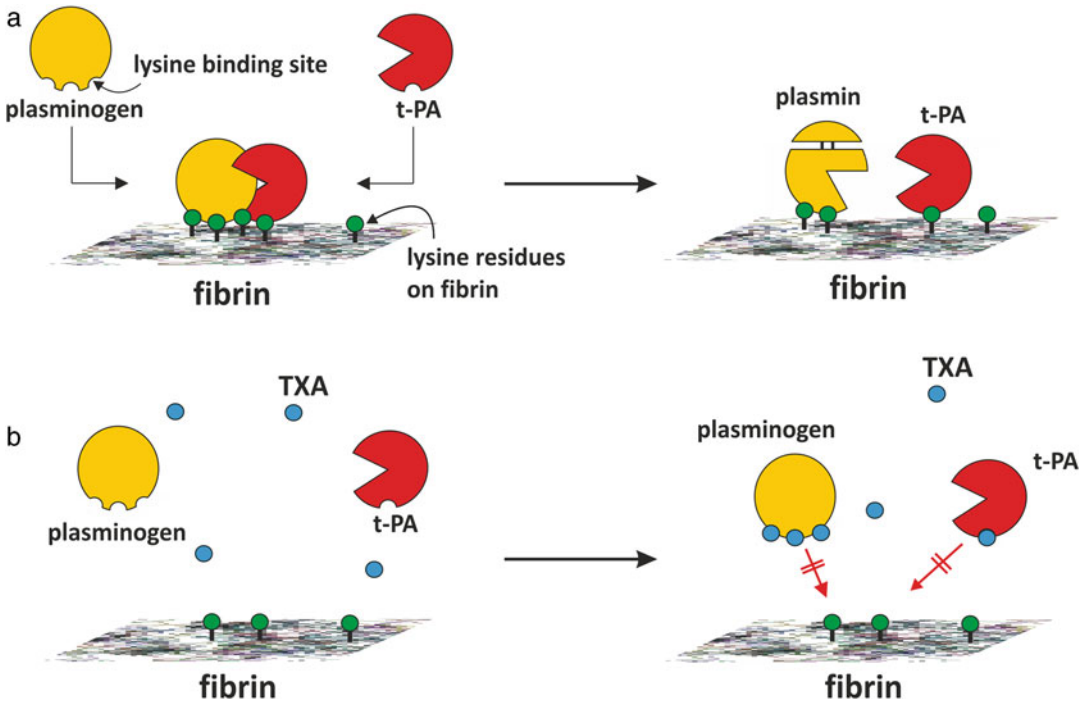

Fig. 25.1 Panel **a**: Plasminogen and tPA both contain lysine binding sites that bind to lysine residues on fibrin, facilitating the proteolytic activation of plasminogen to plasmin and subsequent degradation of fibrin [1]. Panel **b**: TXA, a lysine derivate, blocks the lysine binding sites on plasminogen and tPA, thereby inhibiting their co-localisation on fibrin and plasmin generation [6]

mechanism of regulation is maintained by Thrombin-activatable Fibrinolysis Inhibitor (TAFI), a carboxypeptidase that by proteolytic removal of lysine residues on fibrin can drastically reduce plasmin generation [1]. The mechanisms leading to plasminogen activation into plasmin, and the regulation of this system is reviewed in detail in Chap. 3.

Despite being clearly recognised for its role in fibrinolysis and clot removal, the plasminogen activating system has now been implicated, unexpectedly, as having a critical role in the central nervous system (CNS) [2, 3]. It is not the purpose of this chapter to extensively review this unforeseen role for this enzyme system, as the involvement of the plasminogen activating system in various physiological and pathophysiological processes, including the CNS (learning and behaviour, neuronal modulation, blood–brain barrier permeability, addiction), as well as tissue repair, inflammation and metastatic spreading has been extensively reported in recent times [4, 5]. What is not known, however, is the extent (if any) to which over-activation of this system (i.e. hyper-fibrinolysis) or its inhibition with anti-fibrinolytic agents influences these "non-fibrinolytic" processes.

Hyper-fibrinolysis

Hyper-fibrinolysis is the accelerated degradation of fibrin, resulting in increased likelihood for bleeding [6]. The first entry mentioning hyper-fibrinolysis in PubMed is from 1957 in an article dealing with bleeding disorders of newborn [7]. Hyper-fibrinolytic activity has also been described in malignant diseases, such as prostatic cancer [8] and leukaemia [9] as well as infectious diseases [10]. Pathological hyper-fibrinolysis was first described by Starzl et al. in 1963 in the context of liver transplantation [11].

Hyper-fibrinolysis in Trauma-Induced Coagulopathy

Trauma-induced coagulopathy was long thought to occur due to a simple depletion or dilution of procoagulant factors. However, with the identification of trauma induced coagulopathy (TIC) [12, 13], it was found that an additional mechanism was based on disturbances in the balance of pro- and anticoagulant factors, platelets, the endothelium, and the fibrinolytic system. TIC develops quickly, within minutes after tissue systemic hypoperfusion. Factor V deficiency can be detected, probably due to activation of protein C (aPC), resulting in systemic anticoagulation [14]. The activated vascular endothelium seems to be critically involved in the generation of aPC, which not only inactivates the clotting factors V and VIII, but also the potent fibrinolytic inhibitor, PAI-1. As tPA is also released from endothelial cells under these conditions it is likely to be more active due to the reduction of PAI-1 activity in the presence of aPC [14, 15]. Nowadays, hyper-fibrinolysis is widely acknowledged as being an important participant in the pathophysiology of TIC.

Trauma induced coagulopathy occurs in 10–25 % patients with severe trauma [16, 17], yet, referring to a recent meta-analysis of publications between 1966 and 2007, one out of three patients with traumatic brain injury (TBI) suffers from coagulopathy [18]. A recent report investigating the fibrinolytic profile of severely injured patients revealed a comparable distribution of patients with physiological fibrinolytic activity and hyper-fibrinolysis (approx. 18 %). The majority (60 %), however, suffered from fibrinolysis shutdown, associated with multiple organ failure [19]. Raza et al. observed minimal clot lysis as evaluated by thromboelastography (TEG), but increased levels (fivefold) of plasmin–antiplasmin complex in 57 % of trauma patients [20] suggesting that TEG may actually underestimate the extent of hyperfibrinolysis. However, others believed that the elevated plasmin–antiplasmin complex levels might be an artefact due to methodological issues [21], and that this group would in fact represent patients with a shutdown of fibrinolysis [19]. On the other hand, plasmin–antiplasmin complex formation is a reflection of plasmin generation, and not necessarily of "fibrinolysis" per se. For example, the misfolded protein response that occurs following tissue injury can also promote tPA-mediated plasminogen activation [22, 23] and this is also likely to produce plasmin–antiplasmin complexes. Noteworthy, the presence of hyper-fibrinolysis in trauma patients is associated with significantly increased overall mortality [24, 25] and death due to exsanguination [19]. For further description of the fibrinolytic system, particularly during trauma and haemorrhagic shock, please refer to Chap. 9.

Interestingly, the incidence of TIC is significantly higher in patients with TBI compared to patients with injuries to the trunk or limbs. This occurs despite the fact that blood loss is usually not as high in this condition and therapeutic fluid replacement is restricted in such patients [26]. High levels of tissue factor within the brain tissue and excessive release after TBI might be a major contributor to this observation [27].

While other chapters in this book have adequately covered in more detail the fibrinolytic system in general and the occurrence of hyper-fibrinolysis as well as diagnostic evaluation of TIC, this chapter will present an overview of the history and use of antifibrinolytic drugs in the treatment of patients with severe trauma, including TBI.

Anti-fibrinolytic Drugs

Two anti-fibrinolytic drugs are currently available: TXA and ε-aminocaproic acid (EACA), both discovered by Okamoto et al. around 1960 [28, 29]. These agents were first used in the treatment of gynaecological complications as recently reviewed [6]. A third anti-fibrinolytic agent, aprotinin (trasylol), a synthetic direct plasmin inhibitor, first described over 80 years ago was used as an anti-fibrinolytic drug in patients with pancreatitis and in more recent times during cardiac surgery and many other indications associated with bleeding [30]. However, aprotinin was recently withdrawn from the market as it was shown to be associated with significantly incre-

ased mortality from myocardial infarction, heart failure and cardiogenic shock in patients undergoing high-risk cardiac surgery [31].

TXA and EACA both are analogues of the amino acid lysine. By reversibly binding to lysine binding sites within plasminogen and thereby competing with the binding to fibrin and other targets containing exposed lysine residues, these analogues efficiently prevent plasmin formation on the lysine bearing substrate (Fig. 25.1). It is also important to mention that the interaction of plasmin itself with fibrin depends on lysine-binding and is also inhibited by TXA [6].

Tranexamic Acid

TXA is the most widely used anti-fibrinolytic drug and binds to plasminogen 10- to 16-fold more efficiently than EACA [32]. Its licenced indications for clinical use are broad and vary significantly between different countries. The maximal concentration of TXA in the plasma is reached 15 min after intravenous administration while the terminal elimination half-life of TXA has been stated to be approximately 2–3 h [6]. Maximal inhibition of plasmin activity ranges from 30 min in plasma to 2 h in muscle and heart referring to data from animal studies [33]. TXA has been reported to sufficiently suppress fibrinolysis at plasma concentrations of 5–10 or 10–15 mg/L [6, 34], and despite a serum half-life of only ~3 h an adequate inhibition of fibrinolysis can be expected for up to 8 h [35]. Metabolism in the liver is minimal and excretion happens mainly via the kidney, which implicates the need of dose adjustment in patients with renal impairment in order to avoid toxic accumulation [6].

The use of TXA has been extensively studied in cardiac surgery as well as hepatic, pancreatic and also orthopaedic, spinal and cranial surgery. Furthermore, hypermenorrhoea, postpartum haemorrhage and bleeding due to implanted contraceptives are typical indications for TXA in gynaecology [6]. Dose regimens vary depending on indication and also the country in which it is prescribed. TXA can be administered by intravenous, intramuscular or intraosseous injection, orally or topically [6]. TXA is stable and can be stored at a wide range of temperatures with a long shelf-life. Although no major drug interactions are known, it is often recommended that TXA should not be given in combination with thrombin, Factor IX Complex concentrates or Anti-inhibitor Coagulant concentrates in order to avoid the risk of thrombotic complications [6, 36].

In general TXA is considered a safe drug. Historically the greatest concern has related to the induction of seizures, because it is capable of crossing the blood–brain barrier. Cases of seizures after TXA administration have been described in particular in patients with impaired renal function [36, 37]. The cause of the TXA-induced seizure is not known and might relate to the inhibition of non-fibrinolytic effect of plasmin activity on neurons in the brain although this is speculative and remains to be determined. More common adverse effects of TXA are diarrhoea, nausea and vomiting, dizziness, hypotension and allergic dermatitis [36]. In order to avoid hypotension it has to be administered slowly over 10 min (100 mg/min) [38]. The use of TXA during pregnancy and lactation period is, in principle, possible. However, as it passes the placenta and can also be detected in breast milk, its use should be carefully considered [36]. A recent meta-analysis on the safety and efficacy of antifibrinolytics in paediatric surgery found it appears to be safe for use in children [39].

ε-Aminocaproic Acid

EACA is also a lysine analogue and can be administered intravenously as well as orally [6, 30]. Peak plasma levels after oral intake are reached after approximately 1.2 h. The clearance happens mainly via the kidney and only a small fraction gets metabolised [32, 40]. The plasma half-life of EACA has been stated as 2 h [30] while 85 % of the intravenously injected drug is cleared after 3 h. However, as EACA can reach the entire extracellular space, it is likely to be detectable in the urine for up to 36 h. Plasma concentrations of 131.4 μg/mL (1 mM) sufficiently suppress fibrinolysis [35]. Indications for EACA are similar as

for TXA and include cardiac surgery, hepatic cirrhosis, gynaecological complications and also bleeding associated with malignant diseases [40]. EACA, like TXA is a well-tolerated drug, although muscle necrosis has been described after prolonged use, with consequences ranging from mild myalgia to rhabdomyolysis and renal impairment. Caution has been advised for its use if there is evidence of intravascular clotting, in which it should be used only in combination with heparin, and the combination of EACA with Factor IX Complex concentrates or Anti-Inhibitor Coagulant concentrates might increase the thrombotic risk [40].

Other adverse reactions that have been observed with EACA include bradycardia and hypotension, gastrointestinal problems, seizures, delirium and syncope. Moreover, pruritus and rash, headache, oedema and malaise have been reported [40]. The use of EACA in pregnant women and nursing mothers has not been well studied, but, like TXA, EACA has been used in children without apparent undue harm [39].

Aprotinin

Aprotinin was also widely used as an anti-fibrinolytic drug and acts by directly inhibiting plasmin activity [6]. It has been used in various bleeding conditions, particularly in surgical interventions, such as cardiac surgery [41]. However, after the BART trial was published in 2008 revealing a significant association of aprotinin with death of cardiac causes [31] it was withdrawn from the market. However, in Canada it was soon re-authorised for use in cardiac surgery as only patients undergoing high risk surgical procedures were included in the BART trial and mortality rates were clearly higher than in other studies [6, 30]. This led to the conclusion that aprotinin might still be beneficial in non-high-risk cardiac surgery [30]. In order to avoid hypersensitivity reactions, the use of aprotinin is contraindicated if already administered within the last 12 months. In addition to its thrombogenic potential, aprotinin increases the risk of renal impairment [41]. Its use during pregnancy has been tested in animal studies and considered safe [41].

Safety and Efficacy of Tranexamic Acid in Non-trauma Applications

Considerable experience exists with the use of anti-fibrinolytics in elective surgery, especially cardiac and orthopaedic surgery [6]. A meta-analysis conducted by the Clinical Randomisation of an Antifibrinolytic in Significant Haemorrhage (CRASH-2) investigators, in which TXA was compared with no TXA in elective and emergency surgery concluded that the need for blood transfusion was reduced by one third if TXA was administered [42], and that this effect seems to exist for all surgical specialties. Uncertainty remained regarding the effect of TXA on thromboembolic events, such as myocardial infarction (MCI), stroke, deep vein thrombosis (DVT) and pulmonary embolism (PE) [42].

The Use of Tranexamic Acid in Trauma

Despite it being recognised for its effectiveness at reducing bleeding in elective surgery, TXA was used little in trauma until publication of the CRASH-2 study [43, 44]. Said to be conceived on the helipad at Royal London Hospital in a discussion between clinicians awaiting a trauma patient's arrival, the CRASH-2 Study tested the effectiveness of early in-hospital administration of 2 g TXA (as 1 g bolus over 10 min in 100 ml saline, followed by an 8 h infusion of 1 g in 1 l of saline), versus placebo, on all cause in-hospital mortality within 4 weeks of injury. The clinical trials network of the London School of Hygiene and Tropical Medicine was mobilised to enrol 20,211 patients in 274 hospitals in 40 countries. Inclusion criteria were broad—adult patients who were assessed by the treating clinician to be bleeding or at risk of bleeding, as indicated by systolic blood pressure of <90 mmHg or heart rate >110 beats per minute. Drug and placebo were matched, and both patients and staff were

blinded to treatment allocation. Secondary outcomes included death due to specific causes, and the need/frequency for blood transfusions.

The study found that TXA, compared with placebo, was associated with 9 % reduced risk of all-cause mortality. Subsequent publications have reported subgroup analyses, cost-effective analyses and mechanistic hypotheses [45–48]. Of particular importance, the risk of death due to bleeding, a secondary outcome, was temporally related—the earlier TXA was given the more effective it appeared to be, with 32 % reduction, compared to placebo, in relative risk of death due to bleeding if given within an hour of injury, 21 % if administered 1–3 h after injury, and, surprisingly, a 44 % increased risk of death if given >3 h after injury [43].

The findings of CRASH-2 were widely promulgated with a clear message that all trauma patients who were at risk of bleeding should be given TXA within 3 h of injury. In the UK TXA became the first drug to be fast-tracked for use in the National Health Service under the Government's "medicines innovation scheme", and case-based payments in the new trauma system were tied to TXA administration. Some European guidelines were quickly amended to recommend administration of TXA in trauma patients who are bleeding or at risk of haemorrhage as early as possible [49]. Many institutions throughout the world incorporated TXA into their massive transfusion protocols for bleeding trauma patients. The implications for pre-hospital administration have been widely canvassed [50], and many physician-led pre-hospital ambulance services have begun to administer it on the field [51].

The Military Application of Tranexamic Acid in Trauma Emergency Resuscitation (MATTERs) study was a retrospective observational trial published in 2012 [52]. Eight hundred and ninety-six patients suffering from combat-related injuries and receiving at least 1 unit of packed red blood cells have been included, of whom 293 were treated with TXA. Despite a significantly higher injury severity score (ISS) (25.2 vs 22.5) the TXA group presented lower mortality rates than the non-TXA group (17.5 % vs 23.9 %), with a more pronounced benefit in patients receiving massive transfusion (≥10 unit of packed red blood cells; 14.4 % vs 28.1 %). In contrast to the CRASH-2 trial, however, this retrospective study identified a significantly increased rate of thromboembolic events in patients administered TXA. However, the authors emphasise that the higher injury severity of the TXA group has to be taken in consideration, which might be associated with thrombotic complications independent of TXA administration [52]. Inconsistent dosing and the lack of standardised indication criteria for TXA treatment are obvious limitations of the MATTERs trial [53].

A summary of clinical studies performed in recent years to explore the use of TXA in trauma patients is provided in Table 25.1.

Unresolved Issues in the Use of Tranexamic Acid After Trauma

Editorials questioned whether CRASH-2 provided sufficient evidence for widespread adoption in civilian and military settings [16]. The question of whether the addition of TXA to the complex management regime currently practised in advanced trauma systems is effective and efficient still remains unanswered. Further unanswered questions surround the interaction of TXA with components of massive transfusion guidelines and whether it predisposes to thromboembolic adverse events—would such adverse events be additive or multiplicative [54]? It has, moreover, to be emphasised that the interactions of TXA with age-related co-morbidities and pharmacotherapy are not well understood and certainly require further investigation [16].

Furthermore, the ideal dosage regimen and possible interactions with other drugs, such as fibrinogen concentrates, prothrombin complex concentrates and recombinant activated factor VIIa are still unknown and will hopefully be revealed by prospective trials [55]. Severe conditions like polytrauma and shock can lead to impaired renal function within 1 h, as demonstrated in animal experiments [56], which certainly has to be considered regarding dose

Table 25.1 Clinical trials investigating the use TXA in trauma patients

Published trials	Study design	Main findings	References
CRASH-2	International, prospective, randomised, double-blind, placebo-controlled multicentre trial investigating the effect of TXA in trauma patients bleeding or at risk to bleed	TXA administration was associated with a reduction in RR of all-cause mortality of 9 % and moreover with RR reduction of death due to bleeding of 21 % if administered within 3 h after injury. However, later administration was associated with an increase in RR for death of 44 %	Shakur et al. [44] Roberts et al. [43]
MATTERs	Retrospective observational study investigating TXA administration vs no TXA in patients with combat injuries who received at least 1 unit of packed red blood cells	TXA was associated with decreased mortality (17.4 % vs 23.9 %) with a pronounced effect in individuals receiving massive transfusion (14.4 % vs 28.1 %)	Morrison et al. [52]
Valle et al. (2014)	Retrospective study comparing treatment with TXA vs no TXA in a subset of the most severely injured ICU patients	In a population of most critically injured patients referring to ISS, TXA is detrimental regardless of the time post trauma it was administered and associated with increased mortality (27 % vs 17 %)	Valle et al. [38]
Cole et al. (2015)	Prospective single centre cohort study investigating the use of TXA in severely injured patients with or without shock	TXA was independently associated with a decrease in MOF (OR 0.27) and adjusted all-cause mortality (OR 0.16) in shocked patients. However, no independent association was found with respect to any outcome difference in the overall or non-shocked population	Cole et al. [65]
Harvin et al. (2015)	Retrospective single centre study assessing the effect of TXA treatment in trauma patients with diagnosed hyper-fibrinolysis	TXA administration was associated with increased unadjusted mortality (40 % vs 17 %). After multivariate regression it was found to be an independent risk factor for 24 h mortality, but not for in-hospital mortality	Harvin et al. [64]

TXA tranexamic acid, *RR* relative risk, *OR* odds ratio, *ISS* injury severity score, *MOF* multiple organ failure

adjustment in order to avoid toxic accumulation of TXA [55]. A recently performed pilot trial compared two different dosing schemes and placebo in patients undergoing cardiac surgery with cardiopulmonary bypass [57]. No significant difference in amount of fibrinolysis (defined by the authors as percent clot lysis 30 min after maximum amplitude of the clot (LY30) of ≥7.5 % assessed with TEG) and no significant difference in outcomes was found between the two TXA groups (30 mg/kg bolus followed by 16 mg/kg/h infusion in the high dose group and a 5 mg/kg bolus followed by 5 mg/kg/h in the low dose group) and placebo. A significant increase in D-dimer levels, however, was detected in the placebo group in comparison to both TXA groups. The authors stated that a higher number of cases will be necessary to clearly identify differences in fibrinolysis and outcome. In order to determine the optimal dose of TXA in trauma patients, clinical trials also testing different dosing regimens are underway [58].

Another question that still needs to be clarified is why early TXA administration shows a beneficial effect, but late administration was associated with a detrimental effect *due to bleeding* in the CRASH-2 trial. It has been speculated that the differential effects of TXA in relation to time after trauma observed might be due to PAI-1-mediated suppression of fibrinolysis and the conversion of the early coagulopathy into a disseminated intravascular coagulopathy with thrombotic phenotype (i.e. disseminated intravascular coagulation can manifest as bleeding) [47, 59]. This notion is supported by a recent study indicating that 60 % of severely injured patients actually present with fibrinolysis shutdown as determined with TEG [19]. The authors hypothesised that fibrinolysis shutdown might be the "missing link" in post-injury venous thromboembolic complications. They furthermore stated that haemorrhagic shock might be the promoting factor for hyper-fibrinolysis, whereas tissue injury and subsequent PAI-1 release could be the cause for fibrinolysis shutdown.

Interestingly this observation is further supported by a retrospective observational single centre study, conducted by Valle et al. exploring effects of TXA administration to patients with severe trauma [38]. These authors studied patients who were subjected to emergency surgery at arrival to the hospital; TXA treated patients were compared to patients not administered TXA by nearest neighbour matching assessed with propensity scores involving sex, age, mechanism of injury, TBI, transfusion requirements, systolic blood pressure and ISS. Those in the TXA group received 1 g of TXA as a bolus followed by an infusion of another gram over 8 h. Surprisingly, regardless of the administration time after injury, TXA was associated with increased mortality and increased requirements for total fluid, packed red blood cells and plasma, compared with patients not administered TXA. The authors therefore supported the use of TEG in order to identify those trauma patients who suffer from hyperfibrinolysis and would hence most likely benefit from an anti-fibrinolytic therapy. Using the cut-off by LY30 of more than 3 % fibrinolysis has been determined to represent relevant hyperfibrinolysis and to predict the need for anti-fibrinolytic therapy [60, 61]. However, some disagree with the necessity for viscoelastic clot assays in this regard [62]. Referring to the CRASH-2 trial where TEG was not used [44], it is argued that the time required to conduct this assay would result in an unnecessary delay for patients in receiving TXA, given that earlier use was also shown to be more beneficial [62]. Nevertheless, it has to be mentioned in this regard that TXA did not significantly reduce the necessity of blood products in the CRASH-2 trial, yet the mortality due to bleeding was still decreased by ~15 % (RR 0.85) [44]. Subgroup analysis showed that the beneficial effect of TXA was most prominent in the group with severe shock, as defined by a systolic blood pressure <75 mmHg with respect to all cause 28-day mortality [43, 63]. Like Valle et al, a study by Harvin et al. also did not find a survival benefit of TXA treatment after trauma [64]. In this retrospective single centre study, TEG was performed on 1032 trauma patients with hyperfibrinolysis (LY30>3 %). Of those, 10 % received TXA (1 g as a bolus followed by a 1 g infusion over 8 h) and 90 % did not. Significant baseline differences between

TXA and non-TXA groups were also described, whereby individuals of the treatment group were older (37 vs 32 years median age), more severely injured (ISS 29 vs 14), had lower systolic blood pressure (103 mmHg vs 125 mmHg), and presented with a higher base deficit (5 mEq/L vs 2 mEq/L). The unadjusted mortality was significantly higher in the TXA group (40 % vs 17 %). The authors acknowledged the obvious selection bias resulting from the study design, and after multivariate regression adjusting for sex, age as well injury severity, they found that TXA was not an independent predictor of in-hospital mortality but rather of 24 h mortality. Yet, no differences between the two groups were observed with respect to thromboembolic complications. However, the authors themselves indicated that this was not a definitive study and further studies will be required to substantiate these findings.

A recent study by Cole et al. compared TXA with no TXA in severely injured patients (ISS > 15) with or without shock admitted to the ICU [65]. Shock was defined by a base deficit of ≥6 mEq/L. Patients in the TXA group received 1 g as a bolus within 3 h after injury, followed by a 1 g infusion over 8 h. In this prospective cohort study the authors identified TXA treatment to be beneficial with respect to mortality and multiple organ failure outcome in patients with shock. Importantly, no significant benefit was observed in the cohort without shock. No increased risk of thromboembolic events was reported.

The studies outlined above support the opinion that careful assessment may be necessary to distinguish those trauma patients who are likely to benefit from TXA treatment from those who will not, but this remains a contentious issue.

In order to explain the contradictory effects of TXA treatment with respect to time of administration after injury it also remains a possibility that TXA treatment may have a counterintuitive effect at promoting bleeding by paradoxically *promoting* fibrinolysis. This unexpected consideration is based on the fact that urokinase (uPA) mediated plasminogen activation is actually promoted by TXA [66]. While TXA does indeed inhibit fibrinolysis initiated by tPA or uPA on the fibrin surface, a different scenario occurs in solution. This is because the binding of TXA to kringle domains in plasminogen in the solution phase conformationally alters plasminogen in a manner that renders it more susceptible to uPA-mediated activation; hence TXA can therefore promote plasmin formation when uPA is present. A recent publication investigating the role of the fibrinolytic system in a mouse model of TBI revealed that both tPA and uPA levels were increased in the cerebrospinal fluid (CSF) after TBI (Fig. 25.2), but there were marked differences in the temporal profile of each protease: tPA levels

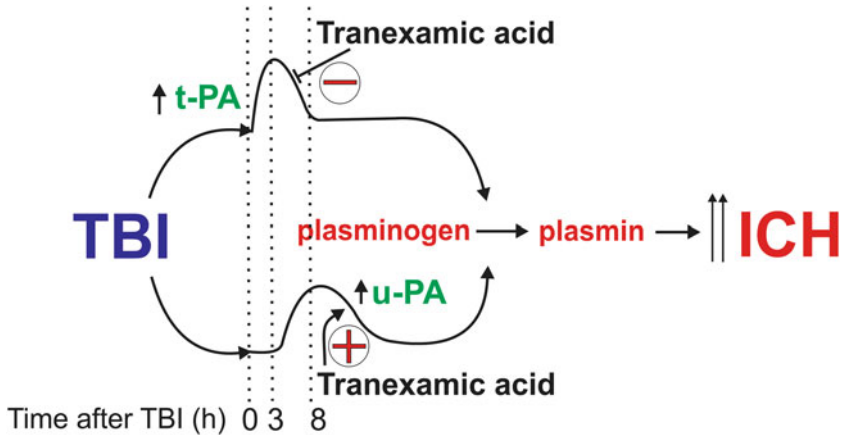

Fig. 25.2 TXA inhibits tPA-mediated, but enhances uPA-mediated plasmin formation. CSF levels of tPA peak ~3 h, those of uPA ~8 h post TBI, as shown in mice [48] (*TBI* traumatic brain injury, *ICH* intracranial haemorrhage)

increased rapidly (within 3 h) and returned to base line by ~8 h [48]. In contrast, uPA levels increased later peaking at ~8 h. Moreover, administration of TXA immediately after TBI resulted in reduced haemorrhage in this mouse model, consistent with its blocking effect against tPA-mediated fibrinolysis. In stark contrast, administration of TXA 8 h post-TBI, where uPA levels were maximal, resulted in an *increase* in haemorrhage, reminiscent of the CRASH-2 trial. This finding, albeit in mice, is consistent with the notion that the increase in deaths due to bleeding in severe trauma patients administered TXA after 3 h from injury as seen in the CRASH-2 trial, may be due to acceleration of uPA-mediated plasminogen activation, the very process that TXA was thought to stop [67]. Hence, an interesting question now would be to determine the time course of uPA activity in cerebrospinal fluid and in plasma of patients following severe trauma. If an increase in uPA can be detected in patients with severe trauma, future anti-fibrinolytic approaches should be used that target both tPA and uPA-mediated plasmin formation. Indeed, this also raises the prospect of whether aprotinin could be reconsidered in the trauma setting given that it directly blocks plasmin activity, regardless of the activation of plasminogen by either tPA or uPA.

What Other Effects Do Anti-fibrinolytic Drugs Have?

Given the broad implication of the fibrinolytic system in various physiological and pathophysiological processes, notably in the CNS, it might be worthwhile to ask what else could be affected by TXA administration. For example, inhibition of the blood–brain barrier permeability associated with plasminogen activation [68] might further contribute to the preventive effects on bleeding, particularly in TBI [5]. The plasminogen activating system is also involved in wound healing [69], which could potentially become impaired by TXA. The wound healing capacity after trauma is obviously important, however, with dosing regimens suggested so far, pointing to a short term use rather than a prolonged use of this drug, the effect on wound healing might not be a major concern.

The effects of TXA on the inflammatory response have already been elucidated in cardiac surgery [70]. This phenomenon is also being addressed in the setting of trauma by some of the currently running clinical trials [58]. As the need for red blood cell transfusion was not significantly different between TXA and placebo group in the CRASH-2 trial it has been speculated that the anti-inflammatory effects of TXA might be involved in the favourable outcome [63].

The Thromboembolic Risk of Tranexamic Acid

If TXA is able to reduce bleeding in trauma patients on the one hand, does it increase the risk of thrombosis on the other hand? The CRASH-2 study did not reveal significant differences between TXA and placebo with respect to thromboembolic events [44]. However, concerns have been raised, as those complications may not have actively been sought in many of the participating hospitals in CRASH-2, as reported events were rare [16] and diagnostic modalities such as computerised tomography and ultrasound were unlikely to have been extensively used [54].

Noteworthy, the MATTERs trial, a retrospective study investigating the effect of TXA in patients after combat injury, who received at least 1 unit of packed red blood cells found an increased incidence of deep vein thrombosis and pulmonary embolism in TXA-treated patients [52]. Nevertheless, this study did confirm reduced mortality in the TXA group. The survival benefit was most compelling in patients receiving massive transfusion and treatment of TXA compared with no TXA. The authors suggested prospective clinical trials to further elucidate the thromboembolic risk profile of TXA in trauma patients [52]. Later studies did not describe increased rates of thromboembolic complications [64, 65].

Interestingly, TXA not only inhibits the generation of plasmin and its ability to bind to fibrin, but also blocks binding of α2-antiplasmin to

plasmin [6]. In animal models, it was shown to increase thrombus formation and thrombus weight in a dose-dependent manner [71]. Reports on indications other than trauma have described a significant increase in thromboembolic complications in TXA treatment of subarachnoid haemorrhage [72], as well as a non-significant trend of increased vascular events when used in hip fracture surgery patients [73].

Controversy and Discussion

Without a doubt the findings of the CRASH-2 trial are compelling. However, concerns about the applicability of those results for different subgroups of trauma patients have been raised by several investigators [50, 60, 63]. A prospective randomised trial performed in a controlled environment with laboratory monitoring of coagulation and standardised transfusion protocols before TXA becomes standard of care in trauma has been suggested [16]. Moreover, some recommend the proof of existing hyper-fibrinolysis before the administration of TXA [60, 63]. Yet, the authors of the CRASH-2 study highlight that TXA might also be of benefit in trauma patients with fibrinolytic activity considered normal [20, 47] and hence, it would be necessary to identify those patients who are at risk of bleeding rather than to search for hyper-fibrinolysis [47]. Furthermore, it has been suggested that TXA should be used only in severe haemorrhagic shock, when systolic blood pressure drops below 75 mmHg [63]. The CRASH-2 investigators, however, argue that early administration was shown to be most effective and therefore waiting for such a severe decompensation would be an unnecessary delay [47].

There was also a suggestion to use TXA only in adults [63]. Children were not included in CRASH-2, as it is logistically difficult and the fixed dose was preferable to the body weight adjusted dose necessary for children [47]. However, referring to the ability of TXA to reduce bleeding complications in paediatric surgery, it has been suggested to apply the CRASH-2 study results in paediatric trauma. An adult TXA dose could thereafter be used in children over 12 years of age and weight adjustment should be done for younger children (15 mg/kg) [47]. The CRASH-2 authors argued that generalisation of results is widely possible, as differences in patient characteristics do not interfere with the pharmacological mechanism of the drug [47]. The results of CRASH-2 are certainly intriguing. However, further evidence needs to be provided to confirm a significant contribution of TXA to a favourable outcome in developed countries which have advanced trauma systems and a lower risk of preventable death, given that trials performed in such countries already questioned a clear benefit [38, 64].

Ongoing Trials Addressing Unresolved Issues

Several studies are currently being performed or are in the design phase, aiming to address these open questions. For summary of these studies please see Table 25.2. One of these is the CRASH-3 trial which will include 10,000 patients with TBI to whom either TXA or placebo will be administered within 8 h after injury [74]. Patients will be included and randomised if they have an imaging-proven intracranial haemorrhage or a Glasgow Coma Scale <13. However, patients with relevant extracranial bleeding will be excluded from the study. Patients will receive 1 g of TXA or placebo as a bolus of followed by a 1 g infusion administered over 8 h. The Resuscitation Outcomes Consortium (ROC) is a clinical trial network providing infrastructure and support for research on cardiorespiratory arrest and severe traumatic injury [75]. After CRASH-2 had been published the ROC committee also suggested a randomised, double-blind, placebo-controlled trial to further elucidate the effect of TXA on TBI. This trial will soon begin enrolment and will evaluate neurological outcomes 6 months after injury, 28-day mortality, as well as other recovery-related information such as hospital-free, intensive care unit-free, and ventilator-free days. Additionally, thromboembolic events and seizures will be assessed in both the TXA and the placebo group.

Table 25.2 Clinical trials planned or currently conducted to address the open questions regarding the use of TXA in trauma patients

Ongoing/prospective trials	Study design	Open questions addressed	References
CRASH-3	International, prospective, randomised, double-blind, placebo-controlled multicentre trial investigating the effect of TXA administration within 8 h after TBI	Is TXA administration beneficial for TBI patients?	Dewan et al. [74]
ROC	Prospective, randomised, double-blind, placebo-controlled trial exploring the effect of TXA on TBI	Is TXA administration beneficial for TBI patients?	ROC report [75]
PATCH	Prospective, randomised, double-blind, placebo-controlled multicentre trial investigating the early, mainly prehospital use of TXA in severely injured patients, likely to suffer from ATC	Is there a significant net benefit of TXA treatment for patients in countries with advanced trauma care? How does TXA influence parameters of ATC?	Mitra et al. [54]
STAAMP	Prospective, randomised, double-blind, placebo-controlled multicentre trial exploring the effects TXA administered during air medical transport in trauma patients at risk of bleeding	What is the best dose regimen for the use of TXA in trauma patients? How does TXA administration affect parameters of coagulation and inflammation?	Brown et al. [58]

TXA tranexamic acid, *RR* relative risk, *TBI* traumatic brain injury, *ATC* acute traumatic coagulopathy

The "Pre-hospital Anti-fibrinolytics for Traumatic Coagulopathy" (PATCH) trial, another randomised, prospective multicenter study is currently being conducted to further explore unresolved questions, such as the effect of TXA administration on parameters of trauma induced coagulopathy [16, 54]. Severely injured patients managed through developed trauma systems, and likely to have trauma induced coagulopathy, will be included and the effectiveness and safety of early TXA administration (pre-hospital) will be evaluated by comparison of 6-month outcomes and adverse effects [54].

The "Study of Tranexamic Acid during Air Medical Prehospital Transport" (STAAMP) Trial, a randomised, placebo-controlled, double-blind multicentre trial is underway [58]. Patients will be included if they are between 18 and 90 years old, within 2 h after injury and present with a systolic blood pressure <90 mmHg and a heart rate >110 bets per minute. TXA or placebo will be administered as a bolus of 1 g followed by a 1 g infusion over 8 h. The study is aiming to further investigate the effect of TXA administration during air medical transport in patients at risk of bleeding after trauma. Different dose regimens will be tested pre- and in-hospital and 30-day mortality will be evaluated. This study will also assess the effects of TXA on coagulation and inflammation post-injury.

Concluding Remarks

The plasminogen-activation system is broadly involved in physiological and pathophysiological processes. With respect to trauma its inhibition could possibly be beneficial not only due to the prevention of haemorrhage, but also due to inhibition of blood–brain barrier disruption and subsequent brain oedema, as well as its anti-inflammatory properties [5] but this remains to be formally evaluated. TXA is likely to be beneficial but others have argued for more trials to confirm a net benefit in advanced trauma care systems [50, 64] and to identify which trauma patients should be considered for anti-fibrinolytic therapy [74]. The detrimental effects of TXA when administered later than 3 h after injury observed in the CRASH-2 trial [43] limits its therapeutic window. This may be explained by a rise in uPA levels at later time points after TBI (referring to an animal model of TBI and evaluated in CSF), as uPA mediated plasmin generation is enhanced by TXA [48, 67] (Fig. 25.2).

Nonetheless, despite the controversies in the clinical literature and the growing appreciation of the broader effect of the fibrinolytic system in physiology, the use of anti-fibrinolytic agents in the setting of trauma has advanced substantially in the past 5 years. While the CRASH-2 trial has set the stage for the use of TXA, the outcome of the latest series of trials evaluating TXA in trauma is eagerly awaited.

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Part V

Post-injury Hypercoagulability

Venous Thromboembolism

26

Steven R. Shackford and C. Beth Sise

Introduction

On November 9, 1945, General George S. Patton, the brilliant field general and strategist of the European Theater in World War II, suffered a broken neck and quadriplegia in an automobile crash on his way to go pheasant hunting in Bavaria. He was kept immobilized and eventually placed in a total body plaster cast on November 20th. He did well until the afternoon of November 21st when his condition dramatically worsened. In the words of his physician, Major General Albert W. Kenner:

> … he had a shower [of] emboli that hit his right chest and he started to fill up with his own sputum. We managed to get that pretty well under control, only to see him die very suddenly as a result of another shower of emboli. As a matter of fact, he went out like a light and certainly suffered no pain. The service lost its best field commander and I lost a damn good friend [1].

The association of pulmonary embolism (PE) with trauma was made by J.S. McCartney 11 years before Patton's untimely death [2]. McCartney emphasized the importance of immobilization as a contributing factor to fatal PE, demonstrated the increased frequency associated with fractures of the spine, and noted that most emboli occurred in the second week after trauma. Despite the passage of 80 years since McCartney's clarion call, venous thromboembolism (VTE), including PE and deep venous thrombosis (DVT), remains a significant cause of morbidity and mortality following trauma.

Incidence of Venous Thromboembolism

Much of the current literature describing the incidence and epidemiology of VTE following trauma lists only "symptomatic" DVT and "symptomatic" PE. However, symptomatic VTE represents only the "tip of the iceberg." Most cases of DVT are asymptomatic and most PE are clinically silent. In addition, all of the symptoms of extremity DVT (e.g., swelling, pain with passive motion, erythema) are also characteristic of an injured limb. An accurate assessment of the incidence of post-traumatic VTE thus requires an objective test both to confirm symptomatic thromboses and to detect asymptomatic cases. Accordingly, Geerts and colleagues used serial plethysmography and venography to prospectively evaluate a cohort of 349 trauma patients

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(median Injury Severity Score [ISS][1] of 26) for VTE [4]. None of the patients received any prophylaxis. Overall, 201 (58 %) were diagnosed with DVT. Only three (1.5 %) were symptomatic or had clinical features suggesting DVT. Proximal (above knee) thrombosis occurred in 63 (18 %) patients, while the rest were located below the knee in the tibial and calf veins. These results demonstrate that the vast majority of DVT are asymptomatic, and that objective diagnostic imaging of asymptomatic patients (i.e., screening and surveillance) is essential to an accurate assessment of the incidence of DVT. Reports of DVT only in patients who are symptomatic will grossly underestimate the magnitude of the disease.

The incidence of DVT is also subject to surveillance bias. Sometimes called detection bias, surveillance bias occurs when variation in the use of screening and detection influences the frequency of an outcome, or "the more we look, the more we find [5]." Pierce and colleagues documented evidence of surveillance bias in DVT reporting among hospitals in the National Trauma Data Bank [5]. The hospitals were separated into quartiles according to the use of duplex ultrasound, an objective imaging test to diagnose DVT in symptomatic and asymptomatic patients. The DVT rate observed in the highest quartile of ultrasound use was sevenfold higher than the average combined DVT rate for the other three quartiles. These findings demonstrate that variability in the use of duplex ultrasound results in variability in the reported rates of DVT, further underscoring the impact of screening and surveillance in DVT case finding.

The use of prophylactic measures also affects the incidence of VTE following trauma. Thus, Geerts and colleagues followed their sentinel work on the incidence of VTE in trauma with a study to determine the effect of chemical prophylaxis on the reduction of thromboembolic events [6]. Patients were randomized to receive subcutaneous low-dose unfractionated heparin [UFH] or low-molecular-weight heparin [LMWH]. Venography was again used to assess symptomatic and asymptomatic patients. Of the 265 patients included in the study, 100 (38 %) developed a DVT. Proximal DVT occurred in 11 %. Subsequent studies using duplex surveillance of the lower extremity in patients receiving a combination of mechanical and chemical prophylaxis have reported rates of DVT in the range of 2.5–9 % [7–9].

The incidence of VTE following trauma is also a function of the risk level of the patient population or subgroup in question. The prevalence of VTE risk factors, such as older age and obesity (see Section "Risk Factors for VTE Following Trauma"), may result in a higher reported rate. In a study stratifying trauma patients based on the presence of VTE risk factors (see Table 26.1), Bandle and colleagues found a DVT rate of 17 % despite prophylaxis among the group at highest risk [9]. Patients in the groups at moderate risk and high risk had a combined DVT rate of 5 %.

Compared with DVT, the incidence of PE reported in studies is relatively low, around 0.3 % [10–13]. It is nonetheless a formidable problem with a mortality rate between 17 % and 26 % [10, 11, 14, 15]. Indeed, PE is a leading cause of death after the first 24 h of admission following injury and is thought to be responsible for approximately 12 % of all deaths due to trauma [16, 17].

Like DVT, surveillance bias affects the incidence of PE. With the advent of noninvasive diagnostic imaging, more specifically, contrast-enhanced thin-slice multidetector computed tomography (CT), more patients are evaluated for PE. As a result, the incidence of PE in trauma patients is increasing. Schultz and colleagues prospectively studied 90 consecutive patients with moderate-to-severe injury (ISS $\geq$ 9) who had no PE or DVT symptoms [18]. Utilizing multidetector helical CT scanning, the authors found a PE in 22 (24 %) of the 90 patients. Knudson and colleagues examined data from the National Trauma Data Bank to compare the incidence of PE reported between 1994 and 2001 (historical cohort) to that reported between 2007 and 2009

[1] Injury Severity Score (ISS) is an anatomical scoring system used to describe patients with multiple injuries [3]. The range is 0–75: 0 is no injury and 75 indicates an unsurvivable injury. An ISS of 9 indicates injury considered moderately severe but survivable.

Table 26.1 VTE Risk Stratification (reproduced with permission, Bandle et al. [9])

Highest-risk patient (any one of these criteria)
Spinal cord injury with neurological deficit
Unstable spine fracture (requiring fixation)
History of DVT or PE
Hypercoagulable state
Factor V Leiden, protein C/S, or antithrombin deficiency,
hyperhomocystinemia, prothrombin mutations, anticardiolipin antibody
Four or more "high-risk" criteria
High-risk patient (any one of these criteria)
ISS ≥ 10
Head AIS score ≥ 3
Pelvic fracture
Age > 70 years (with additional "moderate-risk" criteria)
Mechanical ventilation for ≥ 3 days
Major venous injury/repair
Long-bone fracture of lower extremity
Presentation in shock (SBP on arrival of ≤80 mmHg or base deficit ≥ 5)
Insertion of a femoral venous catheter
Central line placement
Active malignancy (not in remission)
Varicose veins
Recently post partum (1 month) or current pregnancy
Extrication from MVA at scene
Myeloproliferative disorder
Sickle cell disease
Obesity (BMI ≥ 30)
Stable spine fracture
Operative time ≥ 2 h
Transfusion of ≥ 4 U of PRBC
Moderate-risk patient
ISS ≤ 9
Age ≥ 70 years (without additional risk factors)
Age 40–70 years (with one additional "moderate-risk" factor)
Foot/ankle/fibula fracture
Inflammatory bowel disease (Crohn's or ulcerative colitis)
Additional "moderate-risk" factors
Supplemental estrogen use/hormone therapy
Prior diagnosis of cancer
History of myocardial infarction
Congestive heart failure
Chronic obstructive pulmonary disease
Current smoking

ISS Injury Severity Scale, *AIS* Abbreviated Injury Scale, *BMI* body mass index, *MVA* motor vehicle accident, *PRBC* packed red blood cell, *SBP* systolic blood pressure

(current cohort) [13]. Patients in the historical cohort were younger and less severely injured than those in the current cohort. PE occurred in 0.21 % of patients in the historical cohort and in 0.49 % ($p < 0.01$) in the current cohort. Despite the significant increase in injury severity and age, the mortality rate associated with PE decreased from 15 % in the historical cohort to 11 % in the current cohort. Only 20 % of the patients with PE had an associated DVT. This could be explained, at least in part, by centers that only reported on symptomatic DVT; thus, DVT associated with PE would be underreported because asymptomatic DVT was not detected.

In summary, the incidence of VTE reflects the aggressiveness of the pursuit of the diagnosis. Studies reporting the incidence of symptomatic DVT without screening or regular surveillance will underreport the true incidence. Studies that utilize screening and surveillance in combination with mechanical and pharmacologic prophylaxis will report an incidence of DVT between 2.5 % and 17 %, depending on the level of risk for VTE in the study population. Proximal DVT represents about 30 % of DVT diagnosed in trauma patients, while below-knee DVT represents the remainder. The incidence of PE appears to be increasing, but the mortality associated with PE is decreasing. This increase may be due to surveillance bias owing to the recent use of more sensitive multidetector CT scanners. Symptomatic PE occurs in about 1–2 % of *severely* injured trauma patients and is highly dependent on the clinical threshold for obtaining a diagnostic study.

Pathogenesis of Venous Thrombosis

The etiology of thrombus formation in the venous system differs from that in the arterial system. Arterial thrombus contains erythrocytes, fibrin, and platelets. Venous thrombus, in addition to erythrocytes, fibrin, and platelets, also contains leukocytes, the presence of which plays an important role in the pathogenesis of VTE. Historically, the finding of leukocytes in the thrombus led many of the early physicians to suspect inflammation as a cause of thrombosis,

particularly when they observed thrombus in proximity to an abscess [19].

The traditional paradigm for the pathogenesis of venous thrombosis, "Virchow's Triad" (stasis, venous injury, and hypercoagulability), is attributed to Rudolf Virchow [19]. Virchow did not actually describe the conditions necessary for thrombus *generation*; however, he did elucidate the conditions necessary for thrombus *propagation*. Based on a thorough review, historians Bagot and Arya [19] credit Ludwig Aschoff with summarizing the elements that predispose thrombus *generation*:

> [C]hanges in the blood plasma (diminished or increased coagulability)
> [C]hanges in blood elements (increased or diminished powers of agglutination)
> Changes in the blood flow (slowing and formation of eddies)
> Changes in the vessel wall itself (endothelial damage) [20].

Although Aschoff's description comports with current thinking, Virchow's contribution to the understanding of venous thrombosis is nonetheless substantial. Virchow coined the terms "thrombosis" and "embolus," [19] and his triad of factors provides a construct that has served clinicians well for over 156 years. Recent advances in molecular biology identify important cellular processes involved in venous thrombosis and, in a general sense, validate Virchow's (and Aschoff's) paradigm.

The trauma patient represents the "perfect storm" of causative factors for thrombogenesis—the patient is injured, often immobilized, and hypercoagulable.

Venous Stasis

It should come as no surprise that stasis plays a major role in thrombus generation. Even the casual observer will notice a clot forming in stagnant blood. Early support for stasis as a risk factor for DVT was provided by Gibbs, who observed that DVT was strongly associated with the duration of bed rest in hospitalized patients and in the paralyzed limbs of patients suffering a stroke [21]. These observations were corroborated by the seminal autopsy series of 756 trauma and burn patients conducted by Sevitt and Gallagher [22]. They found the highest frequency of venous thrombosis in the veins of the soleus and gastrocnemius muscles as well as the tibial veins of the leg. They also noted from the hospital records that DVT was associated with prolonged bed rest. At the time of their report, two views on the pathogenesis of DVT prevailed. One view was that the thrombus originates in the small soleal and gastrocnemius veins, where flow velocity dramatically diminishes when the muscle(s) is not contracting. The thrombus then propagates proximally into the popliteal vein where it either remains or embolizes to the pulmonary circuit. The other view was that venous thrombus could arise de novo, even in larger veins with relatively high flow velocity, such as the iliac or femoral vein. Thus, Sevitt and Gallagher found continuous thrombus on necropsy extending from the soleal plexus all the way to the iliac vein. They also found discontinuous clot and isolated thrombi in injured and uninjured limbs. This demonstrated that both of the prevailing views on the pathogenesis of DVT were correct and, by extension, that stasis was not necessary to initiate DVT.

The pathogenesis of stasis-associated DVT was further elucidated by work reviewed by López and Chen [23]. They posited that low venous flow or stagnation facilitates the local accumulation of procoagulants, such as thrombin, which typically are washed out by muscle contraction with leg movement. In the absence of leg muscle contraction during bed rest or immobilization, procoagulants accumulate in valve pockets or the small sinusoids in the gastrocnemius and the soleus. Stasis also results in rapid desaturation of hemoglobin in local erythrocytes. The resulting hypoxia then activates leukocytes, platelets, and endothelial cells. Activated endothelial cells release the content of Weibel–Palade bodies which contain von Willebrand factor and membrane bound P-selectin. Both of these proteins remain attached to the endothelial surface and bind leukocytes (a distinguishing pathologic constituent

of the venous thrombus), including monocytes that have the capacity to synthesize tissue factor, particularly during periods of hypoxia. Thrombus formation is further enhanced by local platelet activation.

Venous (Vessel Wall) Injury

Venous or sinusoidal wall injury can occur as a direct result of mechanical trauma, such as an adjacent fracture or muscle contusion. This exposes the subendothelial matrix, a rich source of tissue factor, which initiates the process of thrombin generation [24]. When injury is severe enough to occlude the vein and/or reduce flow, velocity conditions are sufficient for the initiation of DVT. Venous thrombus is also noted to occur at vein junctions, where the vein wall is relatively thin and more likely susceptible to injury and subsequent exposure of the subendothelial matrix [23, 25]. However, endothelial injury adjacent to a fracture does not always produce DVT [22]. Thrombus can also occur in an uninjured limb, in a larger vein segment more proximal to the injury, or in a vein completely remote from the injury.

Thrombus remote from the site of injury was demonstrated by Schaub and colleagues, who investigated the early events that might contribute to postoperative DVT [26]. They performed transmission and scanning electron microscopy on the jugular and femoral veins of canines exposed to one of three types of surgery: splenectomy, hysterectomy, or intestinal division followed by reanastomosis. Each dog was followed for 4 h while paralyzed and anesthetized. The response of the venous endothelium was assessed by examining it for injury and the adhesion of blood elements and debris to the luminal surface. Responses were compared between the three experimental groups and control animals that had no operation, but remained anesthetized and paralyzed for 4 h. Control veins (both femoral and jugular) showed a smooth endothelial carpet with intact cellular junctions that had only rare cells and very small amounts of noncellular debris attached to luminal surface. The endothelium of veins from splenectomized animals had raised endothelial junctions with pseudopod formation and attachment of cells, mostly leukocytes and platelets, and noncellular granular and particulate matter. The femoral veins from these animals also showed microthrombi. Endothelial changes and attachment of cells, granular material, and fibrinous debris were more frequent and more severe in the animals having either hysterectomy or intestinal anastomosis. In areas free of cells or debris, the endothelial surface was similar to that of the control animals. However, in areas where leukocytes and platelets were attached, endothelial alterations were also present. These included indistinct cellular junctions and pseudopod formation.

It appears from Schaub's [26] work that remote abdominal operative trauma can produce a systemic effect manifested on the jugular and femoral venous endothelium—perhaps changing its "phenotype" to a procoagulant one. If this is indeed the case, it provides a mechanism to explain the observations of Sevitt and Gallagher [22]; namely, that DVT can occur in veins remote from the sites of injury or stasis. It also suggests that the vehicle or agent for this occurrence exists in the circulation. The attachment of leukocytes in the presence of endothelial alterations implicates a chemotactic stimulus in the apparently uninjured endothelium, not unlike that which occurs during inflammation. One may speculate that the attached leukocytes produce local endothelial cell injury and activation. The normal-appearing endothelium in the control (anesthetized and paralyzed) animals further suggests that relatively brief periods of stasis without trauma have little effect on remote veins.

Hypercoagulability

There is now substantial evidence that trauma patients are initially hypercoagulable following injury. Under conditions of protracted shock, acidosis, and hypothermia, trauma patients can rapidly become hypocoagulable [27]; however, most injured patients present-

ing without shock, acidosis, and hypothermia are hypercoagulable.

Several notable studies examined hypercoagulability in trauma patients. In a prospective study of severely injured patients (mean ISS of 23), Owings and colleagues demonstrated that antithrombin activity fell below normal in a majority of patients early in the hospital course [28]. They found that patients who developed DVT had lower antithrombin levels than those who did not. Meissner and colleagues prospectively measured prothrombin fragment 1 + 2 and D-dimer levels in severely injured patients (mean ISS of 27) [29]. They found that these levels were markedly elevated at admission and throughout the hospital course. However, elevation of these markers of activated coagulation was not predictive of DVT. The authors concluded that "increased thrombin generation and intravascular fibrin formation are present in virtually all seriously injured patients [29, pp. 227–228]" and that hypercoagulability persists for at least 30 days after injury. They further proposed that injury results in systemic hypercoagulability that manifests itself locally. Utter and colleagues prospectively studied monocyte expression of tissue factor in severely injured patients (mean ISS of 24) [30]. They found that monocytes express tissue factor, a procoagulant, within hours of injury and this continues for up to 3 days following admission. More recently, Selby and colleagues prospectively studied the sequential changes in coagulation markers and their relationship to VTE in 135 severely injured trauma patients (mean ISS of 24) [31]. They examined several markers indicative of thrombin generation at 24 h, 2 days, 5 days, and 12–14 days following injury. All patients underwent venography to detect both symptomatic and asymptomatic DVT. Of 101 evaluable patients, VTE occurred in 59 %. Markers of thrombin generation were significantly elevated in all patients after injury (not just those who had VTE). Of note, despite the increase in thrombin generation, no compensatory increase in Tissue Factor Pathway Inhibitor was observed. Acquired protein C resistance and hypofibrinolysis did not appear to contribute to the hypercoagulability. However, the authors were not able to predict which patients would develop VTE based on the plasma markers. They concluded that "[i]t may be more appropriate to focus our collective energy on implementing universal, timely, pharmacologic prophylaxis that has proven efficacy in the prevention of VTE in these high-risk patients rather than attempting to risk stratify them on the basis of laboratory markers [31, p. 286]." Researchers have also confirmed hypercoagulability following trauma using global assays of coagulation (rather than plasma markers), such as thromboelastography (TEG) and overall hemostatic potential [32–35]. Using TEG, Harr and colleagues found that platelets contribute more to this hypercoagulable state than previously held, thus advocating for the addition of antiplatelet agents to the current chemoprophylaxis regimens [34].

In summary, injury strongly invokes all three of the components identified by Virchow and Aschoff as factors contributing to venous thrombosis. Indeed, it is surprising that not all severely injured patients develop DVT.

Pathogenesis of Pulmonary Embolus

Until recently, most trauma clinicians held that a PE arose from a preexisting extremity DVT. However, imaging for PE has evolved from radionuclide scanning and pulmonary arteriography to noninvasive high resolution CT, and more trauma patients (known to be at high risk) are studied at a lower index of suspicion. As a result, more PE are now discovered and fewer are found to have an associated DVT [13, 36, 37], challenging the traditional paradigm that all pulmonary clots arise as an embolus from a DVT.

Recent data support this paradigm shift. In a study examining the incidence of PE using the National Trauma Data Bank, Knudson and colleagues noted that some of the risk factors associated with PE in trauma patients differed from the risk factors for DVT, suggesting that not all

PE are embolic [13]. The authors found that patients with a severe chest injury had a 42 % increased risk of PE with little likelihood of DVT. In contrast, patients with a major head injury were more at risk for DVT than for PE. Velmahos and colleagues, in a review of patients from their institution, found only 15 % of PE with an associated DVT, calling into question the relationship between PE and DVT [36]. Both Knudson [13], and Velmahos [36] hypothesized that some PE may arise de novo as a result of chest injury or local pulmonary inflammation which are diagnosed incidentally on a chest CT done for reasons other than clinical suspicion of PE. They theorized that PE without DVT might constitute a different disease from PE associated with DVT. Support for this theory was offered by Van Gent and colleagues [37]. They studied 2881 trauma patients at high risk for VTE who received duplex screening and surveillance of the lower extremities from ankle to groin. Thirty-one (1.08 %) patients had a PE, only 12 (39 %) of whom had an associated DVT. Patients with a PE but no DVT were younger, incurred more chest injuries ipsilateral to the PE, and had more peripheral clot. The authors surmised that local phenomena associated with injury or inflammation initiated the thrombus, thus coining the term "de novo pulmonary thrombosis."

Based on the foregoing, it appears that PE following trauma can arise from a disease entity distinct from PE associated with DVT. Indeed, the developing consensus is that PE may represent a composite of true emboli from peripheral DVT (representing 20–40 % of cases) and de novo pulmonary vascular thrombosis (representing 60–80 % of cases) associated with local injury or infection.

Risk Factors for VTE Following Trauma

Among hospitalized patients, those with traumatic injury are at the highest risk for VTE [38]. Without chemical or mechanical prophylaxis, the risk of DVT approaches 50 % and PE occurs in up to 1 %. The risk, however, is not equivalent among all injured patients. Data reveal a number of factors that are associated with a higher incidence of VTE [4, 9, 16, 39]. Currently, there is a strong evidence base to support only spinal cord injury with paralysis as a major risk factor for VTE [39].

The identification of VTE risk factors is dependent upon surveillance. Because studies utilizing objective imaging for screening and surveillance will have a higher incidence of DVT [5], they are likely to reveal more risk factors. Knudson and colleagues examined the National Trauma Data Bank (NTDB) to determine those factors associated with VTE and assigned odds ratios based on the strength of the association (Table 26.2) [14]. While identifying important risk factors, the strength of the association with VTE was limited because the NTDB dataset lacked specific data on screening, surveillance or prophylaxis. More recently, Bandle and colleagues applied risk factors identified by Knudson [14] and systematic reviews [39, 40] to classify patients into categories of moderate-, high- and highest-risk for VTE (Table 26.1) [9]. All patients in each risk category received screening and weekly surveillance as well as mechanical and chemoprophylaxis. A DVT was found in 9 % of the total

Table 26.2 Risk factors associated with VTE (univariate analysis) (reproduced with permission) (Knudson et al. [14]).

Risk factor (number with risk)	Odds ratio (95 % CI)
Age ≥ 40 year ($n = 178{,}851$)	2.29 (2.07–2.55)
Pelvic fracture ($n = 2707$)	2.93 (2.01–4.27)
Lower extremity fracture ($n = 63{,}508$)	3.16 (2.85–3.51)
Spinal cord injury with paralysis ($n = 2852$)	3.39 (2.41–4.77)
Head injury (AIS ≥ 3) ($n = 52{,}197$)	2.59 (2.31–2.90)
Ventilator days > 3 ($n = 13{,}037$)	10.62 (9.32–12.11)
Venous injury ($n = 1450$)	7.93 (5.83–10.78)
Shock on admission (BP < 90 mmHg) ($n = 18{,}510$)	1.95 (1.62–2.34)
Major surgical procedure ($n = 73{,}974$)	4.32 (3.91–4.77)

$p < 0.0001$ for all factors
AIS Abbreviated Injury Scale score, *BP* systolic blood pressure

2169 patients; 17 % of 778 patients in the highest-risk group, 5 % of 1173 patients in the high-risk group, and <1 % of 218 patients in the moderate-risk group. Based on the risk assessment, the authors also concluded that screening and surveillance of patients in the highest-risk group offered the greatest value.

An assessment for VTE risk should be performed on every trauma patient admitted to the hospital, and the intensity of prophylaxis should be commensurate with the assessed risk, unless contraindicated [41]. Cognizant of the pathogenesis of VTE, the surgeon managing the individual patient must consider the contribution of each of the following: prolonged bed rest (stasis); mechanical ventilation (stasis: increased intra-thoracic pressure resulting in a reduction in mean venous flow velocity); lower extremity, pelvic, and unstable spine fracture with or without paralysis (stasis, local inflammation, possible venous injury); severe head injury (stasis, release of tissue factor); and, age greater than 50 years (while age is identified as a risk factor in virtually all studies, the mechanism is unclear).

Two models or tools are available to assess the magnitude of risk for the individual trauma patient. Each one is based on the sum of a "weighted" score that is related to the strength of the association of the risk factor with VTE events. The older Risk Assessment Profile (RAP) utilizes 17 variables (Table 26.3) [42], and has been used both retrospectively and prospectively to determine its effectiveness in identifying patients at risk for VTE [42–47]. The newer Thromboembolic Scoring System (TESS) has five variables (Table 26.4) [48], and has been used to assess the risk of symptomatic VTE events. Zander and colleagues recently compared the two models to assess their validity in trauma patients for both symptomatic and asymptomatic VTE; both groups had received VTE prophylaxis [47]. Results revealed TESS had a sensitivity of 49 % and a specificity of 72 % while RAP had a sensitivity of 83 % and a specificity of 37 %. Each of the two models was limited in accuracy, which might be remedied by incorporating additional, yet unidentified, risk factors.

Table 26.3 RAP scoring tool used at admission to characterize patient risk factors for VTE (reproduced with permission) (Proctor et al. [42])

	Weight	Score
Underlying conditions		_____
Obesity (>120 % Metropolitan Life Table)	2	_____
Malignancy	2	_____
Abnormal coagulation factors at admission	2	_____
History of thromboembolism	3	_____
Iatrogenic factors		_____
Central femoral line > 24 h	2	_____
Four or more transfusions during first 24 h	2	_____
Surgical procedures > 2 h	2	_____
Repair or ligation of major vascular injury	3	_____
Injury related factors		_____
AIS* > 2 for the Chest	2	_____
AIS* > 2 for the Abdomen	2	_____
Spinal fractures	2	_____
AIS* > 2 for the head	3	_____
Coma (GCS < 8 for > 4 h)	3	_____
Complex lower extremity fracture	4	_____
Pelvic fracture	4	_____
Spinal cord injury with para or quadriplegia	4	_____
Age		
≥40 but <60	2	_____
≥60 but <75	3	_____
≥75	4	_____
	Total	_____

AIS Abbreviated Injury Scale score, *GCS* Glasgow Coma Scale score

In summary, each trauma patient presents with a unique constellation of demographic characteristics, injuries, and risk factors. These interact to produce an outcome, which may well include VTE. Clinical judgment based on the calculus of harm is required, balancing the risks and benefits with regard to symptoms, use of screening and surveillance, and implementation of prophylactic measures.

Table 26.4 Significant prediction factors for VTE in multivariate model ($n = 16{,}383$) (reproduced with permission, Rogers et al. [48])

Predictor	Coefficient	OR	p	95 % CI	TESS score
Age, years					
18–29	Referent	1.00			0
30–64	0.480556	1.62	0.039	1.03–2.55	1
≥65	0.827127	2.29	0.001	1.44–3.64	2
ISS					
1–9	Referent	1.00			0
10–16	1.42017	4.14	<0.001	2.32–7.38	3
17–25	1.62973	5.10	<0.001	2.79–9.33	3
>25	2.18105	8.86	<0.001	4.91–15.97	5
Preexisting obesity					
No preexisting obesity	Referent	1.00			0
Preexisting obesity	0.557299	1.75	0.041	1.02–2.98	1
Ventilation days					
No ventilations days	Referent	1.00			0
Ventilation days	1.88068	6.56	<0.001	4.33–9.94	4
Lower-extremity fracture					
No lower-extremity fracture	Referent	1.00			0
Lower-extremity fracture	0.925682	2.52	<0.001	1.72–3.70	2
Constant	−7.06655				

Hosmer–Lemeshow $p = 0.101$
Area under the ROC curve, 0.89

ISS Injury Severity Score

Prophylaxis

Two modes of VTE prophylaxis are available: mechanical and chemical (pharmacologic).

Mechanical Prophylaxis

Pneumatic compression hose (PCH) contain an inflatable bladder that intermittently expands and compresses the gastrocnemius and soleus muscles to achieve an increase in venous flow velocity, thereby reducing stasis [49, 50]. The effects of PCH may not be solely mechanical [49]. These devices may also increase fibrinolysis; given transient increases in tissue plasminogen activator (tPA) levels; however, this effect appears to decay within minutes of their discontinuation [51, 52].

Studies generally show that PCH devices are effective VTE prophylaxis in hospitalized patients [53–55]. However, these studies did not examine trauma patients in detail. Dennis and colleagues demonstrated that PCH were significantly better than no DVT prophylaxis in a prospective study of 395 patients with an ISS > 9 [56]. In contrast, Velmahos and colleagues, using meta-analysis, found that PCH were no better than no DVT prophylaxis [57].

There are minimal risks associated with the use of PCH. Although skin necrosis and peroneal palsy have been reported [58, 59], these complications are exceedingly rare and may be related to improper application. PCH devices should be used continuously until ambulation [40]. However, there is a high rate of noncompliance, particularly in patients on general hospital units [60], which may limit their efficacy [60, 61]. The utility of PCH is further limited in patients with lower extremity fracture, external fixators, plaster immobilizers, or significant open wounds, which can occur in up to 35 % of patients [62]. In these situations, foot PCH can be used [39, 63, 64].

In summary, because of their low risk and potential benefit, PCH are an option for patients

who are not ambulatory following trauma, particularly those over the age of 50. PCH should be used when pharmacologic measures are contraindicated.

Pharmacologic or Chemical Prophylaxis

The regimen of 5000 units (U) UFH administered once subcutaneously in the immediate preoperative period and continued every 8 h postoperatively is very effective in reducing the incidence of VTE in patients undergoing elective surgery [65–68]. Given in this fashion, UFH significantly increases the ability of antithrombin to inhibit activated factor Xa [39]. In addition, UFH can inhibit thrombin [39]. Low-molecular-weight heparin (LMWH) can be administered in a similar regimen in surgical patients with similar efficacy, but at a lower dosing frequency of every 12 h [69]. LMWH has a mechanism similar to UFH, but has less ability to inactivate thrombin [70].

The success of UFH and LMWH in elective surgical patients made these agents a logical choice for pharmacologic prophylaxis in trauma patients. Geerts and colleagues compared LMWH (30 mg subcutaneously, twice daily) with UFH (5000 U, *twice* daily) in a prospective randomized clinical trial involving 344 patients, all of whom had significant injury (ISS $\geq$ 9) [6]. Patients underwent bilateral lower extremity contrast venography between 10 and 14 days after admission or just before discharge if discharged earlier. Of the 265 patients with venograms adequate for analysis, VTE was confirmed in 40 (31 %) in the LMWH group compared to 60 (44 %) in the UFH group ($p = 0.014$). No mechanical prophylaxis was used in concert with either agent in this study, which could account for the relatively high incidence of VTE. Velmahos and colleagues conducted a meta-analysis to evaluate the methods of VTE prevention in trauma patients [57]. Using PE as the outcome, they combined data from the two randomized clinical trials comparing low-dose UFH and LMWH with data from the one nonrandomized clinical trial that used the same methods of prophylaxis. No difference in PE rates was seen. Further comparisons among the existing methods of VTE prophylaxis (mechanical prophylaxis, low-dose UFH, LMWH) demonstrated that no one method was more effective than another and no better than no prophylaxis at all. However, the confidence intervals on many of the pooled results were wide, thus a clinically significant effect could still exist.

Both LMWH and UFH administered subcutaneously increase the risk of bleeding and heparin-induced thrombocytopenia (HIT) [70]. Bleeding associated with these agents can occur in up to 2 % of trauma patients receiving them [16]. HIT is rare with both agents, but is thought to be higher with UFH. In a meta-analysis to determine the incidence of HIT in surgical and medical patients receiving thromboprophylaxis with either LMWH or UFH, the absolute risk for HIT was only 0.2 % with LMWH but was 2.6 % with UFH [71].

There are other clinical considerations as well. Because the kidneys clear LMWH, renal function should be assessed prior to administration [40]. LMWH cannot be used safely in the presence of renal insufficiency. In addition, a recent finding that platelets contribute to the post-injury hypercoagulable state has led to the recommendation to add aspirin to the prophylactic regimen [34].

There are patients, such as those with intracranial injuries, in whom the use of anticoagulants is absolutely or relatively contraindicated. This has led to the recommendation for the use of inferior vena cava (IVC) filters as prophylaxis [72, 73], and the use of filters in this role has increased significantly [74]. In a recent meta-analysis, Haut and colleagues found a consistent reduction of PE and fatal PE in trauma patients with IVC filter placement, with no difference in mortality (or DVT) [75]. However, the strength of the evidence was low, and the use of IVC filters for PE prophylaxis remains controversial [76].

In summary, injured patients with any of the noted risk factors deserve some form of prophylaxis. None of the existing measures is superior as VTE events continue to occur despite prophylaxis. As Owings and colleagues

suggest [28], the hypercoagulable state associated with decreased antithrombin activity may significantly diminish the efficacy of heparin (UFH and LMWH). Moreover, one must consider the possibility of preexisting and undiagnosed thrombophilia.

Treatment of Venous Thromboembolism

Once established, VTE should be treated with therapeutic anticoagulation to prevent recurrence [77], which can occur in up to 30 % of patients within 10 years [78]. In the setting of a contraindication to anticoagulation, such as a high risk of bleeding, or in patients with recurrent VTE despite effective doses of anticoagulation, an IVC filter is indicated. Otherwise, therapy is initiated with a heparin (UFH or LMWH) "bridge" followed by a transition to warfarin. This is to avoid the risk of creating a paradoxical hypercoagulable state because the regulatory anticoagulant proteins C and S are susceptible to warfarin [77].

There are several anticoagulant strategies available for the treatment of VTE. In a recent systematic review and meta-analysis, Castellucci and colleagues demonstrated that LMWH-warfarin was associated with a slightly lower rate of VTE recurrence compared to the UFH-warfarin combination [79]. The authors also showed that the Xa inhibitors rivaroxaban and apixaban (rather than warfarin) were associated with a lower risk of bleeding. However, it is important to note that most of the patients in the studies reviewed were medical patients rather than trauma patients.

Controversy exists as to whether the duration of anticoagulation therapy should exceed 3 months. As with prophylaxis, the duration of therapy must be considered in the context of the individual patient. If the temporary trauma is the only risk factor in a young and active patient, 3 months of warfarin should be considered. If other factors (e.g., prolonged inactivity or ongoing infection) are present, 3–6 months is reasonable.

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Congenital and Acquired Hypercoagulable States

27

Joseph Emmerich

Introduction

Venous thrombosis, which includes deep-vein thrombosis (DVT) and pulmonary embolism (PE), affects approximately 1 in 1000 individuals in industrialized countries [1]. The terms "hypercoagulability" or "thrombophilia" are often used to define this condition. Thrombophilia can be congenital (constitutional, genetic, or hereditary) or acquired. Congenital thrombophilia is mainly associated with the occurrence of venous thrombosis, while acquired thrombophilia can be associated with either venous or arterial thrombosis.

In 1856, Virchow described that thrombosis occurs due to stasis of the blood, endothelial injury, and with "changes in the composition of blood" (i.e., hypercoagulability). It was only in 1965 that Egeberg published the first case of inherited antithrombin deficiency, which led to an increased interest in thrombophilia, with the description of protein C (PC) and protein S (PS) deficiencies 15 years later [2]. Thrombophilia was initially considered a rare monogenic disorder, but this view was challenged in the mid-1980s with the evidence of more frequent genetic risk factors of thrombophilia, demonstrating that venous thrombosis is a multicausal disease [3]. At this same time, acquired risk factors such as the antiphospholipid syndrome (APS) were described with the occurrence of venous and arterial thrombosis.

We describe here the main congenital and acquired hypercoagulable states associated with thrombosis, and their implication in clinical practice according to current evidence and guidelines.

The main risk factors of venous thrombosis are summarized in Table 27.1. Acquired causes of thrombosis, such as trauma or surgery, can trigger the occurrence of venous thrombosis alone or when the basal risk of thrombosis is increased by congenital or hypercoagulable states. Thrombosis must be considered as a multifactorial disease. For further description of hypercoagulability and venous thromboembolism after trauma please refer to Chap. 26.

Description of Congenital Thrombophilia

Antithrombin Deficiency

Antithrombin (AT) is a 58 kDa plasma protein that regulates coagulation by inhibiting procoagulant serine proteases such as thrombin, activated (a) factor X, and factor IXa. The reaction between AT and thrombin involves the reactive center loop and the protease active site serine, and is increased

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Table 27.1 Main risk factors for venous thrombosis

Acquired risk factors	Genetic risk factors	Other risk factors[a]
Age	Antithrombin deficiency	Hyperhomocysteinemia
History of venous thrombosis	Protein C deficiency	High levels of factor VIII
Surgery	Protein S deficiency	High levels of factor XI
Cancer	Factor V_{Leiden}	High levels of factor IX
Hormonal treatment	Factor II G20210A	High levels of factor VII
Antiphospholipid syndrome	Dysfibrinogenemia	High levels of TAFI
Myeloproliferative disorders	Fibrinogen γ 10034T	Low levels of TFPI
Trauma	Non-O blood group	APCR in the absence of FV_{Leiden}
Plaster cast		
Obesity		

[a]Possible genetic regulation. *TAFI* thrombin activatable fibrinolysis inhibitor, *TFPI* tissue factor pathway inhibitor

approximately 1000-fold by heparin and other glycosaminoglycans (e.g., heparan sulfate) that are present on the endothelial surface [4]. AT is synthesized by the liver and circulates at a concentration of approximately 2.5 μM; levels are decreased by estrogen and heparin therapy. For further description of AT and its role during physiologic hemostasis as well as during trauma-induced coagulopathy, please refer to Chap. 2.

AT deficiency is transmitted as an autosomal dominant disease (0.02 % prevalence in the general population). Among congenital thrombophilias, AT deficiency is undoubtedly the one bearing the highest risk of VTE (approximately 1 % per year) [5–7]. There are two types of hereditary AT deficiency. Type I or quantitative deficiency is the most frequent and is characterized by decreased activity and a decreased protein concentration. Type II or qualitative deficiencies are caused by functional defects, protein concentrations being normal or near normal in immunoassays. The dysfunction may affect the reactive site (type II_{RS}) or the heparin-binding site (type II_{HBS}) or both (type II_{PE}—pleiotropic effect) [8]. Homozygosity is mostly present in patients with type II_{HBS}, with the exception of one patient with type II_{PE} deficiency. Venous and arterial thrombosis may occur during infancy in such patients [9].

The ability of AT deficient plasma to inhibit bovine thrombin and human FXa in the presence of heparin can be assessed by chromogenic heparin cofactor assays. Concentrations of less than 80 % (without heparin or estrogen treatment) call for further investigation, although only patients with severe deficiencies (<60 %) are at high risk of thrombosis. Chromogenic assays distinguish type II deficiencies, in that type II_{HBS} has normal activity whereas type II_{RS} has low activity; the risk of VTE is very low in type II_{HBS} deficiency [8, 10].

Protein C and Protein S Deficiencies

PC is a vitamin K-dependent protein that is activated at the endothelial surface when thrombin binds to thrombomodulin. This reaction transforms thrombin from a procoagulant enzyme into an inhibitor, by activating PC to activated protein C (APC). In the presence of its cofactor PS, APC degrades activated FV (FVa) and FVIIIa, thereby impeding further thrombin generation [11]. FV inactivation occurs after a rapid cleavage at Arg506, followed by slower cleavage at Arg306 (see below factor V Leiden). PS markedly stimulates the second phase of the inactivation process, by a 20-fold enhancement of Arg306 hydrolysis [12]. The mechanism of FVIIIa inhibition by APC is also biphasic, with cleavage at Arg562 and then at Arg336. FVIIIa inactivation by APC is increased by PS and FV, which act synergistically as cofactors for the reaction.

PC is synthesized by hepatocytes and circulates at a concentration of approximately 70 nM, with a half-life of approximately 8 h [1].

APC forms inactive complexes with serine protease inhibitors, mainly protein C inhibitor (PCI), but also protease nexin 3, α_1 antitrypsin, and α_2 macroglobulin. Although PS is mainly produced by hepatocytes, it is also detected in endothelial cells and platelets. In the circulation, PS forms inactive complexes with C4b-binding protein (C4b-BP) [13]. Free PS represents approximately 40 % of the total circulating level, and only this fraction has APC cofactor activity. PS plasma levels are lower in women younger than 45 years and in those who are pregnant or are using oral contraceptives [14]. For further description of PC and its role during physiologic hemostasis as well as during trauma-induced coagulopathy, please refer to Chap. 6.

PC and PS deficiencies are transmitted, like AT, as autosomal dominant traits. Hereditary PC deficiency was first identified in subjects with a family history of thrombosis. In prospectively studied asymptomatic members of thrombophilic families, the incidence of VTE was approximately 0.5 % per patient-year in patients with PC deficiency and between 0.5 % and 1.65 % in patients with PS deficiency [5–7].

The thrombotic risk associated with PC levels less than 67 % was confirmed in a case–control study of unselected patients who developed DVT before 70 years of age, with a relative risk (RR) of approximately 3.1 [confidence interval (CI) 1.4–7.0] [15]. Free PS of less than the 90th percentile of control values are also associated with an increased risk of developing VTE [16]. In heterozygous subjects belonging to families with PC or PS deficiencies the probability of being free of thrombotic events at 45 years of age is approximately 50 %; they are also at risk of recurrent thromboembolic disease in adulthood. Homozygous PS deficiency, or homozygous PC deficiency, are rare thrombophilia phenotypes associated with severe thrombosis, including neonatal skin necrosis and purpura fulminans [17–19].

PC activity is measured after PC activation by Protac with synthetic substrates (amidolytic assays) or by measuring the prolongation of the activated partial thromboplastin time (aPTT) (anticoagulant assays). In type I, quantitative deficiency, the plasma concentration and activity are low; this is the case in most PC deficiencies. Type II, qualitative deficiency, is characterized by normal synthesis of a nonfunctional protein that affects concentration and coagulation assays. Patients with PC concentrations less than 70 % may have a hereditary deficiency, although values between 55 % and 70 % must be considered as borderline [17].

The diagnosis of PS deficiency is complicated by the presence of two plasmatic forms, one is free PS and the second is PS complexed with C4b-BP/PS. PS deficiency is characterized by a low free PS concentration measured by a monoclonal antibody-based immunoenzymatic assay. APC cofactor activity can be evaluated in an aPTT assay after adding diluted plasma to PS-depleted plasma in the presence of purified APC and purified FVa. According to the International Society on Thrombosis and Hemostasis (ISTH) standardization subcommittee, three types of PS deficiency have been defined on the basis of total PS levels, free PS levels, and APC cofactor activity. Type I deficiency is characterized by low total PS and free PS antigen levels; type II deficiency by normal free PS and low APC cofactor activity; and type III PS deficiency is characterized by low free PS levels and normal or near normal total PS levels. Type I and type III deficiencies in fact appear to be two phenotypic expressions of the same genetic disease. The lower normal limit of total and free PS levels is 65 % of the level observed in a pool of normal plasmas. However, the reference range in women younger than 45 years is approximately 55 % under the same conditions. Therefore, it is recommended to use both the clotting assay and the monoclonal-based immunoassay specific for free PS to screen patients for PS deficiency.

Factor V_{LEIDEN}

In 1993, Dahlbäck described three families in whom APC did not yield the expected prolongation of the clotting time in an aPTT assay, that defined a new phenotype, called APCR

(Activated PC Resistance) [20]. APCR was found in more than 15 % of patients with DVT and in 2–10 % of control subjects [21, 22]. It was then demonstrated that APCR cosegregated with the *FV* gene and with a single base mutation, guanine to adenine at position 1691 of the *FV* gene, which is responsible for the Arg506 Gln mutation known as FV_{Leiden}, affecting one of the APC cleavage sites [23].

FV is a 330-kDa multidomain single-chain glycoprotein, with a plasma concentration of 20 nmol/L (0.007 g/L) [24]. Thrombin and FXa activate FV by a cleavage at peptide bonds at positions 709, 1018 and 1545, thereby releasing the B-domain, which connects the heavy chain (domains A1-A2) to the light chain (domains A3-C1-C2). Upon activation, FVa is formed by the heavy and light chains that are noncovalently associated by a Ca^{2+} ion. FVa is an essential FXa cofactor; its presence in the prothrombinase complex enhances the rate of prothrombin activation into thrombin by 10^3- to 10^5-fold. Downregulation of the procoagulant activity of FVa is accomplished through its inactivation by APC at positions Arg306, Arg506, and Arg679. Cleavage at Arg506 is essential for optimal exposure of cleavage sites Arg306 and Arg679 but results in partial inactivation of FVa (approximately 40 % of procoagulant activity remains). Therefore, any defect on one or more of these three cleavage sites (i.e., Arg506, Arg306, and Arg679) may potentially affect inactivation by APC. FVa inactivation is enhanced by protein S.

FV_{Leiden} results in a substantially reduced anticoagulant response to APC, because FV_{Leiden} is inactivated about ten times slower than normal FV. This impairment of FVa inactivation increases thrombin generation and explains more than 90 % of clinical APCR phenotypes. The fact that FV_{Leiden} is a much less active cofactor of APC than wild-type FV for FVIIIa inactivation contributes also to the APCR phenotype in subjects with FV_{Leiden}. Clinical states with low APC sensitivity that are not caused by FV_{Leiden} may also be acquired, as is the case during pregnancy, with the use of oral contraceptives, or in patients with lupus anticoagulant or high levels of FVIII.

Most cases of APCR are caused by FV_{Leiden}, but two other FV mutations affect the Arg306 cleavage site. Arg306 replaced by Gly in $FV_{Hong\ Kong}$, and Arg306 replaced by Thr in $FV_{Cambridge}$. $FV_{Hong\ Kong}$ is prevalent (approximately 5 %) among the Chinese in Hong Kong, but neither of these mutations is associated with an increased risk of developing venous thrombosis [25]. FV Ile359Thr (FVLiverpool) and FV Glu666Asp are two rare mutations also associated with thrombosis and APCR [26].

The prevalence of FV_{Leiden} is high in populations of white descent, but low in native populations of Asia, Africa, and Australia [27]. All FV_{Leiden} alleles are carried by the same haplotype, leading to the inference that the mutation occurred only once and spread by a founder effect. The estimated time of the mutation is approximately 30,000 years, implying that it took place after the out-of-Africa divergence that occurred approximately 100,000 years ago [28]. Its spread among Whites and its high prevalence suggests that FV_{Leiden} is associated with a survival advantage, such as a decrease in severe bleeding after delivery.

The frequency of FV_{Leiden} in white populations is approximately 5 % for Caucasians; being present in 15–25 % of Caucasian patients with DVT, and the risk of DVT in heterozygous carriers is approximately fivefold higher than in a control population [29]. The association with pulmonary embolism is much weaker than with deep vein thrombosis [30, 31]. Familial and case–control studies show that FV_{Leiden} is a milder thrombophilic state than heterozygous AT, PC, or PS deficiency [5–7]. The absolute risks of VTE events in subjects with AT, PC, and PS deficiency are between 0.5 % and 1.5 % per year, compared to only 0.1–0.3 % per year in heterozygous carriers of FV_{Leiden}.

The prevalence of FV_{Leiden} homozygosity in the general population is approximately 1 in 2500. The thrombotic complications are far less severe than in homozygous PC and PS deficiencies [32]. FV_{Leiden} homozygosity was found in 4.1 % of 1200 consecutive patients with juvenile VTE [33]. In a pooled analysis of eight case–control studies, we found FV_{Leiden} homozygosity in

1.3 % of 2310 cases and 0.13 % of 3204 controls [28]. In homozygotes, the risk of venous thrombosis is increased 30- to 140-fold, yet an additional environmental or genetic risk factor is often present at the first thrombotic event [32, 33].

Two types of tests can be used for diagnosing APCR: the functional APCR assay and the genetic FV_{Leiden} test. The functional assay is based on aPTT prolongation after the addition of purified APC and is expressed as an APC sensitivity ratio: APTT(+APC)/APTT(−APC). A low APC sensitivity ratio defines APCR. Second-generation APCR tests, which use dilution of test plasma into FV-deficient plasma, have very good specificity and can even be used to test patients taking warfarin [34]. It is mandatory to confirm a positive APCR test by direct detection of FV_{Leiden}. The advantage of genetic testing is that it avoids ambiguous results and determines: heterozygous/homozygous status in patients with a low APC sensitivity ratio.

The FII (Prothrombin) G20210A Mutation

Prothrombin has procoagulant, anticoagulant, and antifibrinolytic activities after its activation into thrombin by the prothrombinase complex [1]. Thrombin acts by activating factors XIII, XI, and VIII, V, PC, and the thrombin-activatable fibrinolysis inhibitor (TAFI), and by cleaving fibrinogen to fibrin. Prothrombin is a 72-kDa multidomain single-chain vitamin K-dependent glycoprotein [35]. Prothrombin activation is mediated by FXa, which cleaves prothrombin at Arg271–Thr272 and Arg320–Ile321 to release the catalytic domain from the carboxy-terminal domain. Prothrombin activation is accelerated approximately 300,000-fold in the presence of FVa, phospholipids and Ca^{2+}. The prothrombin gene is 21 kb in length and is encoded by chromosome 11 (position 11p11-q12); it comprises 14 exons separated by 13 introns, with the 5′ and 3′ untranslated regions that may play regulatory roles in gene expression.

By extensively screening the prothrombin genes of 28 families with unexplained venous thrombosis, Poort et al. found one heterozygous nucleotide transition (G to A) at position 20210 in the 3′ untranslated region in 5 probands (18 %) [36]. This mutation is associated with significantly higher prothrombin levels in heterozygotes than in noncarriers and is an independent risk factor for thrombosis, leading to increased thrombin generation [37].

The prevalence of the FII mutation is high in populations of white descent but low or nil in Asians, American Indians, and African Americans [38]. Similarly to FV_{Leiden}, a founder effect explains the high prevalence of the factor II G20210A mutation in Caucasians [39].

The estimated frequency of FII G20210A in white populations is around 2–3 % [29]. This mutation is more common in southern than in northern Europe, a gradient opposite to that of FV_{Leiden} [36]. The risk of DVT is increased by threefold to fourfold in heterozygous carriers of FII G20210A compared to noncarriers. In the rare FII G20210A homozygotes, the risk of thrombosis is only moderately increased, often associated with other genetic or acquired risk factors [40].

Although FII G20210A heterozygotes have 30 % higher prothrombin levels than noncarriers on average, this phenotype cannot be used to identify carriers of the mutation because of a large overlap of prothrombin levels. To check for the mutation, genetic screening after PCR amplification is the only reliable method.

Other Genetic Risk Factors

Some more rare genetic risk factors of thrombosis have been described. They have drawn much less interest in clinical practice, and they are not part of routine thrombophilia testing.

Blood Group Non-O

It was known since 1969 that non-O blood groups are associated with an increased risk of venous thrombosis [41]. O blood group is associated with decreased levels of factor VIII and vWF, due to increased clearance of vWF [41]. A two-fold increased risk for all non-OO genotypes,

with a synergistic interaction with FV_{Leiden} was confirmed in the Leiden Thrombophilia study [42]. The attributable risk of venous thrombosis to the non-O blood group is high due to the magnitude of the frequency.

X-Linked Thrombophilia (Factor IX Padua)

If all genetic causes of thrombophilia were reported to date to be autosomal dominant, a recent report described X-linked thrombophilia due to a Leu for Arg mutation in the factor IX gene. This mutation was found as a gain of function of factor IX, as the male proband had a ratio of activity to antigen of 8 [43]. This mutation was only found in one family and does not play a role in the epidemiology of the disease. On the other hand it is an excellent paradigm defining thrombophilia as the counterpart of hemophilia (see Introduction). Furthermore, it demonstrates that rare mutations in candidate genes could also play a role in unexplained familial thrombophilia.

A Prothrombin Mutation Conveying Antithrombin Resistance

Recently, another exceptional prothrombin mutation was reported, a substitution of arginine for leucine at position 596. This prothrombin mutation had moderately lower activity than wild type thrombin, but mainly its inactivation by antithrombin was substantially impaired. It thus results in an increase in thrombin generation explaining the thrombophilia observed in this family [44].

Table 27.2 summarizes the main genetic hypercoagulable states. Other polymorphisms in candidate genes have been described but are not described here, as their relation with the occurrence of VTE has not been demonstrated.

Clinical Consequences of Congenital Thrombophilia

There is no consensus on the subsets of subjects who qualify for thrombophilia screening, the types of laboratory test to use, or the clinical treatment of patients with thrombophilia. When to screen for thrombophilia depends mainly from the knowledge concerning the associations between thrombophilia and its clinical consequences. Congenital thrombophilia is mainly associated with venous thrombosis. The most salient recommendations for testing are summarized in Table 27.3. Finally, testing for thrombophilia would be useful if we were able to identify patients who are particularly prone to recurrence; however, this is not always the case as the risk of recurrence is not increased for FV_{Leiden} or FII G20210A, even in homozygous or double heterozygous states [45, 46].

Table 27.2 Main features of hereditary thrombophilia

Protein affected	Antithrombin	Protein C	Protein S	Factor V	Factor II
Gene location	1q23-25	2q13-14	3p11	1q21-22	11p11-q12
Type of mutation(s)	Loss-of-function			Gain-of-function	
	Private mutations			Arg506Gln	G20210A
Frequency in the general population %	0.02	0.2–0.4	0.7–2.3	2–10	2–4
Type of assay	Heparin cofactor activity against FXa	Clotting assay or amidolytic assay	Clotting assay or immunoassay for free PS	APCR (second generation aPTT-based assay) or FV genotyping	FII genotyping
Functional effect	Thrombin and FXa inhibitor	Reduce thrombin generation by inactivating FVa and FVIIIa		FV variant resistant to APC inactivation	Increase the circulating FII concentration
Risk of VTE	×10	×4–5	×4–5	×4–5	×3–4

PS protein S, *APCR* activated protein C resistance, *aPTT* activated partial thromboplastin time, *APC* activated protein C, *VTE* venous thromboembolism

Table 27.3 Recommendations concerning screening for thrombophilia

1. *Thrombophilia screening is recommended in patients with*:
– A history of recurrent VTE
– A first VTE at younger than 50 years of age
– A first unprovoked VTE at any age
– A first VTE at an unusual anatomic site, such as the upper limb, cerebral, mesenteric, portal, or hepatic
– A first VTE related to pregnancy, the puerperium, contraceptive or hormone replacement therapy
– Women with two or more unexplained pregnancy losses
2. *Testing for thrombophilia is controversial in*:
– Young women smokers (age <50 years) with a myocardial infarction
– Elderly patients (age >50 years) with a first provoked VTE event in the absence of cancer or an intravascular device
– A first VTE related to SERMs or tamoxifen
– Selected cases of women with unexplained severe preeclampsia, placenta abruptio, or intrauterine growth retardation
3. *Testing for thrombophilia may be indicated in*:
– Asymptomatic adult family members of probands with known coagulation inhibitor deficiency and maybe in FV_{Leiden} families (especially those with a strong family history of thrombosis at a young age)
– Asymptomatic female family members who are pregnant or are considering oral contraceptives or pregnancy
4. *Thrombophilia testing is not recommended*:
– As a general population screen
– As a routine initial test prior to or during oral contraceptive use, hormone replacement therapy, or SERMs therapy
– As a prenatal test, newborn initial test, or as a routine test in asymptomatic prepubescent children
– As a routine initial test in patients with arterial thrombotic events; however, testing can be considered in certain unusual situations, such as in patients with unexplained arterial thrombosis without atherosclerosis or in young patients

VTE venous thromboembolism, *SERMs* selective estrogen receptor modulators, FV_{Leiden} Factor V_{Leiden}

The NICE guidelines, issued in 2012, concerning the management of VTE and the role of thrombophilia testing made the following recommendations concerning thrombophilia screening (www.nice.org.uk/guidance/cg144). These guidelines are for main indications and do not take into consideration detailed situations.

1. Do not offer thrombophilia testing to patients who are continuing anticoagulation treatment.
2. Consider testing for hereditary thrombophilia in patients who have had unprovoked DVT or PE and who have a first-degree relative who has had DVT or PE if it is planned to stop anticoagulation treatment.
3. Do not offer thrombophilia testing to patients who have had provoked DVT or PE.
4. Do not routinely offer thrombophilia testing to first-degree relatives of people with a history of DVT or PE and thrombophilia.

Probably, the most evidence based and detailed information concerning thrombophilia screening and clinical consequences are those from the British Society of Haematology [47]. A partial summary is reproduced below that are applicable to trauma patients (criteria used to quote levels and grades of evidence are indicated in bracket):

- Initiation and intensity of anticoagulant therapy following a diagnosis of acute venous thrombosis should be the same in patients with and without heritable thrombophilia (1B).
- Indiscriminate testing for heritable thrombophilias in unselected patients presenting with a first episode of venous thrombosis is not indicated (1B).
- Decisions regarding duration of anticoagulation (lifelong or not) in unselected patients should be made with reference to whether or not a first episode of venous thrombosis was provoked or not, other risk factors, and risk of anticoagulant

therapy-related bleeding, regardless of whether a heritable thrombophilia is known (1B).

- Testing for heritable .thrombophilias in selected patients, such as those with a strong family history of unprovoked recurrent thrombosis, may influence decisions regarding duration of anticoagulation (C). It is not possible to give a validated recommendation as to how such patients should be selected.
- Testing is not recommended in unselected patients with upper limb venous thrombosis (1B).
- Testing is not recommended in patients with central venous catheter (CVC)-related thrombosis (1C).
- Testing for heritable thrombophilia after a first episode of cerebral vein thrombosis (CVT) has uncertain predictive value for recurrence (C). Decisions regarding duration of anticoagulant therapy in relation to the results of testing are not evidence-based.
- Testing is not indicated in patients with retinal vein occlusion (1B).
- Testing for heritable thrombophilia after a first episode of intra-abdominal vein thrombosis has uncertain predictive value for recurrence (C). Decisions regarding duration of anticoagulant therapy in relation to the results of testing are not evidence-based.
- Neonates and children with purpura fulminans should be tested urgently for protein C and S deficiency (1B).
- A variety of functional methods may be required to identify specific severe type 2 functional defects when levels of protein C or S are not <5 % (1B).
- It is suggested that adults who develop skin necrosis in association with oral vitamin K antagonists (VKAs) are tested for protein C and S deficiency after VKA treatment is withdrawn (2B).
- Case finding of asymptomatic relatives with low risk thrombophilia, such as F5G1691A (FVR506Q, factor V Leiden) or F2G20210A, is not indicated (1B).
- Case finding of asymptomatic relatives with high risk thrombophilia, such as deficiency of antithrombin, protein C or protein S, should only be considered in selected thrombosis-prone families (1B). If testing is performed, the risks, benefits and limitations of testing should be discussed in the context of explained inheritance and disease risk. It is not possible to give a validated recommendation as to how such patients and families should be selected.
- Case finding for very rare homozygosity or compound heterozygous heritable thrombophilia is not indicated as these defects are so rare, they are not predicted by family history, and the risk of unprovoked thrombosis is low (2C).
- If a first-degree relative with venous thrombosis has not been tested then suggest that women consider an alternative contraceptive or transdermal hormone replacement therapy (HRT). Testing for heritable thrombophilia will provide an uncertain estimate of risk and is not recommended (1C).
- If a first-degree relative with venous thrombosis has been tested and the result is negative then suggest that a woman considers an alternative contraceptive or transdermal HRT. Testing for heritable thrombophilia will provide an uncertain estimate of risk and is not recommended (1C).
- If a first-degree relative with venous thrombosis has been tested and the result is positive then suggest that women consider an alternative contraceptive or transdermal HRT before offering testing as a negative test result does not exclude an increased risk of venous thrombosis. Testing for heritable thrombophilia may assist counselling of selected women particularly if a high risk thrombophilia has been identified in the symptomatic relative (C).
- Women should be assessed for risk of pregnancy-associated venous thrombosis primarily in relation to clinical risk factors (1B).
- Most pregnant women with a previous unprovoked venous thrombosis (1B) or pregnancy or combined oral contraceptive (COC)-related thrombosis (2C) will qualify for thromboprophylaxis on clinical risk alone and so testing for heritable thrombophilia is not required.
- Pregnant women with a previous event due to a major provoking factor, e.g., surgery or

major trauma, would not usually require prophylaxis or testing (2B).
- Pregnant women with a previous event due to a minor provoking factor, e.g., travel, should be tested and considered for prophylaxis if a thrombophilia is found (2C).
- In the asymptomatic pregnant woman with a family history of venous thrombosis, testing is not required if the clinical risks alone are sufficient to result in thromboprophylaxis (2C).
- It is suggested that asymptomatic pregnant women with a family history of venous thrombosis be tested if an event in a first-degree relative was unprovoked, or provoked by pregnancy, COC exposure or a minor risk factor (2C). The result will be more informative if the first-degree relative has a known thrombophilia.
- Antithrombotic therapy should not be given to pregnant women with a history of pregnancy complications based on testing for heritable thrombophilia. Randomized controlled trials with a no-treatment or placebo arm in women with a history of pregnancy complications are in progress. If these studies indicate a benefit in women with pregnancy complications and heritable thrombophilia, as compared with women without thrombophilia, only then would there be a rational basis for recommending that antithrombotic therapy is given to pregnant women with a history of pregnancy complications based on testing for heritable thrombophilia.
- Testing asymptomatic women before assisted conception and those with ovarian hyperstimulation syndrome is not indicated (1B).
- Thrombophilia screening of hospitalized patients to identify patients at risk of hospital-acquired venous thrombosis is not indicated (1A).
- All hospitalized patients should be assessed for risk of venous thrombosis regardless of heritable thrombophilia based on a clinical risk assessment (1B). The presence of a previously known heritable thrombophilia may influence the assessment of risk.
- Testing for heritable thrombophilia is not indicated in patients with arterial thrombosis (1B).
- It is suggested that testing for heritable thrombophilia is not indicated in children with stroke (2C).

Finally for patients with trauma or undergoing surgery, prophylaxis recommendations are similar for patients with or without thrombophilia. Patients with history of VTE and a thrombophilic defect need short-term thromboprophylaxis to cover periods of increased thrombotic risk, for example, surgery, trauma, plaster casts, or immobilization. Affected asymptomatic relatives of patients with thrombophilia merit consideration for short-term thromboprophylaxis to cover periods of increased thrombotic risk.

Acquired Thrombophilia

Acquired thrombophilia is mainly represented by two general conditions: the antiphospholipid syndrome and myeloproliferative neoplasms. Cancer is associated with a well-known increased risk of venous thrombosis and of recurrence despite oral anticoagulation, but it is not discussed here.

Antiphospholipid Syndrome

The antiphospholipid syndrome (APS) is an autoimmune systemic thrombophilic disorder characterized by recurrent thrombosis and/or obstetrical morbidity in the presence of at least one persistent antiphospholipid antibody [48, 49]. Several other clinical manifestations can be associated with APS. The clinical and biological criteria for the diagnosis of APS were updated in 2006 and are indicated in the Table 27.4 [50]. The diagnosis of APS is considered if at least one of the clinical criteria is associated with at least one of the laboratory criteria.

APS can be primary or secondary to systemic lupus erythematosus (SLE). In SLE, approximately one third of patients have antiphospholipid antibodies and approximately half of those (10–15 %) will have complications due to APS.

APS is also associated with many other clinical manifestations, also known as “non-criteria

Table 27.4 International consensus criteria for the diagnosis of APS (revised classification of 2006)

Clinical criteria
1. *Vascular thrombosis*
One or more clinical episodes of arterial, venous, or small-vessel thrombosis, in any tissue or organ
Thrombosis should be supported by objective validated criteria (unequivocal findings of appropriate imaging studies or histopathology). For histopathological support, thrombosis should be present without substantial evidence of inflammation in the vessel wall
2. *Pregnancy morbidity, defined by one of the following criteria*:
(a) One or more unexplained deaths of a morphologically healthy fetus at or beyond the 10th week of gestation, with healthy fetal morphology documented by ultrasound or direct examination of the fetus, or
(b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of: (1) eclampsia or severe preeclampsia defined according to standard definitions or (2) recognized features of placental insufficiency[a], or
(c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded
Laboratory criteria
3. *Lupus anticoagulant (LA)* present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies)
4. *Anticardiolipin (aCL)* antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e., >40 GPL or MPL, or >the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA
5. *Anti-β_2 glycoprotein-I antibody* of IgG and/or IgM isotype in serum or plasma (in titer >the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures

Antiphospholipid antibody syndrome (APS) is present if at least one of the clinical criteria and one of the laboratory criteria that follow are met

[a](1) Abnormal or non-reassuring fetal surveillance test(s), e.g., a nonreactive non-stress test, suggestive of fetal hypoxemia, (2) abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxemia, e.g., absent end-diastolic flow in the umbilical artery, (3) oligohydramnios, e.g., an amniotic fluid index of 5 cm or less, or (4) a postnatal birth weight less than the 10th percentile for the gestational age

clinical manifestations" that are listed in Table 27.5. The most severe but rare manifestation of APS is the "catastrophic antiphospholipid syndrome". The severity of this manifestation is related to multiorgan failure related to widespread small-vessel thrombosis characterized histologically by acute thrombotic microangiopathy. Criteria for the diagnosis of catastrophic APS have been edicted [51]. The definite diagnosis requires all four criteria:

1. Evidence of involvement of three or more organs, systems and/or tissues.
2. Development of manifestations simultaneously or in less than 1 week.
3. Confirmation by histopathology of small vessel disease occlusion in at least one organ or tissue.
4. Laboratory confirmation of the presence of antiphospholipid antibodies.

A high index of suspicion of this diagnosis of catastrophic APS requires aggressive and immediate treatment in order to reduce the mortality, which is approximately 50 %.

The biological diagnosis of APS requires the presence of antiphospholipid antibodies. Both lupus anticoagulant (LA) and anticardiolipin (aCL) antibodies of isotype G (IgG) and isotype M (IgM) were initially part of the diagnosis. In the revised criteria anti-β2 glycoprotein I (anti-β2GPI) antibody of IgG and/or IgM isotype were included. The presence of at least one antiphospholipid antibody (with at least one clinical criteria) is necessary for the diagnosis of APS.

Lupus anticoagulants are detected by inhibition of phospholipid-dependent coagulation reaction. They can be suspected by a spontaneous increase of the activated partial thromboplastin time (aPTT), which is not corrected by mixture

Table 27.5 Other clinical manifestations, "noncriteria clinical manifestations," associated with the antiphospholipid syndrome (some of these clinical manifestations can be secondary to unusual arterial occlusions)

1. *Neurologic manifestations*
Migraines, seizures, chorea, Guillain–Barré syndrome (transverse myelitis), transient global amnesia, dementia, peripheral neuropathy, orthostatic hypotension
2. *Cardiovascular manifestations*
Premature atherosclerosis, heart valve disease (valve thickening/vegetations)
3. *Pulmonary manifestations*
Pulmonary artertial hypertension, acute diffuse alveolar damage
4. *Hepatic and gastrointestinal manifestations*
Esophageal necrosis, pancreatitis, colonic ulceration, acalculous acute cholecystitis, mesenteric venoocclusive disease, primary biliary cirrhosis, Budd–Chiari syndrome
5. *Renal manifestations*
Nephropathy, renal artery stenosis, minimal change/focal segmental glomerulonephrosis, membranous nephropathy, mesangial C3 nephropathy, pauci-immune crescentic glomerulonephritis
6. *Skin manifestations*
Leg ulcers, digital gangrene, skin necrosis, livedo reticularis, erythematous macules, purpura ecchymoses, painful nodules, subungual splinter hemorrhages, anetoderma, discoid lupus erythematosus, cutaneous T-cell lymphoma
7. *Ophthalmological manifestations*
Retinal vein or arterial occlusion, amaurosis fugax, optic neuropathy
8. *Hematological manifestations*
Thrombocytopenia, hypothrombinemia, acquired APC resistance, protein S deficiency, acquired von Willebrand syndrome, inhibitors to specific coagulation factors, leukopenia, autoimmune hemolytic anemia
9. *Other manifestations*
Acute adrenal insufficiency due to bilateral adrenal hemorrhage, osteonecrosis, acute sensorineural hearing loss

with normal plasma. More specific tests must be used to screen for the presence of LA: the dilute Russell Viper Venom Time (dRVVT) is a sensitive test to detect the presence of inhibitor of phospholipid dependant coagulation, but false positives exist in case of anticoagulation or other anticoagulation abnormalities. Different aPTT tests with LA-sensitive reagent versus LA-insensitive reagent, and their correction with hexagonal phase array phospholipids or frozen washed platelets (neutralization procedures) can also be used.

The anticardiolipin immunoassay is expressed in IgG phospholipid units (GPL) or IgM phospholipid units (MPL); with 1 unit corresponding to the cardiolipin-binding activity of 1 μg/ml of affinity-purified antibodies. This test has a high sensitivity but a poor specificity mainly in asymptomatic patients, as aCL antibodies can also be associated with infectious diseases. For this reason, positive aCL antibodies as well as LA or anti-β2GPI always need to be confirmed 12 weeks after the initial screening.

Triple positivity for LA, aCL and anti-β2GPI antibodies is strongly associated with the occurrence of the clinical symptoms of APS. Anti-β2GPI is the main target of aPL antibodies and are usually present in association with aCL antibodies, but some patients can have isolated anti-β2GPI antibodies. High levels of antibodies or LA are also more strongly associated with complications. Antiphospholipid antibodies bind to endothelial cells, platelets and monocytes in a β2GPI-dependent manner. Several pathogenic mechanisms explain the occurrence of thrombosis [48, 52]:

- Inhibition of endogenous anticoagulant and fibrinolytic mechanisms.
- Activation of platelets.
- Injury and activation of endothelial cells.
- Complement activation.

Long term anticoagulation with oral anticoagulants is the cornerstone for treatment of APS once thrombosis has occurred. Intensity of antivitamin K is recommended at the usual doses (INR between 2 and 3), but in some severe cases recurrence can be observed and higher anticoagulation levels are then necessary [52, 53]. The new oral anticoagulants (direct inhibitors of thrombin or factor Xa) are of potential interest for long term treatment, but their evaluation in this indication is still ongoing. There can be one exception for anticoagulation in thrombosis associated with APS, it is the secondary prevention of stroke in patients without other history of thrombosis.

In the APASS study, a randomized trial comparing warfarin (INR between 1.4 and 2.8) to aspirin (325 mg) in patients with APS and ischemic stroke, no differences were observed in the recurrence rates after a follow-up of 2 years [54]. This negative result could be explained by the fact that the majority of patients had low or moderate levels of LA or ACL antibodies, by the low intensity of warfarin treatment and also because APS could not be ascertained in this population as repeat testing was not performed.

Hydroxychloroquine (HCQ) is a synthetic antimalarial drug currently used in SLE. It has anti-inflammatory and immunomodulative properties without causing immunosuppression. It increases lysosomal pH, interferes with antigen processing, and modulates the immune response mediated by toll-like receptor 9. Furthermore, HCQ has an antithrombotic effect by inhibition of platelet aggregation, and also by reduction of the attachment of anti-β2GPI complexes to phospholipids and cells, by reversing the binding of antiphospholipids antibodies to syncitiotrophoblasts, and by restoring annexin 5 expression. For these reasons HCQ is a well established drug used for treatment of SLE, and it has been demonstrated that in secondary APS it reduces arterial and venous thrombosis events [53]. A recent prospective non-randomized study compared anticoagulation alone versus HCQ (400 mg daily) plus anticoagulation in primary APS. Six recurrent thrombotic events were observed in the 20 patients treated with anticoagulation alone, compared to none in the HCQ plus anticoagulation group [55]. A major limitation of this study is that only venous thrombosis events were included, and its results do not represent the effects of this treatment modality on arterial thrombotic events.

Statins, beside their hypocholesterolemic and preventive effects on coronary artery disease also display pleiotropic and anti-inflammatory properties [52, 53]. Since statins have been beneficial in this setting, clinical studies remain necessary to fully establish their role in APS.

For the treatment of the catastrophic APS, a life-threatening disease, aggressive treatment is required, which is a combination of anticoagulation, glucocorticoids, plasma exchange, cyclophosphamide, intravenous immunoglobulins and antiplatelet agents. New therapeutic approaches have been tested in small series of patients using rituximab (anti-CD20 antibody), eculizumab (inhibitor of complement terminal pathway), and defibrotide.

In pregnant women, the goal of treatment is the prevention of adverse maternal and fetal outcomes such as: maternal thrombosis, fetal loss, eclampsia, placental insufficiency, fetal growth restriction, and preterm birth. Subcutaneous low-molecular weight heparin (LMWH) and low dose aspirin are the treatment of APS in pregnancy with introduction of LMWH during the first trimester and aspirin prior to conception due to its beneficial effect on early stage of implantation [49]. There is no more room for glucocorticoids or venous immunoglobulins in the prevention of APS related complications associated with pregnancy.

Myeloproliferative Neoplasms

Myeloproliferative neoplasms (MPN) comprise three classic entities: polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF); which are clonal expansion of an abnormal stem/progenitor cell (BCR-ABL negative) [56–58]. They are associated with the JAK2 (V617F) mutation in exon 14, which is found in >95 % of PV and around 60–70 % of ET and PMF patients. In patients with ET or PMF without JAK2 mutations, somatic mutations in the endoplasmic reticulum chaperone CALR (Calreticulin) are also frequent, associated with 15–25 % of ET or PMF [59]. Other mutations have also been identified in MPN, like Myeloproliferative Leukemia Virus oncogene (MPLW515L is an acquired mutation that induces constitutive activation of the JAK-STAT pathway) or in exon 12 of JAK2. MPN are rare diseases, as for each of them, their yearly incidence is between 1 and 2/100,000. The usual median age at diagnosis is around 70 years, but around 20 % of cases occur in younger individuals. Their natural history is a predisposition to

thrombo-hemorrhagic complications (for PV and ET) but also the risk to transform into myelofibrosis and acute leukemia. It is not rare that the diagnosis is made after an arterial or venous thrombotic event, based on anomalies found in the systematic hemogram performed at this occasion or on hemogram performed in asymptomatic patients.

Endogenous in vitro erythroid or megakaryocyte colony formation, in the absence of growth factors, is a characteristic of MPN. Erythropoietin-independent proliferation is observed mainly in PV but also in ET and PMF.

The diagnosis of PV, ET, and PMF is based on the World Health Organization diagnostic criteria from 2008, summarized in Table 27.6.

PV must be suspected in the presence of hemoglobin levels above 18.5 g/dl in men and 16.5 g/dl in women. The second major criteria is the presence of the JAK2 V617F mutation or JAK2 exon 12 mutation. Three minor criteria are bone marrow trilineage proliferation, subnormal levels of erythropoietin (EPO) and endogenous erythroid colony growth. In clinical practice when the diagnosis of PV is suspected EPO measurement and search for JAK2 V217F must be performed:

- If EPO is subnormal and JAK2 V217F mutation is positive the diagnosis is done.
- If EPO is normal or elevated and JAK2 V217F mutation is negative, the diagnosis of PV is unprobable.
- If EPO is subnormal and JAK2 V217F mutation is negative, screening for JAK2 exon 12 mutation and bone marrow biopsy are required.

The diagnosis of PV must be suspected in the presence of clinical symptoms often insidious and non specific such as pruritus increased by water, reddened face, headaches, dizziness, weakness, fatigue, unexplained weight loss, excessive sweating, double or blurred vision, and gouty arthritis. Nowadays, diagnosis is often suspected after reading a hemogram performed for another reason or at the time of a complicated event such as arterial or venous thrombosis or more rarely hemorrhage.

The diagnosis of ET must be suspected by non specific symptoms such as weakness, headache, dizziness, erythromelalgia and of course in case of thrombotic complications or hemorrhage. In the case of an increased platelet count, the first

Table 27.6 Criteria for polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF)

	Major criteria	Minor criteria
PV	1. Hb > 18.5 g/dl for men and 16.5 g/dl for women	1. BM trilineage proliferation
	2. Presence of JAK2V617F or JAK2 exon 12 mutation	2. Subnormal EPO levels
		3. Endogeneous erythroid colony growth
ET	1. Platelet count > 450×10^9/L	
	2. Megakaryocyte proliferation with large and mature morphology	
	3. Not meeting WHO criteria for CML, PV, PMF, myelodysplasia or other myeloid neoplasm	
	4. Demonstration of JAK2V617F or other clonal marker or no evidence of reactive thrombocytosis	
PMF	1. Megakaryocyte proliferation and atypia accompanied by either reticulin and/or collagen fibrosis	1. Leukoerythroblastosis
	2. Not meeting WHO criteria for CML, PV, PMF, myelodysplasia or other myeloid neoplasm	2. Increased serum LDH level
	3. Demonstration of JAK2V617F or other clonal marker or no evidence of reactive marrow fibrosis	3. Anemia
		4. Palpable splenomegaly

BM bone marrow, *Hb* hemoglobin, *EPO* erythropoietin, *CML* chronic myeloid leukemia, *LDH* lactate dehydrogenase

approach is to exclude reactive thrombocytosis secondary to infection, tissue damage, chronic inflammation, cancer, renal disorders, hemolytic anemia, blood loss, or post-splenectomy or iron deficiency (ferritinemia). Other causes of primary thrombocytosis that must be excluded to fulfill diagnosis of ET are chronic myeloid leukemia, acute leukemia, myelodysplastic syndrome, and myeloid neoplasm.

The diagnosis of ET is then based on four major criteria: platelet count above 450×10^9/L, megakaryocyte proliferation with large and mature morphology, absence of other myeloid neoplasm and demonstration of JAK2V617F or other clonal marker and no reactive thrombocytosis (Table 27.5). It should be noted that bone marrow (BM) examination in ET is often necessary to make an accurate morphologic diagnosis of ET and distinguish it from pre-fibrotic PMF or other myeloid neoplasms, with differences in the appearance of megakaryocytes. In the absence of JAK2V617F, the possibility of CML is addressed by screening for BCR-ABL1 mutation.

PMF is a rare disease, compared to PV or ET. Usually it can be discovered in the screening for anemia, a deterioration of the general status or painful and palpable splenomegaly. Hemogram revealed anomalies on the three cell lineages with the presence of nucleated red blood cells with teardrop-shaped erythrocytes, granulocytes precursors and megakaryocytes. However this myelophthisic smear is not specific and can be associated with myeloid malignancy, bone marrow fibrosis, or an infiltrating process. Thus, a BM biopsy with a careful morphologic evaluation and cytogenetic tests are necessary for the diagnosis of PMF.

The epidemiology of thrombosis in MPN has been recently reviewed [57]. Thrombotic events are more frequent in PV than in ET and PMF. Arterial thrombosis accounts for two thirds of all thrombotic events. Large arteries (cerebral, coronary, and peripheral vessels) are frequently involved. In around 30 % of patients with PV, and in 20 % of patients with ET, the diagnosis is made after the occurrence of an initial thrombotic event, while during follow-up recurrence of thrombosis is observed in 20–33 % of patients.

Other unusual sites of thrombotic occurrences, mainly Budd–Chiari syndrome, and portal or mesenteric vein thrombosis, are also clinical features of MPN. Even in the absence of patent MPN the search for "occult MPN" is mandatory as it is present in one third of Budd–Chiari syndrome patients, as well as in non-malignant, non-cirrhotic, portal vein thrombosis patients [60]. In Budd–Chiarri syndrome the prevalence of MPN is 40.9 % and the prevalence of JAK2V617F mutation is 41.1 %; in portal vein thrombosis the prevalence is 31.5 % and 27.7 % respectively. PV is more prevalent in Budd–Chiari syndrome than in portal vein thrombosis. These results validate the systematic screening for JAK2V617F mutation in the diagnostic workup of patients with splanchnic vein thrombosis and the notion of occult MPN. In a meta-analysis, JAK2 exon 12 and MPL515 mutations were extremely rare, and hence, should not be used when screening splanchnic vein thrombosis patients for thrombophilias [61].

In the pathogenesis of MPN several parameters must be taken into consideration; those clinical risk factors of the patient that are not specific to MPN and the disease-related risk factors [56, 57]. Age (>60 years old) and a history of thrombosis have consistently proven to be independent predictors of thrombosis in PV, ET, and PMF, with a 1.5 and 1.9 hazard ratio after a follow-up of 6 years, respectively [57]. Conventional risk factors for atherosclerosis (hypertension, hypercholesterolemia, diabetes, smoking) have also been associated with arterial thrombosis in MPN. Male gender is also associated with a higher risk of thrombosis, mainly myocardial infarction in patients with PV. In ET male gender is also associated with a higher risk of venous thrombosis.

Concerning disease-related risk factors, mainly two parameters are taken into account: the JAK2V617F status and blood cell counts. JAK2V617F status could influence the risk of thrombosis in ET with a doubling of the risk and possibly also in PMF [57]. In PV a higher JAK2V617F burden (>75 % V617F allele) should allow to discriminate patients with a higher risk of thrombosis. Red cell count is a major cause of

morbidity and mortality in PV and is a major determinant of blood viscosity and thrombosis. A hematocrit target below 45 % is associated with a significant lower incidence of thrombosis and cardiovascular deaths. At variance, no study demonstrated a significant correlation between platelet count or function and thrombosis in PV and ET. Instead, extreme thrombocytosis seems to reduce the risk of thrombosis (maybe by an acquired von Willebrand disease) and is even associated with a significant increase risk of hemorrhage (almost tenfold higher above 1.000×10^9/L) [56–58]. Leukocytosis was also found to be to be an independent risk factor for arterial thrombosis in PV when white blood cell count exceeds 15×10^9/L [57]. In ET, leukocytosis was also an independent predictor of both thrombosis and survival.

Finally, by taking into account these risk factors, the IPSET (International Score of thrombosis in WHO—Essential Thrombocythemia) thrombosis score was developed (Table 27.7) [57, 58]. This simple score includes age, cardiovascular risk factors, previous thrombosis, and JAK2V617F and allows a prognostic model comprising three classes: low, intermediate, and high risk. Low-risk patients had a thrombosis-free survival of 1.03 % patients/year, the intermediate-risk 2.35 %, and the high-risk 3.56 %.

Beside the identification and appropriate management of cardiovascular risk factors and the promotion of a healthy lifestyle, as in the general population, specific measures are recommended. Nevertheless, the supporting evidence is generally weak as there are only few prospective and interventional studies.

The efficacy of aspirin has been demonstrated in PV in a randomized trial, with a RR of 0.4 in the treated group compared to placebo for combined endpoint including nonfatal myocardial infarction, stroke and major thromboembolism [62]. In ET the efficacy of aspirin has not been tested and is used to control clinical symptoms like erythromelalgia and transient ocular or neurologic episodes before cytoreduction. When platelet count is high, aspirin is not recommended due to the risk of bleeding.

All PV patients should also be treated by phlebotomy to lower the hematocrit, and high-risk patients should also receive cytoreduction, mainly with hydroxyurea which is more effective in reducing thrombotic complications than phlebotomy alone. The goal of treatment is to achieve a target hematocrit ≤45 %, as it has been demonstrated that, compared to a less intensive treatment with an hematocrit of 45–50 %, the risk of thrombosis was decreased fourfold (2.7 % versus 9.8 % after a mean follow-up of 31 months) [63]. In ET, cytoreduction therapy is not indicated in patients without or with controlled cardiovascular risk factors; in high-risk patients, hydroxyurea or interferon α are first line therapy when cytoreduction is recommended [64]. New generation drugs, with JAK2 inhibitory activity (ruxolitinib, lestaurtinib, pacritinib) have been found to be effective for the treatment of splenomegaly or adult patients with myelofibrosis, but no data are available concerning their efficacy on thrombosis.

Table 27.7 The IPSET-thrombosis score

Risk factor	Hazard ratio	Score
Age > 60 years	1.50	1
Cardiovascular risk factors	1.56	1
Previous thrombosis	1.93	2
JAK2V617F	2.04	2

Score: 0–1 = low risk; 2 = intermediate risk and ≥3 = high risk

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Part VI

Organ-Specific Coagulopathy

Coagulopathy of Traumatic Brain Injury (TBI)

28

Marc Maegele

Traumatic brain injury (TBI) is commonly associated with hemocoagulative disorders but incidence rates vary considerably between studies (10–90 %) due to heterogeneity in patients and study design, inconsistency in the definition of coagulopathy and laboratory assays (e.g., prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), platelet count), diversity in the magnitude of injury, and the mix between early and delayed disturbances [1, 2]. A meta-analysis of 34 studies on the frequency of coagulopathy after civilian TBI has demonstrated that one out of three patients suffering from TBI displays signs of coagulopathy [1]. These results were confirmed by a systematic review conducted by Epstein et al. that included 22 studies [2]. By using a random effect model the pooled proportion of patients with acute coagulopathy in the setting of isolated TBI was 35.2 % (95 % CI: 29.0–41.4). According to most recent data from the TR-DGU® (TraumaRegistry® of the German Society for Trauma Surgery) database, studying the 2010–2013 period, the prevalence of coagulopathy in patients with TBI was similar to that in injured but without a TBI at 23 % (Fig. 28.1a). While hemocoagulative disorders may occur in >60 % of patients with severe TBI [3], in mild head injury coagulopathy is uncommon (<1 %) [4]. Among injury types, coagulopathy is more common in patients suffering from penetrating head injuries, most likely due to more severe tissue damage and disruption of the blood–brain barrier (BBB).

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Risk Factors for Hemocoagulative Disorders After TBI

Wafaisade et al. have retrospectively assessed the TR-DGU® database for frequency, outcome, and risk factors of coagulopathy in isolated TBI [5]. Out of 3,114 patients, 706 (22.7 %) were coagulopathic upon emergency room (ER) arrival and stepwise logistic regression analysis identified the following independent risk factors for the development of acute coagulopathy after TBI:

1. Magnitude of head trauma as reflected by AIS_{head} ≥3 (Abbreviated Injury Scale for head)
2. Glasgow Coma Scale (GCS) at scene ≤8 points
3. Systolic blood pressure ≤90 mmHg at scene or upon emergency room (ER) arrival
4. Pre-hospital intravenous fluid administration ≥2000 ml
5. Age ≥75 years

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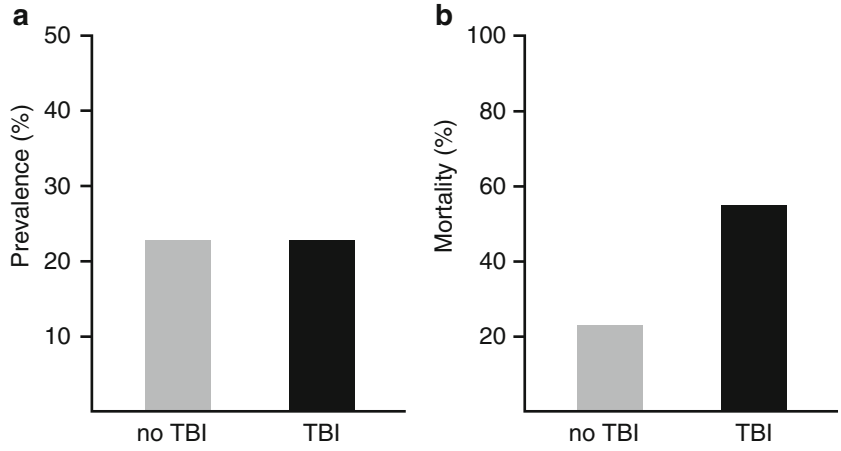

Fig. 28.1 Prevalence of coagulopathy and mortality in non-TBI versus TBI trauma patients according to most recent data from the TR-DGU® (TraumaRegistry® of the German Society for Trauma Surgery) database studying the 2010–2013 period. The prevalence of coagulopathy upon emergency room (ER) admission in patients with and without TBI was similar at 23 % (**a**). If TBI is coincidenced with coagulopathy mortality is threefold higher compared to non-TBI trauma patients with coagulopathy (**b**). Data from the German TR-DGU®-database 2010–2013: $n = 37.423$ patients; Injury Severity Score (ISS) ≥16; Quick's value <70 % (INR >1.2); platelets $<100 \times 10^9$/l

Chhabra et al. have prospectively studied a cohort of 208 patients identifying head injury severity, elevated d-dimers, low hemoglobin, and affected cisterns on head CT as risk factors for the development of coagulopathy after acute TBI [6]. This risk association is further supported by prospective data from Talving et al. who identified the presence of cerebral edema, subarachnoid hemorrhage, and midline shift as additional risk factors for the development of coagulopathy in the context of isolated TBI [7]. Similar findings have been reported in the military setting [8].

Disease Progression Over Time

It has been observed that the number of patients with isolated TBI that develop coagulopathy may double within the first 24 h after injury and that hemostatic abnormalities reflected by impaired global coagulation parameters may continue until the third day after injury or even longer [9]. Lustenberger et al. studied 127 patients with isolated severe TBI in which coagulopathy defined as thrombocytopenia, and/or elevated INR, and/or prolonged aPTT occurred at a mean of 23 ± 2 h [range 0.1–108 h (0–4.5 days)] after ER admission with a mean duration of 68 ± 7.4 h [range 2.6–531 h (0.1–22.1 days)] [10]. In this study, the time interval to the onset of coagulopathy decreased substantially with increasing magnitude of injury. Early hemocoagulative abnormalities occurring within 12 h after admission along with markers of devastating head injury (AIS_{head} 5), penetrating mechanism, subdural hematoma (SDH), and low GCS were independent risk factors for mortality. Those patients developing coagulopathy within 24 h of injury had a mortality rate of 55 % versus 23 % in those developing abnormalities at a later stage after 24 h [10]. Uncorrected coagulopathy in the context of severe TBI has also been identified as an independent risk factor for and related to an 11.5-fold increased risk of intraoperative severe brain swelling [11]. Figure 28.2 depicts a clinical example of dramatic disease progression and development of severe acute coagulopathy in a 64-year-old male patient with severe TBI after a fall. Cranial computed tomography (CT) upon ER arrival revealed a severe open TBI and conventional coagulation assays indicated borderline coagulopathy. After 2 h of observation on the intensive care unit (ICU) the patient developed anisocoria and a repeated CCT showed a

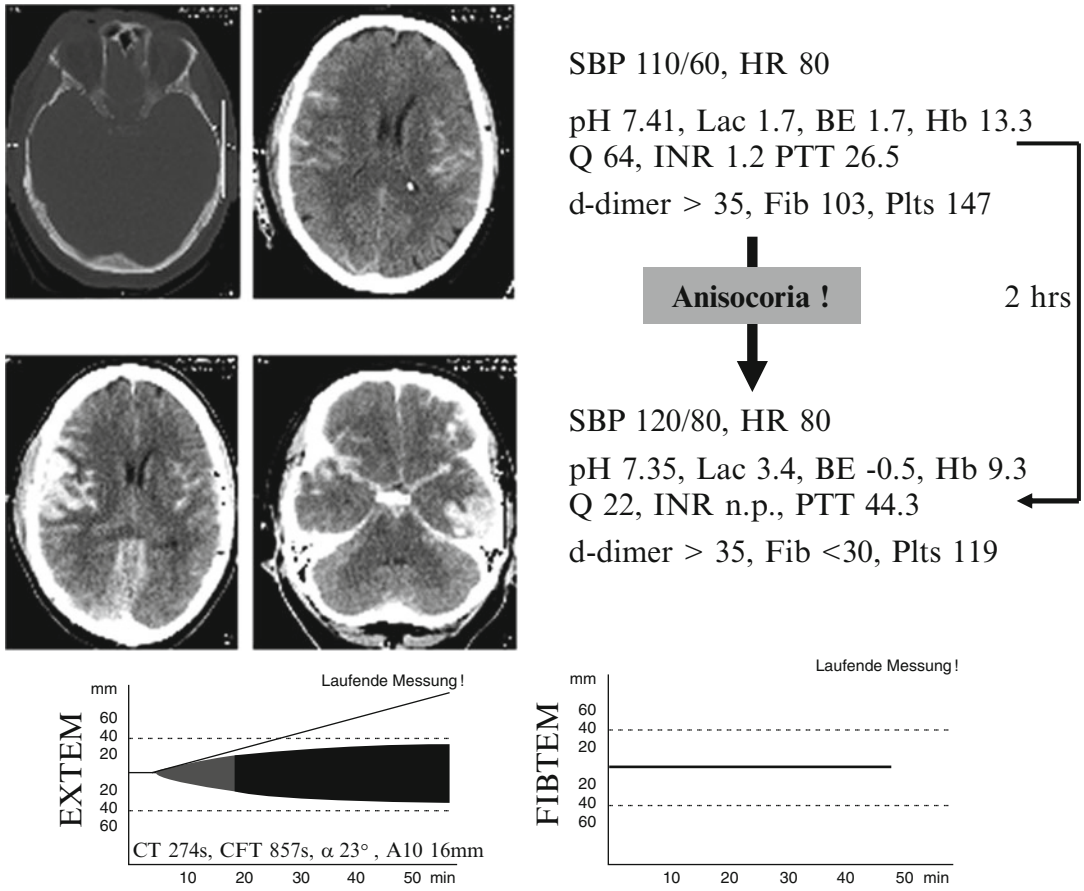

Fig. 28.2 Disease progression and development of severe acute coagulopathy in a patient with severe traumatic brain injury (TBI). A 64-year-old male was admitted to the emergency room (ER) after a fall from a flight of stairs onto his head. He arrived intubated and mechanically ventilated with stable vital signs and absence of any history of anticoagulants or platelet aggregation inhibitor intake. Cranial computed tomography (CCT) revealed a severe open TBI with multiple fractures to the skull combined with mixed subdural and subarachnoidal hemorrhage and generalized brain edema. Standard coagulation tests indicated borderline coagulopathy and the patient was admitted to the intensive care unit (ICU) for further observation. Two hours later, the patient developed anisocoria and repeated CCT showed a dramatic hemorrhage progression together with signs of severe coagulopathy as measured by standard coagulation tests. Additionally performed viscoelastic testing (thromboelastometry (ROTEM®)) further specified the quality of the clotting problem. The EXTEM test result indicated a substantial delay in the initiation of the clotting process as reflected by prolonged clotting time (CT) and clot formation time (CFT) as well as a dramatically reduced clot firmness at 10 min after initiation of the assay (MCF A 10: maximum clot firmness). The FIBTEM test result showed absence of any fibrin polymerization due to a substantial lack of fibrinogen as substrate for clotting. *BE* base excess, *Fib* fibrinogen, *Hb* hemoglobin, *HR* heart rate, *hrs* hours, *INR* international normalized ratio, *Lac* lactate, *Plts* platelets, *PTT* partial thromboplastin time, *Q* Quick's value, *SBP* systolic blood pressure

dramatic hemorrhage progression along with laboratory signs of severe coagulopathy. Additionally performed thromboelastometric testing (ROTEM®) indicated insufficient clotting as well as a dramatically reduced clot firmness. The FIBTEM test result showed absence of any fibrin polymerization due to a substantial lack of fibrinogen as substrate for clotting.

Coagulopathy and Secondary Injury in TBI

Coagulopathy plays a major role in the development of secondary injury as demonstrated by the occurrence of new or progression of initial lesions on follow-up CCT, further compounding the sequelae of TBI. In a review of 253

patients with serial CCT scans after TBI the risk of developing delayed insults was 85 % if at least one coagulation test upon ER admission (PT, aPTT, or platelet count) was abnormal versus 31 % if coagulation tests upon ER admission were within reference ranges [12]. Similarly, Allard et al. have reported intracranial hemorrhage (ICH) progression in 80 % of TBI patients if an abnormal coagulation test was present upon admission versus 36 % in non-coagulopathic patients; all patients with an abnormal aPTT experienced progression [13]. Intracranial hemorrhage progression carried a fivefold higher odds of death; 32 % of patients with progression died versus 8.6 % without. Within their study cohort of 142 TBI patients, Oertel et al. reported progressive intracranial hemorrhage in 48.6 % of patients undergoing repeated scanning within 2 h of injury, with the initial PTT being the best predictor of intracranial hemorrhage progression on logistic regression [14].

Vice versa, neuropathological studies in humans as well as in experimental models have demonstrated the formation of microthrombi in smaller vessels and within the microcirculation, thus causing ischemia with secondary injury both locally and at more distant sites from the injury [15]. The mechanisms behind these findings remain unclear but may involve both local and systemic states of hypercoagulability as well as alterations in blood flow at injury sites that promote stasis. In a clinical study, Chen et al. have assessed the influence of trauma-induced hemocoagulation disorders on the development of post-traumatic cerebral infarction (PTCI) in 265 patients with moderate or severe TBI within the first week after injury [16]. In this study, PTCI was observed in 10 % ($n=28$) of the patients and subsequent multivariate analysis identified thrombocytopenia (OR 2.2; CI 95 % 1.1–4.7), an abnormal PT (OR 3.2; CI 95 % 1.1–7.6), d-dimer <2 mg/l (OR 7.3; CI 95 % 1.8–28.1), or disseminated intravascular coagulation (DIC) scores ≥5 (OR 4.7; CI 95 % 1.8–12.5) to be independently associated with an increased risk of PTCI.

Coagulopathy as a Predictor of TBI Prognosis

Coagulopathy upon ER arrival in TBI patients is almost uniformly associated with worse outcomes and, therefore, represents a powerful predictor of prognosis [1, 2, 5, 6, 9]. Unfavorable neurological outcome after isolated TBI is largely determined by early hemorrhagic progression of brain contusions (HPC) and coexisting coagulopathy [17]. The risk of dying in patients with coagulopathy after TBI is about ten times higher than in patients without coagulopathy and the risk of unfavorable outcome in surviving patients is even more than 30 times higher if coagulopathy is present upon ER arrival [1]. In their recent review, Epstein et al. reported mortality rates up to 86 % when TBI is coincidenced with acute coagulopathy [2]. In a prospective observational cohort of adult patients ($n=345$) admitted to a Canadian Level I Trauma Center the presence of coagulopathy (OR 5.61; CI 95 % 2.65–11.86, $p<0.0001$) and isolated severe TBI (OR 11.51; CI 95 % 3.9–34.2, $p<0.0001$) were independent risk factors for in-hospital mortality [18]. Even in the absence of differences in the initial severity of TBI, in-hospital mortality is doubled in the presence of coagulopathy in patients with traumatic subdural hematoma [19]. Most recent data from the German TR-DGU® database revealed a threefold increase in mortality if TBI is coincidenced with coagulopathy compared to non-TBI trauma with coagulopathy (Fig. 28.1b). A previous analysis from the same database revealed an overall hospital mortality for isolated blunt TBI with coagulopathy of 50.4 % versus 17.3 % for patients without coagulopathy [5]. Likewise, higher blood transfusion rates, longer in-hospital and ICU stays, decreased ventilator-free days, higher rates of single and multiple organ failure, and higher incidence rates of delayed injury and disability at discharge have been reported among TBI patients with acute coagulopathy [2, 17].

Several authors have claimed that laboratory parameters for coagulation may be a better predictor for outcome and mortality than midline shift or pupillary reactivity [20] and a variety of

parameters have been suggested, for example fibrinogen degradation products (FDPs), aPTT, plasmin/alpha 2-antiplasmin complex, and/or decreased fibrinogen levels. Recently, the IMPACT (International Mission for Progression and Clinical Trial) proposed PT as a powerful independent prognostic factor after TBI [21]. Abnormalities in mean PT, aPTT, and platelet counts upon ER arrival have been associated with the development of delayed injury defined by new intracranial lesions or lesion progression. Von Willebrand factor (vWF) and thrombomodulin (TM) have been suggested as indicators of cerebral endothelial injury and increased TM levels to predict delayed brain lesioning [22]. A recent analysis of 591 isolated TBI patients emphasized the role of standard bedside coagulation parameters at admission in predicting outcomes after blunt TBI [23]. In this study, platelet counts of $\leq 100 \times 10^9$/l have been advocated as the strongest predictor for progression of initial insults on repeated CCT (OR 4; CI 95 % 1.7–10), need for neurosurgical intervention (OR 3.6; CI 95 % 1.2–6.1), and mortality (OR 2.6; CI 95 % 1.1–4.8).

In general, alterations in almost every single coagulation parameter after TBI have been associated with poor prognosis but the interpretation of the predictive value of these parameters and outcome of different studies is complex as the result of heterogeneity in study design/size, cohorts assessed, and definitions for coagulopathy used [1]. Moreover, hematologic studies from peripheral studies may not entirely reflect a coagulopathy localized to the cerebral circulation [24]. However, the strong prognostic value of coagulopathy during the first 24–72 h after TBI warrants early and repetitive coagulation monitoring as well as adequate control of it.

Diagnosing Coagulopathy in TBI

An overview of laboratory tests currently available to assess coagulopathic states after TBI is shown in Fig. 28.3. Diagnostic tests and criteria for the coagulopathy of TBI are still not commonly defined but usually include a clinical condition consistent with coagulopathy, for example severe injury, together with thrombocytopenia, i.e., platelet counts $<100 \times 10^9$/l, and abnormal standard coagulation tests, i.e., INR >1.2 and/or prolonged aPTT >35 s (reference range may vary between laboratories) [1, 5, 7, 10, 20, 21]. For further detail on the methodology and interpretation of PT, INR, and aPTT refer to Chapter #(PT and PTT—Ness). Real-time viscoelastic tests, for example thrombelastometry (ROTEM®) or thrombelastography (TEG®), allow the assessment of both hypocoagulable and hypercoagulable states in one single set of tests as they provide detailed information on clot formation kinetics and clot stability during dynamic clot formation [25]. These tests also provide real-time information of the effects of therapeutic interventions, for example blood product transfusion and/or factor substitution therapy, and may thus be used for monitoring. For further description of viscoelastic assays refer to Chapter #(TEG-Gonzalez, ROTEM-Gorlinger). Thresholds for treatment according to these viscoelastic measures as well as for all coagulation laboratory parameters are not well defined and require further investigation. Recently, a consensus panel issued recommendations regarding viscoelastic thresholds for triggering the initiation of specific treatments including fibrinogen, platelet, plasma, and prothrombin complex concentrates in bleeding trauma patients (Table 28.1) [26]. Although these thresholds were developed for the general trauma population they may also hold potential to be integrated into the management of bleeding TBI patients with coagulopathy.

There is an increasing use of antithrombotic and antiplatelet medications in elderly patients for various medical reasons and available data suggest that pre-injury intake of these agents may increase the risk of an unfavorable outcome, especially in cases of severe TBI [27, 28]. Grandhi et al. reported that pre-injury use of warfarin is associated with decreased survival and need for neurosurgical intervention in elderly TBI patients with intracranial hemorrhage [29]. Standard laboratory investigations are insufficient to evaluate

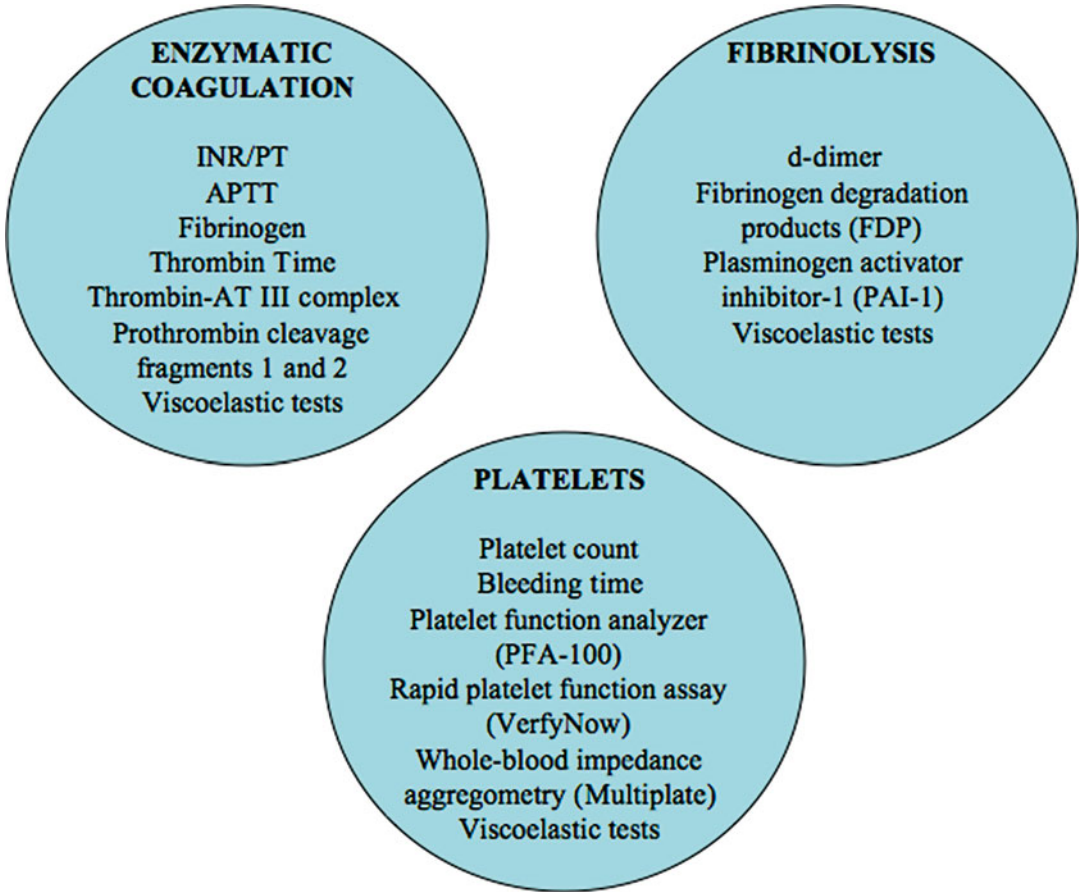

Fig. 28.3 Laboratory tests currently available to assess hemostatic disorders after TBI. *APTT* activated partial thromboplastin time, *AT* antithrombin, *INR* international normalized ratio, *PT* prothrombin time, (modified from [34])

Table 28.1 Consensus panel recommendations regarding viscoelastic (ROTEM®) thresholds for triggering the initiation of specific treatments including fibrinogen, platelet, plasma, and prothrombin complex concentrates in bleeding trauma patients (modified from [26])

Clinical decision	Interpretation		
	EXTEM		**FIBTEM**
Consider administering fibrinogen (cryoprecipitate or fibrinogen concentrate)	A10 < 40 mm or MCF < 50 mm	+	A10 < 10 mm or MCF < 12 mm A10 < 7 mm or MCF < 9 mm
Consider administering plasma (or prothrombin complex concentrate) *Caution: low platelets and low fibrinogen may also prolong CT!*	CT ≥ 80s **and** A10 ≥ 40 mm/MCF ≥ 50 mm	+	Normal A10 or MCF
Consider administering platelets	A10 < 40 mm or MCF < 50 mm	+	Normal A10 or MCF
Consider administering antifibrinolytic drugs	Any evidence of lysis		Any evidence of lysis
Consider withholding transfusions	Abnormal high A10/MCF		

platelet activity, but new assays for monitoring platelet activity have been developed (Fig. 28.3).

As there are no specific guidelines yet developed for the management of bleeding and coagulopathy in TBI, treatment strategies usually follow the same principles as for the general trauma population except for targeting at higher mean arterial pressure (MAP) ≥80 mmHg, higher platelet counts ($>100 \times 10^9$/l), and the avoidance of hypotonic solutions during initial resuscitation [30]. Commonly used interventions to restore platelet activity include platelet transfusion and the application of hemostatic drugs such as desmopressin, tranexamic acid (TXA), recombinant factor VIIa (rFVIIa), and prothrombin complex concentrates (PCC). For further description of these medications please refer to Chapter # and # (Factor replacement-Franchini, Anti-fibrinolytics-Gruen). For the emergency reversal of vitamin K-dependent oral anticoagulants, e.g., warfarin, the early use of PCC is recommended [30]. The early use of the anti-fibrinolytic TXA in TBI is currently under investigation with a clinical trial by the Resuscitation Outcomes Consortium (ROC) group and another one by the CRASH-3 consortium. Pre-injury clopidogrel therapy should warrant the need for a routine repeated CCT scan in patients with TBI given its high risk of intracranial hemorrhage progression [31]. For patients with blunt head trauma on warfarin or a warfarin-aspirin combination, a repeated CCT scan after an initial one being negative is acceptable [32]. For further description of the management of chronically anticoagulated patients refer to Chapter #(anticoagulated patients-Levy).

Pathogenesis of Coagulopathy in TBI

The complex pathophysiological mechanisms behind the coagulopathy of TBI are multifactorial and remain poorly defined. Brain injury is one of the leading causes of trauma deaths and the development of coagulopathy after TBI translates into dismal outcomes for these patients [33]. Moreover, the nature of the coagulation abnormalities differs between TBI and non-TBI patients with multiple somatic injuries and may follow distinct mechanistic patterns. In healthy individuals, coagulation and fibrinolysis are well balanced to control hemorrhage and thrombosis. Traumatic brain-injured patients are at risk of developing abnormalities in both coagulation and fibrinolysis and the loss of this tightly regulated equilibrium can either result in hypercoagulation with thrombotic phenotypes including microthrombosis and ischemia or in hypocoagulation with substantial bleeding and progression of hemorrhagic lesions (Fig. 28.4) [34, 35]. To date, there is no precise definition of what constitutes a coagulopathy after TBI. The likely mechanisms discussed at the moment have been summarized as follows [34–37]:

1. Massive release of tissue factor (TF) (TF hypothesis and TF-bearing microparticles)
2. Platelet hyperactivity/dysfunction (and affected platelet-endothelial interactions)
3. Altered protein C homeostasis (the "protein C" pathway)
4. Hyperfibrinolysis

The Tissue Factor Hypothesis and TF-Bearing Microparticles

Tissue factor (TF) is an integral membrane lipoprotein found in higher concentrations in the central nervous system compared to other tissues. This protein consists of three domains: an extracellular domain, a transmembrane domain, and a cytoplasmatic tail [38, 39]. The extracellular domain functions as the high-affinity cell surface receptor for active coagulation factor VII (FVIIa), preferentially on phosphatidylserine (PS)-rich surfaces of platelets and platelet-derived microparticles [40]. The binding of FVIIa to negatively charged phospholipids greatly enhances the protein–protein binding of FVIIa and TF in the presence of calcium. The resulting complex provides a catalytic event for the initiation of the coagulation cascade by activating coagulation factor IX (FIX) and catalyzing the conversion of coagulation factor X (FX) into the active protease FXa (Fig. 28.5). For further description of TF

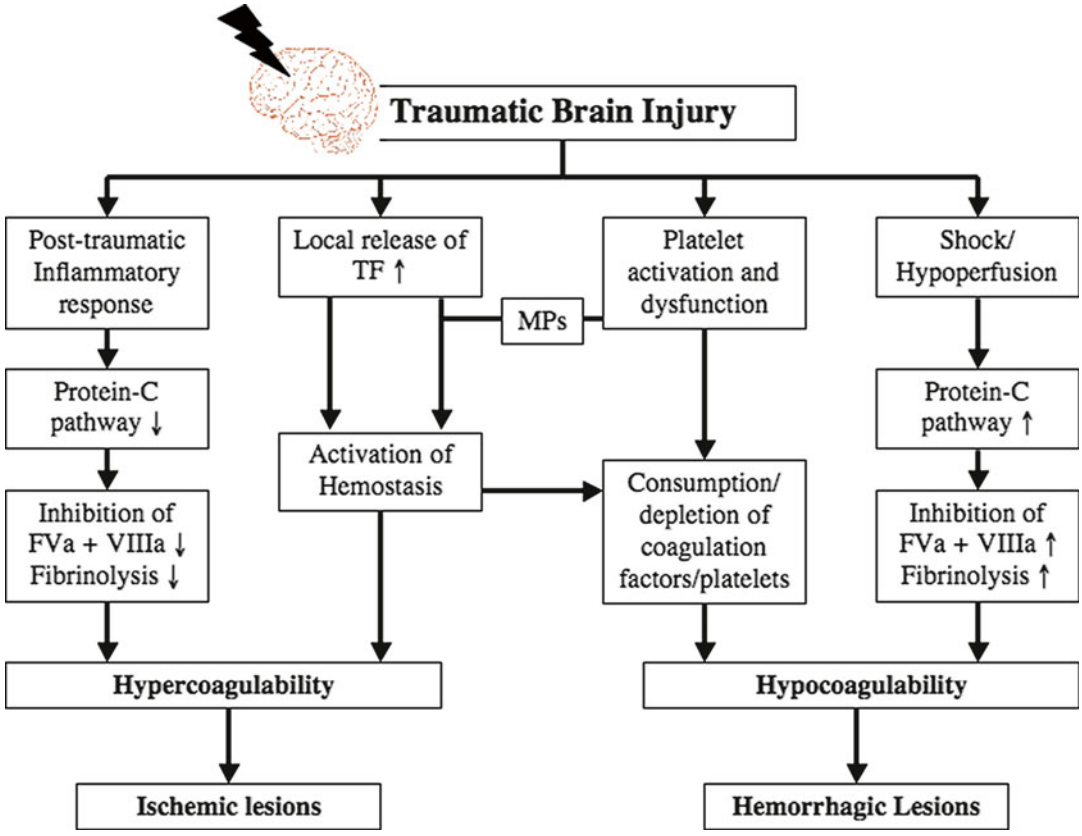

Fig. 28.4 Current understanding of the mechanisms underlying the coagulopathy of TBI. TBI patients may additionally suffer from hypothermia and acidosis which contribute to the further deterioration of hemostasis. *FVa* coagulation factor V active, *FVIIIa* coagulation factor VIII active, *MP* microparticles, *TF* tissue factor

and its role in the cell-based model of hemostasis refer to Chapter #(CBM-Hoffman).

Under physiological conditions, TF is not exposed to circulating blood cells as it is rather expressed on fibroblasts surrounding blood vessels, vessel-bound subendothelial smooth muscles cells, as well as nonvascular cells such as astrocytes, epidermal cells, and renal glomeruli. However, this can change in case of blood vessel damage by injury or plaque rupture. The exposure of TF-expressing cells during injury allows the complex formation with TF and FVIIa. Monocytes and endothelial cells can also express TF, but this is restricted to the presence of stimuli such as injury and inflammation. There is also blood-borne soluble TF at approximately 1–10 ng/ml. This blood-borne TF does not trigger coagulation under physiological conditions [41] but may contribute to the ongoing amplification of coagulation after initial injury [42].

How TF is integrated into the surface membranes of activated platelets to trigger the coagulation pathway has yet to be characterized. One possible explanation may be through the interaction with monocytes as well as through delivery via microparticles (Fig. 28.5). If activated, for example in response to injury, cells such as platelets, monocytes, endothelial cells, and other cell types shed microparticles via vesiculation or apoptosis by membrane phospholipid redistribution and disruption of the membrane skeleton [43–45]. Neural cells, in particular, are highly sensitive to apoptosis and thus produce TF-bearing neural microparticles. These microparticles are enriched in microdomains with high concentrations of cholesterol, sphingolipids,

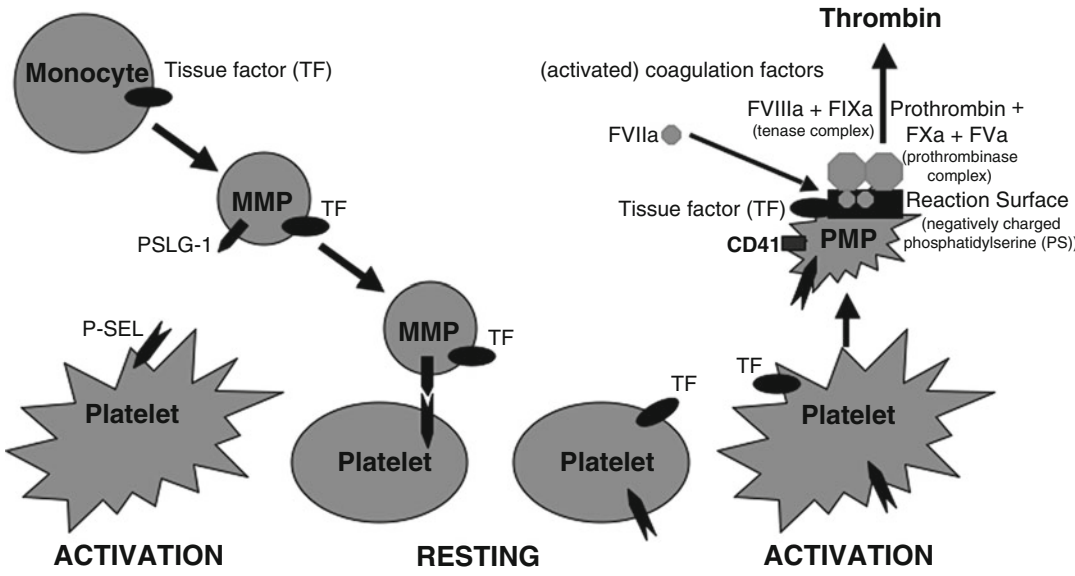

Fig. 28.5 Possible origin of tissue factor (TF) resulting from monocyte and platelet interaction. Platelet-monocyte binding is increased under stimulation in trauma and sepsis [77]. If activated, for example in response to injury, both monocytes and platelets shed microparticles via vesiculation or apoptosis by membrane phospholipid redistribution and disruption of the membrane skeleton. P-selectin glycoprotein ligand 1 (PSGL-1) expressed on monocytes could link TF-bearing microparticles with activated platelets and endothelial cells that express P-selectin. Platelet-derived microparticles are also enriched in phosphatidylserine (PS) providing a negatively charged reaction surface linking platelet activation to thrombin generation by connecting to specific binding sites on FXa, FVa, and prothrombin. The exposure of PS facilitates the binding of (activated) coagulation factors to the membranes enabling the formation of tenase and prothrombinase complexes. P-selectin triggers TF expression on monocytes. *FVa* coagulation factor V active, *FVIIa* coagulation factor VII active, *FVIIIa* coagulation factor VIII active, *FIXa* coagulation factor IX active, *FXa* coagulation factor X active, *MMP* monocyte-derived microparticle, *PMP* platelet-derived microparticle, *PS* phosphatidylserine, *P-SEL* P-selectin, *PSLG-1* P-selectin glycoprotein ligand 1, *TF* tissue factor

TF, and adhesion receptors [46, 47]. The latter, in particular, could link TF to cells expressing ligands or counter receptors. For example, P-selectin glycoprotein ligand 1 (PSGL-1) expressed on monocytes could link TF-bearing microparticles with activated platelets and endothelial cells that express P-selectin. Nekludov et al. have recently demonstrated that the pattern of circulating microparticles is altered after TBI and that platelet-derived microparticles expressing P-selectin as well as endothelial-derived microparticles expressing TF seem to be generated in the injured brain [48]. These microparticles could deliver additional tissue factor to activated platelets, thus enhancing coagulation. Tissue factor-bearing microparticles may also fuse with or may be endocytosed by other target cells spreading out the coagulation process onto other cells that, under physiological conditions, do not participate in the coagulation process [49–51].

Platelet-derived microparticles are also enriched in PS and therefore provide a 50- to 100-fold increase in pro-coagulant activity as compared to activated platelets [51, 52]. Under resting conditions, PS is usually located in the inner leaflet of the cell but may be externalized onto the outer leaflet if cells are activated or undergo apoptosis. Phosphatidylserine provides a negatively charged reaction surface linking platelet activation to thrombin generation by connecting to specific binding sites on FXa, FVa, and prothrombin [53], enabling the formation of the tenase and prothrombinase complexes (Fig. 28.5). Phosphatidylserine has also been found on microparticles derived from ATP-stimulated microglial cells and injured neurons. Platelet-derived PS

along with other anionic phospholipids such as phosphatidylethanolamine (PE) have been shown to synergistically promote thrombin generation [54]. Morel et al. have reported high levels of procoagulant microparticles in the cerebrospinal fluid (CSF) and in the peripheral blood at the onset of TBI [43]. The release of endothelial-derived microparticles mirrored the extent of the vascular damage and sustained generation of these pro-coagulant microparticles in the CSF correlated with poor clinical outcome.

It has been postulated that the abovementioned mechanisms involving TF, and PS-bearing microparticles [38, 55], result in a widespread activation of coagulation with possible consumptive coagulopathy by depletion of coagulation factors and platelets [56, 57]. Furthermore, the temporospatial pattern of TF release has been associated with alterations in the blood–brain barrier [58, 59].

Platelet Hyperactivity/Dysfunction and Platelet Endothelial Interactions

Hemostasis is usually initiated when platelets adhere to the exposed subendothelial matrix at sites of injury to generate platelet-rich plugs which are further stabilized by fibrin cross-linking. The simultaneous occurrence of low platelet counts and spontaneous platelet aggregation in the absence of significant hemorrhage suggests platelet hyperactivity in TBI, either due to (partial) activation or priming. The frequent observation of intravascular microthrombosis in TBI further supports the assumption of platelet hyperactivity under these circumstances [60–62]. Interestingly, such microthrombi, consisting of platelets, fibrin, and vWF, have mainly been found within the peri-contusion cortex.

A platelet count $<100 \times 10^9$/l has been associated with a ninefold adjusted risk of death, and a platelet count $<175 \times 10^9$/l has been identified as a significant predictor of intracranial hemorrhage progression [63, 64]. However, bleeding tendency can be present even with a platelet count within normal range [63]. The observed platelet dysfunction in TBI patients appears to involve the cyclooxygenase and adenosine diphosphate (ADP) pathways as evidenced through modified thrombelastography [i.e., platelet mapping (TEG-PM)].

However, the mechanisms for platelet dysfunction after TBI still remain speculative and multiple factors that cause platelet activation have been considered [34]. Platelet-activating factor (PAF), a classical autacoid and potent platelet agonist, has been suggested as a key factor in the development of TBI-associated coagulopathy. Platelets have two types of PAF receptors and upon binding to the high-affinity receptor, PAF triggers intracellular pathways that release arachidonic acid, cytosolic calcium, and protein kinase C [65]. For further description of platelet physiology refer to Chapter #(Platelets-Diamond). The brain has been reported as a richer source for PAF and neural cells may release PAF during ischemia and hypoxia. PAF levels have been reported to increase up to 20-fold in experimental models of ischemia and reperfusion to the spinal cord [66]. In this context, PAF may contribute to the hypoxia-induced breakdown of the blood–brain barrier, which would promote the release of additional PAF and other brain-derived procoagulants into the systemic circulation [67]. Vice versa, experimental PAF antagonism has been shown to attenuate ischemic edema, early post-ischemic hyperemia, and microvascular thrombosis [60].

The role of platelet ligands in the development of TBI-associated coagulopathy has yet to be defined. For example, von-Willbrand factor (vWF), commonly known as a marker for endothelial injury and stored in Weibel-Palade bodies of endothelial cells and in the α-granules of megakaryocytes/platelets, facilitates the initial capture of platelets to pertubated endothelium or exposed subendothelial matrix. In complex, vWF protects FVIIIa from inactivation. The stored vWF multimers are enriched in ultra-large (UL) and prothrombotic forms which are released upon stimulation and then cleaved by the zinc metalloprotease ADAMTS-13 into smaller hemostatically active multimers [68, 69]. In the

absence of proteolytic activity ultra-large vWF multimers capture platelets and leukocytes to the endothelium resulting in local in situ thrombosis and thromboembolism in the downstream microvasculature. Endothelial cells in the cerebral vascular beds synthesize significantly higher amounts of vWF compared to other organs and plasma vWF levels are increased in TBI, while ADAMTS-13 activity is reduced in trauma and surgical injury [68]. Experimental data using a standardized model of TBI has shown that intravascular microthrombosis most frequently develops within the boundary zone of the inflicted lesion containing substantial amounts of vWF, fibrin, and platelets [70]. Reduction of the plasma vWF level and platelet activity by atorvastatin decreased intravascular microthrombosis after experimental TBI.

Altered Protein C Homeostasis (The "Protein C" Pathway)

Several studies suggest that a maladaptive protein C pathway in response to injury and hemorrhagic shock causes an immediate activated protein

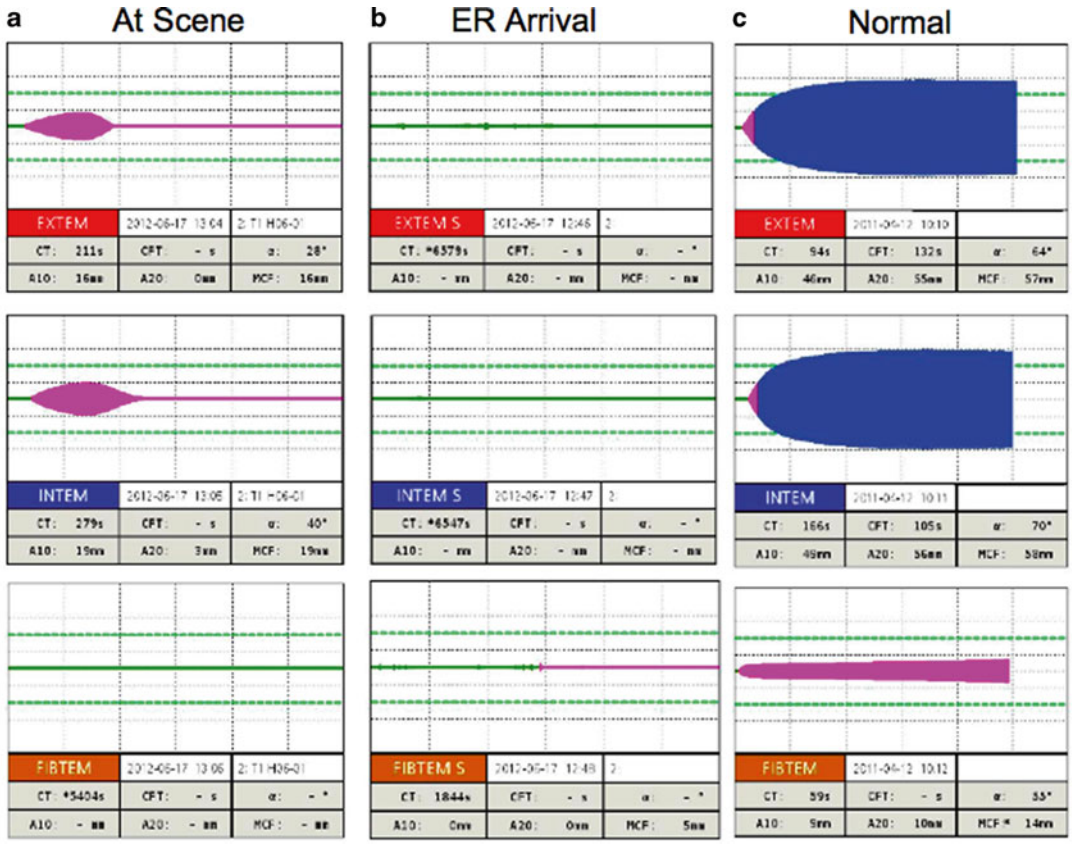

Fig. 28.6 3-Channel ROTEM® from a trauma patient with severe TBI. Evolvement of fulminante hyperfibrinolysis (HF) during the pre-hospital phase of care (**a**) until ER admission (**b**). Fulminante HF in EXTEM and INTEM with no fibrin generation in FIBTEM (defibrinogenation). (**c**) Shows a reference 3-channel ROTEM® from a healthy volunteer. EXTEM measures the activation of coagulation by thromboplastin (tissue factor) with factors VII, X, V, and I, platelets, and fibrinolysis. FIBTEM records the activation as for EXTEM with the addition of the platelet-blocking agent cytochalasin. The resultant clot is thus formed purely by fibrin formation and fibrin polymerization; the fibrin level and the degree of fibrin polymerization can be evaluated. INTEM activates the contact phase of haemostasis, and, in the absence of heparin, this test is used as a screening test for the hemostasis system

C-mediated coagulopathy and a chronic protein C depletion-mediated enhanced susceptibility to infectious and thromboembolic events [34, 71]. The combination of TBI and hemorrhagic shock results in an immediate activation of coagulation and complement systems with subsequent endothelial shedding, protein C activation, and inflammation [72]. Combined hypo- and hypercoagulable states most likely triggered by the extent of injured brain tissue will lead to secondary injury via ischemic and hemorrhagic lesioning [34]. Other inflammatory mediators, for example cytokines and complement, may also contribute to the pathogenesis of the acute coagulopathy of TBI.

Hyperfibrinolysis

Hyperfibrinolysis has been suggested as a potential cause of bleeding diathesis after TBI. However, its incidence is low, reported at 2.5–7 % of all trauma/TBI patients. Its presence, however, is associated with high mortality [73, 74]. A clinical example of the rapid evolvement of fulminant hyperfibrinolysis in a patient with severe TBI from pre-hospital care to the ER is shown in Fig. 28.6. A percent clot lysis of ≥15 % has been suggested as a criterion for diagnosing hyperfibrinolysis when using viscoelastic testing [75]. However, lower thresholds than this have been proposed for diagnosing fibrinolysis. For further description of hyperfibrinolysis and its diagnosis refer to Chapter #(Fibrinolysis-Moore). Increased d-dimers and FDPs as well as low concentrations of alpha 2-antiplasmin have been associated with negative outcomes after TBI and the level of plasma d-dimer after TBI has been suggested as a predictor of progressive intracranial hemorrhage [76].

Conclusion

Coagulopathy after TBI is frequent and an important independent risk factor related to prognosis. The complex mechanisms driving coagulopathy after TBI remain poorly defined and the earliest pathophysiologic changes occurring after injury require further study. An overlap of hypo- and hypercoagulability in TBI exists. The use of functional assays, i.e., viscoelastic tests, is advocated in order to identify the phenotypes of coagulopathy associated with TBI and to guide timely targeted therapy. Trials providing evidence-based treatment thresholds for patients with coagulopathy after TBI are needed.

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Coagulopathy of Liver Disease

29

Shahzaib Ahmad and Beverley J. Hunt

Organ-Specific Coagulopathy: Liver Disease

Objective of Chapter

To review the biology of haemostasis and thrombosis in liver disease, and provide a practical guide on principles of management, allowing the clinician to better care for the injured patient with underlying or new-onset liver disease, as well as to provide a knowledge base for those interested in research of coagulopathies associated with liver disease.

Introduction

Acute liver failure is characterised by a rapid decline in liver function, which manifests itself by a prolonged prothrombin time (PT), hypertransaminasemia and jaundice. If accompanied by encephalopathy, the term fulminant hepatic failure is applied, which may be subdivided into hyperacute if encephalopathy occurs within 7 days of onset of jaundice, acute if within 8–28 days and subacute if within 5–26 weeks [1]. It is the final common pathway from diverse injuries to the liver, which may be delivered by infection, drugs, vascular, metabolic, autoimmune disease and/or sometimes indeterminate causes. Chronic liver failure implies decompensated chronic liver disease which is characterised by jaundice, ascites, coagulopathy and encephalopathy. The liver has diverse functions; one of these is the production of thrombopoietin (TPO) and most haemostatic proteins except for von Willebrand factor (VWF), thrombomodulin and tissue-plasminogen activator (t-PA), all of which are produced by endothelial cells. It is not surprising therefore that liver disease has significant effects on primary and secondary haemostasis as well as fibrinolysis. This disturbance in haemostasis can be observed in the laboratory with prolonged screening tests of coagulation and reduced platelet number on a full blood count. Clinically, liver disease is associated with bleeding, previously attributed to portal hypertension and/or coagulopathic changes, causing a bleeding diathesis. However, despite this tendency to bleeding, patients with liver disease are not protected from thrombosis; paradoxically, their risk of thrombosis may even be increased. This chapter first discusses in detail the haemostatic changes of liver disease and how the clinician should approach bleeding in patients with liver disease before moving on to a discussion on the thrombotic tendency seen in patients with liver disease, and its therapeutic implications.

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The Defect in Primary Haemostasis

Primary haemostasis is the formation of a platelet plug immediately following vascular damage. It is this platelet plug that is strengthened by secondary haemostasis, which results in fibrin deposition. In chronic liver disease, patients may develop both quantitative and qualitative platelet defects, resulting in impaired primary haemostasis. In the past thrombocytopenia in liver disease was classically attributed to hypersplenism that occurs as a result of portal hypertension, which increases sequestration with subsequent destruction of platelets. Increased platelet pooling in the spleen and increased destruction have been demonstrated by studies analysing radiolabelled platelets [2, 3]. However, transjugular portosystemic shunt, a procedure which would alleviate portal hypertension and would be expected to decrease the pooling of platelets in the spleen, may not always result in an improvement to thrombocytopenia [4–8], showing that simple pooling in the spleen is not the only mechanism that mediates thrombocytopenia in patients with liver disease.

The spleen is a major site for removal of immunoglobulin-coated blood cells. In chronic liver disease, platelet-associated immunoglobulin (PAIg) targeted against the major platelet antigen GPIIb/IIIa by B cells is increased [9]. PAIg clears platelets from the circulation by sequestration in the spleen and subsequent clearance via the reticuloendothelial system and may also have direct effects on megakaryopoiesis [10].

A tertiary mechanism responsible for thrombocytopenia seen in patients with liver disease is a decrease in production of the hormone TPO. Predominantly produced by the liver, thrombopoeitin (TPO) acts on the bone marrow where it stimulates megakaryopoiesis and platelet maturation. Initially when TPO was discovered, reduced levels were thought to be the major explanation for the thrombocytopenia in liver disease [11]. Whilst later studies showed this to be the case in patients with liver disease of such a severity that required transplantation, TPO levels were normal in mild-moderate cases as a result of a compensatory increase in production by the liver, stimulated by thrombocytopenia from splenic sequestration [12].

While these elegant mechanisms explain the thrombocytopenia observed in liver disease, the exact contribution of each in different types of liver disease is not yet established. In addition, thrombocytopenia may be caused by unique clinical contexts. For example, vitamin deficiencies, sepsis, disseminated intravascular coagulation (DIC) and ethanol poisoning can cause thrombocytopenia that is independent yet contributive to the causes listed above.

Impairment of platelet function further exacerbates thrombocytopenia and causes a defect in primary haemostasis. Bleeding time, the paradigmal test of platelet activity, has shown to be prolonged in patients with cirrhosis. However, the test correlated poorly with platelet number, suggesting that platelet dysfunction rather than reduction in number is important in the bleeding defect of liver disease [13]. Both aggregometry and flow cytometry have shown a clear decrease in platelet function, with decreased aggregation demonstrated in response to all types of stimulation—adenosine diphosphate (ADP), ristocetin and epinephrine to arachidonic acid, thrombin and collagen [14], suggesting widespread platelet defects. The functional defect has also been detected using more global assays of coagulation such as thromboelastography, with a similar outcome [15]. Abnormal granule release, reduced release of calcium from intracellular stores, decreased arachidonic acid availability and upregulation of inhibitory secondary messengers cyclic adenosine monophosphate (cAMP) and guanosine 3′,5′-cyclic phosphate (cGMP) have all been demonstrated as causes of impaired platelet function [16–18].

The Defect in Secondary Haemostasis

Not unusually, patients with liver disease often suffer from bleeding complications of liver disease presenting with epistaxis, menorrhagia/metrorrhagia, purpura and petechiae [19]. Bleeding intra- and post-operatively is common in patients with liver disease and represents a significant cause of morbidity and mortality in this

group of patients [20]. Routine tests of coagulation are frequently abnormal in patients with liver disease, with a prolonged PT and APTT a commonly encountered clinical scenario [21]. This is not at all surprising, given that the liver is the site where most haemostatic proteins are synthesised and reflects declining hepatocyte function as the synthesis of coagulation factors is significantly impaired and circulating levels are reduced. One of the first coagulation factors to be reduced, probably owing to its short plasma half-life of 4–6 h and dependence on vitamin K for normal synthesis, is factor VII (FVII) and levels of FVII are inversely proportional to the severity of disease [22, 23]. This relationship is also observed for other factors, with the levels of factors II (FII), V(FV), IX (FIX), X (FX) and XI (FXI) reducing as the disease progresses [24]. Levels of factor VIII (FVIII) are increased in liver disease possibly owing to extrahepatic production by endothelial cells and decreased degradation owing to protection conferred upon FVIII by the increased levels of VWF which is a carrier of FVIII [21, 25]. Cholestasis, a common occurrence in liver disease, further exacerbates this situation as decreased absorption of the lipid-soluble vitamin K leads to a further decrease in the production of vitamin K-dependent coagulation factors. FII, FVII, FIX, FX and FXI require vitamin K for post-translational modifications that render these factors physiologically active.

One of the factors that particularly suffer in liver disease is factor V. Indeed levels of factor V have been shown to correlate with acute hepatic failure [26]. The aetiology of the fall is not fully resolved, but may relate to the increased fibrinolytic activity seen in liver disease, for factor V is a particular target of plasmin [27]. Thus, liver disease leads to significant derangements in secondary haemostasis.

Impaired hepatocyte function also results in dysfibrinogenaemia owing to the failure of the normal enzymatic cleavage of sialic acid from the fibrinogen molecule inhibiting efficient fibrin polymerisation [28]. However fibrinogen levels can remain normal in those with chronic liver disease and may even be increased in those with chronic inflammatory states such as primary biliary cirrhosis [29].

The Defect in the Fibrinolytic System

Defects of the fibrinolytic system also contribute to the bleeding phenotype observed in patients with liver disease. The changes result in increased fibrinolytic potential which will be exacerbated during a bleeding episode potentially leading to hyperfibrinolysis [22]. Increased t-PA production by endothelial cells with clearance impaired in liver disease, and reduced plasminogen activator inhibitor-1 (PAI-1), produced less by the diseased liver, is skewed in favour of increased fibrinolytic potential in liver disease [30]. Additionally, other inhibitors of fibrinolysis such as α2-antiplasmin and thrombin activatable fibrinolysis inhibitor (TAFI) are both produced in the liver and therefore reduced in patients with liver disease [31, 32], although their production is reduced by the same amount as plasminogen.

Thus defects in primary and secondary haemostasis as well as the fibrinolytic system are responsible for the haemostatic derangement observed in patients with liver disease. These changes are summarised in Fig. 29.1.

Management of Bleeding

Low platelet count is well tolerated in most patients and expert opinion recommends that the trigger for prophylactic platelet transfusions to prevent spontaneous bleeding in stable patients with chronic liver disease is a platelet count of 10,000/mm^3 [33, 34]. This threshold may be raised to 15,000–20,000/mm^3 if the patient develops concomitant infection [35]. Although not evidence based, platelet counts of 50,000/mm^3 are considered safe for performing invasive procedures. Neurological and spine procedures, cardiac surgery and great vessel surgery, as well as ocular surgery, may warrant platelet counts greater than 100,000/mm^3; again, this is based on expert opinion. It has been common practice to correct the coagulation defect in the bleeding patients with liver disease with fresh frozen plasma (FFP), monitoring the response with the prothrombin time (PT). The use of FFP has major

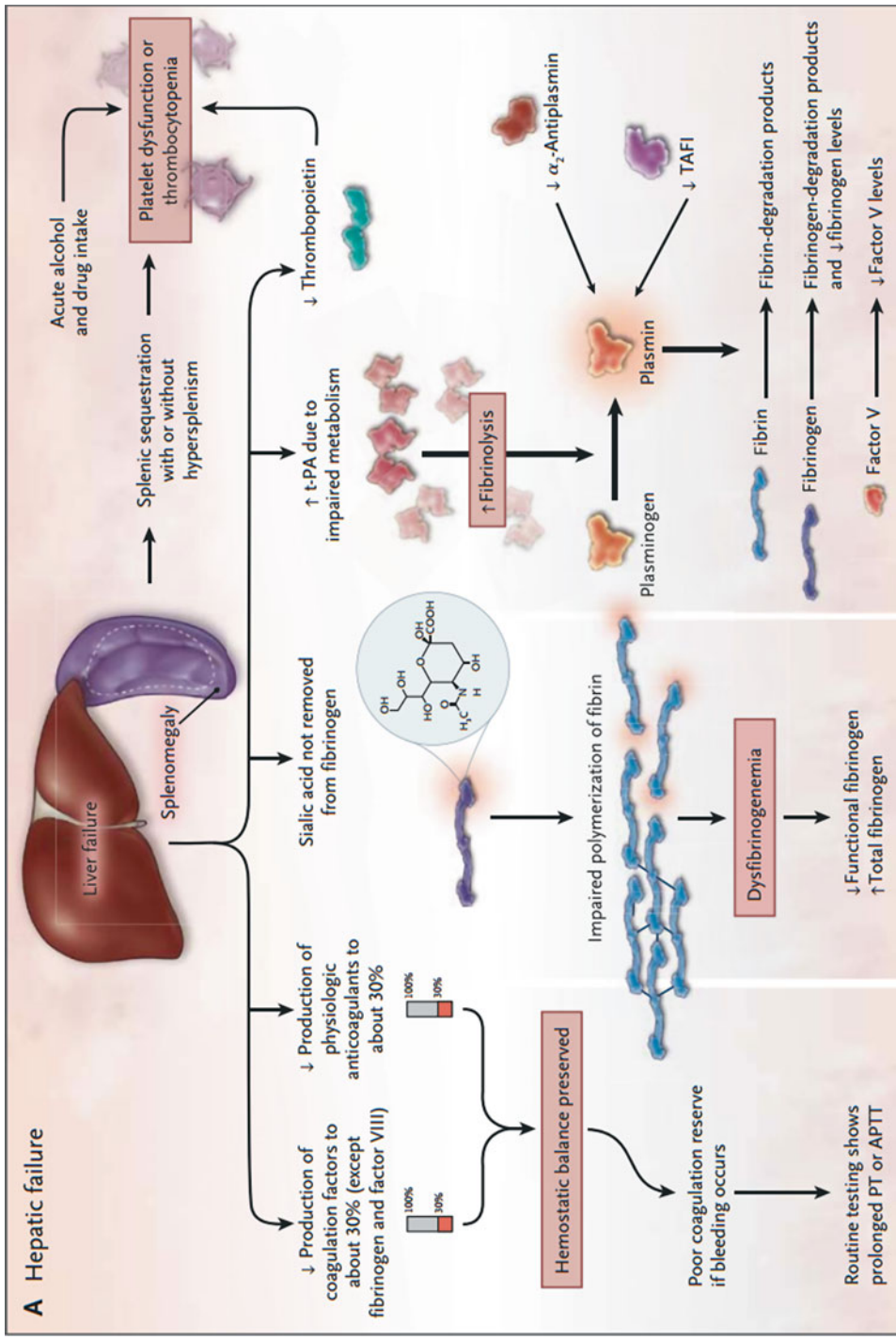

Fig. 29.1 Liver failure leads to complex haemostatic changes, since the liver is the producer of coagulation factors, physiologic anticoagulants and thrombopoietin, as well as the site of the metabolism of sialic acid residues from fibrinogen, activated coagulation factors and tissue-plasminogen activator. These defects result in poor coagulation reserve, dysfibrinogenaemia and increased fibrinolytic potential

advantages; it contains the coagulation factors in physiological quantity [36]. In addition, FFP contains already functional factors lessening the impact of impaired vitamin K absorption [37]. The British Committee for Standards in Haematology recommended the use of FFP in liver disease when the patient is actively bleeding or prophylactically for surgery. FFP is normally titrated against the PT/INR. However, FFP is unlikely to fully correct all aspects of the haemostatic defect seen in liver disease, and its routine use in clinical practice in the non-bleeding patient is not evidence based [19]. Indeed, frequently FFP will not correct the prolonged screening tests of coagulation seen in liver disease. Moreover the use of FFP can cause harm because the complications of portal hypertension may be exacerbated by the expansion of plasma volume when plasma expanders are used [38]. The chances of developing acute lung injury (transfusion-related acute lung injury (TRALI)), as well as other complications of transfusion such as the risk of transfusion-transmitted disease and volume overload, are increased; therefore the use of FFP should be limited to the bleeding patient alone [39].

The dangers of volume overload associated with the use of FFP may be overcome with prothrombin complex concentrates (PCC), which deliver vitamin K-dependant coagulation factors (usually factors II, VII, IX and X, protein C and protein S) in a very small volume, thus lessening the effect on portal hypertension which may be responsible for a significant proportion of bleeding in patients with liver disease [37]. PCC has been shown to be effective at stopping active bleeding in patients with liver failure; the effect was transient providing a time-critical window to deliver more definitive treatment [40]. However the use of PCC is accompanied with a prothrombotic state for several days [41]. The utility, safety and cost-effectiveness of PCC as a treatment and prophylaxis for bleeding are currently undefined; one clinical trial in orthotopic liver transplantation is currently underway to address these questions [42].

Another targeted approach to a bleeding patient with liver failure has been the use of recombinant FVIIa (rFVIIa). Originally developed for managing haemophiliac patients with inhibitors, rFVIIa can normalise the PT time in patients with liver failure, although the effect on bleeding risk is not clear [37]. An initial clinical trial showed a benefit of using rFVIIa for managing bleeding; however mortality was not improved [43]. When trials were replicated, no difference was seen even for managing bleeding [44]. Thus, the utility of rFVIIa in liver disease is not proven, whilst the high rate of arterial thrombotic complications and the high cost of rFVIIa remain major deterrents [37].

Cryoprecipitate is a source of FVIII, fibronectin, VWF, FXIII and fibrinogen [37]. As mentioned above, patients with liver disease demonstrate dysfibrinogenaemia due to coating with sialic acid. Although cryoprecipitate provides physiologically normal fibrinogen, the exact amount needed is not established, and moreover may be subject to sialic acid coating. Additionally, unless a Clauss fibrinogen assay is requested (a test of functional fibrinogen), usual laboratory assessment of fibrinogen quantity is an overestimation of the levels for many routine fibrinogen assays are not able to distinguish between functionally normal and abnormal fibrinogen. One US group uses a fibrinogen level of 1.0–1.2 g/L as a target for the treatment of active bleeding or prophylaxis for surgery [37]. However modern practice is to set a higher target of 1.5 g/L in major bleeding [45] due to recognition of the importance of fibrinogen in coagulation and platelet function.

A mechanism for targeting the deranged fibrinolytic phase of coagulation is to use antifibrinolytic agents. Two most commonly used antagonists are tranexamic acid (Europe) and in North America aminocaproic acid, synthetic derivatives of lysine, which act as competitive inhibitors of tissue plasminogen activator (tPA) binding to plasminogen. The effectiveness of these antifibrinolytic agents has been demonstrated in many bleeding scenarios. The benefit of using tranexamic acid on trauma patients was demonstrated in the Clinical Randomisation of an Antifibrinolytic in Significant Haemorrhage-2 (CRASH-2) trial. All-cause mortality and death from bleeding were significantly reduced, when tranexamic acid was used within the first 3 h from injury. Additionally, there is no increase in the risk of thromboembolic events.

A systematic review in 2012 [46] found that there were 129 trials using tranexamic acid in surgical patients, totaling 10488 patients, carried out between 1972 and 2011. In this meta-analysis tranexamic acid reduced the probability of receiving a blood transfusion by a third (risk ratio 0.62, 95 % confidence interval 0.58–0.65; $P<0.001$). This effect remained when the analysis was restricted to trials using adequate allocation concealment (0.68, 0.62–0.74; $P<0.001$). Fewer deaths occurred in the tranexamic acid group (0.61, 0.38–0.98; $P=0.04$), although when the analysis was restricted to trials using adequate concealment there was considerable uncertainty (0.67, 0.33 to 1.34; $P=0.25$). The authors concluded that cumulative meta-analysis showed that reliable evidence that tranexamic acid reduces the need for transfusion has been available for over 10 years.

A meta-analysis of 252 randomised controlled trials with over 25,000 participants has shown that the use of tranexamic acid in surgical patients has the potential to reduce blood transfusion requirements by one-third [47]. However the utility of tranexamic acid in patients with upper gastrointestinal bleeds from causes such as oesophageal varices and peptic ulcers has been poorly studied [48]. The efficacy of tranexamic acid on mortality in patients with upper gastrointestinal bleeds is being studied in the Haemorrhage Alleviation with Tranexamic acid—InTestinal system [(HALT-IT) clinical trial (NCT01658124)].

Patients with liver failure are often malnourished and may be vitamin K deficient. Vitamin K is needed for post-translational modifications, such as the conversion of glutamic acid to γ-carboxyglutamic acid, of coagulation FII, FVII, FIX, FX and FXI. Thus, vitamin K deficiency leads to a decrease in production of functional coagulation factors. Repletion of vitamin K aims to correct this imbalance. However supplementation does not always lead to full reversal of coagulation changes. One study showed only a modest decrease in PT and APTT [49]. In theory at least, vitamin K repletion should help increase the haemostatic potential in patients with liver disease, and owing to variable gut absorption, the intravenous route is better than oral route [50].

Managing Bleeding Risk in Invasive Procedures

There is an increased risk of bleeding in those with abnormal liver tissue/cirrhosis in comparison to those patients with normal liver tissue undergoing liver biopsy [51]. Among patients with established liver disease, there is no difference in the risk of bleeding between those with an abnormal and normal PT [52] or between those patients with mild thrombocytopenia and normal platelet counts. However, a platelet count of <60,000/mm^2 was associated with a significantly increased risk of bleeding from percutaneous liver biopsies [53]. Based on these studies, there is no evidence to support correction of coagulopathy in patients with prolonged screening tests of coagulation undergoing liver biopsy with the exception that a platelet count of <60,000/mm^2 will require platelet transfusion. Safer fine-gauge needles and the use of ultrasound have made this procedure even safer [54].

Central venous cannulation complications also show no difference amongst patients with cirrhosis and prolonged screening tests of coagulation and those with normal coagulation parameters [55]. Rather strikingly, out of 259 central venous cannulation procedures carried out on liver transplant recipients, 200 of which were performed without correcting the underlying coagulopathy, no serious bleeding complications developed [56], and an experienced set of hands may be all that is needed to achieve central venous access safely. Again, ultrasound guidance may play a significant role in reducing bleeding complications [55].

Paracentesis is another common procedure that is used both diagnostically and therapeutically in liver disease. Again, there was no difference noted in the bleeding risk of those patients with a prolonged PT and a platelet count of >50,000/mm^2 in comparison to those patients with normal coagulation parameters [57]. In another study, patients who were given FFP as prophylaxis against haemorrhage did not show a decreased rate of bleeding than those who were not given FFP [58].

The Prothrombotic State in Liver Disease

The dogmatic approach to liver disease sees it as a bleeding diathesis. However the liver is also the site of synthesis of physiological anticoagulants, which are decreased by the same amount as the coagulation factors in chronic liver disease. These natural anticoagulant factors include protein C, protein S and antithrombin, and patients with liver disease have low levels of such proteins which tightly regulate the process of haemostasis. Thus liver disease is haemostatically balanced but this is set at a lower level and thus is more delicate and less stable than in normal individuals who have a larger reserve of procoagulant and anticoagulant factors. Therefore, the deficiency of both procoagulant and anticoagulant factors creates a fragile balance which may be tipped towards haemorrhage or thrombosis depending on the clinical context [59].

Studies have shown that patients with liver disease, who were thought to be "auto-anticoagulated," are not protected from the development of venous thromboembolism (VTE) [60]. Not only are patients with liver disease not protected from the development of VTE, but they may also even be at a significantly increased risk when compared to the general population [61]. This raised risk also manifests itself particularly but not exclusively with increased rate of portal vein thrombosis [62]. Even post-transplantation hepatic artery thrombosis, long considered a complication of transplant surgery, may be a result of this hypercoagulability [63]. Using a global assay of coagulation known as endogenous thrombin potential, it has been found that patients with cirrhosis produce as much thrombin as healthy individuals, even when you control for the varying platelet counts [64].

Prothrombotic Changes in Haemostasis

A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) is produced in the liver; levels of ADAMTS13 are reduced in liver disease. As its role is to cleave ultra-large multimers of VWF (UL VWF) into smaller molecules both in circulation and within a growing thrombus, levels of UL VWF multimers are increased in some patients with liver disease [65, 66]. It has been demonstrated that despite both quantitative and qualitative defects in platelets, these UL WVF multimers are able to stimulate platelet activation [67]. A raised level of large VWF multimers is an independent risk factor for the development of thrombosis [68]. This has been demonstrated in multiple large-scale studies and applies to both arterial and venous thromboembolism. In the Prospective Epidemiological Study of Myocardial Infarction (PRIME) analysis, a threefold increase in the risk of myocardial infarction was observed in those with VWF levels in the top quartile [69]. Contrary to this postulate, patients with severe von Willebrand disease (VWD) are not protected from the development of atherosclerosis [70]. Therefore, raised levels of VWF may significantly contribute to the thrombogenic event, but not increase the actual risk of this event occurring [68]. The evidence for VTE is more scant; the Longitudinal Investigation of Thromboembolism Etiology (LITE) study found that the risk increased in a dose-dependent manner as VWF levels were increased [71]. However, another study also found an increased risk of VTE with increasing VWF levels, but this was abolished when adjusting for FVIII levels [72].

One mechanism for this may be the raised level of FVIII in patients with liver failure, which may potentiate the generation of thrombin [73]. Levels of FVIII may be raised by a dual mechanism: (1) raised levels of VWF confer protection upon FVIII from degradation by forming VWF-FVIII complexes that occur naturally as a transport mechanism for FVIII and (2) owing to impaired hepatocyte function, there is a reduced expression of low-density lipoprotein receptor-related protein which mediates the uptake and degradation of FVIII [25]. A raised level of FVIII is a weak independent risk factor for the development of VTE [73].

Therapeutic Implications

As liver disease has classically been seen as a bleeding diathesis in the past, it was seen as a contraindication to thromboprophylaxis with

low-molecular-weight heparin (LMWH) to prevent hospital-acquired thrombosis. There is biological plausibility to this stance, as bleeding complications are common in liver disease; heparins are partially metabolised by the liver and so will accumulate in liver disease. The case has been erroneously further strengthened by abnormal screening tests of coagulation [61]. It is perhaps due to this that there are no specific guidelines for thromboprophylaxis in patients with liver failure [74]. However, since thrombotic events are more prevalent in these patients than previously thought, thromboprophylaxis may be of benefit, especially when additional risk factors for thrombosis, such as immobilisation, surgery or hospitalisation, can compound this risk. This has to be a cautious approach however, as the clinician must also take into account additional risk factors for bleeding such as uraemia and sepsis; in these patients, grade I graduated compression stockings, if there are no other contraindications, should be used. Trials are required to answer the question about the benefits of pharmacological thromboprophylaxis in liver disease.

Treating thrombosis is equally tricky and lacking in evidence base. Using LMWH can have unpredictable outcomes, as it exploits the action of antithrombin on thrombin, and levels of antithrombin are variably reduced in patients with liver disease [75]. Indeed, trials assessing the use of LMWH in these patients show both benefit and harm; in one study analysing patients with portal vein thrombosis, recanalisation was achieved in 42 % without bleeding complications but another showed bleeding complication rates as high as 83 % [76, 77]. Certainly, bleeding complications are a hindering factor for the use of anticoagulants in patients with liver disease, especially given reports of intracranial haemorrhage in this subset of patients [78]. The use of vitamin K antagonists, such as warfarin, is difficult because the INR is frequently prolonged in liver disease and the correct target INR for these patients is not known [75]. Newer agents such as the direct oral anticoagulants (DOACs) provide a safer option over vitamin K antagonists and heparin in patients without liver disease, but as yet there is no data in those with liver disease. Additionally, they are metabolised by the liver and so there is a risk of accumulation in liver disease [79]. Future studies may reveal the best options. Low-molecular-weight heparin is often preferred to vitamin K antagonists in treating venous thromboembolic disease in patients with liver disease. Monitoring of anti-Xa activity seems a sensible approach to ensure that there is appropriate anticoagulation when using unfractionated or low-molecular-weight heparin. It is important to use an anti-Xa assay where no supplementary antithrombin is added, because patients with liver disease will have lower levels of antithrombin and the total effect of both this low level of antithrombin and heparin will be different from that of an assay where antithrombin is added.

Interpreting Coagulation Tests in Liver Disease

Abnormal screening tests of coagulation (PT/APTT/TT/platelet count) are a frequently encountered scenario in clinical practice. Prophylactic use of FFP is not indicated unless there is bleeding, for use may frequently fail to correct the results and there is significant evidence for the poor correlation between abnormal screening tests of coagulation and risk of bleeding during procedures apart from platelet count $<60{,}000 \times 10^9$/L [80]. Abnormal screening tests of coagulation are also unreliable in predicting gastrointestinal bleeding, the archetypal haemorrhagic event in severe liver disease [81]. A significant piece of evidence for the poor correlation between abnormal screening tests of coagulation and bleeding comes from the changes seen in the management of bleeding risk during liver transplantation; there has been a substantial reduction in the use of blood products as bleeding has become less frequent, despite persisting abnormal coagulation profiles [82]. One reason for the discrepancy between a raised INR and bleeding risk may be due to the fact that INR has never been standardised for patients with liver disease; it was designed to calculate the degree of anticoagulation in patients treated with vitamin K antagonists [59]. To standardise the INR for patients with liver disease, the INR would have to be calibrated using

plasma from patients with liver disease. This suggests that the bleeding diathesis seen in patients with liver disease is not due to hypocoagulability, as the haemostatic mechanism is in balance. Instead, other biologically plausible explanations should be sought, such as haemodynamic changes in portal hypertension (risk of bleeding increases with volume expansion). Additionally, renal failure also contributes to bleeding, and is common in patients with liver disease [83]. Finally, patients with chronic liver disease are susceptible to infection, and sepsis is a significant risk factor for bleeding as the use of antibiotics has been shown to decrease the risk of bleeding [84, 85].

Standard screening tests of coagulation are excellent for detecting an isolated defect in the haemostatic mechanism, such as that seen in haemophilia. However, in patients with liver disease, the haemostatic defect is global, with effects on primary and secondary haemostasis as well as the fibrinolytic system. Therefore, these tests fail to reflect the true state of haemostasis in these patients. Global tests of coagulation such as thromboelastography may help overcome this problem which has shown hypercoagulability in certain cases of liver disease (maximal clot strength reflected by an increased α angle and maximum amplitude as well as shortened r times), despite prolonged screening tests of coagulation [59, 86].

Conclusion

Prolonged screening tests of coagulation have contributed in establishing the dogma that liver disease results in a bleeding diathesis. Thrombocytopenia and thrombocytopathy, laboratory detection of changes in secondary haemostasis, changes in the fibrinolytic system as well as clinical evidence of bleeding further convinced the clinician of this understanding. However, these tests did not correlate with the risk of bleeding, with haemorrhage occurring far less frequently than the tests would suggest and patients with liver disease are susceptible to thrombosis. The emerging concept is one of rebalanced haemostasis, where an equal reduction in both procoagulant and anticoagulant proteins is seen. This has significant implications for the management of both bleeding and thrombosis in liver disease, expanding the already diverse challenges of managing patients with liver disease.

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Coagulopathy of Renal Disease

30

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The coagulation disorders associated with renal disease present a complex clinical picture owing to the seemingly paradoxical mixture of bleeding and thrombotic predispositions observed in these patients. Diagnosis, prophylaxis, and treatment of acute hemorrhagic or thrombotic complications are thus challenging and require a nuanced understanding of the underlying pathogenic mechanisms as well as diagnostic and therapeutic options. The traditional view of the chief coagulation disorder in renal disease being that of a bleeding diathesis attributable to uremia is overly narrow and indeed antiquated.

In the modern era, the majority of renal disease presents as a chronic condition which is managed electively before acute uremia sets in, and the underlying pathophysiology responsible for the development of chronic kidney disease (CKD) and end-stage renal disease (ESRD) governs the patient's coagulation status. However, acute kidney injury (AKI) is also frequently observed in the complex settings of critical illness and acute traumatic or surgical insult, and these patients may indeed become acutely uremic as well as suffering other coagulation dysfunction associated with their critical illness. Thus, it is important to view the hemostatic abnormalities of a patient with renal disease in terms of the distinct pathophysiologies associated with acute or chronic onset.

Additionally, iatrogenic factors are a key consideration involved in coagulation management of patients with renal disease. Patients with CKD generally suffer from multiple comorbidities including diabetes mellitus (DM), hypertension, atherosclerotic disease, obesity and the metabolic syndrome and the pharmacologic therapy of these disorders can profoundly impact many aspects of hemostasis. These patients are also frequently treated for anemia with erythropoietin and transfusion of blood products, with the associated impact on hemostasis. Moreover, the surgical construction of hemodialysis and peritoneal dialysis access, and dialysis itself, have critical implications for coagulation management both in terms of bleeding risk and access failure due to thrombosis and fibrosis. The impact of coagulation disorders on the success of renal transplantation, and vice versa, are also evident, but are beyond the scope of this chapter.

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The focus of this review will be both the well-known risk of bleeding in patients with renal disease and the perhaps under-recognized risk for thrombosis. Part of this seeming contradiction stems from the apparent heterogeneity of renal disease, with acute uremia, the nephrotic syndrome, and CKD treated as separate entities in the traditional coagulation literature. Our evolving understanding of renal disease, however, is revealing that the increased bleeding and thrombotic tendencies exist simultaneously in these patients. Moreover, this paradox has parallels in other systemic disease processes, such as end-stage liver disease and trauma, and should be thought of as a global derangement of hemostasis which manifests as a risk of bleeding from disrupted vasculature and a risk of thrombosis in intact vasculature.

Pathogenesis

Coagulopathic Bleeding

The manifestations of the bleeding diathesis of renal disease have been recognized for over 200 years, and range from mild (such as ecchymoses and gingival bleeding) to life-threatening (such as spontaneous gastrointestinal bleeding or intracranial hemorrhage) [1–3]. Spontaneous bleeding may also occur in the pericardium, pleural spaces, retroperitoneum, intraocularly or into the subcapsular space of the liver [3–7]. Alarmingly, rates of serious bleeding are quite high in these patients with gastrointestinal bleeding reaming the second leading cause of death in renal failure, and incidence of pericardial and intracranial hemorrhage remaining stubbornly high at up to 3 % with significant associated mortality [4, 8–10]. Menorrhagia is another frequent complaint and may require intervention in up to 10 % of all women on hemodialysis [11]. Obviously, excessive bleeding from surgical or other invasive procedures is a feared complication, but in practice is usually preventable with adequate monitoring of coagulation, correction of uremia by dialysis, and avoidance of aspirin and other antiplatelet agents in the perioperative period [12–14].

These hemorrhagic events are associated chiefly with uremia, although a clear correlation with the degree of uremia (i.e. blood urea nitrogen level and creatinine clearance) and bleeding has yet to be demonstrated [12]. Other than an increased level of activated protein C (aPC),most soluble coagulation factors and prothrombotic mediators are normal to elevated in renal disease, and the chief mechanism responsible for the hemorrhagic tendency in uremic patients appears to be an intrinsic platelet defect, coupled to abnormal platelet–endothelial interactions [13, 15–18]. This platelet dysfunction manifests both as impaired aggregation and adhesiveness, with derangements of the interaction of glycoprotein IIb/IIIa with both fibrinogen and von Willebrand Factor (vWF) [19–22]. Platelet response to stimulation by ADP and epinephrine is also impaired, and the uremic platelet demonstrates a global derangement of its metabolism (particularly prostaglandin synthesis), granule trafficking and degranulation and an altered transcriptome [23–25].

Fundamentally, the proposed toxic factors responsible for these platelet defects remain to be identified, and azotemia itself (i.e. urea or creatinine exposure) has been shown to have no impact on platelet function [24, 26]. The reversal of platelet dysfunction by dialysis, however, strongly suggests that some such soluble “uremic toxins” do exist [24, 27]. Additionally, the uremic platelet dysfunction phenotype can be transferred to healthy platelets by incubation with uremic plasma and, conversely, platelets transfused into uremic patients acquire the dysfunctional phenotype of uremia, suggesting that a soluble toxic factor accumulating in renal dysfunction is at least partly responsible for the observed platelet defect [28–32]. One plausible candidate factor in the platelet dysfunction of uremia is overproduction of nitric oxide (NO), possibly stimulated by uremic toxins such as guanidinosuccinic acid and methylguanidine [24]. While uremic plasma has been shown to increase NO synthesis in cultured endothelial cells, there is also considerable competing evidence that the global inflammatory state of renal disease impairs the production of NO [33–37].

The typical normocytic, normochromic anemia of CKD (secondary to reduced erythropoietic signaling form the diseased kidney) has a

direct mechanical effect on coagulation [38]. Indeed, maintenance of a more normal hematocrit has been shown to reduce bleeding time in uremic patients [39]. From a rheologic standpoint, the bulk flow of erythrocytes is necessary for the radial displacement of platelets to the vessel wall necessary for adhesion and aggregation [40, 41]. Additionally, erythrocytes act in the amplification phase of coagulation both by adding to the available catalytic membrane surface area and stabilizing the platelet plug and enhancing sheer-induced platelet activation via the release of ADP [42, 43].

Thrombosis, Hypercoagulability, and Impaired Fibrinolysis

Thrombotic complications occur at an alarmingly high rate in patients with renal disease, with a combined incidence between 9 and 35 % [44, 45]. In contrast to the purely uremic patient, those with the nephrotic syndrome are at markedly increased risk of deep vein thrombosis, renal vein thrombosis, and arterial thrombosis [44, 46–50]. While the mechanism remains uncertain, patients with a protein-losing membranous nephropathy were demonstrated to be at a 2.5-fold increased risk of venous thromboembolism (VTE) if their serum albumin was ≤2.8 g/dL [51]. It is uncertain how to apply these findings to the more general population of all nephrotic patients, far less the entire spectrum of patients with renal disease.

Other more general contributors to hypercoagulability may be inferred from elevated levels of fibrinopeptide A and thrombin–antithrombin complexes in nephrotic patients, suggesting a baseline state of subclinical intravascular coagulation [52]. Specific factors contributing hypercoagulability may include elevated circulating levels of fibrinogen, tissue factor, coagulation factors VIIa, VIII, XIIa, and von Willebrand Factor (vWF) with a concomitant decrease in antithrombin [29, 45, 53–55]. While these derangements of coagulation mediators are most pronounced in patients with the nephrotic syndrome, they are found to some extent in all forms of CKD [29, 55]. Platelet dysfunction presents a more confusing picture. Platelet hyperaggregability is observed in nephrotic patients, whereas in uremic patients an intrinsic platelet adhesion defect is compensated to near normality by elevated levels of vWF [29–31, 45].

Fibrinolysis, conversely, is universally impaired in renal disease by a variety of mechanisms. Tissue plasminogen activator (tPA) is decreased, and plasminogen activator inhibitors (PAI-1 and -2) are increased both in CKD and in DM, possibly due to increased signaling via the renin–angiotensin–aldosterone axis [16, 18]. Other less evident inhibitors of fibrinolysis are increased as well in renal disease. Circulating antibodies are found against alpha-enolase, which is critical for cell-surface activation of plasminogen [56, 57]. Lipoprotein(a) is increased, which is linked to impairment of the fibrinolytic system and to cardiovascular events and hemodialysis access failure [58–63].

Vascular endothelial dysfunction likely also plays a key role in the global thrombotic predisposition in renal disease. Vascular stiffening and impaired relaxation are merely the most easily demonstrable signs of endothelial failure, and are known to be predictive of cardiovascular thrombotic events [64]. While the exact mechanisms by which endothelial dysfunction is mediated in CKD are unclear, systemic inflammation and increased oxidative stress with resultant reduced nitric oxide bioavailability due to endothelial nitric oxide synthase dysfunction (mediated in part by excessive peroxynitrite production) are likely contributors [35–37]. It is difficult to disentangle the direct impact on the endothelium of uremic toxins and renal disease from the shear stresses and advanced glycation end products of the patient's underlying hypertension and diabetes mellitus [65, 66].

Given the ubiquity of hemodialysis for ESRD in the United States, this potential iatrogenic contributor to coagulation dysfunction must be considered. Apart from the obvious exposure to anticoagulants such as heparin associated with extracorporeal blood circulation, the impact of hemodialysis on coagulation function remains poorly understood. Most studies show activation of the fibrinolytic system, but data are conflicting

as to whether platelets are stimulated, inhibited, or unaffected by passage through the dialyzer circuit [67–69]. It seems likely that sheering forces in the ultrafiltration device activate platelets and cause loss of granule contents, with the net effect being either pro- or anticoagulant based upon a number of factors including flow rates, dialysis time and frequency, and circuit materials [70–73]. Exploration of these questions largely constitutes a bioengineering challenge as the materials and other design elements of the dialyzer circuit govern the effect of dialysis on hemostasis [74].

Dialysis Access Failure

Complications leading to dialysis access failure are a significant cause of morbidity, hospitalization or even mortality and worthy of separate consideration. Dialysis access problems account for between 16 and 48 % of all hospitalization of ESRD patients [75, 76]. Primary patency rates remain dismal at around 50 % after 2 years [77]. Elevated levels of lipoprotein(a), serum fibronectin, and comorbid diabetes mellitus have been identified as predisposing risk factors for access failure [60]. Apart from avoidance of synthetic graft materials, and management of the underlying disease states, little has been proven effective in the way of prophylaxis of graft and fistula thrombosis [78, 79]. Peritoneal dialysis is also susceptible to fibrotic complication. Encapsulating peritoneal sclerosis is a rare complication which not only causes dialysis failure but may progress to fibrotic bowel obstruction. Interestingly, this devastating complication of peritoneal dialysis is linked to low serum plasmin and high PAI-1 and -2 levels, identical to the failure of fibrinolysis associated with thrombotic vascular events [80].

Diagnostic Testing

The presumption that an acquired platelet dysfunction underlies the coagulopathy of renal disease has made bleeding time the traditional test of choice for evaluating bleeding risk in these patients. Unfortunately, bleeding time has never been shown to correlate well with bleeding risk, and the test is increasingly difficult to obtain, requiring specially trained personnel and considerable time to perform [26]. Conventional coagulation tests such as PT, PTT, and platelet count are also of little utility, as they are usually normal or only minimally perturbed, though if found to be abnormal should raise the suspicion of an underlying coagulopathy of another etiology [13, 15]. These studies should nonetheless be performed as well as obtaining a hematocrit to diagnose anemia before undertaking invasive procedures on a uremic patient [81]. Since the bleeding disorder in renal disease is primarily due to platelet dysfunction, specific evaluation of platelet dysfunction via whole blood aggregometry is a logical metric of uremic coagulopathy [23, 24, 82]. The role of viscoelastic hemostatic assays (VHAs) such as thrombelastography (TEG) or rotational thromboelastometry (ROTEM) remains to be elucidated but shows promise. For further description of the viscoelastic assays TEG and ROTEM, please refer to chapters # and # (TEG-Gonzalez, ROTEM-Gorlinger). The coagulation picture reported by these assays reflects the fundamentally paradoxical nature of hemostasis in renal disease, with normal to prolonged clot initiation, but rapid propagation and supernormal final clot strength with impaired fibrinolysis [83, 84]. Preliminary TEG data from our center, comparing 40 ESRD patients to 154 healthy volunteers, are shown in Table 30.1. These data indicate that a hypercoagulable phenotype dominates in CKD/ESRD, typified by elevated fibrinogen levels and final clot strength, coupled to profound resistance to thrombolysis by exogenous tPA. Notably, the TEG-activated clotting time was found to be essentially normal in these patients, calling into question the utility of coagulation tests such as PT, PTT and bleeding time which interrogate only the initiation phase of coagulation. This finding suggests that VHAs may be a more appropriate modality for evaluating hypercoagulability in renal disease.

Table 30.1 Comparison of thrombelastography parameters in healthy volunteers and ESRD patients

	Rapid TEG-activated clotting time (seconds)	Rapid TEG alpha angle (degrees)	Rapid TEG maximum amplitude (mm)	Low-dose tPA-challenged TEG 30-minute clot lysis (%)	High-dose tPA-challenged TEG 30-minute clot lysis (%)	TEG functional fibrinogen level (mg/dL)
Healthy volunteers	113 (105—121)	74 (71—76)	65 (62—68)	8.2 (4.8—14.8)	52.6 (38.2—63.8)	471 (401—529)
End-stage renal disease	113 (105—123)	81 (79—82)	72 (68—75)	3.5 (1.4—7.2)	28.6 (16.6—41.0)	611 (557—702)

Values are expressed as medians with interquartile ranges in parentheses. All parameters were significantly different between healthy controls and ESRD except for activated clotting time. ESRD patients were generally hypercoagulable with elevated fibrinogen and suppressed fibrinolytic capacity (as demonstrated by exogenous tPA challenge) compared to healthy volunteers

Prophylaxis and Treatment of Hemorrhagic Complications

Prophylaxis

No specific prophylactic modality is accepted for the prevention of either bleeding or thrombotic patients in renal disease. Rather, the approach is supportive, aimed at preventing acute uremia by the timely initiation of renal replacement therapy, and the prevention of anemia through the appropriate use of erythropoiesis stimulating agents (ESAs). In CKD, renal replacement therapy should be initiated using either peritoneal or hemodialysis in symptomatic patients with and those with an estimated glomerular filtration rate (eGFR) of <8–10 mL/min per 1.73 m^2 of body surface area, according to the 2012 Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines [85–87]. These guidelines, however, remain somewhat subjective and open to interpretation. Its aim is a balance between the risks of hemodialysis and the risks of life-threatening uremia. Cardinal symptoms such as evidence of coagulopathic bleeding are obviously indications for emergent initiation of dialysis. Therefore, per the 2008 clinical practice guidelines of The Society for Vascular Surgery, all CKD patients with an eGFR of 20–25 mL/min per 1.73 m^2 should undergo elective dialysis access construction to forestall the potential need for emergent access [88].

Similarly, anemia should be corrected prophylactically or acutely via transfusion, in the event of suspected acute bleeding or in anticipation of invasive procedures. Maintenance therapy for anemia relies on with ESAs such as erythropoietin, as well as normalization of iron stores. Patients on hemodialysis routinely lose 1–2 g of iron per year [89]. Transferrin saturation and serum ferritin levels should be corrected to at least 30 and 500 ng/mL respectively, but even at these levels, functional iron deficiency may exist in CKD patients [90, 91]. Patients with refractory resistance to ESAs thus may benefit from a trial of intravenous iron, even if serum iron markers are normal [92]. Once anemia is corrected to the target level, iron supplementation should be maintained at 250–500 mg every 3 months, with intravenous therapy strongly preferred to oral by most authors [93–96]. Iron sucrose or ferric gluconate is preferred to iron dextran, owing to the greatly lower risk of anaphylaxis or other side effects [97, 98].

Recommendations from various bodies including the National Kidney Foundation as well as KDIGO guidelines target hemoglobin concentrations of between 10 and 12 g/dL and discourage correction above 13 g/dL [99–104]. These recommendations reflect the balance between the increased risk of adverse events and death at both the high and low ends of target range, and are not specifically aimed at improvement of coagulation parameters [103]. Nevertheless, these guidelines are consonant with studies indicating an optimization of bleeding time in uremic patients by correction of hemoglobin levels by either transfusion or erythropoietin therapy [39, 105]. Interestingly, treatment with erythropoietin improved intrinsic

platelet function in adhesion studies, irrespective of the hematocrit of the ex vivo perfusate, suggesting a possible direct hormonal effect or neutralization of a uremic toxin by the increased erythrocyte mass or improved tissue oxygen delivery [106]. This stimulatory effect of erythropoietin on platelets may not be entirely benign. Several studies have implicated ESAs as prothrombotic, including one large randomized clinical trial of erythropoietin use in critical illness and trauma in which the hazard ratio for thrombotic events was 1.41 in the erythropoietin group, compared to placebo [107, 108]. The even greater thrombotic risk from erythropoietin use in individuals with other underlying prothrombotic tendencies (e.g. oncologic patients) would suggest that use in CKD patients would be likewise hazardous [108, 109]. However, scant evidence regarding this risk exists one way or the other. In fact, the salutary effects on the rheologic properties of blood from the correction of anemia may even help ensure hemodialysis access graft patency, and the cautious use of ESAs remains standard practice in renal disease [110].

Acute Therapy

While maintenance dialysis and correction of anemia can forestall most bleeding events, desmopressin (DDAVP) remains the mainstay of therapy for acute coagulopathic bleeding and emergent peri-procedural prophylaxis in renal disease [88]. This analog of arginine vasopressin is fast acting and has low potential for toxicity [111]. It acts by releasing endothelial stores of high-molecular-weight fVIII: vWF multimers [112, 113]. These partially offset the intrinsic platelet dysfunction of gpIIb/IIIa interaction with vWF, however this therapy is prone to tachyphylaxis due to depletion of these finite endothelial reserves [28, 30]. In a hemorrhagic emergency or for preprocedural prophylaxis, a dose of 0.3–0.4 mcg/kg may be given intravenously diluted in 50 mL of normal saline, over 10 min. The onset of effect is within 30–60 min and lasts 4–8 h [113]. A second dose may be given, but tachyphylaxis renders subsequent doses beyond this of little utility [111, 112]. There is a theoretical contraindication for use of DDAVP in patients with a creatinine clearance <50 mL/min; its use remains off-label in patients in renal failure. Notably, there have been case reports of thrombotic events associated with the use of DDAVP in uremic patients due to spontaneous platelet aggregation driven by the intravascular release of high-molecular-weight vWF multimers. Therefore, this agent should be used with caution, particularly in patients with known atherosclerotic disease [114].

Anemia should also be corrected by packed red blood cell transfusion to a hemoglobin of at least 10 mg/dL, in the face of active bleeding or in preparation for a non-elective invasive procedure [39]. Dialysis obviously must be initiated or maintained in order to prevent worsening uremic coagulopathy, and regional citrated hemodialysis or peritoneal dialysis (described above) offers good alternatives in the actively bleeding patient. As an adjunct, in cases of refractory coagulopathy, conjugated estrogens given either orally or transdermally may be of utility (albeit via an unknown mechanism) and have a longer period of efficacy (up to 14 days) than DDAVP [115]. ESAs may also be used for their direct effect on platelet function, possibly through upregulation of gpIIb/IIIa and enhanced signal transduction [116–118].

Prevention of Thrombotic Complications

While therapies to ameliorate bleeding risk in renal patients are well described, management of thrombotic risks is less well understood. No specific guidelines for thromboprophylaxis exist for patients with renal disease, largely owing to a paucity of prospective randomized clinical trials [119]. Despite their prothrombotic tendencies, there is no current evidence that routine screening for DVT or RVT is beneficial in renal disease [29, 120]. Prophylactic oral anticoagulation therapy has been proposed for the extremely high risk subset of nephrotic patients with membranous nephropathy, but these recommendations

are based on scant evidence and are not generalizable [121]. Potential novel therapeutic targets for prophylaxis of thrombotic events and dialysis access failure are suggested by the specific mechanisms of hypercoagulability in renal disease. For instance, fibrinogen lowering (a secondary effect of fibrate therapy) or lipid-lowering agents may be of use, as could inhibitors of PAI-1 [17, 122–124]. Blockade of the renin–angiotensin–aldosterone axis (already employed to slow the progress of CKD) might also serve to improve fibrinolysis through decreases in PAI-1 and lipoprotein(a) levels [125–127]. Despite the lack of consensus on the utility of thromboprophylaxis, the authors' current practice is to utilize systemic heparinization during construction of dialysis access, followed by maintenance therapy with low-dose aspirin, in patients with TEG-proven hypercoagulability.

During the performance of hemodialysis, which requires extracorporeal blood circulation, anticoagulation is also necessary to prevent clotting of the dialyzer device. Anticoagulation is routinely achieved with unfractionated heparin, either given as standardized doses or targeted to an activated clotting time (ACT) of between 200 and 250 s [128–130]. Alternatively, in patients with a history of heparin-induced thrombocytopenia (HIT) or at high risk of bleeding, regional citrate anticoagulation can utilized, and is gaining wider acceptance. In this methodology, the dialysis circuit is citrated and the citrate anticoagulation is reversed by a continuous infusion of calcium into the return limb of the dialyzer [131–134]. Hemodialysis circuit thrombosis should raise the suspicion of a worsening thrombotic tendency in the patient and indeed may be a sign of evolving HIT [134]. Citrate anticoagulation may have other advantages, reducing the activation of both platelets and neutrophils during extracorporeal circulation, presumably owing to attenuation of transmembrane calcium flux in response to contact stimuli [135]. The use of other systemic anticoagulants such as warfarin, argatroban, and hirudin for hemodialysis circuit protection in cases of suspected or proven HIT remains in its infancy, but shows promise [136–139]. Low-molecular-weight heparins have no proven advantages over unfractionated heparin in hemodialysis, are extremely expensive and have been implicated in platelet activation [11, 140, 141].

Conclusion

While the early diagnosis of CKD and ready availability of dialysis in both ESRD and AKI have markedly reduced the risk of uremic bleeding in the modern era, renal disease still presents numerous challenges with regard to coagulation management and these patients are still at grave risk of serious bleeding and thrombotic complications. A limited understanding of the pathogenesis of both the hyper- and hypocoagulable aspects of hemostatic disorder in renal disease continues to hamper diagnosis, prophylaxis, and therapy. The need for further clinical and basic scientific research regarding the coagulation disorders in the varied and heterogeneous population of patients with renal disease is clear. For the moment, coagulation management in these patients remains largely empiric and symptomatic. A high degree of vigilance and a high index of suspicion for both bleeding and thrombosis are warranted when treating these patients. Clinicians should avail themselves of the best available coagulation assays, including viscoelastic hemostatic assays and platelet function tests, before embarking on invasive procedures, subjecting the renal patient to immobilization or in any circumstance where the risk of bleeding or thrombosis is incurred. Therapies and prophylaxis should be carefully titrated in a goal-directed manner to the immediate need, preferably with the consultation of a coagulation specialist. With appropriately cautious monitoring and intervention, life-threatening complications of either a thrombotic or hemorrhagic nature can usually be avoided, even in these complicated patients.

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Part VII

Trauma Induced Coagulopathy in Special Populations

Pediatrics

31

Robert I. Parker

Introduction

The development of coagulopathy following trauma appears to be independent of patient age [1–6], although trauma-induced coagulopathy (TIC) is reported less often and appears to be less severe in children in comparison to that noted in adult patients [7–9]. However, this may be an artifact of reporting rather than a true difference. The coagulopathy of trauma involves multiple elements of hemostasis, and consequently may be manifest as either the development of a bleeding diathesis or of increased incidence of thromboembolism [9–11]. Most studies on TIC have involved either animal models or clinical studies in adult patients. As the factors involved in the regulation of hemostasis in adults and in children (those beyond newborn/early infancy) are essentially identical, it may not be unreasonable to apply insights gained from these studies to children. However, there are differences in how hemostasis is regulated in neonates and young infants in comparison to older children and adults that may potentially affect how TIC develops and is manifest; laboratory studies clearly linking these differences to TIC do not exist. Currently, there are little data addressing trauma-induced hemostasis defects in pediatric trauma with the exception of the coagulopathy noted to occur in association with head trauma. Consequently, this discussion will focus on reviewing differences in hemostasis in young infants, pointing out where these differences may affect the development of TIC, reviewing our knowledge regarding the coagulopathy noted in pediatric head trauma, and summarizing the available data on the management of TIC in pediatric patients.

Regulation of Hemostasis in Newborns and Young Infants

The most common tests employed to measure in vitro clot formation are the prothrombin time (PT) and activated partial thromboplastin time (aPTT, PTT). The normal range for these tests in neonates is prolonged compared to older children and adults due in part to the higher hematocrit (and therefore lower plasma volume) in newborns resulting in an over-anticoagulation of blood specimens unless the amount of anticoagulant is adjusted accordingly. However, this prolongation of both the PT and aPTT is also due to differences in important clotting factors that affect the ability to generate a fibrin clot in vitro. Based on these prolonged values, one

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might conclude that newborn infants have an intrinsic coagulopathic state predisposing them to hemorrhage. However, it has been well documented in the literature that the hemostatic balance in newborns and infants is different than that noted in older children and adults. This balance favors thrombosis and has been documented in both premature and full-term newborns as well as in ill and well newborn infants [12–19]. How this difference could affect the coagulopathy of trauma in infants is not at all clear. However, any investigation into this concept must start with a thorough understanding of how the hemostatic system in newborn and young infants differs from that of older children and adults. At birth, several plasma proteins necessary for clot formation or involved in the regulation of hemostasis are essentially at those levels noted in healthy adults. These include the clotting factors fibrinogen, factor VIII and factor XIII, the inhibitors of coagulation Protein S and alpha-1-antitrypsin, the coagulation promoting cofactors high molecular weight kininogen, prekallekrien and heparin cofactor-II, and the fibrinolytic pathway proteins plasminogen and its inhibitor alpha-2-antiplasmin. While levels of anti-thrombin-III levels may be normal, they are more often low in newborn infants. The levels of clotting factors II, V, VII, IX, X, XI, and XII are lower than in older children and adults, as is the important natural anticoagulant protein C. Finally, levels of von Willebrand factor (vWf), alpha-2-macroglobulin (α2M), and c_1-esterase inhibitor are above normal adult levels at birth (Table 31.1). In the case of von Willebrand factor, this likely

Table 31.1 Levels of various hemostasis factors at birth compared to adult levels

At/near normal adult levels		Lower than adult levels		Higher than adult levels	
Protein	Function	Protein	Function	Protein	Function
Fibrinogen	Promotes clot formation; precursor of fibrin	FII (prothrombin)	Precursor of thrombin; promotes clot formation; activates platelets; activates fibrinolysis	von Willebrand factor	Promotes hemostasis by enhancing platelet adhesion
FVIII	Promotes clot formation	FV	Promotes clot formation	α_2-macroglobulin	Inhibits coagulation
FXIII	Stabilizes fibrin clots	FVII	Promotes clot formation	C_1-esterase inhibitor	
Protein S	Inhibits coagulation	FIX	Promotes clot formation		
HMWK	Promotes clot formation	FX	Promotes clot formation		
Prekallekrein	Promotes clot formation	FXI	Promotes clot formation		
HC-II	Allows for normal clot formation	FXII	Promotes clot formation		
Plasminigen	Active in fibrinolysis; precursor of plasmin	Protein C	Inhibits coagulation		
α_2-AP	Inhibits fibrinolysis	Antithrombin-III (low/near normal)	Inhibits coagulation		
α_1-antitrypsin	Inhibits clot formation				

is due to birth-induced release of vWf from endothelial cells rather than from an increase in synthesis. The most common laboratory studies employed to assess coagulation (i.e., PT and aPTT [also referred to as the PTT]) were developed to be sensitive to decreased levels of those clotting factors identified in the "clotting cascade." For the most part, these are the same clotting factors that are present in lower levels in neonates when compared to adults and older children. Consequently, the normal ranges for both the PT and aPTT are more prolonged than the normal ranges in adults (Table 31.2) [12–19]. This prolongation of in vitro clot production does not appear to reflect a decreased potential of neonate plasma to generate thrombin in vivo. Studies investigating this question have produced variable results; some have shown normal thrombin generation while others have shown it to be slightly reduced [20–22]. When global hemostasis has been investigated (i.e., clot production, fibrinolysis, and platelet aggregation/adhesion), the general consensus has been that the overall balance in the neonate is "neutral" or favors clot production in spite of the observed prolongation of PT and PTT [23, 24]. This is an important point to take into consideration when evaluating PT and/or aPTT results in neonates. One must realize that a prolongation of either the PT or aPTT does not necessarily reflect a hemostatic balance predisposing the infant to hemorrhage.

While all of the factors involved in producing this prothrombotic milieu in newborn infants have not been identified, both the decreased AT-III and elevated von Willebrand factor activity have been shown to be important. In newborn infants, AT-III is decreased with a compensatory increase in α2-macroglobulin [24, 25]. However, the affinity of α2M for thrombin is much lower than of AT-III for thrombin, and, as a result, the capacity to inhibit the strongly procoagulant molecule thrombin is significantly less in neonates and young infants when compared to that of older infants and children [17, 19, 25]. The resultant effect is a hemostatic balance that favors clot formation.

Studies investigating platelet reactivity in neonates have relied largely (but not exclusively) on evaluation of cord blood platelets. Even when platelet responsivity has been demonstrated to be deficient, aggregation and platelet secretory responses have been shown to recover to normal levels within 24–48 h of birth [26–29]. Consequently, it is very likely that platelets obtained from cord blood may have an element of "exhaustion" from the birth process explaining any reduced aggregation capabilities noted [30]. This possibility is supported by the lack of studies demonstrating any abnormalities in activation pathways in newborn's platelets, and the finding of normal, even shortened, bleeding time in neonates [28–30]. The presence of natural platelet inhibitors, such as prostaglandin E_2, in the plasma of cord blood may also play a role in the transient platelet aggregation defects noted in neonatal platelets [31]. In newborns as in older children and adults, von Willebrand factor is critical in the process of platelet adhesion. When compared to adults, vWf activity is increased in neonates due to both an increase in the amount of vWf in circulation and a shift in the vWf multimers present in plasma characterized by an increase in the very large and ultra-large multimers known to have a more pronounced effect promoting platelet adhesion [32, 33]. Possible mechanisms explaining the increase in the amount of circulating vWf have included a decrease in the vWf cleaving metalloprotease ADAMTS13 but this has been found to be normal in cord and neonate blood [29, 33]. Consequently, it has also been proposed that the abnormal vWf multimeric profile in neonates at least in part represents a consequence of the birth process as it reverts to normal in a relatively short period of time. However, the abnormal vWf multimer pattern noted in newborns may reflect an intrinsic difference in vWf processing at the cellular level in newborns as this abnormality persists for up to 2 months [32]. As a result of the increased vWf activity in the plasma of neonates and young infants, platelet adhesion and hence primary hemostasis is enhanced during the time when the infant's ability to generate fibrin clots may be most compromised [34]; a point that should be taken into account when deciding on transfusion support in neonates (see below). As stated previously, how

Table 31.2 Age-dependent normal range for hemostasis-related parameters (from Andrew M: Am J Pediatr Hematol Oncol 1990;12:95–104 [17])

Test	Birth (premie[a])	Birth (term[b])	1 Month (premie)	1 Month (term)	3 Months (premie)	3 Months (term)	6 Months (premie)	6 Months (term)	Adult
PT (sec)	04.6–16.2	10.1–15.9	10.0–13.6	10.0–14.3	10.0–14.6	10.0–14.2	10.0–15.0	10.7–13.9	10.8–13.9
aPTT (sec)	27.5–79.4	31.3–54.5	26.9–62.5	32.0–55.2	28.3–50.7	29.0–50.1	27.1–53.3	28.1–42.9	26.6–40.3
TT (sec)	19.2–30.4	18.7–28.3	18.8–29.9	19.4–29.2	19.4–30.8	20.5–29.7	18.9–31.5	19.8–31.2	19.7–30.3
Fibrinogen (mg/dL)	150–373	167–700	150–414	162–378	150–352	150–379	150–360	150–387	156–400
D-dimer[c] (ng/mL)		716–1133							41–50
Platelets (k)	150–400 k	150–400 k							150–400 k
FII (%)	20–77	26–70	36–95	34–102	30–106	45–105	51–123	60–116	70–146
FV (%)	41–144	36–108	48–156	62–134	59–139	48–132	58–146	55–127	62–150
FVII (%)	21–113	28–104	21–145	42–138	31–143	39–143	47–151	47–127	67–143
FVIII (%)	50–213	52–148	50–199	50–157	58–188	50–125	50–187	50–109	50–149
FIX (%)	19–65	15–91	13–80	21–81	25–93	21–113	50–120	36–136	55–163
FX (%)	11–71	12–68	20–92	31–87	35–99	35–107	35–119	38–118	70–152
FXI (%)	8–52	10–66	15–71	27–79	25–93	41–97	46–110	49–134	67–127
FXII (%)	10–66	13–93	11–75	17–81	15–107	25–109	22–142	39–115	52–164
FXIIIa (%)	32–108	27–131	51–147	39–147	71–155	36–127	65–161	50–170	57–137
HMWK (%)	9–89	26–102	16–112	33–121	32–124	30–146	41–125	36–128	50–136
PK (%)	9–57	5–69	31–87	23–91	37–121	41–105	40–116	56–116	62–162
Plasminogen (U/mL)	1.12–2.48	1.25–2.65	1.09–2.53	1.26–2.70	1.58–3.18	1.74–3.22	1.19–3.59	2.21–3.81	2.48–4.24
α2-AP (U/mL)	0.40–1.16	55–115	0.55–1.23	0.76–1.24	0.64–1.48	0.76–1.40	0.77–1.53	0.83–1.39	0.68–1.36
tPA (ng/mL)	3.0–16.7	5–18.9	2.0–7.8	1.0–6.0	2.0–5.1	1.0–5.0	2.0–5.85	1.0–6.0	1.4–8.4
PAI-1 (IU/mL)	0–12.2	2–15.1	0–10.9	0–8.8	1.0–11.8	1.0–15.3	1.0–10.2	6.0–13.0	0–11.0
Protein C (%)	12–44	17–53	15–59	21–65	23–67	28–80	31–83	37–81	64–128
Protein S (%)	14–38	12–60	22–90	33–93	40–112	54–118	44–120	55–119	60–124
AT-III (%)	14–62	49–137	37–81	48–108	45–121	73–121	52–128	84–124	79–131

Test	Birth (premie[a])	Birth (term[b])	1 Month (premie)	1 Month (term)	3 Months (premie)	3 Months (term)	6 Months (premie)	6 Months (term)	Adult
α_2M (%)	56–182	95–183	72–204	106–194	120–266	126–226	110–321	149–233	52–120
HC-II (%)	10–93	0–93	15–71	10–87	20–111	10–146	45–140	50–190	66–126
C_1est-inh (%)	31–99	36–108	40–124	47–131	60–168	71–159	96–204	89–193	71–131
α_1-AT (%)	36–144	49–137	38–112	36–88	49–113	42–102	96–204	47–107	55–131
vWf Ag (%)	78–210	50–287	66–216	50–246	75–184	50–206	54–158	50–197	50–158
vWf Multimers[d]	(+) Ultra-large multimers	+/− Ultra-large multimers	(+) Ultra-large	+/− Ultra-large	+/− Ultra-large	Normal pattern	Normal pattern	Normal pattern	Normal pattern

TT thrombin time, *HMWK* high molecular weight kininogen, *PK* prekallekrein, *α2AP* alpha-2-antiplasmin, *tPA* tissue plasminogen activator, *PAI-1* plasminogen activator inhibitor type-1, AT-III-antithrombin-III, *α2M* alpha-2-macroglobulin, *HC-II* heparin cofactor-II, *C1 est Inh* c1-esterase inhibitor, *α1-AT* alpha-1-antitrypsin, *vwf* von Willebrand factor

[a]Gestational age 30–36 weeks

[b]Gestational age ≥37 weeks

[c]From Siow-Ping, T: Blood Coagul Fibrinolysis 2003;14:125–129 [18]; newborn infant results obtained on cord blood

[d]from Katz JA: Blood 1989;73:1851–58 [33]

these findings affect the development of TIC is not clear. While there are no data suggesting an increased risk of a consumptive coagulopathy in young infants with trauma, the reduced capacity to neutralize thrombin and the presence of ultra-large vWf multimers in circulation potentially creates an environment that may promote the development of microvascular thrombosis and over-production of thrombin—both events that have been associated with the development of a consumptive coagulopathy (i.e., disseminated intravascular coagulation [DIC]).

Coagulation Abnormalities with Pediatric Head Trauma

Altered hemostasis is well described following traumatic brain injury (TBI) in both adults and children [35]. Studies in pediatric TBI have shown that the severity of injury (as assessed by Glasgow Coma Scale; GCS) is associated with the incidence of a coagulopathic state, and that the presence of a coagulopathy is associated with a worse outcome in TBI [36–38]. Additionally, abnormalities in the protein C pathway and in platelet function have also been found to be abnormal in the setting of TBI [39–41]. The mechanism(s) by which these abnormalities develop is not clear, but hypo-perfusion (and presumably local/regional acidosis) may play an important role [39]. Other studies have linked TBI with parenchymal brain injury (including severe edema or hemorrhage) with the development of an activated coagulation system (i.e., a consumptive coagulopathy) and an increased risk of mortality [42–47]. Brain pathology (e.g., TBI, mass lesions) has also been associated with an increased incidence of venous thromboembolic events (VTE) [48].

Management of Hemostasis in Pediatric Patients

Patients with pre-existent coagulopathies such as hemophilia (A, B, or C), von Willebrand disease, hypo- or dysfibrinogenemia, should receive appropriate factor concentrate to support hemostasis as would be given for uncomplicated surgery or for trauma. Currently, there are recombinant or plasma-purified products available for clotting factors VII, VIII, IX, XIII, and fibrinogen (recombinant von Willebrand factor has recently been FDA approved but availability may be limited due to a lack of familiarity with the product). By convention, the amount of each clotting factor in 1 mL of human plasma is defined as 1 U/mL or 100 % activity. For patients with either Hemophilia A (factor VIII deficiency) or Hemophilia B (factor IX deficiency), the goal of transfusion support in a patient with major bleeding is to raise their clotting factor to approximately 80–100 % of normal (i.e., 0.8–1.0 U/mL). For factor VIII, infusion of 1 U/kg body weight will increase the circulating factor VIII level by 2 % (0.02 U/mL); for factor IX, 1 U/kg increases factor IX level by 1 % (0.01 U/mL). As an illustration, let us use a 10 kg child with hemphilia A and trauma. In the case of severe hemophilia A (i.e., factor VIII activity <1 %), to achieve a final factor VIII activity level of 80 %, one would have to infuse 40 factor VIII U/kg = 400 U (10 kg × (80 U kg 1/2) = 400). In the case of hemophilia B (factor IX deficiency), the dose for this same 10 kg child would be 800 factor IX units (10 kg × (80 U kg 1/1) = 800). The risk of bleeding in children with factor VIII or factor IX deficiency is directly proportional to the plasma factor activity. Individuals with "severe" hemophilia (<1 % clotting factor activity) are at risk for "spontaneous" hemorrhage in joints, soft tissue, or closed spaces. These are bleeds that occur without obvious trauma. Individuals with "moderate" severity (1–5 % factor activity) will experience bleeding that is more than anticipated for the degree of trauma, while those with "mild" deficiency (5–50 % activity) may bleed more than anticipated with moderate to severe trauma. In general, it is desired to correct an individual's clotting factor to 75–80 % normal in the case of major surgery. For surgery that carries a moderate risk of bleeding, correction to 30–40 % are recommended while levels of 20–30 % are generally sufficient for surgery that carries a low risk of hemorrhage. For life-threatening bleeding, correction of FVIII or FIX

to 100 % or greater is recommended. All patients who present with a clotting factor deficiency should be evaluated for the presence of an inhibitor, even in the setting of a positive family history or established diagnosis. These guidelines are largely empiric and have not been validated by control studies [49].

Von Willebrand factor can be replaced with infusions of plasma-derived FVIII that contain high molecular weight vWf multimers (e.g., Humate-P, Wilate, Alphanate); it is important to note that not all plasma purified FVIII concentrates and no recombinant FVIII concentrates contain high molecular weight von Willebrand factor. As noted, a recombinant vWf product has recently been approved by the FDA. Advantages of this recombinant product over plasma-derived vWf products is a higher specific activity due to the absence of other plasma proteins, reduced risk of pathogen transmission and the elimination or reduction in a need for transfused FVIII to treat severe von Willebrand disease. Lyophylized fibrinogen concentrates are now available in the United States and have been shown to be effective in correcting bleeding in individuals with hypofibrinogenemia (acquired or congenital) or dysfibrinogenemia. An infused dose of 70 mg/kg has been shown to be effective and safe in clinical studies. For other factor deficiencies, FFP or solvent-detergent plasma can be administered; dosing is arbitrary and adequacy should be monitored by appropriate blood tests (e.g., PT, aPTT, clotting factor levels) as clinically indicated. As the concentration of specific clotting factors in FFP or SD plasma is no more than 1 U/mL (and frequently lower), the ability to give large amounts of the desired clotting factor is limited by volume constraints. Consequently, when plasma is utilized for hemostatic support, a dose of 10–20 mL/kg body weight is frequently utilized and the effect of bleeding control is often limited and transient. The half-life of plasma purified or recombinant clotting factors and typical doses are shown in Table 31.3.

Individuals on anticoagulant therapy with a vitamin K antagonist should receive a 4-factor prothrombin complex concentrate (4-PCC) if immediate correction of the elevated INR is desired, as it is in the setting of hemorrhagic shock or intracranial hemorrhage; however, for prolongations of INR from vitamin K antagonists where no bleeding is evident vitamin K replacement should be administered [50, 51]. Vitamin K deficiency can also occur in the absence of vitamin K antagonist therapy. Patients at risk are those with malabsorption secondary to gastrointestinal disorders (e.g., α_1-antitrypsin deficiency, cystic fibrosis, celiac disease, short gut syndrome

Table 31.3 Half-life of plasma purified and recombinant clotting factors

Factor	Source	*t*1/2	Dosing	Reference
VIII	Plasma	12–14 h	1 U/kg increases plasma level by 2 %	[103–105]
	Recombinant	12–14 h		
	Modified*	19 h		
IX	Plasma	16–18 h	1 U/kg increases plasma level by 1 %	[106–108]
	Recombinant	17–20 h		
	Modified*	82 h		
VII	Plasma (FFP)	6–7 h		[109–111]
VIIa	Recombinant	1.5–2.5 h	35 mg/kg	
	Modified*	6–10 h		
XIII	Plasma	6–7 days	40 U/kg	[112–114]
	Recombinant	11–15 days	35 U/kg	
Fibrinogen	Plasma	77–100 h	70 mg/kg	[115, 116]
vWf	Plasma	12–18 h	1 vWf:RCoF U/kg increases plasma vWf activity 2 U/mL	[117, 118]
	Recombinant	21–25 h		

*protein modified synthetically to prolong in vivo half-life

including bariatric surgery), chronic diarrhea, malnutrition, or those who have been exposed to broad spectrum or non-absorbable antibiotics. Newborn infants present a special group of patients at risk for vitamin K deficiency. With the widespread administration of prophylactic vitamin K to neonates in the delivery room or newborn nursery, the incidence of "classic" neonatal vitamin K deficiency (that which develops at 2–7 days of life) has nearly disappeared. While the administration of either intramuscular (0.5–1.0 mg) or oral (2 mg) vitamin K appear equally effective in preventing "classic" vitamin K deficiency syndrome, those children who did not receive parenteral vitamin K at birth are at risk for developing "late" vitamin K deficiency (developing 2–8 weeks after birth) particularly in exclusively breast fed infants or those infants who have other clinical factors that predispose to poor vitamin K absorption. Overall, the risk of "late" vitamin K deficient bleeding (VKDB) ranges from 1.4 to 7.2 per 100,000 infants receiving only oral vitamin K prophylaxis [52]. Patients who are vitamin K deficient should receive vitamin K (IV or PO) if not bleeding to correct a significantly elevated INR, or, if bleeding, a 4-factor PCC to rapidly correct the INR toward the normal range.

While it is common practice to infuse fresh frozen plasma (FFP) or recombinant activated FVII in patients with a prolonged PT (INR [International Normalized Ratio]), there are no studies demonstrating a benefit for this practice in the non-bleeding patient. Consequently, the "prophylactic" infusion of FFP or recombinant FVIIa when bleeding is not present is discouraged. Studies in pediatric patients assessing mortality with the use of plasma or clotting factor concentrates have failed to offer conclusive evidence in support of these products [39, 53, 54]. Indeed, aggressive support with plasma or pRBCs has been associated with worse outcome in critically ill pediatric patients [54–56]. However, in the setting of a major central nervous system hemorrhage, treatment with plasma is acceptable although with a greater cardiovascular complication risk than with other products [57].

Use of recombinant factor VIIa (rFVIIa) has been employed as an adjuvant to blood component therapy in achieving hemostasis in neonatal and pediatric patients with coagulopathic bleeding and TBI who did not have hemophilia [58, 59]. A retrospective study including 135 children who received rFVIIa for off-label use (currently the only approved indication is for management of bleeding in hemophilia patients) demonstrated significantly decreased blood-product administration but was also associated with thromboembolic events [60]. In adult studies, recent evidence has discouraged the routine role of this hemostatic agent, due to lack of data on efficacy, increased incidence of thromboembolic events, and prohibitive cost; reserving it for salvage therapy. In 2011 a systematic review of 16 randomized controlled trials, 26 comparative observational studies, and 22 non-comparative observational studies on the off-label use (intracranial hemorrhage, cardiac surgery, trauma, TBI, liver transplantation) of rFVIIa concluded that there was no evidence that the use of rFVIIa changed overall survival, and that there was evidence for an increased risk of thromboembolic events. Given that the incidence of thromboembolic events associated with rFVIIa use was not significantly high when first studied in hemophiliac patients, it is thought that this high incidence is due to its off-label use. In other words, hemophiliac patients will always have an underlying hemostasis defect, relatively protecting them from thromboembolisms when exposed to this pro-coagulant; however, when used in patients with normal hemostasis, or in those who after surviving bleeding will become hypercoagulable (i.e., trauma patients and neonates), thromboembolic events occur.

Prothrombin complex concentrates (PCC) were initially developed for the management of bleeding in patients with hemophilia. To date, there are no studies reporting benefits of off-label use in pediatric patients. They are currently reserved for those patients on vitamin K antagonist therapy who present with severe bleeding, TBI, or intracranial hemorrhage, as they contain vitamin K dependent factors (II, VII, IX, X). Concerns also exist for an increased risk of thromboembolic events. Four-factor PCCs (4-PCC) are the preferred product for correcting the INR in

individuals who are on vitamin K antagonist therapy (e.g., warfarin) because of the higher concentration of FVII contained in these products when compared to traditional 3-factor PCCs [50, 51].

Use of the antifibrinolytic tranexamic acid (TXA) in pediatric patients has not been prospectively studied. Although in a relatively older pediatric patient population, a retrospective review of pediatric combat casualties in the Afghanistan conflict (mean age of 11 years, 73 % penetrating injury, 10 % required a massive transfusion) 9 % received tranexamic acid. The only independent predictors of TXA use were severe abdominal or extremity injury and a base deficit of greater than 5. TXA use was independently associated with decreased mortality (odds ratio, 0.3; $p=0.03$). There was no significant difference in thromboembolic complications or other cardiovascular events [61].

Transfusion Support

Special considerations are present when resuscitating bleeding pediatric patients. Estimates of volume loss and volume replacement are based on weight; children over the age of 3 months have an estimated blood volume of 70 mL/kg, and younger infants have an estimated blood volume of 90 mL/kg [62–64]. Clinical manifestations of hemorrhagic shock in children vary from adults due to a substantial physiologic reserve [65], and initial vital signs may not be good predictors of the degree of hemorrhage [64, 66, 67]. Changes in pulse pressure may be a more sensitive sign of hypovolemia than tachycardia or systolic hypotension [64]. Unique to children, substantial bleeding may occur due to closed head trauma [64].

Regarding dosing of blood products, dosing must be weight-based for pediatric patients, particularly those who weigh <50 kg. Dosing of resuscitation during massive transfusion is described in Table 31.4. In general, during massive transfusion, a dose of 10–20 mL/kg of blood products should be administered, adjusting within this range based on the severity of bleeding and coagulopathy. Consequently, studies reporting transfusion ratios in pediatric patients <50 kg of weight should do so in dose ratios rather than unit ratios; e.g., 10:10:20 mL/kg of plama:platelets:RBC, rather than 1:1:2 units of plama:platelets:RBC.

Table 31.4 Dosing of resuscitation for pediatric trauma patients during massive transfusion (for those <50 kg weight)

Product	Dose
Crystalloid[a]	10 mL/kg
RBC	10–20 mL/kg
Plasma	10–20 mL/kg
Platelets	10 mL/kg

Packed red blood cells (RBC); milliliter per kilogram (mL/kg)

[a]During massive transfusion, blood products should be prioritized over crystalloid

The decision of when and with what product to transfuse a child who has suffered trauma is largely based on the clinical assessment of that child. While several strong studies in adult and pediatric critically ill patients have demonstrated that a restrictive pRBC transfusion regimen is not inferior to a more liberal transfusion regimen (i.e., hemoglobin trigger of 7 g/dL vs. 10 g/dL), these studies have only been carried out on hemodynamically stable ICU patients [68–72]. None of these studies included patients who were actively bleeding. Consequently, the application of this lower hemoglobin threshold to trigger pRBC transfusion in trauma patients is untested. The goal in trauma resuscitation is to provide enough pRBCs to ensure adequate oxygen delivery to the tissues, adequate (functional) platelets to support primary hemostasis, and adequate clotting factors to allow for the formation of a stable, fibrin clot. This generally requires transfusion of pRBCs, plasma, and platelets in variable quantities.

Historically, the majority of transfused blood product in trauma has been RBCs in the form of either whole blood or (more commonly) packed RBC units (pRBCs). Transfusion regimens that minimize or delay the inclusion of plasma (fresh frozen plasma; FFP) or platelets (e.g., 1 U of FFP or platelets for every 4–5 U of pRBCs transfused) increase the risk of an iatrogenic coagulopathy.

Aggressive transfusion support through the implementation of a formal transfusion protocol that would provide multi-product support with RBCs, plasma, and platelets in a ratio that is closer to 1:1:1 has been hypothesized to improve outcome in these children [67, 73, 74]. However, to date, prospective studies have not clearly demonstrated an improvement in outcome, and concern for an increase in transfusion-related organ dysfunction (e.g., transfusion-related acute lung injury; TRALI) exists. Only two single-center studies have investigated outcomes with the use of a predefined massive transfusion protocol in pediatric patients. Hendrickson et al. [73] conducted a study in pediatric trauma patients (median age of 5.5 years) comparing outcomes after implementation of an institutional massive transfusion protocol to a historic cohort. Their weight-based massive transfusion protocol had an intended plasma:RBC ratio of 1:1, and every other package of blood products delivered also contained apheresis platelets and cryoprecipitate units. Only 49 % of the patients received a massive transfusion (defined as >70 mL/kg total blood products transfused). Patients transfused after implementation of this protocol received twice the ratio of plasma:RBC as the historic controls (1:1.8 vs. 1:3.6). The median time to FFP transfusion decreased fourfold after implementation of the protocol. These investigators did not find a statistically significant reduction in mortality (38 % vs. 23 %, $p=0.35$) after their massive transfusion protocol implementation when taking injury severity and coagulopathy into account in a multivariate analysis. Chidester et al. [67] conducted a prospective (non-randomized) study of 55 injured children (mean age of 9.6 years), in which 22 of the children received blood transfusions according to an established massive transfusion protocol and 33 were transfused at physician discretion. The investigators defined massive transfusion as ≥1 blood volume (weight-adjusted to age) transfused within 24 h or half of a blood volume in 12 h. Even though the total number of blood products transfused was greater in the massive transfusion protocol group, the actual blood product ratio between the groups was similar with a mean ratio plasma:RBC of 1:3 in both groups. No significant difference in mortality was found between the two groups (45 % vs. 45 %).

Institutionalization of such massive transfusion protocols has demonstrated prioritization of blood products, and in some studies, decreased blood product use as well as decreased blood bank-related costs. However, concern for the adverse consequences of blood product transfusions still exist, particularly in more vulnerable populations such as neonates in whom volume overload is less tolerable [75]. In a retrospective study of 43 pediatric trauma patients (median age of 8 years), Pieracci et al. reported that early RBC transfusion (<6 h from admission) was associated with increased mortality, and that late RBC transfusion (≥6 h from admission) was associated with an increased length of stay in the ICU and more ventilator days compared to non-transfused patients [56]. Similarly, Stone et al. identified an association between transfusion of RBC within the first 24 h after admission and increased mortality, prolonged duration of mechanical ventilation and ICU admission in a larger retrospective study of pediatric trauma patients [76]. In critically ill pediatric patients, a dose-dependent relationship between the number of RBC transfusions and mortality was observed by Kneyber et al. [77]. However, as the "excess" mortality was determined by comparison of actual mortality with expected (anticipated) mortality as determined by severity of illness scores on PICU admission, these studies cannot conclusively exclude the possibility that the increased mortality in the transfusion cohort was not due to their severity of illness. While earlier studies demonstrated that transfusion of RBC units stored for more than 14 days was association with increased incidence of multiple organ failure (MOF) and longer intensive care unit stays in critically ill children [78, 79], a more recent randomized trail failed to show such an association [80]. Plasma transfusions have also been found to be independently associated with an increased occurrence of new or progressive MOF, nosocomial infections. and prolonged hospitalization of critically ill children [55]. Some centers have investigated the use of whole blood viscoelastic

assays, such as thrombelastography (TEG) and rotational thromboelastometry (ROTEM), to guide blood product transfusion in trauma patients [3, 81–83]. While this methodology offers the potential advantage of evaluating several different aspects of hemostasis and not merely fibrin clot formation (as is measured by the PT and aPTT), these methods have not yet been widely accepted in clinical practice. Injured pediatric patients in whom an admission TEG was performed were retrospectively reviewed by Vogel et al. [81]. In a cohort of 88 patients with a median age of 8 years, when controlling for age, gender, and ISS, a regression analysis demonstrated that the TEG variables ACT, r-value, α-angle, and maximal amplitude predicted red blood cell and plasma transfusion within 6 h as well as mortality. In a prospective study on the use of intraoperative thromboelastometry in pediatric cardiac surgery to guide transfusions, 50 patients were compared with 50 procedure- and age-matched control patients. Those patients in the TEG group received intraoperative transfusion of fewer RBC and plasma units, but more platelets and fibrinogen concentrates compared to the control group. The authors reported no significant differences in outcomes or postoperative blood loss [84]. Age-specific reference values have been reported for both TEG and ROTEM in pediatric patients [83, 85]). From data based on 359 children aged 0 months to 16 years, an attempt to establish age-dependent reference values for the ROTEM assay revealed that children aged 0–3 months, despite having prolonged standard plasma coagulation test results (PT and aPTT), exhibited accelerated coagulation times compared to older children and adults, and a strong clot firmness within the range of adults [85] suggesting this assay is more reflective of the overall balance of hemostasis in neonates and infants than as measured by the plasma assays PT and aPTT.

The American Association of Blood Banks (AABB) has published guidelines for the transfusion of blood products in infants and children [86]. While these guidelines represent a consensus opinion, many rely on "expert opinion" rather than data developed from randomized clinical studies. Those recommendations relying primarily on expert opinion are so noted in the tables (Tables 31.5, 31.6 and 31.7). For a more thorough discussion of pediatric transfusion practices, I refer the reader to a recent review of the topic [87].

As in adults, excessive resuscitation with crystalloids exacerbates coagulopathy through hemodilution and potential exacerbation of fibrinolysis [88–91]; hence, restrictive use ofcrystalloids, while prioritizing blood product administration, during massive transfusion of pediatric patients

Table 31.5 AABB transfusion guidelines for RBCs in infants less than 4 months of age (from: Josephson CD. Neonatal and pediatric transfusion practice. AABB Technical Manual [86])

1. Hematocrit <20 % with low reticulocyte count and symptomatic anemia (tachycardia, tachypnea, poor feeding)
2. Hematocrit <30 % and any of the following:
(a) On <35 % oxygen hood[a,b]
(b) On oxygen by nasal cannula[a,b]
(c) On continuous positive airway pressure and/or intermittent mandatory ventilation on mechanical ventilation with mean airway pressure <6 cm of water
(d) With significant tachycardia or tachypnea (heart rate >180 beats/min for 24 h, respiratory rate >80 breaths/min for 24 h)
(e) With significant apnea or bradycardia (>6 episodes in 12 h or 2 episodes in 24 h requiring bag and mask ventilation while receiving therapeutic doses of methylxanthines)[b]
(f) With low weight gain (<10 g/day observed over 4 days while receiving ≥100 kcal/kg/day)
3. Hematocrit <35 % and either of the following:
(a) On >35 % oxygen hood[a]
(b) On continuous positive airway pressure/ intermittent mandatory ventilation with mean airway pressure ≥6–8 cm of water
4. Hematocrit <45 % and either of the following:
(a) On extracorporeal membrane oxygenation[a]
(b) With congenital cyanotic heart disease[a,c]

[a]These represent weak recommendations with limited supporting data and a wide range of clinical practice. A lower transfusion threshold may be appropriate after careful evaluation of any individual patient

[b]Data from the Kirpalani PINT study [69] would support a lower Hb/Hct threshold of 8 g/dl &/or 24 %

[c]Cholette data [72] may support a lower transfusion threshold

Table 31.6 AABB transfusion guidelines for plasma products in neonates and older children (from: Josephson CD. Neonatal and pediatric transfusion practice. AABB Technical Manual [86])

Fresh frozen plasma (FFP)
1. Support during treatment of disseminated intravascular dissemination
2. Replacement therapy:
a. When specific factor concentrates are not available, including, but not limited to, antithrombin; protein C or S deficiency; and Factor II, Factor V, Factor X, and Factor XI deficiencies
b. During therapeutic plasma exchange when FFP is indicated (cryo-poor plasma, plasma from which the cryoprecipitate has been removed)
3. Reversal of warfarin in an emergency situation, such as before an invasive procedure with active bleeding[a]
Note: FFP is not indicated for volume expansion or enhancement of wound healing
Cryoprecipitate
1. Hypofibrinogenemia or dysfibrinogenemia with active bleeding[b]
2. Hypofibrinogenemia or dysfibrinogenemia while undergoing an invasive procedure[b]
3. Factor XIII deficiency with active bleeding or while undergoing an invasive procedure in the absence of Factor XIII concentrate[c]
4. Limited directed-donor cryoprecipitate for bleeding episodes in small children with hemophilia A (when recombinant and plasma-derived Factor VIII products are not available)
5. In the preparation of fibrin sealant
6. von Willebrand disease with active bleeding, but only when both of the following are true:
(a) Deamino-D-arginine vasopressin (DDAVP) is contraindicated, not available, or does not elicit response
(b) Virus-inactivated plasma-derived Factor VIII concentrate (which contains von Willebrand factor) is not available

[a]Current recommendations for reversal of warfarin in emergency situations or for bleeding is to infuse 4-factor prothrombin complex concentrates (PCC) when available
[b]Lyophylized fibrinogen is preferred when available due to decreased risk of viral transmission
[c]Factor XIII concentrate is now the preferred product for support of FXIII-deficient patients

Table 31.7 Transfusion guidelines for platelets in neonates and older children with thrombocytopenia (from: Josephson CD. Neonatal and pediatric transfusion practice. AABB Technical Manual [86])

1. Platelet count 5,000–10,000/μL due to decreased platelet production
2. Platelet count <30,000/μL in neonate due to decreased platelet production
3. Platelet count <50,000/μL in a clinically stable premature infant:
(a) With active bleeding, or
(b) Before an invasive procedure, with decreased platelet production[a]
4. Platelet count <100,000/μL in sick premature infant:[a]
(a) With active bleeding, or
(b) Before an invasive procedure in patient with DIC
Without thrombocytopenia
1. Active bleeding in association with qualitative platelet defect[b]
2. Unexplained excessive bleeding in a patient undergoing cardiopulmonary bypass[a]
3. Patient undergoing ECMO with:[a]
(a) A platelet count of <100,000/μL, or
(b) Higher platelet counts and bleeding

DIC disseminated intravascular coagulation, *ECMO* extracorporeal membrane oxygenation
[a]These represent controversial recommendations with little data supporting platelet transfusions under these conditions. A lower transfusion threshold may be appropriate after careful evaluation of any individual patient
[b]Target platelet count post-transfusion is not established

should also be emphasized. The adverse effects of hemodilution in children (mean patient age 11 years old) were described by Hussmann et al., who found that increased prehospital crystalloid volume replacement was associated with increased transfusion requirements, prolongation of the PT, and demonstrated a tendency toward increased mortality and multiple organ dysfunction rates [92].

VTE Risk and Prophylaxis

The incidence of venous thromboembolic events (VTE) in critically ill medical and surgical pediatric patients has been shown to have substantially increased over the past few decades [93–97]. While this increase has been documented across the entire pediatric age spectrum, it is most pronounced at the extremes of the pediatric age range (i.e., <1 year and >13 years of

age). Among the clinical settings that have been shown to be associated with an increased risk for VTE, the strongest association has been with the presence of a central venous catheter (CVC), surgery/trauma, immobilization, and obesity [93–95, 98, 99]. Currently, there is no consensus on when or for whom pharmacologic thromboprophylaxis should be administered. However, individual institutions have developed risk assessment tools that take into account those risk factors for VTE that have been shown to be significant on retrospective case series analyses (e.g., age >13–15 years, obesity, major surgery and/or trauma, a prolonged period of immobilization, presence of a CVC, and anticipated long length of stay) [100–102]. While preliminary, these risk assessment tools show promise in the identification and subsequent targeting of those patients most at risk for VTE for pharmacologic thromboprophylaxis. In an evidence-based review of the literature, Streck et al. [98] identified that the mean age of pediatric injured patients with a VTE was 16.6 years vs. 12.1 years ($p<0.01$) in those who did not develop VTE, independent of other risk factors. By multivariate logistic regression those <14 years of age had a decreased risk of VTE (OR 0.2, 95% CI 0.1–0.9). With the limitations of the data available, this study identifies an age cut-off (≥14 y.o.) at which VTE prophylaxis in pediatric trauma patients could be beneficial.

Summary

The acute coagulopathy of trauma is well described across all age groups including the entire pediatric age spectrum. While the exact incidence in pediatric trauma is likely less than that in adult trauma patients, it is no less real and no less a contributor to mortality and morbidity. Management of the coagulopathy requires a firm understanding of the processes involved in hemostasis, and specifically the normal developmental changes in these processes that occur during childhood. Principles of treatment also require a knowledge of these processes in order to choose appropriate treatment.

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Pregnancy

32

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Introduction

Trauma in pregnancy is a condition that is estimated to affect 1/12 of all pregnancies and 1 % of trauma admissions are pregnant women [1, 2]. It represents 46 % of all maternal deaths and is considered to be the leading cause of non-obstetrical maternal death [1, 2]. The mechanisms of injury are various, from motor vehicle accidents (MVA) to domestic abuse, falls, burns, and toxic exposure. Penetrating injuries are frequent in developed countries [1]. Delivery-related trauma, i.e., ureteral and bladder injuries, perineal tears, and postpartum hemorrhage, will not be discussed as they are out of the scope of this chapter.

As pregnancy is associated with a hypercoagulable state for the mother, trauma-induced coagulopathy in this special population requires special knowledge of the hemostatic state of the pregnant patient. Moreover, specific pathologies, intrinsic to pregnancy, such as hypertensive disorders and thrombocytopenia might complicate the management of the pregnant injured patient. Regarding the fetus, injured pregnant patients have an increased risk of placental abruption, spontaneous abortion, preterm birth [3], and fetal loss [4].

Trauma in pregnancy can be a life-threatening state for the mother as well as the fetus. Special attention, rapid assessment, and multidisciplinary management are needed. Prevention of injuries in pregnant women is crucial. Indeed, prevention campaigns and obstetrical counseling should focus on seat belt use as one of the most important protective factors for MVA [5], and on the development of psychological help centers for women that are victims of violence.

In this chapter, we first discuss the physiological changes in coagulation during pregnancy, next we resume the available data on the prevalence and incidence of trauma-related complications in pregnant women focusing on specific settings leading to coagulopathy, and finally we review practical emergency management.

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Physiological Hemostasis Changes in Pregnancy

A physiological switch of the hemostatic balance that favors a hypercoagulable state is observed throughout normal pregnancy [6]. The global effect of such variations, affecting the primary (platelet), secondary (coagulation), and tertiary (fibrinolysis) hemostasis, is thought to promote placental function and decrease excessive bleeding at delivery. Hemostatic changes of normal pregnancy can affect the management of trauma in pregnant women and should be taken into account very carefully. A list of hemostatic changes seen in pregnancy is reported in Table 32.1 and briefly discussed in this paragraph.

Concerning primary hemostasis, gestational thrombocytopenia occurs by definition in the third trimester of pregnancy and is by far the most frequent cause of pregnancy-related thrombocytopenia. Its prevalence by the end of pregnancy ranges between 6.6 and 11.6 % [7]. Physiological hemodilution is thought to be the main cause [8], although an increased platelet turnover has also been suggested [9]. Regarding secondary hemostasis, during pregnancy the levels of fibrinogen, factors VII, VIII, and IX [10], as well as von Willebrand factor rise significantly [6] whereas there is a slight decrease of FXIII activity [11]. There is conflicting data on changes in factors II, V, X, and XI; however, these variations are minor [10]. Protein C and antithrombin appear to be unaffected by gestation while a significant free and total proteins S fall is observed [12]. Acquired activated protein C resistance has also been reported [13]. As to fibrinolysis, changes in proteins that regulate this system, i.e., tissue plasminogen activator, endothelial derived plasminogen activator inhibitor-1, placenta-derived plasminogen activator inhibitor-2, and thrombin activatable fibrinolysis inhibitor, result in a state of decreased fibrinolytic activity [12]. This overall hypercoagulable state is also supported by the increase of the markers of activation of coagulation such as D-dimer [14], prothrombin fragments 1 + 2 [15], fibrin monomer complexes [16], as well as thrombin-antithrombin complexes [17]. Several global assays of hemostasis assays such as thrombin generation assays [16, 18], thrombelastography [19], and euglobulin clot lysis time [20] also suggest a biological hypercoagulable state throughout the pregnancy. Tissue factor, one of the initial catalysts of coagulation, is constitutively expressed by trophoblasts and is also highly concentrated in the amniotic fluid, explaining the great coagulation potential of the feto-placental unit.

Table 32.1 Physiological changes in hemostasis factors during normal pregnancy

Platelet count	↓
Fibrinogen, FVII, FVIII, FIX, VWF	↑
FII, FV, FX, FXI	=/↑/↓
FXIII	↓
AT, PC	=
PS	↓
rAPC	↑
t-PA	↓
PAI-1, PAI-2, TAFI	↑
DD, F1 + 2, FMC	↑

VWF von Willebrand factor, *AT* antithrombin, *PC* protein C, *PS* protein S, *rAPC* activated protein C resistance, *tPA* tissue plasminogen activator, *PAI-1* plasminogen activator inhibitor-1, *PAI-2* plasminogen activator inhibitor-2, *TAFI* thrombin activatable fibrinolysis inhibitor, *DD* D-dimer, *F1 + 2* prothrombin fragments 1 + 2, *FMC* fibrin monomer complexes

Causes and Implications of Trauma During Pregnancy

A recent systematic review estimated the incidence and prevalence of injury by mechanism during pregnancy [2], which are described in Table 32.2. A summary of studies reporting the prevalence of maternal and fetal complications related to trauma is presented in Table 32.3. In a retrospective study of women hospitalized for MVA in Washington State from 1989 to 1997, severely ($n = 84$) and non-severely injured women ($n = 309$) were at increased risk of cesarean delivery as compared to pregnant women who had not been hospitalized for an MVA [21]. Interestingly in this study women involved in an MVA that did not have significant injury were also at risk of

Table 32.2 Prevalence of mechanisms of trauma in pregnancy (adapted from [2])

	Estimated prevalence[a]
Domestic violence	8307
Motor vehicle accident	207
Falls	48.9
Toxic exposure	25.8[b]
Penetrant injuries	3.27
Homicides	2.9
Suicides	2
Burns	0.17

[a]Per 100,000 live births
[b]100,000 person-years

adverse pregnancy outcomes, suggesting that the forces of the collision can stimulate uterine contractions even without provoking direct injuries to the patient [21]. Their infants were also at increased risk of respiratory distress syndrome and fetal death [21]. In a population-based Australian cohort of women admitted to the hospital due to an MVA, outcomes for those giving birth immediately were poor with an increased risk of antepartum hemorrhage, preterm birth, cesarean section, and perinatal death [22]. A Swedish population-based retrospective study that included pregnant women between 1991 and 2001 reported that MVA during pregnancy caused 1.4 maternal fatalities per 100,000 pregnancies and a fetus/neonate mortality rate of 3.7 per 100,000 pregnancies [23]. A more recent retrospective study of 728 pregnant women injured by MVA in Kuwait reports 100 (13.7 %) maternal and 78 (10.7 %) fetal deaths [5]. The major risk factor for adverse outcomes during MVA is inadequate seat belt use. A retrospective cohort study of 878,546 pregnant women from North Carolina showed that stillbirth rates were elevated following crashes involving unbelted pregnant drivers compared to belted pregnant drivers (relative risk of 2.77, 95 % CI = 1.22–6.28) [24]. In addition, a retrospective cohort study demonstrated that airbags do not increase the risk of adverse outcomes [25].

Fall is a frequent cause of trauma in pregnancy. In a population-based cohort study of 3997 women, 1070 (27 %) reported falling at least once [26]. Likewise, in a prospective study, the incidence of falls was 4.1 cases per 1000 exercise hours [27]. Falls are also associated with adverse maternal and fetal outcomes. In a retrospective study major falls were associated with an increased risk of preterm labor, placental abruption, fetal distress, and fetal hypoxia [28]. Decrease in postural stability and weight gain during the third trimester probably influences the risk of fall [28].

Data on penetrating trauma such as gunshot and stab wounds are limited to few retrospective studies. Petrone et al. reported outcomes of 321 women admitted to two level 1 trauma centers from 1996 to 2008 including 30 (9 %) victims of penetrating trauma. Of the penetrating injuries, 22 (73 %) were gunshot wounds, 7 (23 %) stab wounds, and 1 (4 %) shotgun injuries. Adjusted multivariate analysis showed that the penetrating trauma group experienced higher maternal and fetal mortality and significantly higher maternal morbidity than the non-penetrating injured women [29]. Awwad et al. reported the experience of a single center over 16 years of civil war in Lebanon where 14 pregnant women had penetrating abdominal trauma resulting in uterine injuries secondary to projectiles. Two maternal deaths occurred and perinatal deaths occurred in half of the cases [30]. More recently, a retrospective study of 1075 pregnant women admitted to a trauma center in South Africa reported an increase in the prevalence of penetrating injuries [31].

Data on miscellaneous trauma including burns [32], electrocution [33], thermal injury [34], and poisoning [35] are scarce but also associated with adverse outcomes. A retrospective study conducted on women at 23 weeks of gestation or more studied the effect of minor trauma (defined as any trauma that was not life threatening) on pregnancy outcomes. There was no difference between pregnant women with minor trauma ($N = 512$) and non-trauma pregnant women ($N = 1024$) in terms of placental abruption, preterm birth, and birth weight <2500 g [36].

Finally, domestic violence and intimate partner violence are frequent depending on the cohort investigated [2]. Several risk factors have been identified and include substance abuse, low maternal educational level or socioeconomic status,

Table 32.3 Maternal outcomes reported in studies published since 2000

Author	Mechanism of injury	Design	Sample size	Preterm labor *n* (%)	Placental abruption *n* (%)	Labor induction *n* (%)	Cesarean delivery *n* (%)	Blood transfusion *n* (%)	Maternal death *n* (%)
Schiff et al. [50]	All types of injuries	Population-based case-control	294	28 (9.5)	20 (6.8)	70 (23.8)	68 (23.1)	NA	NA
Schiff et al. [21]	MVA	Population-based case-control	Severe[a] injury: 84 Nonsevere injury: 309 Uninjured: 189	11 (13.1) 76 (24.6) 97 (51.3)	11 (13.1) 23 (7.4) 16 (8.5)	13 (15.5) 71 (23) 46 (24.3)	26 (31) 77 (25) 45 (23.8)	NA	NA
El Kady et al. [82]	Fracture injuries	Population-based case-control	Fracture injury at delivery: 497 Fracture injury during pregnancy: 3292	61 (13.9) 118 (7.8)	75 (17.7) 25 (1.5)	10 (2.1) 41 (2.6)	226 (83.4) 415 (34.6)	32 (6.9) 6 (0.3)	13 (2.7) 0
Weiss et al. [83]	All types of injuries	Population based	7350	813 (11.1)	123 (1.7)	2423 (33)	1426 (19.4)	NA	NA
Schiff et al. [25]	MVA	Population based	3348	325 (9.7)	41 (1.2)	958 (28)	868 (25.9)	NA	NA
Vivian-Taylor et al. [22]	MVA	Population-based case-control	Delivery at time of MVA: 72 Delivery subsequently MVA[b]: 1950	29 (40.1) 31 (1.6)	18 (25) 15 (0.8)	NA	44 (61.1) 482 (24.7)	8 (11.1) 16 (0.8)	NA
Chibber et al. [5]	MVA	Hospital based	728	291 (40)	428 (58.8)	NA	438 (60.2)	NA	100 (13.7)

MVA motor vehicle accidents

[a]Injury severity score (ISS) >9

[b]>20 Weeks of gestation

unplanned pregnancy, and history of witnessed violence as a child by mother or intimate partner [37–39]. A population-based study in New Zealand showed that intimate partner violence was significantly associated with both induced and spontaneous abortion [40]. An increase in preterm delivery has been described in abused pregnant women [41]. These women are also at increased risk for depression [42] and suicide [43].

Hemostasis in Pregnant Women with Trauma

As aforementioned, trauma during pregnancy could be a trigger for several hemostasis-related complications. The hypercoagulable state of pregnancy may increase the risk of disseminated intravascular coagulation (DIC) following placental injury and the risk of thromboembolism. In this section we overview hemostatic complications following trauma in pregnancy as well as the risk of venous thromboembolism in this setting.

Few studies have evaluated the predictive role of DIC in maternal and infant outcomes after injury with conflicting results. In a retrospective study of 20 severely injured pregnant women the most significant predictor of fetal mortality was the presence of DIC. The authors hypothesized that DIC may result from placental products entering the maternal circulation [44]. In a retrospective case-control study of 188 pregnant women admitted to a trauma center the prothrombin time (PT) and the partial thromboplastin time (PTT) both correlated significantly with maternal outcome [45]. On the other hand, in a retrospective study of 233 cases of non-catastrophic blunt abdominal trauma, coagulation values were not predictive of fetal or maternal mortality or morbidity [46]. Similarly, in a prospective cohort study of 317 pregnant patients with minor trauma, a fibrinogen level lower than 2 g/L was not statistically associated with adverse outcomes [47].

In placental abruption DIC occurs due to sudden release of procoagulant substances to the maternal circulation secondary to rupture of the uterine spiral arteries [48]. It has also been suggested that decidual hypoxemia generates vascular endothelial growth factors that induce aberrant expression of tissue factor, resulting in widespread activation of coagulation [49]. Moreover, the injured placenta and myometrium also release abundant tissue factor [48]. The reported incidence of placental abruption after trauma is high. In a population-based study of women hospitalized for injury in Washington State from 1989 to 1997, non-severely injured ($n=266$) and severely injured women ($n=28$) had an increased risk of placental abruption compared to non-injured pregnant women [50]. Likewise, in the aforementioned study of Schiff et al., women hospitalized for MVA had an increased adjusted relative risk of placental abruption as compared to pregnant women who were not hospitalized for a motor vehicle crash [21]. Even minor traumatic placental injury can result in feto-maternal hemorrhage. In a prospective cohort study of 85 women who suffered various degrees of injuries, feto-maternal hemorrhage was significantly more frequent than in a control group of pregnant women matched for gestational age [51]. Feto-maternal hemorrhage was more likely to occur in women with anterior placenta location than in other positions [51]. In a retrospective study of 71 pregnant women, the Kleihauer-Betke (KB) test, which measures the amount of fetal hemoglobin transferred from a fetus to the mother's bloodstream, was associated with a risk of preterm labor after trauma [52]. However in a recent prospective study of 317 patients evaluated for minor trauma the KB test was not related to maternal adverse outcomes [47].

Another cause of obstetrical DIC is amniotic fluid embolism (AFE). AFE is defined by the entry to maternal circulation of procoagulant material such as tissue factor [53], phosphatidylserine [54], endothelin 1 [55], arachidonic acid [56], and plasminogen activator inhibitor type-1 [57]. Recently, activation of complement has also been reported as one of the main triggers of DIC in AFE [58]. Trauma-associated AFE is rare and only some case reports have been published [59, 60]. MVA and improper seat belt use could be a risk factor for AFE [61].

Venous thromboembolism can also be a complication of trauma in pregnant women, since the

venous thrombosis risk associated with pregnancy is increased at baseline. In a retrospective case-control study including 114 pregnant women admitted to a trauma service, 2.2 % of women in the trauma group experienced venous thrombosis whereas none were observed in the control group [62]. Similarly in a retrospective case-control study among 2795 women who sustained a fracture there was a 9.2-fold increased risk of thrombotic events. Therefore, thromboprophylaxis, either mechanical or by antithrombotic agents, should be carefully considered in cases of trauma in pregnant women.

Management

General Management

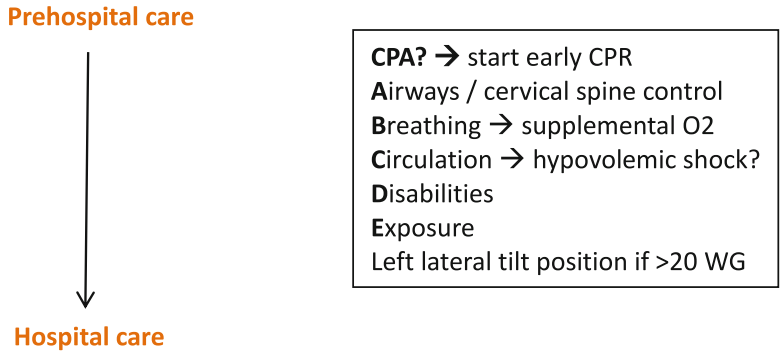
Obstetric trauma is a special situation that implicates two individuals: the mother and the fetus. The stability and survival of the mother remain the first priority of this specific management [63]. Assessment and resuscitation of the pregnant trauma patient should follow the ABCDE (Airways, Breathing, Circulation, Disability, and Exposure) approach from the advanced trauma life support (ATLS) protocols. When gestational age is >20 weeks of gestation, early left lateral tilt position should be adopted to reduce the risk of supine hypotensive syndrome linked to the compression of the vena cava by the gravid uterus [64]. Hospital general management is described in Fig. 32.1. A rapid general physical exam, followed by a specific obstetrical assessment of the mother, should be performed, focused specially on uterovaginal and abdominal exams. Localization of the bleeding, determination of its quantity, and repercussion on the hemodynamics of the patient are critical. Then, fetal assessment includes obstetrical ultrasound that will determine more precisely the gestational age of the fetus, viability, and signs of placental problems. Abdominal screening sonography for blunt abdominal trauma remains sensitive and specific even in pregnant patients, with a sensitivity of 80 % and specificity of 100 % for detecting the necessity of major abdominal surgery [65]. Cardiotocographic monitoring of the fetus will assess the fetal heart rate. In certain cases, computerized tomography scanning may be helpful, especially in cases of penetrating injuries to the back or flank. Because of the toxicity of radiation on the fetus, this procedure should be considered only when the benefit for the mother outweighs the risk of the fetus. Blood tests should be run as quickly as possible, including a complete blood cell count, electrolytes, glucose, coagulation profile, blood type and Rhesus, and Kleihauer-Betke test. Depending on the degree of hemorrhage, transfusion should be considered (see management of severe hemorrhage). Moreover, diagnostic peritoneal lavage in the unstable patient can be safely performed in pregnancy in order to diagnose intraperitoneal and uterine hemorrhage [66, 67]. Some obstetrical conditions, such as preterm birth or severe hemorrhage, may necessitate specific treatment as described below. Screening for psychosocial conditions should also be considered, especially in case of domestic violence, toxic exposure, depression, and suicidal risk (Fig. 32.1).

Specific Management of Obstetrical Conditions

Some conditions seen during pregnancy may complicate the management of pregnant trauma patients. Here we discuss the management and treatment to be considered in case of preterm birth, hypertensive disorders, thrombocytopenia, as well as some other conditions.

Preterm Birth

According to the World Health Organization, the viability of the baby is defined by a birth >22 weeks' gestation or a baby weight ≥550 g. Preterm birth is defined by a birth before 37 weeks' gestation, and considered to be severe when ≤32 weeks' gestation. It is the leading cause of fetal death, which increases with the degree of prematurity. A 6-month survival study conducted in trauma centers over a 10-year period showed a 54 % (30–72 %) survival of the baby born at 25 weeks' gestation, 23 % (0–33 %) at 23, and 0 % at 22 [3]. Trauma increases preterm birth and low birth weight by about twofold



1. Physical exam	Normal physical exam should be combined with gynaecological and obstetrical exam simultaneously Specific: vaginal bleeding, uterine contraction, abdominal or uterine tenderness
2. Fetal monitoring – prenatal ultrasonography	GA, placental location, amniotic fluid assessment, biophysical profile Uterine rupture? Fetal trauma? Intra-abdominal fluid?
3. Laboratory work	CBC, electrolytes, glucose, coagulation profile, blood type and Rhesus, Kleihauer-Betke test
4. Diagnostic peritoneal lavage	Intraperitoneal hemorrage: splenic rupture, hepatic rupture, uterine rupture Retroperitoneal hemorrage: rupture of the pelvic venous plexus Intrauterine fetal demise
5. Surgical exploration	For penetrating trauma
6. Medications	Preterm birth? → ATB if group B streptococcus status unknown, antenatal CS when 24-34 WG, tocolytic agents (MgS, calcium channel blockers…) Bleeding? → fluid resuscitation, blood transfusion, fibrinogen replacement
7. To be considered	Screening for domestic violence, toxic exposure, depression and suicidal risk CT-Scan (only when the benefit of the mother outweighs the fetal risk) Tetanus prophylaxis

CPA: cardiopulmonary arrest; CPR: cardiopulmonary resuscitation; WG: weeks of gestation; GA: gestational age; CBC: complete blood count; ATB: antibiotics; CS: corticosteroids; MgS: Magnesium sulfate

Fig. 32.1 General management of trauma pregnant women [63, 64]. *CPA* cardiopulmonary arrest, *CPR* cardiopulmonary resuscitation, *WG* weeks of gestation, *GA* gestational age, *CBC* complete blood count, *ATB* antibiotics, *CS* corticosteroids, *MgS* magnesium sulfate

(relative risk, 1.9; 95 % confidence interval, 1.1–3.3 and relative risk, 1.8; 95 % confidence interval, 1.04–3.2, respectively) [68].

As shown in Fig. 32.1, specific therapeutics should include antibiotic administration if vaginal Streptococcus B status is unknown and two doses of intramuscular corticosteroids for fetal lung maturity when premature delivery is suspected between 24 and 34 weeks gestation. In select cases, perimortem C-section should be considered

in case of cardiopulmonary arrest with a viable fetus after 24 weeks' gestation, following the "4-min rule": maternal cardiopulmonary resuscitation for 4 minutes, infant should be delivered by the fifth minute [69].

Hypertensive Disorders

Hypertension is a common disorder that affects up to 20 % of all pregnancies. Hypertension can be pre-existent to pregnancy and defined as chronic or limited to pregnancy and defined as gestational. When associated with proteinuria and after 20 weeks' gestation, it defines a special condition named preeclampsia that affects up to 8 % of all pregnancies [70, 71]. Its complications include severe preeclampsia, eclampsia, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), placental abruption, and birth of small-for-gestational-age neonates. High risk of feto-maternal morbidity and mortality is intrinsic to these conditions. Hypertension as well as trauma are two risk factors for placental abruption [72] which can lead to fetal death, with a threefold increase of perinatal mortality in patients with severe trauma. Preterm birth, intrauterine growth restriction by placental vascular insufficiency, and fetal death can also occur.

For preeclampsia complications, delivery is the best treatment. Relatively safe antihypertensive drugs in pregnancy include labetalol, nifedipine, methyldopa, and hydralazine. Prophylactic and therapeutic strategies of eclampsia also include intravenous magnesium sulfate infusion [73].

Thrombocytopenia

In case of trauma, treatment of thrombocytopenia should be adapted to the etiology and its severity. Transfusions of platelets are useful in case of hemorrhage or severe thrombocytopenia when a surgery is planned. Immune thrombocytopenic purpura can be treated with immunoglobulin and corticosteroids, and rarely splenectomy [74]. The physiologic thrombocytopenia of pregnancy can complicate coagulopathic bleeding of injured pregnant patients and should be considered early on during routine trauma assessment [75].

Other Conditions

More rare conditions can also be found in pregnant trauma patients and physicians should be aware of these, especially of hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). Considered as a thrombotic microangiopathy, the diagnosis of HUS includes (1) thrombocytopenia, (2) hemolytic anemia, and (3) acute renal failure [76]. Pathophysiologically, HUS is associated with complement pathway dysregulation. In addition to HUS associated with sepsis, a possible association between pregnancy and HUS has been described [77]. TTP has a similar clinical presentation and differentiation with HUS might be difficult. TTP is commonly linked to acquired ADAMTS13 deficiency. ADAMTS13 is a protein that cleaves von Willebrand factor that induces platelet aggregation and thrombus formation on the damaged endothelium [78]. Specific therapeutics include plasma exchange treatment. New therapies targeting the complement cascade such as anti-C5 antibodies have been recently studied for the treatment of HUS [76].

Management of Severe Hemorrhage

Trauma can precipitate hemorrhage during pregnancy. As for pregnant women, the definition of severe hemorrhage (SH) is not well defined in the setting of trauma. To date, SH is defined as the transfusion of ten or more units of red blood cells (RBC) within 24 h of emergency department admission and termed "massive transfusion" [79]. Specific conditions during pregnancy might complicate SH linked to trauma (Fig. 32.2). Among them, vaginal bleeding disorders are most frequently seen during the third trimester of pregnancy and include placenta praevia and placental abruption. Placenta praevia can be defined as a malposition of the placenta, low in the uterus, covering partly of entirely the cervix. In placental abruption, bleeding is caused by the detachment of the placenta from the uterine wall before or during labor [80].

For the mother, the main risks of SH include preterm delivery and death. For the fetus, the risks of maternal SH include fetal anemia, hypoxia, neurological disorders, and fetal death. The general management of pregnant patients with SH is described in Fig. 32.2. The cause and estimation of the quantity of bleeding need to be determined quickly.

Changes in blood volume as part of the physiology of pregnancy should also be considered when assessing hemorrhage and resuscitating the injured pregnant patient. A healthy woman bearing a normal-sized fetus, with an average birth weight of about 3.3 kg, will increase her plasma volume by an average of about 1250 ml, a little under 50 % of the average nonpregnant volume for white European women of about 2600 ml. There is little increase during the first trimester, followed by a progressive rise to a maximum at about 34–36 weeks, after which little or no further increase occurs.

Pregnant trauma patients with SH should receive the same medical care as nonpregnant patients, giving priority to the resuscitation of the mother. Resuscitation should follow the same principles as adult nonpregnant patients. When needed, hemostatic resuscitation should be prioritized through massive transfusion protocols. Hemostatic medications (i.e., factor replacement agents and anti-fibrinolytic agents) should be administered under the same criteria as for nonpregnant patients [78, 81]; however, pregnancy-related differences in primary, secondary, and tertiary hemostasis described in this chapter should be considered.

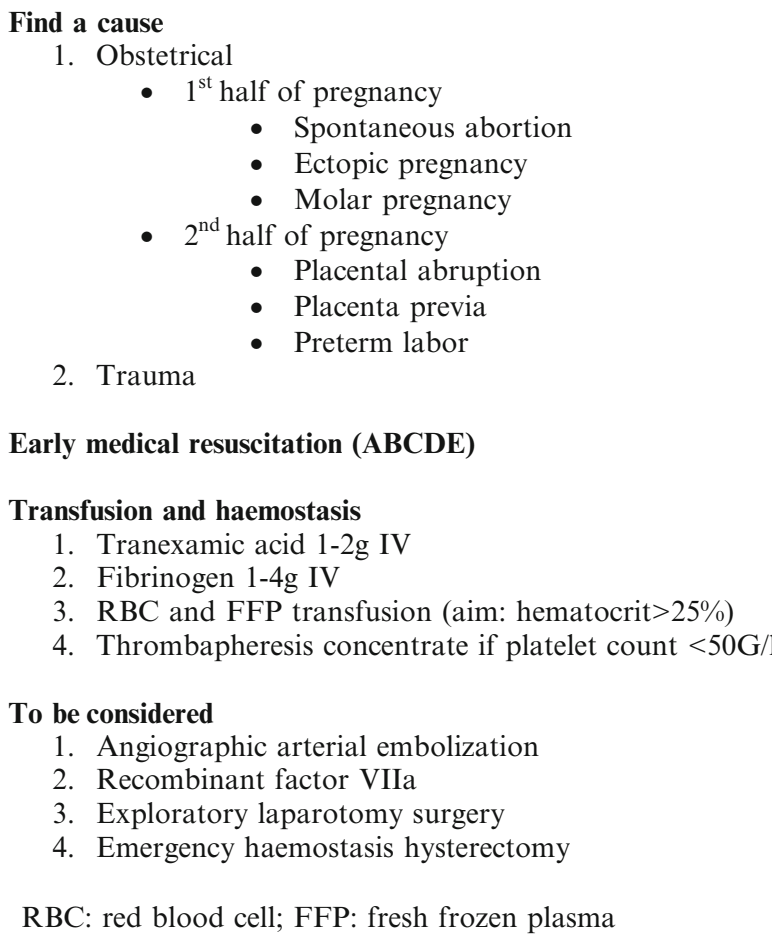


Fig. 32.2 Management of pregnant patients with severe hemorrhage [79, 84]. *RBC* red blood cell, *FFP* fresh frozen plasma

Conclusion

Trauma during pregnancy is a highly morbid condition that needs specific and rapid assessment of both the mother and the fetus. Mechanisms of injury affecting pregnant patients are numerous, with motor vehicle accidents being the most prevalent. In addition, intentional trauma should be taken into account and specific screening for domestic violence and suicidal risk should not be forgotten.

Pregnancy leads to a hypercoagulable state due to an increase in procoagulant factors resulting in a potential risk of DIC and venous thromboembolism. However, the decreased platelet count seen in pregnancy can lead to an increased risk of bleeding in cases of severe trauma.

General management of trauma in pregnancy first follows the ABCDE principles and full assessment of feto-maternal risks. Then, specific therapeutics should take into account preterm labor, hypertensive disorders, and thrombocytopenia pathologies. Severe hemorrhage during pregnancy is a major concern in pregnant trauma patients and complicates the prognosis of the pregnant patient and the fetus.

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Management of Chronically Anticoagulated Patients

33

Jerrold H. Levy

Introduction

Critically ill patients who present following trauma or are in an intensive care unit (ICU) often have received or require anticoagulation for multiple reasons. These include stroke prevention, atrial fibrillation (AF), mechanical valves, and venous thromboembolic disease [1, 2]. The need for therapy and type of anticoagulant used vary depending on whether patients have arterial or venous thromboembolic issues. Both these issues are important following traumatic injury and have significant implications for patient management. There are also important interactions and links between coagulation and other physiologic responses, including inflammation, that are beyond the scope of this review.

Many types of anticoagulant therapy are available, e.g., oral anticoagulants (vitamin K antagonists [VKAs, e.g., warfarin] and the new target-specific oral agents rivaroxaban, apixaban, and dabigatran), and platelet inhibitors (thienopyridines [e.g., clopidogrel, prasugrel] and nucleoside analogs [e.g., ticagrelor]) [3–5]. Occasionally, patients may also be receiving low-molecular-weight heparin (LMWH). Patients may be receiving a complex array of anticoagulation agents for thromboprophylaxis. In addition, many patients have concomitant coronary artery disease and are chronically treated with antiplatelet therapy.

All of the available anticoagulant therapies prevent thrombus formation in pathologic states, but can also exacerbate bleeding following traumatic or surgical injury. Patients tend to have significant morbidity and mortality owing to their procoagulant diseases, but the anticoagulant therapy they receive creates an important acquired hemostatic defect due to alterations in the physiologic procoagulant/anticoagulant equilibrium [1]. Trauma alone produces a complex coagulopathic state, which is the subject of this book. The acquired hemostatic defect in the anticoagulated patient may further contribute to the trauma-induced coagulopathy that occurs following trauma or during emergency surgery.

Critical to understanding the effects of chronic anticoagulation is that hemostasis is far more complex than the simplified coagulation cascades that most clinicians have learned in medical school [6–10]. The complex equilibrium between blood cells, platelets, coagulation factors, natural inhibitors of coagulation, and the fibrinolytic system is altered by a procoagulant state, and anticoagulant therapy is instituted to alter the pathologic responses of hypercoagulability [11]. Patients may also have arterial disease and may have stents in their coronary or cerebral circulation, adding to the complexity of the situation. Arterial

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thrombi are mediated by platelet responses, although coagulation, hemostasis, and thrombus formation involve important cross talk between endothelial cells, platelets, and coagulation proteins [8, 12].

Arterial injury serves as a procoagulant focus for clot formation due to platelet adhesion, activation, and aggregation. This is a normal physiologic process, but can be pathologic in the case of plaque rupture, myocardial infarction, and/or stroke [13, 14]. Platelets normally circulate in an inactivated state, but following activation they express glycoprotein IIb/IIIa (GpIIb/IIIa) receptors, allowing fibrinogen to bind, cross-link, and aggregate platelets to form a thrombus [14, 15]. Vascular injury causes thrombin formation but also platelet activation and the platelet–fibrinogen plug are formed. Since platelets have a pivotal role in the pathogenesis of thrombosis after plaque rupture, antiplatelet agents including aspirin, thienopyridines (clopidogrel, prasugrel), nucleoside analogs (ticagrelor), and the GpIIb/IIIa inhibitors reduce adverse events that are associated with plaque rupture [16, 17].

Patients may present with underlying hemostatic disorders following trauma, during surgery, or in the ICU because of chronic anticoagulation and/or antiplatelet therapy [18]. All therapies that prevent clot formation in pathologic states also interfere with normal hemostasis, an important mechanism that protects patients from excessive bleeding [19, 20]. Therefore, in cases of trauma, or prior to emergency surgery, these patients require rapid anticoagulation reversal. It is worth noting that a patient's anticoagulant therapy will need to be reinitiated post-trauma and surgery; prophylaxis for deep vein thrombosis (DVT), pulmonary embolism (PE), and venous thromboembolism (VTE) may also be required once hemostasis is established and hemorrhage is controlled [7].

This chapter focuses on current anticoagulant therapies and the therapeutic approaches used to treat or prevent bleeding in patients receiving these therapies in cases of trauma or emergency surgery.

Anticoagulation

Anticoagulation is based on inhibiting both thrombin and platelet activation [4, 10, 21–23]. Thrombin is a potent procoagulant that generates fibrin from soluble fibrinogen, activating coagulation factors (F) V and VIII, and platelets [8]. Activated platelets adhere to injured vascular endothelia, express GpIIb/IIIa receptors, aggregate, and further increase generation of thrombin [24]. Due to the complex humoral amplification system linking both hemostatic and inflammatory responses, there are multiple pathways to produce thrombin and prothrombotic effects [10, 19]. Anticoagulants currently used to prevent clot formation are considered in the following sections.

Oral Anticoagulants

VKAs: Warfarin

Warfarin is the only oral VKA available in the USA [5]. It is an effective anticoagulation agent that acts by inhibiting vitamin K epoxide reductase, the enzyme that posttranslationally modifies the vitamin K-dependent coagulation proteins (FII [prothrombin], FVII, FIX, and FX), converting them to their active forms. Warfarin's onset of action is slow and it takes several days to decrease coagulation factors to the ~20–40 % level that is required for a therapeutic international normalized ratio (INR) of 2–3 [25]. Despite the increased availability of newer agents, warfarin and other VKAs continue to be widely used due to the extensive experience that has been accrued with these agents in a broad spectrum of indications, including mechanical cardiac valves.

New Oral Agents: Dabigatran, Apixaban, and Rivaroxaban

The new target-specific oral anticoagulation agents have a rapid onset of action, and therapeutic anticoagulation is achieved within hours of

administration. Dabigatran is a direct thrombin (FIIa) inhibitor, while apixaban and rivaroxaban (and edoxaban not yet approved) are direct FXa inhibitors; in contrast to LMWH, FXa inhibitors exert their anticoagulant effect independently of antithrombin [5]. The newer oral anticoagulants (NOACs) require dose adjustments in patients with renal failure. These agents will be considered separately in the following sections.

Dabigatran Etexilate (Pradaxa®, Boehringer Ingelheim Pharmaceuticals Inc, Ridgefield, CT, USA)

Dabigatran etexilate is an oral, direct thrombin inhibitor currently approved in the USA to reduce the risk of stroke and systemic embolism in patients with non-valvular AF; for the treatment of DVT and PE in patients who have been treated with a parenteral anticoagulant for 5–10 days; and to reduce the risk of recurrence of DVT and PE in patients who have been previously treated [26]. Dosing should be adjusted for patients with renal dysfunction [26]. The capsule is specially formulated and cannot be altered or crushed for administration in an ICU setting.

Apixaban (Eliquis®, Bristol-Myers Squibb Company, Princeton, NJ, USA)

Apixaban is an FXa inhibitor anticoagulant approved in the USA to reduce the risk of stroke and systemic embolism in patients with non-valvular AF; for the prophylaxis of DVT, which may lead to PE, in patients who have undergone hip or knee replacement surgery; and for the treatment of DVT and PE, and the reduction in the risk of recurrent DVT and PE following initial therapy [27]. The apixaban formulation can be crushed and given through a feeding tube if needed in the ICU setting.

Rivaroxaban (Xarelto®, Janssen Pharmaceuticals Inc., Titusville, NJ, USA)

Rivaroxaban is a direct-acting oral, FXa inhibitor that, unlike heparins, does not require antithrombin [28]. Rivaroxaban has the broadest indications of all of the NOACs and is approved in the USA for reducing the risk of stroke and systemic embolism in patients with non-valvular AF; for the treatment of DVT and PE, and for the reduction in the risk of recurrence of DVT and of PE; and for the prophylaxis of DVT, which may lead to PE in patients undergoing knee or hip replacement surgery [29].

Measurement of NOAC-Induced Anticoagulation

One of the major advantages of the NOACs is that coagulation monitoring is not necessary for their management. In addition, overall dosing recommendations are simple, based on renal function and indication, i.e., VTE prophylaxis, thromboembolism treatment, or prevention of stroke in patients with AF. Nevertheless, specific quantitative testing to determine the anticoagulant effect of NOACs may be required following trauma, prior to emergency surgery, and/or in cases of significant bleeding. When managing a patient in one of these scenarios, it is helpful to determine when the last dose of anticoagulant therapy was given, especially in cases of suspected drug overdose; this is also useful in assisting with the interpretation of coagulation test results. However, obtaining an accurate medical history can sometimes be difficult for a trauma patient or in the emergency department (ED). Knowing the renal function at the time of coagulation testing is also essential, since renal excretion is a major component of the elimination mechanism for some of these agents, particularly dabigatran (Table 33.1) [28, 30, 31].

Overall, as screening and qualitative assessments of effect but not definitive tests, the activated partial thromboplastin time (aPTT) is prolonged by dabigatran, while the prothrombin time (PT) is prolonged with apixaban, rivaroxaban, and other FXa inhibitors [32, 33]. It is important to understand that these tests should be considered screening tests, as they are relatively insensitive as quantitative assays and, following trauma, multiple factors influence the PT so that it is frequently prolonged even in the absence of FXa inhibitors. Specific quantitative tests for NOACs exist, but may not be readily available in

Table 33.1 Pharmacokinetics and monitoring of NOACs [28, 31]

	Dabigatran	Apixaban	Edoxaban	Rivaroxaban
Plasma peak level	2 h after ingestion	1–4 h after ingestion	1–2 h after ingestion	2–4 h after ingestion
Plasma trough level	12–24 h after ingestion	12–24 h after ingestion	12–24 h after ingestion	16–24 h after ingestion
PT	Not for routine monitoring	Cannot be used	Prolonged but needs calibration	Prolonged: may indicate excess bleeding risk but local calibration required
INR	Cannot be used	Cannot be used	Cannot be used	Cannot be used
aPTT	At trough > 2× ULN suggests excess bleeding risk	Cannot be used	Prolonged but no known relation with bleeding risk	Cannot be used
dTT	At trough >200 ng/ml or >65 s: excess bleeding risk	Cannot be used	Cannot be used	Cannot be used
Anti-Fxa chromogenic assays	Not applicable	Quantitative: no data on threshold values for bleeding or thrombosis	Quantitative; no data on threshold values for bleeding or thrombosis	Quantitative: no data on threshold values for bleeding or thrombosis
ECT	At trough: ≥3× ULN: excess bleeding risk	Not affected	Not affected	Not affected

aPTT activated partial thromboplastin time, *dTT* diluted thrombin time, *ECT* ecarin clotting time, *F* factor, *INR* international normalized ratio, *N/A* not applicable, *PT* prothrombin time, *ULN* upper limit of normal. Table adapted from Levy JH, et al. Anesthesiology 2013;118:1466–74 and Heidbuchel H, et al. Europace 2013;15:625–51

the urgent situation of trauma or in the ED. Recommendations suggest that point-of-care testing should not be used to assess INR in patients receiving NOACs [34].

The coagulation tests used to manage NOACs in trauma or emergency situations are summarized in Table 33.1 and are discussed further in the following sections.

Measuring and Monitoring Dabigatran

The anticoagulant effects of dabigatran can be monitored using thrombin clotting time/thrombin time (TT) and aPTT values, although TT is generally preferred [35]. aPTT may provide an estimate of dabigatran effect, but a normal aPTT does not exclude its presence. Furthermore, there is a curvilinear relationship between dabigatran and the aPTT [32]. The INR and PT are not affected by dabigatran at therapeutic concentrations, and should not be used for assessing its anticoagulant effect. However, in cases where dabigatran levels are several times higher than therapeutic levels, the PT/INR can be elevated. The ecarin clotting time can be used to assay direct thrombin inhibitors, but this is not used in most centers, especially in the USA. Most centers can measure TT, and a normal TT excludes a dabigatran effect. A specialized assay that uses a diluted TT is helpful for specific measurement of dabigatran levels when needed [28]. This assay is available in some centers as the Hemoclot® test, and can exclude a clinically relevant anticoagulant effect.

Measuring and Monitoring Apixaban and Rivaroxaban (FXa Inhibitors)

Therapeutic levels of FXa inhibitors will slightly prolong the PT (~1.3 fold), but multiple factors can also prolong the PT/INR in trauma patients. The effects of FXa inhibitors on PT prolongation

also depend on the type of assay, the particular agent, and the PT reagents used. All clotting tests use a specific tissue thromboplastin. INR is used specifically for warfarin and other VKAs, and cannot be used for determining the effects of apixaban, rivaroxaban, or other FXa inhibitors. The aPTT is also not appropriate for determining the effects of the FXa inhibitors. The most sensitive assay to monitor FXa inhibitors is a specific anti-Xa chromogenic assay. This is similar to the anti-FXa assay used to monitor LMWH therapy, but is modified slightly when used for the FXa inhibitors [33].

Emergency Management of Patients on Oral Anticoagulants

Following trauma or prior to emergency surgery, urgent anticoagulation reversal or management plans are needed, especially in patients with acute bleeding. While major concerns have been expressed about the lack of specific reversal therapy for NOACs, there is also no antidote for LMWH and it can accumulate in patients with renal failure. Specific reversal therapies for the NOACs are in clinical development, but these are not yet commercially available; therefore, effective therapeutic strategies are important for managing these emergencies. Anticoagulation reversal strategies are discussed in the following sections.

Managing Bleeding Associated with Warfarin and Other VKAs

Various treatment options for VKA reversal exist [25]. Vitamin K supplementation is important in the trauma and/or bleeding patient; however, it will not immediately reverse the anticoagulant effect of VKAs. Therefore, additional therapies are needed in emergency situations, as detailed in the American College of Chest Physicians (ACCP) guidelines for perioperative management of antithrombotic therapy [36]. Fresh frozen plasma (FFP) is often used for VKA reversal. However, transfusion of FFP is unable to restore the INR to baseline, and usually only reduces INR to ~1.4–1.6. Furthermore, transfusion of FFP is associated with various safety issues, such as volume overload. Prothrombin complex concentrates (PCCs) can also be used for urgent VKA reversal. In the USA, PCCs are available as 4-factor (4F-PCC; Kcentra®, CSL Behring GmbH, Marburg, Germany) or 3-factor (3F-PCC; Profilnine® SD, Grifols Biologicals Inc. Los Angeles, CA, USA and Bebulin®, Baxter AG, Vienna, Austria). The 3F-PCCs do not contain clinically relevant amounts of FVII.

The management of warfarin-associated bleeding in trauma or emergency surgery settings has been greatly facilitated by the advent of balanced PCCs that contain all four vitamin K-dependent factors [25]. Although recent ACCP guidelines recommend the use of 4F-PCCs for urgent warfarin reversal [36], these agents are not readily used by clinicians in the USA. Therefore, warfarin reversal remains a clinical challenge.

Sarode et al. conducted a prospective clinical trial comparing a non-activated 4F-PCC (Kcentra®, containing coagulation factors II, VII, IX, and X and proteins C and S) with plasma for urgent VKA reversal in patients with acute major bleeding [37]. Their primary analyses examined whether 4F-PCC was non-inferior to plasma for two co-primary endpoints of hemostatic efficacy (assessed over 24 h from start of infusion) and rapid INR correction (INR $\leq$ 1.3 at 0.5 h after end of infusion). The intention-to-treat efficacy population consisted of 202 patients (4F-PCC: $n=98$; plasma: $n=104$). Baseline characteristics, including baseline INR values, were similar between groups. 4F-PCC was non-inferior to plasma for hemostatic efficacy and both non-inferior and superior to plasma for rapid INR reduction. Effective hemostasis was achieved in 72.4 % and 65.4 % of patients in the 4F-PCC and plasma groups, respectively (Fig. 33.1). Rapid INR reduction was achieved in 62.2 % and 9.6 % of patients in the 4F-PCC and plasma groups, respectively (Fig. 33.1). Mean plasma levels of vitamin K-dependent factors were higher in the 4F-PCC group than in the plasma group from 0.5 to 3 h after the start of infusion ($p<0.02$). There was no significant difference in the occurrence of adverse

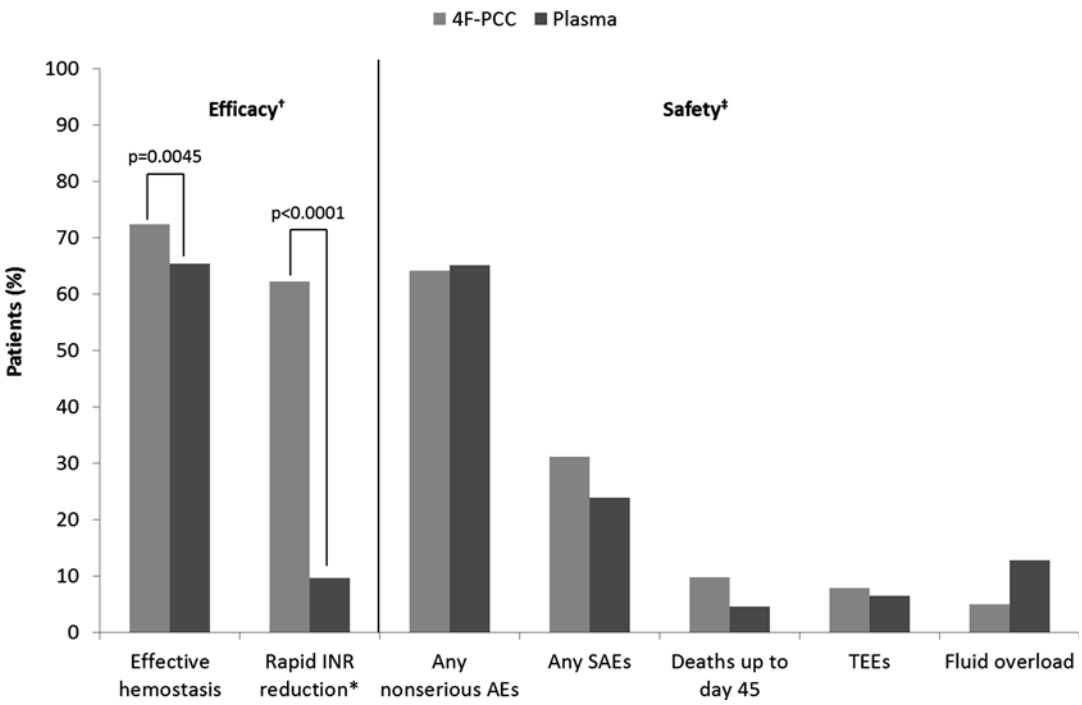

Fig. 33.1 Urgent warfarin reversal in patients with acute major bleeding: results from a randomized controlled trial. *4F-PCC* four-factor prothrombin complex concentrate, *AE* adverse event, *INR* international normalized ratio, *SAE* serious adverse event, *TEEs* thromboembolic events. *Rapid INR reduction: INR ≤ 1.3 at 0.5 h post-infusion start; †intent-to-treat efficacy population (4F-PCC: $n = 98$; plasma: $n = 104$)—reported p-values are Farrington–Manning p-values for non-inferiority; ‡intent-to-treat safety population (4F-PCC: $n = 103$; plasma: $n = 109$). Figure adapted from Sarode R, et al. Circulation. 2013;128:1234–43

events, serious adverse events, thromboembolic events, and deaths between treatment groups, although fluid overload events occurred more frequently in plasma-treated than in 4F-PCC-treated patients (12.8 % and 4.9 %, respectively) (Fig. 33.1). The authors concluded that 4F-PCC is an effective alternative to plasma for urgent VKA reversal in patients with major bleeding events [37].

Managing Bleeding Associated with NOACs

As previously discussed, routine monitoring of NOACs is not standard, but specialized tests can be implemented to evaluate their anticoagulant effects in bleeding patients. To reiterate, for dabigatran, TT and diluted TT (Hemoclot® assays) are the most sensitive, while aPTT is only a qualitative assessment of anticoagulation. The anticoagulant effects of FXa inhibitors are more difficult to measure and specific calibrated anti-FXa assays, such as those used for LMWH, are required.

With NOACs, as with any anticoagulant agent, bleeding complications are a concern and anticoagulation reversal may be required. Nevertheless, most studies suggest that patients fare better on NOACs compared with warfarin [38]. In one study of 27,419 patients treated with either dabigatran or warfarin for up to 3 years, 1034 patients had 1121 major bleeds. The 30-day mortality after the first major bleed was 9.1 % in the dabigatran group compared with 13.0 % in the warfarin group, and dabigatran-treated patients required a shorter ICU stay compared with patients who received warfarin [38]. Studies have also shown that bleeding profiles with NOACs, in particular those of intracranial and

other life-threatening bleeding, are more favorable than those with warfarin [39].

Specific antidotes to antagonize the anticoagulant effect of NOACs are currently available for dabigatran, an Fab fragment idarucizumab (Praxbind), and currently under development for the other agents (andexanet and aripazine). However, NOACs have a relatively short duration of effect in patients with normal renal function; therefore, discontinuation of NOACs can be sufficient in cases of mild bleeding or where surgery can be delayed. Nevertheless, in cases of major bleeding or where emergency surgery is required, reversal strategies should be implemented. Patients receiving NOACs and presenting with bleeding should be hemodynamically and hemostatically resuscitated, and therapy should be multimodal, as outlined in Fig. 33.2 [28].

Increasingly, information is now also becoming available to facilitate bleeding management in NOAC-treated patients [28]. Emerging evidence suggests that the application of PCCs may be able to reverse the anticoagulant effects of NOACs. Successful reversal of rivaroxaban-induced anticoagulation with PCCs has been reported in healthy volunteers, and there are increasing numbers of reports on their off-label use in bleeding cardiac surgical patients [2, 40, 41]. Nevertheless, it should be noted that restoring hemostatic function may not necessarily equate to good clinical outcomes.

A recently published survey of NOAC reversal practices reported by physicians from the Hemostasis and Thrombosis Research Society and US Hemophilia Center directors offers perspectives on the management and effectiveness of therapeutic strategies for dabigatran- and rivaroxaban-associated bleeding [42]. Dabigatran-associated bleeding was controlled in all 43 reported cases, with hemodialysis considered the most effective strategy in patients with renal failure. Activated PCCs and recombinant activated factor VII (rFVIIa) were considered effective in 50–80 % of dabigatran-related bleeding episodes, while co-administration of PCC and rFVIIa was effective in the two cases where this strategy was used. Only five cases of rivaroxaban-related bleeding were reported. The authors noted that withholding treatment, local measures, and administration of activated PCC (in one case) were all considered effective strategies to manage bleeding by the treating physicians.

Currently, recommendations on NOAC-associated bleeding management are based more on experts' opinions or laboratory endpoints, rather than clinical experience. These recommendations will be considered in the following sections.

Managing Moderate-to-Severe Bleeding in NOAC-Treated Patients

Standard bleeding management strategies, such as hemodynamic/hemostatic support and mechanical/surgical hemostasis, can be implemented to treat NOAC-associated bleeding. Moreover, due to the relatively short half-lives of the NOACs compared with VKAs, coagulation is usually restored within 12–24 h of the last dose in patients with normal renal function. However, renal function may be acutely impaired in patients with shock. Special precautions should also be taken with elderly patients, especially those with intracoronary stents and with patients concomitantly receiving NOACs and antiplatelet drugs. Early utilization of a massive transfusion protocol restoring intravascular blood volume should be considered in these patients.

Exposure to dabigatran can be reduced using dialysis. However, this management strategy is not feasible in patients in shock, although it can potentially be conducted in patients with stable blood volumes and life-threatening bleeding such as intracranial hemorrhage. It is important to note that if placing large-bore dialysis catheters is necessary, a femoral insertion should be used due to the potential risk of bleeding. Dialysis is not considered to be effective in patients receiving FXa inhibitors due to the high plasma binding of most FXa inhibitors (Table 33.1). The advent of the specific reversal agent for dabigatran further minimizes the need for dialysis.

Managing Life-Threatening Hemorrhage in NOAC-Treated Patients

In cases of major bleeding, it is critical that clinicians employ a massive transfusion protocol.

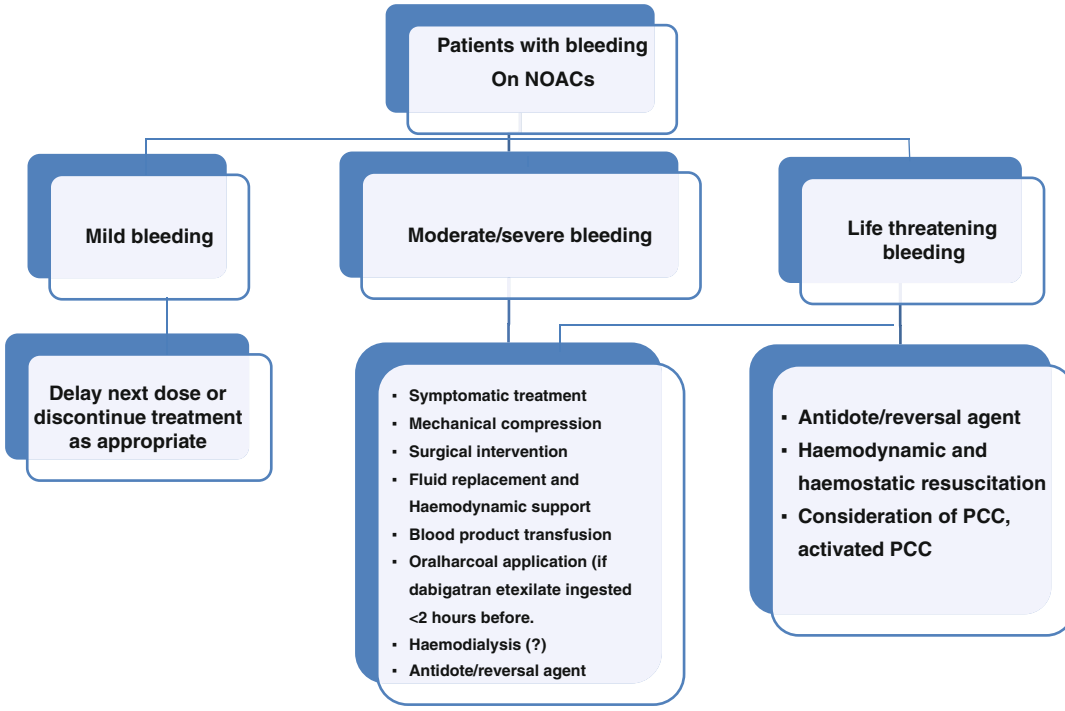

Fig. 33.2 Bleeding management strategies for NOAC-treated patients. In cases of mild bleeding, stopping or delaying the next dose should be considered. The new agents, including dabigatran, rivaroxaban, and apixaban, have relatively short half-lives, so when stopping the drug in patients with normal renal function, the anticoagulant effect rapidly decreases compared with warfarin. *In patients with moderate-to-severe bleeding*, standard therapeutic approaches should be considered, including supportive care that includes volume resuscitation, hemodynamic support with vasoactive therapy, blood product transfusions as determined by testing, and identification of bleeding source that may require surgical or another intervention. If the agents were taken within ~2 h of admission, administration of oral activated charcoal should be considered. For dabigatran, hemodialysis can remove ~60 % of the drug after several hours of dialysis and should be considered in patients with impaired renal function who are bleeding and will have altered clearance. Apixaban and rivaroxaban are highly protein bound and will not be cleared by hemodialysis. However, emergency access for hemodialysis requires vascular access with large-bore catheters that may pose additional risk in the anticoagulated patient. *For patients with life-threatening bleeding*, hemodynamic and hemostatic resuscitation should be considered, with therapy similar to a trauma patient including the use of a massive transfusion protocol. Based on current data as discussed in the manuscript, the use of either 3-factor or 4-factor prothrombin complex concentrates (PCCs), depending on their availability, should be considered as they have been shown to reverse or partially reverse the anticoagulation effect of the newer agents. In patients receiving dabigatran, the use of an activated PCC may be more effective. However, there are few studies reporting the use of PCCs on actual bleeding in patients, and further studies including the development of specific reversal agents are currently under way. In hypotensive patients, hemodialysis is unlikely to be tolerated, and alternate methods for hemofiltration should be considered if needed. The use of recombinant activated factor VIIa (rFVIIa) decreases bleeding times in animal models, but there are no human studies to determine if this is effective [28]

Although this is second nature to trauma physicians, it may not be the case with others. A specific reversal agent for dabigatran, idarucizumab, is a humanized monoclonal antibody that selectively binds dabigatran and is now approved to reverse dabigatran-induced anticoagulation. In the REVERSE-AD study, idarucizumab reversed the anticoagulant effects of dabigatran in patients with a major bleeding event, or were in need of an urgent invasive procedure, and has recently been approved in the USA and Europe for the reversal of dabigatran related anticoagulation in cases where emergency surgery and or urgent procedures are required, or in cases of life-threatening or uncontrolled bleeding.

Other agents including andexanet alfa are in advanced clinical trials as specific reversal agents of direct and indirect factor Xa inhibitors, including

apixaban, edoxaban and rivaroxaban. Andexanet alfa is a bioengineered human factor Xa decoy protein that has been modified to delete the native catalytic activity, while retaining the high-affinity binding of factor Xa inhibitors within the enzymatic active site. By binding to circulating factor Xa inhibitors, andexanet alfa makes endogenous factor Xa available to contribute to the coagulation cascade. Aripazine is an additional agent under investigation.

In instances of life-threatening bleeding, prohemostatic agents such as PCCs should also be considered, especially for bleeding related to the Xa inhibitors, or as part of a multimodal strategy. (Fig. 33.2) [28]. Emerging data indicate that PCCs effectively reverse the anticoagulant effect of NOACs in animal models and healthy volunteers [40, 43]. Animal data demonstrate that PCCs are able to reverse the anticoagulant effects of NOACs and improve bleeding parameters [44], and therefore should be considered for NOAC reversal as part of a multimodal approach. However, clinical trial data is required to confirm these preclinical observations and only a few case reports and limited series have been published using PCCs for NOAC reversal in patients with life-threatening bleeding [2].

In healthy volunteers, administration of 50 IU/kg of 4F-PCC reversed rivaroxaban-induced PT prolongation, but had minimal effect on dabigatran-induced prolongation of coagulation tests and inhibition of thrombin generation [43]. In vitro testing using blood samples from healthy volunteers taking rivaroxaban, dabigatran, or apixaban suggests that activated PCC (FEIBA® [Factor VIII Inhibitor Bypassing Activity]; Baxter Healthcare Corp, Westlake Village, CA, USA) corrects more coagulation parameters than non-activated PCC [45, 46]. Another study in 35 healthy volunteers has compared the reversal of rivaroxaban-induced effects on PT and thrombin generation using a 3F-PCC (Profilnine®) or a 4F-PCC (Kcentra®/Beriplex®) [40]. Participants received rivaroxaban 20 mg twice daily for 4 days in order to obtain supratherapeutic steady-state concentrations. Four hours after the first dose of rivaroxaban on day 5, the volunteers were randomized to receive a single 50 IU/kg intravenous bolus dose of 4F-PCC, 3F-PCC, or saline. 4F-PCC had a greater effect than 3F-PCC on reversing rivaroxaban-induced PT prolongation. Within 30 min, 4F-PCC administration reduced mean PT by 2.5–3.5 s, whereas treatment with 3F-PCC produced only a 0.6–1.0-s reduction. In contrast, 3F-PCC reversed rivaroxaban-induced inhibition of thrombin generation to a greater extent than 4F-PCC. The study demonstrated the potential of both 3F- and 4F-PCCs to at least partially reverse the anticoagulant effects of rivaroxaban in healthy adults. The different effects of the two PCC preparations on coagulation parameters may reflect the differences in the procoagulant components present in each [40].

Concomitant therapy, for example other coagulation factor replacement and antifibrinolytics (e.g., tranexamic acid), should be considered as part of a multimodal therapeutic approach to manage NOAC-associated bleeding. However, data supporting the effectiveness of these approaches in bleeding patients receiving NOACs are not available [31]. Plasma/FFP may be indicated as part of a massive transfusion protocol along with other factors, but not specifically for NOAC reversal. Neither vitamin K nor protamine has any role in managing NOAC-related bleeding.

Although specific outcome data in actively bleeding patients receiving NOACs is still evolving, based on the information available to date, the administration of PCCs should be considered where immediate reversal is required, for example in patients with life-threatening bleeding [31, 47]. Based on current studies with PCCs in preclinical models and in healthy volunteers, 25 IU/kg would be an appropriate starting dose, and doses can be repeated if clinically indicated. The role of rFVIIa (NovoSeven®, Novo Nordisk A/S, Bagsværd, Denmark) does not seem clinically relevant based on current information. Heidbuchel et al. have an extensive review article on this subject matter for further reading [31]. Clinical trials are also being conducted using specific antagonists for FXa inhibitors (andexanet and aripazine). However,

dabigatran reversal with idarucizumab is currently available and should be considered for emergency reversal of bleeding and/or for emergency surgery.

Platelet Inhibitors

Inhibiting platelet activation is critical for the management of patients with ischemic cardiovascular disease and/or atherosclerotic vascular disease [48]. As with oral anticoagulants, platelet inhibitors/antiplatelet agents pose increased risks for bleeding and should also be considered anticoagulants.

Antiplatelet agents differ in their modes of action, potency, onsets of action, and indications. Aspirin is an irreversible platelet cyclooxygenase and thromboxane A2 inhibitor but is also a relatively weak antiplatelet agent [49] and resistance can occur [50]. More potent antiplatelet agents include the $P2Y_{12}$ receptor inhibitors and GpIIb/IIIa receptor antagonists (abciximab, tirofiban, eptifibatide).

Clopidogrel, prasugrel, and ticagrelor have become the mainstays of antiplatelet therapy. These agents act to inhibit platelets by selectively and irreversibly binding to the $P2Y_{12}$ receptor, inhibiting the adenosine diphosphate (ADP)-dependent mechanism of GpIIb/IIIa receptor expression and platelet activation [49, 51, 52].

Clopidogrel is more commonly used than prasugrel or ticagrelor, but there is limited information available on how to monitor its effects and manage the patients receiving this therapy. Furthermore, resistance can occur because it is a prodrug and requires metabolism to be transformed to its active form. It is worth noting that clopidogrel decreases peak thrombin generation [53], and prolongs R-time on thromboelastography, suggesting a delay in thrombin generation [54]. Prasugrel [55] is one of the newer antiplatelet agents and has the advantage of increased potency and potentially a lower rate of resistance compared with clopidogrel [52]. It also has less pharmacologic variation in activation [56]. Prasugrel strongly inhibits ADP-induced platelet aggregation, P-selectin expression, and thrombin receptor agonist peptide (TRAP)-induced microparticle formation [56]. Ticagrelor is a direct-acting antiplatelet agent that should be considered in the same perspective as clopidogrel [57].

Dual-antiplatelet therapy with aspirin and clopidogrel is standard care following revascularization by percutaneous coronary intervention with stent insertion. This dual therapy is recommended for up to 4 weeks after intervention for bare-metal stents and for 6–12 months after intervention for drug-eluting stents [48]. Thus, patients may be receiving these drugs chronically following trauma.

In non-trauma patients, Vincenzi noted a 45 % complication rate and a 5 % mortality rate in patients undergoing non-cardiac surgery after coronary artery stenting [58]. This high complication rate is likely due to discontinuing antiplatelet drugs, underlying cardiovascular disease, and multiple other factors that have a major influence on outcome. Patients presenting with coronary stents placed within 1 year and requiring surgery are at great risk for adverse events. In the study by Vicenzi et al., conducted in 103 patients who had received a coronary artery stent ≤1 year prior to surgery, 44.7 % developed complications after surgery and 4.9 % of the patients died. All except two (bleeding only) adverse events were of cardiac nature, and most of the complications occurred early after surgery. Patients with recent stents (<35 days before surgery) had a 2.11-fold greater risk of experiencing an adverse event compared with those who had undergone percutaneous cardiac intervention more than 90 days before surgery [58]. The clopidogrel package insert suggests that if a patient is to undergo elective surgery and an antiplatelet effect is not desired, therapy should be discontinued 5 days before surgery. However, if patients bleed, treatment strategies or assays to monitor the effects of clopidogrel have not been established.

Managing Bleeding in Patients Receiving Antiplatelet Agents

Patients taking $P2Y_{12}$ receptor inhibitor/thienopyridine antiplatelet agents present following

trauma or requiring emergency surgery with increasing frequency due to the extensive use of drug-eluting coronary stents. In cases of acute bleeding following trauma, urgent reversal of antiplatelet agents may be required. Despite this concern, there are no approved or validated treatments for rapidly reversing anticoagulation in these patients. As a result, procoagulant therapies may be required in these situations. One commonly employed strategy is to transfuse allogeneic platelets. However, the efficacy and safety of this practice are unknown and there have been no randomized controlled trials conducted to date. One of the problems for managing platelet dysfunction is that most platelet function tests cannot be used in the trauma, bleeding, or postoperative patient [57].

rFVIIa may have potential for reversing $P2Y_{12}$ receptor inhibition. The concept behind this is based on the clinical use and US Food and Drug Administration (FDA) approval of rFVIIa (NovoSeven®) for treating bleeding episodes and perioperative management in patients with Glanzmann's thrombasthenia with refractoriness to platelet transfusions, with or without antibodies to platelets. Glanzmann's thrombasthenia is a rare genetic bleeding disorder characterized by defective or low levels of GpIIb/IIIa, a receptor for fibrinogen, resulting in platelet dysfunction. The defect in platelet function is similarly produced by clopidogrel, prasugrel, and ticagrelor.

rFVIIa has been shown to restore thrombin generation in clopidogrel-treated blood samples, and shorten thrombin generation lag time in patients who had been treated with aspirin and clopidogrel [59], and in blood samples treated with clopidogrel or prasugrel's active metabolite [60]. These findings are all consistent with the desired procoagulant effect of rFVIIa.

Although these in vitro data using rFVIIa are promising, caution must be exercised when extrapolating these data to clinical scenarios. It is possible that the increase in thrombin generation is purely an in vitro phenomenon. Also, the off-label use of rFVIIa has been the subject of persistent safety concerns, with two recent reviews describing an increased risk of arterial thrombosis and stroke in patients receiving off-label rFVIIa [61, 62]. Nevertheless, a slightly increased thrombotic risk may be acceptable in patients who have life-threatening bleeding and require salvage therapy [62]. Thus, rFVIIa might be a useful salvage treatment in patients taking antiplatelet agents who present with life-threatening refractory bleeding. However, extreme caution should be taken when using rFVIIa to reverse the effect of antiplatelet drugs in less acute scenarios.

The Future

Although the new oral target-specific anticoagulation agents are thought to be associated with a lower risk of major bleeding than VKAs, there is a lack of validated reversal strategies available for these agents. Clinical studies are needed to assess the effectiveness of therapeutic approaches for the management of bleeding when it occurs, and some are currently under way. Initial results from the RE-VERSE AD (RE-VERSal Effects of idarucizumab on Active Dabigatran) study for dabigatran reversal in patients with uncontrolled bleeding or who require emergency surgery have been published and is currently approved for use in the US. [63]. A novel agent for FXa inhibitor and LMWH reversal (andexanet alpha) is also in clinical development; trials investigating its potential for rivaroxaban and apixaban reversal are currently in progress (NCT02220725 and NCT02207725) [64]. Although the use of the specific reversal strategies or antidotes will continue to evolve, recommendations for their use are reported.

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Part VIII

Research of Trauma Induced Coagulopathy

Animal Models of Trauma Induced Coagulopathy

34

Ted Bambakidis, Martin Sillesen, and Hasan B. Alam

Introduction

Trauma-induced coagulopathy (TIC) is thought to be the product of a complex interplay between multiple insults (Fig. 34.1). Endogenous coagulopathy is driven by tissue injury and shock, and features elements of platelet and endothelial dysfunction as well as protein C-driven hyperfibrinolysis. In addition, iatrogenic factors such as hypothermia, acidosis, and hemodilution further exacerbate coagulation derangements. Elucidating TIC-related mechanistic pathways as well as the development of novel, targeted treatment regimens therefore require both fundamental knowledge of the underlying pathophysiological mechanisms and a platform for preclinical testing of novel treatment agents.

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While human studies remain a cornerstone trauma research, it is clear that these suffer from a number of shortcomings and logistical challenges that necessitate development of robust and reproducible animal models of TIC. As such, the majority of human TIC studies are retro- or prospective in nature, a necessary constraint that only allows for associations to be observed rather than causality to be established. Furthermore, these studies are prone to numerous bias and confounding factors. In contrast, while randomized controlled trials represent the gold standard for human intervention studies, these studies are not only expensive and logistically challenging, but also do not allow for the preliminary testing of promising drug candidates prior to extensive toxicology studies.

While obvious challenges exist in the use of animal models for TIC research, it is thus clear that well-validated and robust models represent an optimal platform for both the investigation of isolated pathophysiologic mechanisms and preclinical testing of novel therapeutic regimens. Animal models will thus likely continue to play a significant role in TIC research in the years to come.

The ideal animal model depends on the study hypothesis. Some hypotheses focus on the effect of isolated events such as the effect of modality/type of injury on specific pathophysiologic pathways. These models should obviously limit the amount of confounding factors (e.g., resuscitation, hypothermia, hemodilution)

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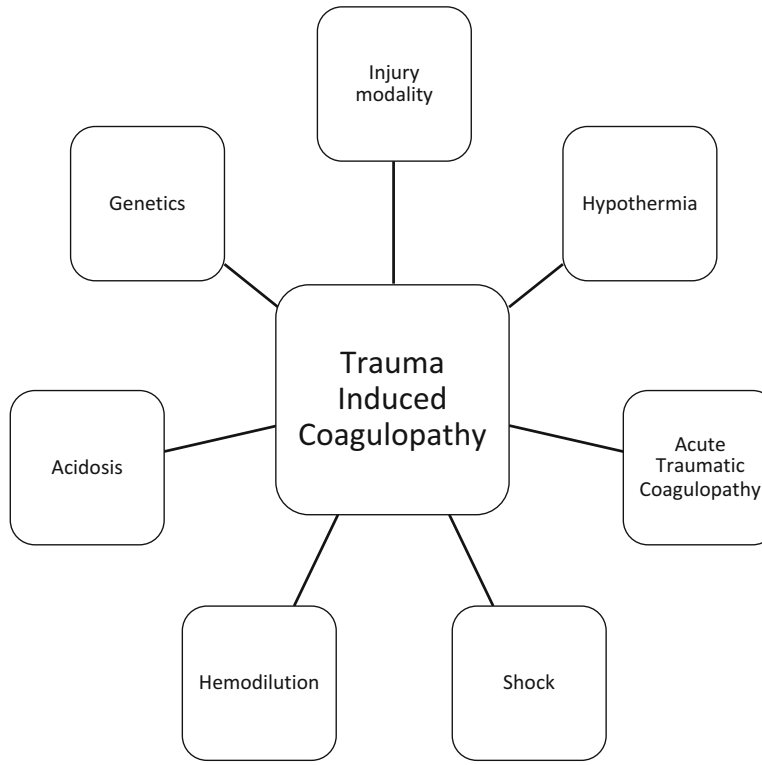

Fig. 34.1 Elements acting in concert to induce and modulate trauma-induced coagulopathy

in order to produce reliable results. These well-controlled models may not be very clinically realistic, but they are crucial for conducting precise mechanistic studies, and for developing and testing new therapies for TIC. In contrast, studies that seek to test the impact of treatments on clinically relevant outcomes such as survival should ideally use a more clinically robust large animal model with realistic insults and more meaningful management strategies in order to facilitate translatability. It is therefore clear that no single ideal animal model of TIC exists. Rather, an ideal model depends on the study question.

> Essentially, all models are wrong, but some are useful.
> —George E.P. Box (1919–2013)

Here we discuss a number of variables that must be considered during the development and selection of an appropriate animal model.

Developing an Animal Model

Ethical Considerations

Establishing a successful model of TIC, or any other disease, centers around not only establishing cause-and-effect relationships between the insult, treatment, and outcome, but ideally also elucidating the underlying mechanisms. Yet in this pursuit, researchers must adhere to special ethical considerations when conducting studies involving animals. This may be particularly important in models involving traumatic injuries, as the relatively acute and severe nature of such insults may draw increased attention from institutional and external regulatory committees. The Guide for the Care and Use of Laboratory Animals, Institute for Laboratory Animal Research (2011), serves as the standard for the development and implementation of animal models. Beyond this benchmark, however, any

Institutional Animal Care and Use Committee (IACUC) compliant animal study must adhere to the concept of the "three Rs": reduction, refinement, and replacement [1]. These ethical guidelines ensure that no animals are used for scientific research questions if alternative options are available. Several steps can be taken to practice the three Rs, such as performing power analyses to calculate the smallest sample size that will reveal intergroup differences, scaling the traumatic injury to minimize unnecessary animal suffering, and intensive collection of real-time data and biomaterial to maximize the yield of data from the fewest number of animals. Finally, investigators should consult with a faculty veterinarian at their institution to assist with protocol development and approval.

Control and Sham Groups

Animal models of TIC are frequently used to test resuscitation fluids and pharmacologic agents. In addition to allocating a group of animals to receive such treatments, investigators must also include control and sham animals. We define controls as animals that receive neither the insult nor treatment—essentially, healthy animals. Instrument and anesthesia controls are advisable to account for the effect of surgical trauma (e.g., craniotomy, femoral cannulation) and prolonged anesthesia. In contrast, sham animals may be defined as receiving the insult without any subsequent therapeutic intervention, therefore representing the "worst-case scenario." This occasionally overlooked group is critically important in developing a well-designed animal model. The investigator must strike an appropriate balance between inflicting a measurable and reproducible injury on the one hand, while ensuring that endpoints are met and suffering is minimized on the other. It is imperative that the injury is severe enough to elicit a measurable physiologic change. For example, differences in circulating markers of coagulation and endothelial function should be statistically significantly different between control and sham groups. Only if the sham animals experience a significant physiologic change can the investigator then evaluate the treatment groups to determine the efficacy of the therapeutic intervention. Additionally, sham animals should receive vehicle controls (e.g., normal saline, dimethyl sulfoxide) to precisely match the volume of fluid administered to treatment groups.

Effects of Anesthesia

The role of anesthesia is an important consideration when designing a model of TIC, as volatile anesthesia agents may affect both the sympathoadrenal response and coagulation derangements following trauma. Traumatic injuries cause an instantaneous surge of catecholamines [2–4], and the resultant sympathetic outflow alters hemodynamics, activates platelets, and induces endothelial release of coagulation and fibrinolytic factors [5, 6]. This sympathoadrenal activation is thought to further induce endothelial damage, glycocalyx shedding, coagulation, and hyperfibrinolysis, as well as subsequent microthrombi formation and secondary shock. Ultimately, a dangerous cycle of hypotension, hypoxia, elevated catecholamine levels, coagulopathy, and shock may develop [7].

A growing body of literature, however, suggests that volatile anesthetics may be protective following trauma. Though halogenated ethers have been shown to cause peripheral vasodilation and hypotension [8, 9], they also decrease sympathetic nervous system activity [10–12]. Moreover, animal models have demonstrated that these agents can protect the endothelial glycocalyx [13] and inhibit ADP-mediated platelet activation [14], ultimately attenuating posttraumatic adhesion of platelets and leukocytes and the ensuing inflammatory response. Taken together, these studies suggest that volatile anesthetics may blunt both the dangerous catecholamine surge following trauma and the subsequent coagulation derangements and inflammatory response.

Yet, our own experience and that of others have shown that halogenated ethers may still be used in clinically realistic models of TIC. The dose of anesthesia can be titrated to balance animal welfare with clinical realism. For example,

in our porcine model of traumatic brain injury (TBI) and hemorrhage, an isoflurane dose of 0.5–1.5 MAC during shock maintains anesthesia while permitting the expected sympathetic compensation to hypovolemia (i.e., tachycardia). Thus, models employing low doses of volatile anesthetics are likely to still allow the study of coagulation derangements, while high-dose models may serve as a promising platform for testing new treatments for TIC.

General anesthesia may also indirectly affect coagulation by inducing intraoperative hypothermia. Core temperature routinely decreases by 3 °C due to a combination of increased peripheral blood flow, systemic vasodilation, as well as decreased cerebral and tissue metabolism [15]. Several studies have implicated increased platelet activation, clot formation and stability, and subsequent consumption as the primary mechanism for the clinical observation of hypercoagulability below 37 °C [15–20]. The situation is even more pronounced at temperatures below 32 °C during cardiac and neurosurgical procedures, when hypothermia-induced hypercoagulability has been shown to cause transient neurological deficits [21] and even be fatal [22]. Thus, unintentional mild hypothermia in animal models of TIC may cause artifacts in coagulation and platelet assays. Active maintenance of normothermia can be achieved by using a heated surgical table and forced-air warming blankets.

Choice of Species

Choice of species is an important aspect of the study design for several reasons. Large animal models remain a cornerstone of translational TIC research, primarily owing to their relative similarity to human physiology. This makes them ideal candidates for studies requiring direct clinical translatability, such as survival studies. Furthermore, the relatively large blood volume allows for multiple aspects of TIC to be investigated simultaneously, such as thrombelastographic evaluation of whole blood coagulation coupled with plasma analysis of biomarkers.

While studies seldom offer justification for their choice of species, porcine models are the most prevalent. Although sheep and dog models have been developed and used widely in the past, the choice of the swine as a model animal in contemporary research is likely due to a combination of ease in procurement of the animals, as well as a desire to avoid the use of companion animals (e.g., dogs). Large animal studies are, however, expensive and logistically challenging. Basic science studies focusing on individual mechanistic pathways or biomarkers are often ill suited for large animal models due to reasons of finance as well as a lack of reliable and validated antibodies and assays when compared with their rodent counterparts. As such, TIC models utilizing rats, mice, or even rabbits represent a much more appealing choice when the study hypothesis is aimed at basic science questions.

Prior to establishing an animal model, investigators should also evaluate the relationship in the coagulation system between the candidate species and humans. While the coagulation system appears to be relatively conserved among the vertebrates [23], evolutionary divergence within the class Mammalia has resulted in interspecific differences in coagulation systems [24, 25]. Several reviews have compared animal coagulation profiles [25–29]. An inverse association between animal size and platelet count is apparent, while coagulation factor concentrations are both species- and factor-dependent. Rapid coagulation assessments (e.g., prothrombin time, activated partial thromboplastin time) reveal that the clotting time of sheep closely mimics that of humans. Likewise, thromboelastic measurements of clotting time and speed show that sheep appear to closely match that in humans. Practical considerations such as animal cost should also be considered (Table 34.1).

Finally, caution must be exercised when interpreting Table 34.1. Coagulation assays and agonists developed for humans have historically been employed in animal models. As a result, species-specific differences in reactivity may in part stem from a lack of structural homology between animals and humans in key factors and platelets [48, 49]. The recent development of

Table 34.1 Key hematologic and hemostatic values and related assays in common laboratory animal species and humans

Parameter	Human (adult)	Swine	Sheep	Rat	Mouse
Blood volume (ml/kg)	68–88 (plasma vol) [30] 55–75 (RBC mass) [30]	56–69 [31]	58–64 [31]	54–70 [31]	60–75 [31]
Platelet count (10[9]/L)	159–376 [30]	350–700 [31]	401 (132–650) [24]	500–1300 [31]	1000–1269 [32, 33]
Coagulation factors					
vWF (%n)	92 (50–158) [34]	140 [27]	117 (30–186) [24]	49 [35]	15 [32]
II (%n)	110 (78–138) [36]	65 (58–74) [37]	40 (36–43) [37]	110 (92–122) [37]	–
V (%n)	118 (78–152) [36]	790 (555–1020) [37]	62 (48–75) [37]	749 (512–781) [37]	292 [38]
VII (%n)	129 (61–199) [36]	58 (42–77) [37]	36 (24–50) [37]	471 (403–586) [37]	–
VIII (%n)	160 (52–290) [36]	555 (380–745) [37]	817 (427–1327) [24]	116 [35]	224 [38]
IX (%n)	130 (59–254) [36]	386 (300–590) [37]	208 (184–224) [37]	28 (24–37) [37]	75 [38]
X (%n)	124 (96–171) [36]	92 (59–120) [37]	15 (13–21) [37]	48 (33–59) [37]	109 [38]
XI (%n)	112 (67–196) [36]	200 (132–270) [37]	15 (12–18) [37]	35 (18–49) [37]	–
XII (%n)	115 (35–207) [36]	747 (670–840) [37]	140 (85–243) [24]	447 (312–512) [37]	–
Anticoagulation factors					
AT (%n)	96 (66–124) [36]	101 (89–111) [37] 101 (98–103) [39]	93 (74–119) [24]	123 [35]	–
aPC (%n)	103 (54–166) [36]	36 (33–39) [39]	49 (30–76) [24]	–	–
Assays					
PT (s)	13.0 (11.5–14.5) [36]	11.4 (9.4–13.1) [37]	13.1 (11.4–15.5) [24]	12.1 (8.9–13.9) [37]	7.3 [40]
aPTT (s) [4]	33.2 (28.6–38.2) [36]	16.6 (13.4–18.1) [37]	29 (19.6–40.8) [24]	64.9 (46.2–76.1) [37]	22 [40]
Fibrinogen (g/L)	3.1 (1.9–4.3) [36]	3.57 (3.38–3.77) [39]	2.61 (1.4–4.3) [24]	1.34 [35]	–
D-dimers (μg/mL)	<0.50 [41]	<0.01 [42]	<0.02 [43]	0.18 [44]	<0.02 [44]
TEG (Kaolin)					
R (min)	3.8–9.8 [45]	2.2–3.0 [29] 5.9 (5.4–6.7) [46]	–	1.0–3.4 [29]	0.9–2.1 [29]
K (min)	0.7–3.4 [45]	0.8–0.8 [29]	–	0.3–1.1 [29]	0.6–1.0 [29]
Angle (degrees)	47.8–77.7 [45]	64.8–81.6 [29] 72.4 (71.5–77.9) [46]	–	77.8–86.2 [29]	80.2–84.2 [29]

(continued)

Table 34.1 (continued)

Parameter	Human (adult)	Swine	Sheep	Rat	Mouse
MA (mm)	49.7–72.7 [45]	80.3 (75.6–82.1) [46]	–	–	59.3 [47]
ROTEM (native)					
CT (s)	595 (476–901) [28]	244 (146–296) [28]	494 (344–1431) [28]	207 (63–352) [28]	–
CFT (s)	200 (104–436) [28]	52 (30–84) [28]	182 (143–532) [28]	55 (35–97) [28]	–
MCF (mm)	58 (49–65) [28]	74 (68–79) [28]	72 (61–77) [28]	75 (70–81) [28]	–
ML (%)	21 (2–24) [28]	17 (12–31) [28]	2 (0–26) [28]	8 (3–13) [28]	–
Cost (USD)	–	200	220	55	45

Mean with range is provided where available. Cost is based on approximate purchase and shipping cost available at the University of Michigan. *vWF* von Willebrand factor, *AT* antithrombin, *aPC* activated protein C, *PT* prothrombin time, *aPTT* activated partial thromboplastin time

species-specific antibodies for common laboratory animals, however, may enhance our ability to quantify and understand animal coagulation. Furthermore, investigators should not select a species based solely on the data presented here and in the literature. Published studies reveal marked variation in animal [25] and human [45] coagulation profiles. In human medicine, assay manufacturers recommend that each institution develops its own reference ranges before drawing clinical conclusions. Investigators would be wise to heed this advice in animal models of TIC.

Clinical Translatability

Before developing an animal research program aimed at improving human health, investigators should be mindful of the clinical translatability and limitations of animal models. Recent advances in genomic, proteomic, and bioinformatic techniques have placed increased scrutiny on the use of animals as model organisms for human disease. For example, Seok et al. [50] provided evidence that murine models of inflammation poorly mimic human disease. Yet, rather than abandoning murine models, other investigators contend that the primary issues lie in model design and data interpretation. Flawed animal models may use inbred, unisex, and immature animals; overlook known differences in physiology; use cells of a single origin (e.g., circulating leukocytes) to draw systemic conclusions; and focus solely on gene expression without also examining protein products [51]. Moreover, investigators must be mindful of evolutionary relationships when making extrapolations from rodents to humans, as species from a more closely related lineage may be more appropriate. Thus, employing a homogeneous and relatively divergent animal model and comparing it with a diverse human population necessarily limits our ability to draw meaningful, clinically relevant conclusions.

Our own experience has taught us to take a stepwise approach in translating basic science into clinical recommendations. Initial hypothesis generation and testing are best performed in vitro. The next step involves proof-of-concept studies in rodent models. Only after substantial hypothesis testing and evidence gathering at this stage do we then perform translational research using clinically relevant large animal models. Human studies are the last step in the sequence, and are only conducted after years of rigorous mechanistic and outcome studies in large animal models. The time frame for the progression of one idea from cell culture through clinical trial may take 5–10 years to complete. Yet, this should be viewed as a continuous process in which new hypotheses are continually generated, progress up the chain, or are filtered out. This stepwise approach is a promising and proven strategy to move novel discoveries from bench to bedside.

Assays and Endpoints

Various different methods have been employed to characterize elements of TIC in animal models. Studies predominantly report conventional coagulation tests such as prothrombin time (PT in seconds or international normalized ratio (INR)) or activated partial thromboplastin time (aPTT) presumably owing to a widespread use of these tests in clinical practice. Activity and concentration of specific coagulation factors, most notably fibrinogen, have also been reported.

Whole blood coagulation test such as thrombelastography (TEG) and thromboelastometry (ROTEM) have been validated in multiple species with commercially available coagulation activators, including swine and rats. Platelet function testing using platelet aggregometry (e.g., Multiplate and Chronolog) has also been utilized in animal models. Some studies have investigated pathophysiological aspects of TIC through histological and plasma-based detection of biomarkers of endothelial injury.

An example timeline for large animal models of TIC is illustrated in Fig. 34.2. Two hours of shock typically induces significant physiologic change in circulating markers. Six hours, however, is appropriate for measuring changes in endothelial markers and brain vasculature. The shock duration can be shortened to 30 min to 1 h for rodent models.

Models of Injury and Resuscitation

Animal models of TIC seldom involve one injury in isolation. Models instead typically incorporate combinations of hemorrhagic shock, soft tissue and organ injuries, and TBI. While such complex, multisystem models may reflect the clinical presentation of trauma, they may limit our ability to elucidate the etiology and mechanisms of the resultant TIC. Moreover, some insults, such as hemorrhagic shock, create systemic hypoperfusion which in turn causes secondary injuries such as hypothermia, metabolic acidosis, and multiorgan system failure. It is therefore difficult to control or eliminate these variables even in carefully constructed animal models of TIC. Yet, some studies have examined coagulopathy from isolated injuries while limiting the role of secondary insults. We begin this section by presenting models of isolated injuries, followed by studies involving TBI and associated insults, and end by

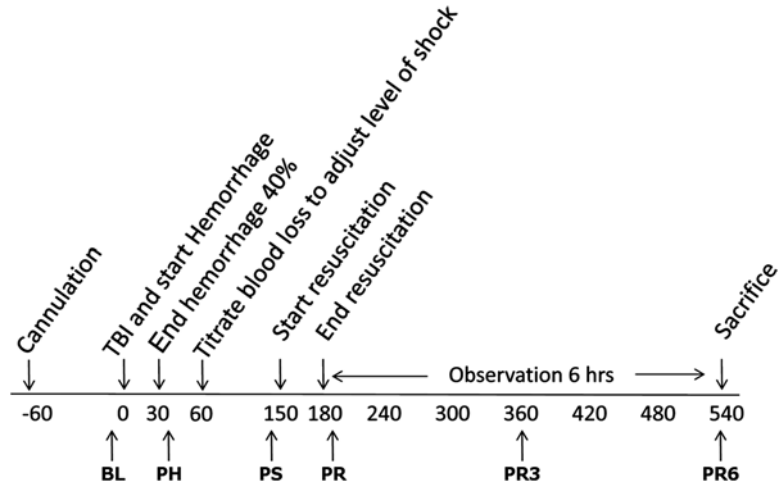

Fig. 34.2 Experimental timeline for a large animal model of trauma-induced coagulopathy. The insults in this example are traumatic brain injury (TBI) and hemorrhagic shock. Shock lasts approximately 2 h, with subsequent resuscitation over approximately 30 min. Blood samples may be collected at baseline (BL), post-hemorrhage (PH), post-shock (PS), post-resuscitation (PR), 3 h post-resuscitation (PR3), and 6 h post-resuscitation (PR6), just prior to sacrifice

discussing relatively complex models of multisystem trauma and resuscitation/hemodilution-induced TIC. Key studies with assays and findings are summarized in Table 34.2.

Models of Isolated Injuries

Models

Several models of isolated hemorrhagic shock (HS) [52, 53], septic shock [47, 54, 55], or tissue injury [56, 57] have examined coagulation derangements. Models of HS in rodents [53] and swine [52] typically used indwelling catheters for controlled exsanguination. Lipopolysaccharide challenge in rodents is routinely employed in models of sepsis [47, 54, 55], while tissue injury has been inflicted in sheep [57] and swine [56] (Table 34.2).

Pathophysiological Aspects of Trauma-Induced Coagulopathy

1. *Hemorrhage*
   Models of isolated HS have demonstrated a hypocoagulable state shortly after hemorrhage. Rapid prolongation of clotting time has been shown as soon as 20 min after hemorrhage, with more than a tenfold delay following 1 h of shock [53]. An evolution of increased clot initiation time and simultaneous weakening of clot strength has also been reported over 6 h [52]. Prothrombin time (PT) and partial thromboplastin time (PTT), as well as ROTEM, have detected hypocoagulability following HS. Interestingly, animal studies have not yet reported measurable changes in the fibrinolytic system during hemorrhagic shock.
2. *Sepsis*
   Rodent models of sepsis have shown conflicting results. Increased clot formation time with simultaneous decreases in clot formation rate and speed, suggesting an overall hypocoagulable state, has been reported [47]. However, a similar study revealed a decrease in the clot formation time and an increase in rate following LPS infusion, with subsequent return to baseline values [55]. Interestingly, there was a delayed decrease in clot strength. Plasma concentrations of thrombin-antithrombin complex (TAT) and plasminogen activator inhibitor-1 (PAI-1) increased in the LPS group. These data suggest rapid and sustained hypercoagulation, with subsequent inhibition of fibrinolysis and consumption coagulopathy, due to LPS challenge. A reduction in platelet function [47, 55] and count [54] has also been demonstrated, although more functional quantification of platelet function, as with platelet mapping or aggregometry, has not been performed.
3. *Tissue Injury*
   Studies of isolated tissue injury have demonstrated the onset of a hypocoagulable state. Giannoudis et al. [57] found that femoral nailing reduced circulating concentrations of factor V and antithrombin III, which was exacerbated when lung contusion and/or HS was added as a second hit. This supported earlier work which showed that repeated muscle trauma prolonged clotting and PT, and induced lung thrombosis [56].
4. *Hypothermia and Acidosis*
   Hypothermia and metabolic acidosis, together with coagulopathy, comprise the "lethal triad" which has been associated with poor outcomes. Mild hypothermia (32 °C) and acidosis have independently been shown to induce a hypocoagulable state in swine by decreasing fibrinogen availability [71, 72] and delaying thrombin generation time [96]. Acidosis decreased the circulating clotting factors, platelet count, rate of clot formation, and clot strength while prolonging the clot generation time [96]. Work by Martini et al. [72] has examined the independent and combined effects of hypothermia and acidosis in swine. Hypothermia independently showed hypocoagulable effects similar to acidosis, although it suppressed thrombin kinetics more severely compared to acidosis alone. Combined acidosis + hypothermia created the most severe hypocoagulable state as measured by splenic bleeding time, circulating fibrinogen concentration, and thrombin kinetics.

Table 34.2 Overview of published animal models in trauma-induced coagulopathy

Modality	Author	Species	Endpoint/assay	Intervention	Result
Isolated shock	Hatch [52]	Swine	ROTEM	None	Significant increases in INR, CT, CFT, and MCF between baseline and 6 h post-injury
	Letson [53]	Rat	PT, aPTT	7.5 % NaCl/ adenocaine/Mg^{2+}	Treatment returned PT and aPTT to baseline levels
	Grier [54]	Rat	Platelet count, PT, PTT	None	Thrombocytopenia, significantly decreased PT and prolonged PTT in trauma + LPS vs. control
	Tsai [55]	Rat	TEG, TAT, PAI-1	None	Significant decrease in R, K, MA, and increase in angle in LPS vs. control. TAT and PAI-1 significantly elevated in LPS vs. control.
	Zhao [47]	Mouse	TEG	SAHA	CLP significantly increases K, decreases angle and MA in CLP vs. sham. SAHA treatment restores K, angle, and MA to control levels
Isolated tissue injury	Hardaway [56]	Swine	PT, aPTT, fibrinogen, fibrin degradation	tPA, urokinase	PT significantly higher, platelet count lower in trauma vs. tPA and urokinase groups. Fibrinogen significantly higher in tPA group vs. urokinase and trauma.
	Giannoudis [57]	Sheep	Antithrombin III, fibrinogen, factor V	LR	Antithrombin III and factor V significantly decreased due to femoral nailing, fibrinogen unchanged
Traumatic brain injury	Castellino [58]	Rat	TEG/PM	None	Significant inhibition of ADP- and AA-mediated platelet function in TBI rats vs. controls
	Dekker [59]	Swine	PF1 + 2, aPC	NS, HEX, FFP	Serum aPC significantly greater in NS vs. HEX. Brain PF1 + 2 significantly greater in NS vs. FFP. Brain E-selectin greater in NS vs. FFP. Serum ICAM-1 greater in NS vs. both HEX and FFP
	Jepsen [60]	Rat	Syndecan-1	Valproic acid	Syndecan-1 significantly elevated in injured vs. uninjured animals. VPA further elevated syndecan-1 levels
	King [61]	Swine	TEG	HEX, NA + mannitol	Survival study: decreased R-time compared to baseline at 240 min due to TBI in all groups. Non-survival study: increased R-time compared to baseline at 72 h due to TBI in all groups
	Patel [62]	Swine	TEG	HBOC-201 + NS, NS	HBOC showed increased angle and MA compared to controls
	Ploplis [63]	Rat	TEG/platelet mapping, P-selectin, tissue factor, fibrinogen	None	Elevated P-selectin, tissue factor, and fibrinogen in TBI vs. control animals Decreased ADP- and AA-mediated platelet aggregation in TBI vs. control animals
	Sillesen [46]	Swine	TEG, platelet aggregometry	None	Significantly lower ADP-mediated platelet aggregation in TBI vs. control animals Significantly lower MA in TBI vs. controls
	Sillesen [64]	Swine	Syndecan-1, PF 1 + 2, aPC	None	Significantly elevated PF1 + 2, syndecan-1, and aPC in TBI vs. control animals
	Imam [65]	Swine	PT, aPTT	NS, FFP, lyophilized plasma	aPTT significantly lower in lyophilized plasma vs. FFP group 2 h after injury

(continued)

Table 34.2 (continued)

Modality	Author	Species	Endpoint/assay	Intervention	Result
Multisystem trauma	Chesebro [66]	Mou`se	aPTT, aPC	aPC antibody	Significantly higher aPTT and aPC levels in trauma + HS mice vs. controls. Selective aPC inhibition restored aPTT to controls
	Frith [67]	Rat	PT, aPTT	None	Significant increase in PT and aPTT in trauma + HS animals vs. sham
	Darlington [68]	Rat	ROTEM, aPTT, fibrinogen, TAT, PAP, soluble fibrin monomer	None	Significantly elevated PT, aPTT, angle, decrease in clot firmness, fibrinogen, and platelet count vs. baseline. Transient increase in TAT and PAP
	White [69]	Swine	TEG, PT, aPTT, fibrinogen	None	Significant decrease in fibrinogen, K, MA, A30, and clot elastic modulus between baseline and shock. Significant increase in angle and EPL30 between baseline and shock
	Mohr [70]	Swine	ROTEM, PT, fibrinogen, platelet aggregometry	Hypothermia	Significant decrease in PT and fibrinogen due to trauma; no effect of hypothermia. Significant increase in CT and CFT, and decrease in MCF following trauma; no effect of hypothermia. Assays performed at 34 °C did show differences in CT and CFT
Hypothermia and acidosis	Martini [71]	Swine	Fibrinogen, TAT, TEG	None	Significant decrease in fibrinogen synthesis and TAT generation at initiation phase between control and hypothermia. R-time significantly increased, angle significantly decreased between baseline and hypothermia
	Martini [72]	Swine	TEG, fibrinogen, PT, aPTT, TAT, bleeding time	None	Significant increase in bleeding time in all groups vs. controls. Fibrinogen significantly decreased in acidotic and combined group. TAT generation onset time decreased in hypothermia and concentration lower in acidosis and combined group. Increase in R-time and increase in angle occurred between groups
	Martini [73]	Swine	TEG, PT, aPTT, activated clotting time, fibrinogen, TAT	THAM	Significant decrease in fibrinogen, platelets, thrombin generation, angle, and MA due to acidosis. Significant increase in PT, aPTT, activated clotting time, and R + K due to acidosis. THAM did not reverse derangements
Hemodilution and resuscitation	Alam [74]	Swine	PT/INR, TEG	Normal saline, HEX, FWB, FFP, PRBC	Hypocoagulable state following crystalloid hemodilution. All blood products significantly restored PT/INR. FFP, and PRBC, and FWB restored R-time. All blood products restored angle and MA
	Dickneite [75]	Swine	PT, thrombin generation, factor II, VII, IX, and X levels	Recombinant factor VIIa (rFVIIa), prothrombin complex concentrate (PCC)	Hemodilution decreased all factor levels, PCC restored II and X. Hemodilution increased PT and PCC and rFVIIa restored it. Peak thrombin time higher in PCC-treated animals vs. control and rFVIIa groups. Hemodilution decreased all factor levels and peak thrombin generation while increasing PT. PCC restored factors II and X as well as peak thrombin time and PCC and rFVIIa restored PT

	Doran [76]	Swine	PT	NS	Higher PT in hypotensive vs. novel hybrid group
	Harr [77]	Rat	TEG	NS	Significant increase in R and SP between baseline and shock, and also from shock to post-resuscitation. Significant increase in thrombin generation between baseline and shock
	Haas [78]	Swine	ROTEM	Hypertonic saline/ hydroxyethyl starch [HS-HES]	Significantly elevated fibrinogen polymerization and decreased blood loss in HS-HES vs. gelatin or HES alone. HS-HES restored ROTEM parameters closer to baseline vs. other groups
	Kheirabadi [79]	Rabbit	Fibrinogen, PT, aPTT, TEG	HEX, Dextran70, albumin	Hemodilution significantly increased PT and aPTT, and decreased fibrinogen in all groups vs. baseline. Albumin maintained baseline levels of TEG parameters better than other groups. Albumin animals had better survival and lower total blood loss
	Martini [80]	Swine	Fibrinogen metabolism, PT, aPTT, TEG	LR	Significant decrease in fibrinogen level in hemorrhage and LR groups vs. controls. Fibrinogen breakdown significantly increased in both groups vs. controls. R and MA significantly decreased in both groups vs. controls
	Martini [81]	Hamster	ROTEM, in vivo microscopy	HEX, fibrinogen	Fibrinogen group restored MCF to baseline levels vs. sham. Microthrombi formation increased in fibrinogen animals vs. sham
	Martini [82]	Swine	TEG, TAT, coagulation factors	LR	Significant decrease in fibrinogen, platelet count, TAT, MA, angle, most factors in LR in days 1–2, then increased to BL level by day 5. Significant increase in R and K in days 2–4, return to control by day 5
	Nielsen [83]	Rabbit	TEG	HEX, albumin	Significant decrease in R in HEX group. TAT significantly lower in HEX and albumin vs. controls. Significant decrease in heparin activity in HEX vs. control and albumin
	Nishi [84]	Rat	Sonoclot	LR	Significant decrease in clot rate in higher hemodiluted animals
	Pragst [85]	Rabbit	Time to hemostasis, blood loss	NS, PCC, rFVIIa	Significantly faster clot time in PCC vs. saline and rFVIIa groups. Reduced blood loss in PCC vs. saline and rFVIIa
	Riha [86]	Swine	TEG, PT, aPTT	Hypertonic saline + dextran, LR, HEX, NS	Significant decrease in MA and fibrinogen in all groups vs. control. Significant increase in PT in all groups (except LR) vs. control. Control had significantly lower blood loss vs. all groups. All R values significantly lower in all groups vs. baseline
	Shuja [87]	Swine	TEG, PT, aPTT, INR, fibrinogen, factor activity	Freeze dried plasma, FFP	No significant differences between dried plasma and FFP

(continued)

Table 34.2 (continued)

Modality	Author	Species	Endpoint/assay	Intervention	Result
	Spoerke [88]	Swine	Coagulation factor activity, blood loss	FFP, lyophilized plasma (LP), PRBC	Lyophilization reduced clotting factor activity by 14 %. LP group had similar coagulation profiles and blood loss as FFP group. 1:1 FFP-PRBC group had similar recovery to 1:1 LDP:PRBC group
	Todd [89]	Swine	TEG, PT, aPTT, fibrinogen	LR, HEX	Significantly higher PT and aPTT, and lower fibrinogen, in HEX vs. LR and controls. Significantly higher R but lower angle and MA in HEX vs. LR and controls
	Torres [90]	Rat	ROTEM, Syndecan-1	HEX, FFP, LR	Decrease in clot firmness and platelet count, and increase in clotting time, in LR and HEX vs. FFP and sham. Glycocalyx significantly lower, and syndecan-1 significantly higher in LR and HEX vs. FFP and sham
	Kozar [91]	Rat	Syndecan-1, microscopy	LR, FFP	Syndecan-1 expression significantly higher in FFP vs. sham and LR. FFP significantly attenuated lung injury vs. sham and LR
	Via [92]	Swine	Bleeding time, PT, aPTT, fibrinogen	Hydroxyethyl starch, LR, albumin	Significant decrease in platelets, fibrinogen, and MA due to dilutional coagulopathy
	Watters [93]	Swine	TEG	NS, hypertonic saline, dextran	Significant decrease in R vs. baseline in all fluids except dextran. Angle significantly increased in all groups except dextran. Significant decrease in MA in dextran animals
	Wohlauer [94]	Rat	TEG	NS	No significant difference in TEG parameters in hemodilution vs. sham groups
	Sillesen [95]	Swine	Platelet aggregometry, TEG	NS, FFP	Significantly increased ADP-induced platelet aggregation, fibrinogen, and MA in FFP vs. NS after resuscitation and after 6 h

TEG thrombelastography/thromboelastometry, *PM* platelet mapping, *PF1 + 2* prothrombin fragment 1 + 2, *aPC* activated protein C, *PT* prothrombin time, *aPTT* partial thromboplastin time, *AL* adenosine (1 mM) + lidocaine (3 mM), *NS* normal saline, *LR* LR, *FFP* fresh frozen plasma, *HEX* HEX, *PRBC* packed red blood cells, *FWB* fresh whole blood, *PCC* prothrombin complex concentrate, *rFVIIa* activated recombinant factor VII, *TAT* thrombin-antithrombin complex, *PAI-1* plasminogen activator inhibitor-1, *SAHA*, suberoylanilide hydroxamic acid, *tPA* tissue plasminogen activator, *PAP* plasmin antiplasmin, *THAM* tris-hydroxymethylaminomethane, *CLP* cecal ligation and puncture, *ICAM-1* intercellular adhesion molecule 1, *E-Selectin* endothelial-leukocyte adhesion molecule-1, *A30* clot strength at 30 min, *EPL30* estimated percentage lysis at 30 min

Comparison to Human

Clinical studies have demonstrated development of a hypocoagulable state following trauma [97], even in the pre-hospital setting [98]. Interestingly, hypercoagulopathy has been reported in trauma patients as well, even in a greater proportion than those presenting with hypocoagulopathy [99]. There is a clear need to further study these phenomena to determine whether these are two distinct pathophysiologic responses to trauma. That is, there may indeed be two separate phenotypes, or one disease with rapid temporal dynamics that shift from hypercoagulopathy to hypocoagulopathy (e.g., disseminated intravascular coagulation and hyperfibrinolysis). Furthermore, the "lethal triad" of hypothermia, acidosis, and coagulopathy has been well documented [74, 100, 101]. The above animal models replicate these clinical conditions. However, the complex interplay between these three phenomena merits further study in animal models.

Models of Traumatic Brain Injury

Models

Local and systemic perturbations of the coagulation system have been reported in swine [46, 59, 61, 62, 64, 65] and rodent [58, 60, 63] models of TBI. Swine models are characterized by the need for a craniotomy prior to injury by either a fluid percussion injury [61, 62] or a computer-controlled cortical impact [46, 59, 64, 65]. In rodent models, a variety of commercially available impactor devices have been utilized, with or without previous craniotomies. All swine models included TBI combined with a secondary hemorrhage, whereas rodent models typically included isolated TBI.

Pathophysiological Aspects of Trauma-Induced Coagulopathy

Several aspects of TIC have been investigated in animal models of isolated TBI or TBI in combination with hemorrhagic shock (Table 34.2), including loss of coagulation cascade potential, platelet and endothelial dysfunction, as well as hyperfibrinolysis. None of these studies have, however, demonstrated an endogenous reduction in the coagulation potential without fluid resuscitation. Immediate activation of the coagulation system within minutes following injury has consistently been demonstrated in swine models of combined TBI and hemorrhagic shock [46, 62, 64, 102]. In these models, activation of the coagulation system, indicating a hypercoagulable state, was assessed by thrombelastography as well as prothrombin fragments 1 + 2 [46, 61, 64]. Interestingly, these changes were not mirrored in a similar model reporting on PT and PTT, potentially indicating different assay sensitivity to detection of a hypercoagulable state in swine. Despite varying periods of either partially resuscitated or unresuscitated shock (1–2 h), none of the studies reported transition to a hypocoagulable state following the shock period.

In contrast, several studies have reported on platelet dysfunction following TBI. A swine model reported a slight but significant reduction in adenosine diphosphate (ADP)-induced platelet aggregation as measured by platelet aggregometry minutes following a combination of TBI and shock [46]. This finding has been mirrored in rodent models of isolated TBI as measured by TEG platelet mapping assays [58, 63].

Systemic endothelial activation and dysfunction as measured by syndecan-1, a marker of endothelial glycocalyx shedding, have been reported in a combined TBI and shock model [64], although this was only associated with a hypercoagulable state. Similar findings have been reported in a rodent model of isolated TBI [60].

Local or systemic activation of natural anticoagulation as measured by activated protein C has been reported in two studies utilizing the same swine model of combined TBI and shock [59, 64], although none of these studies have reported an association with increased fibrinolysis.

Comparison to Human

Perturbations of coagulation, including a reduction in coagulation cascade potential, platelet dysfunction, and activation of natural anticoagulation through the protein C system, have consistently been reported in human TBI patients

[103–105]. Although several clinically relevant large and small animal models of TBI or combined TBI and shock have been developed, none have convincingly demonstrated coagulopathy as measured by conventional coagulation assays or thromboelastography. Several mechanistic aspects of TIC have, however, been reported in animal models of TBI. These include platelet and endothelial dysfunction as well as activation of the protein C system. As such, these models provide an important platform in which pathophysiological mechanisms or targeted interventions can be tested.

Models of Multisystem Trauma

Models

Non-TBI models of TIC include multiple injuries combined with hemorrhagic shock (HS). Laparotomy [66, 68, 106, 107], bone [68, 69, 107], and soft tissue injuries [69, 70] are commonly inflicted in tandem with exsanguination in rodent and swine models that simulate trauma resulting from multiple injuries (Table 34.2).

Pathophysiological Aspects of Trauma-Induced Coagulopathy

Combination of multiple injuries increases the severity of coagulopathy more than individual insults alone. For example, trauma + HS induces a more severe hypocoagulable state as measured by increased aPTT [66, 67] and elevated activated protein C [66] than trauma or HS alone. A more complex model of multiple insults revealed the dynamics of coagulopathy over a 4-h period. ROTEM data revealed an initial hypercoagulable state in the first 2 h following trauma, with a subsequent evolution of hypocoagulopathy by 4 h [68]. The ROTEM findings were supported by data from PT, aPTT, and quantification of clotting factors via enzyme-linked immunosorbent assay.

Several investigators have examined TIC in large animal models of complex trauma. Swine models have demonstrated a reduction in fibrinogen levels [69, 70]. Interestingly, a real-time functional assessment of coagulation revealed conflicting results. One study showed a significant reduction in clot strength and no difference in PT [69], but a similar model demonstrated no differences in any ROTEM parameters but a prolongation of PT [70].

Comparison to Human

Coagulation derangements in animal models resulting from multiple traumatic injuries have reflected clinical findings. Prolonged PT is a well-established predictor of mortality in trauma patients [67, 108]. Furthermore, the severity of traumatic injuries, as measured by injury severity score (ISS) and base deficit, positively correlates with PT and circulating concentrations of d-dimer and PF 1 + 2 [67, 108]. Brohi et al. [108] found that increased soluble thrombomodulin is associated with increased TAFI and protein C activation, as well as decreased fibrinogen utilization, suggesting a central role of thrombomodulin in TIC. Thus, animal models of multisystem trauma mirror findings from clinical studies. However, the hyperfibrinolysis that has been documented in clinical investigations has proven difficult to replicate in animal models.

Models of Hemodilution and Resuscitation-Induced Coagulopathy

Models

The effects of hemodilution and resuscitation have been investigated in multiple different models, predominantly using swine as the species of choice (Table 34.2). Reported models range from simple hemodilution [85] to complex models encompassing a full range of treatment regimens (pre-hospital, operative, and intensive care) including effects of injury modality, hypothermia, and acidosis [74]. Investigated interventions primarily include crystalloids such as normal saline or lactated ringers as negative controls [59, 74, 86, 89, 92, 93] compared to a study fluid of interest. These include synthetic colloids [74, 78, 79, 89, 92] or plasma or whole blood-based regimens [74, 85, 87, 88].

Pathophysiological Aspects of Trauma-Induced Coagulopathy

Models evaluating the effects of large-volume crystalloid resuscitation have reported hemodilution-induced coagulopathy. Studies have thus reported coagulation cascade derangements associated with crystalloid resuscitation as measured by conventional coagulation test [74, 76, 86], thrombin generation [82], fibrinogen metabolism/depletion [80, 82, 86], or coagulation factor levels [82]. Interestingly, these derangements have been associated with accelerated enzymatic coagulation cascade times as measured by the TEG r-time or the Sonoclot clot rate [84, 86, 89, 93]. One study by Wohlauer and colleagues did, however, fail to identify clinically relevant coagulation cascade derangements in a rodent trauma model of resuscitation with crystalloids that resulted in 50 % hemodilution [94]. Large-volume crystalloid resuscitation has also been associated with platelet dysfunction in a swine model [95] as well as disruption of the endothelial glycocalyx in rodent models [90, 91].

Models employing resuscitation with artificial colloids have demonstrated prolongation of conventional coagulation tests [74, 75, 79], reduced overall thrombelastographic measured clot strength [74, 81, 86], as well as prolongation of enzymatic reaction times [79], reduction in coagulation factor levels [65], and increase in fibrinogen polymerization [78, 86, 89, 92].

Interestingly, studies employing models of plasma-based resuscitation have consistently demonstrated reversal of coagulation cascade derangements [74, 87, 88] as well as preservation of platelet function [95] and the endothelial glycocalyx [90, 91].

Comparisons to Human

Animal models of resuscitation and hemodilution have successfully replicated clinically observed adverse effects of large-volume crystalloid resuscitation [109] as well as in vitro-observed coagulation impairment associated with starch-based resuscitation [110]. Clinically observed beneficial effects of plasma-based resuscitation [111] have also been replicated in a variety of animal models. Furthermore, resuscitation models have demonstrated elements of TIC observed in trauma patients, including endothelial glycocalyx shedding and platelet dysfunction [112, 113].

Conclusions

Interest in TIC has grown tremendously in the past decade, with an abundance of animal studies published in the past 4 years alone. This surge in TIC research has necessitated the development of carefully constructed and humane animal models as an important step between in vitro experiments and clinical investigations. Such models have not only improved our understanding of the underlying mechanisms of TIC, but also facilitated the testing of promising agents that may attenuate coagulation derangements. Rodent and swine models in particular have aided our understanding of TIC with the benefits of reproducibility and clinical realism, respectively.

In general, animal models have revealed the development of a hypocoagulable state following non-TBI trauma, but a hypercoagulable state following TBI. Yet, there are temporal dynamics in TIC that we may fail to fully capture and understand, both in animal and clinical investigations. For example, the development of a hypercoagulable state may in fact be common immediately following the insult. Rapid exhaustion of clotting factors, however, may create the appearance of a hypocoagulable state by the time samples are drawn when patients reach the hospital or animal blood is collected.

While animal models have been essential in the study of TIC, they do have certain shortcomings. A number of TIC studies do not collect post-injury data to describe the pathophysiology of TIC, instead choosing to focus on various resuscitation modalities (e.g., [83, 114]). Yet, animal [68] and human [99] studies continue to reveal important dynamics of TIC that are still poorly understood. Therefore, while resuscitation is indeed an important end goal of research in TIC, we suggest that continuing to collect pathophysiological data during injury and/or hemorrhagic shock, and before resuscitation interventions, is essential in improving our

collective understanding of TIC. Moreover, data at the post-injury/pre-resuscitation time point is necessary for demonstrating the establishment of TIC and subsequently measuring the efficacy of the therapeutic agent on restoring normal hemostatic function. In addition, as previously suggested, complex models of multiple injuries may accurately simulate the injuries sustained by trauma patients, but they may also pose difficulties in elucidating the role of each insult in TIC.

Finally, human studies have revealed that hyperfibrinolysis may develop in some patients following severe trauma and cardiac arrest [115–118]. To date, however, few investigators have successfully established animal models of hyperfibrinolysis [119, 120]. We suspect that this may be because most animal models lack the injury severity that may be necessary to induce hyperfibrinolysis. During cardiac arrest or severe hypovolemic shock (>60 % blood loss), continuous blood stimulation of the endothelium slows or ceases altogether. Such circulatory stagnation may signal to the endothelium that systemic hypoperfusion or disseminated intravascular coagulation (DIC) is occurring. To compensate, the fibrinolytic system is activated, which quickly overwhelms the circulatory system in an effort to restore tissue perfusion. In addition, hyperfibrinolysis has been observed in TBI patients, which may be a result of cerebral hypoperfusion, excessive tissue factor release, and subsequent activation of protein C. Yet overall, severe tissue hypoperfusion appears to have a central role in causing fibrinolytic derangements [103]. We therefore believe that a successful animal model of hyperfibrinolysis must involve severe injuries such as lethal or nearly lethal hemorrhage (50–60 %), systemic hypoperfusion (e.g., aortic cross clamp), acute traumatic brain injury, or a combination of these insults. While ethical standards must be upheld, developing animal models with greater injury severity may be the most promising avenue of replicating the hyperfibrinolysis observed in humans.

In summary, animal models of TIC have demonstrated an injury-dependent response of the coagulation system. Increased injury severity leads to more dramatic coagulation abnormalities, while TBI appears to induce unique derangements to the coagulation system. Animal models have proven to be essential in understanding TIC and testing therapies. Yet, future studies may benefit from detailed examination of the temporal evolution of TIC, collection of pre-resuscitation data, and developing models of hyperfibrinolysis.

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Mathematical Models of Hemostasis

35

Keith B. Neeves and Karin Leiderman

Introduction

There is a vast amount of information on the mechanisms of coagulation and its regulation by chemical, cellular, and biophysical processes. The complex and nonlinear nature of the coagulation pathway makes it difficult to intuit how changes in plasma protein levels affect, for instance, thrombin generation. Moreover, biochemical kinetics alone is not enough to characterize in vivo coagulation because its regulation is intimately tied to platelet and endothelial cell activation, transport processes, and the local hemodynamics. Fortunately, coagulation is one of the most studied biochemical networks in terms of network topology (which components interact with each other) and kinetic rate constants (how fast they work). It is these features and complexities that make coagulation particularly well-suited for mathematical modeling.

In this chapter, we present mathematical models of thrombin generation, thrombus formation, and fibrin polymerization. Mathematical models have been used to generate new hypotheses and make novel predictions about the threshold behavior of thrombin generation, some of which have been confirmed experimentally. More recently, some models have been used to identify risk factors for bleeding and thrombosis in retrospective studies. Based on the success of these studies, mathematical models have the potential to guide therapeutic strategies and develop novel approaches to reduce cardiovascular disease. Here, we highlight those models that have made, in our opinion, important contributions not only to revealing the fundamental mechanisms that regulate coagulation, but also that have shown the potential to contribute to clinical science.

As of this writing, the use of mathematical models in the field of hemostasis is limited to a handful of research groups. This is due, in part, to the complexity and sophistication of the models, but also to the fact that most of them are still in their nascent stages. Only a small number of models have been used beyond a few narrow studies and experimental validations have been performed only under very limited conditions. In our opinion, another major impediment is the translation from the developers (primarily mathematicians, engineers, and physicists) to the broader biomedical community. Part of the problem lies with developers, who have yet to build

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interfaces to these models that are accessible to the non-expert. Another problem lies with the fact that these two groups speak different languages. Specifically, the basis of most mathematical models of coagulation are differential equations, a subject that many biomedical researchers and clinicians either do not have exposure to, or, would rather not revisit. While it is not entirely necessary to understand the nuts and bolts of a computational model to use it, it can be instructive at least have an idea of how it works.

In the section "Primer on Differential Equations" we provide a primer on differential equations for the non-expert. Our hope is that this section will give the reader a qualitative understanding of how differential equations are formulated and used in coagulation modeling, without details about the numerical methods with which they are solved. In section "Mathematical Models of Coagulation and Fibrin Formation," we present models of coagulation that consist of systems of ordinary and partial differential equations, particularly highlighting those that contain features of blood flow and platelet-mediated coagulation. The coverage in this section is not intended to be exhaustive nor do we go into the mathematical details of any of the models. The goal here is to give the reader a flavor of the different types of computational models and their salient features. In section "Predictions and Applications of Mathematical Models," we discuss a few case studies of where mathematical models have made notable contributions to the field. In section "Future Challenges," we discuss challenges and futures research needs.

Primer on Differential Equations

Many computational models of hemostasis are based on systems of equations that describe dynamic processes; these processes could be biochemical, biophysical, biomechanical, or a combination thereof. The equations track changes in certain desired quantities (e.g., thrombin concentration, platelet density, blood flow velocity) from their prescribed, or, known initial values. Ordinary differential equation (ODE) models work under the assumption that quantities are well-mixed, that is, changes in these quantities are tracked only in time and there are no spatial variations. Partial differential equation (PDE) models have the ability to track quantities in both space and time so that complex spatial information can be extracted from them, for example, the heterogeneous composition of a clot. In this section, we give a brief mathematical primer on the derivation and meaning of the ODEs and PDEs found in many coagulation models. The aim of this section is to give the reader a qualitative understanding of how differential equations are formulated and used in coagulation modeling, without getting into the methods for solving them.

Spatially Homogeneous Ordinary Differential Equation Models

One type of mathematical model of coagulation simulates well-mixed biochemical reactions in time and not space. To better understand the well-mixed assumption, one can think of a test tube filled with clotting factors in which the concentration of each factor is the same at every spatial location, as if the contents were instantaneously and perfectly mixed. For the model, the initial factor concentrations and chemical reaction schemes that describe the factor interactions are known. The model results are in the form of concentrations tracked in time, for every species in the reaction schemes. To "track" these concentrations, mathematical equations are formulated using the reaction schemes and the law of mass action (LMA). For example, consider the following reaction schemes:

$$A + B \underset{k^-}{\overset{k^+}{\rightleftharpoons}} C, \tag{35.1}$$

$$F \xrightarrow{k} G. \tag{35.2}$$

The reversible reaction in Scheme (35.1) states that two reactants, A and B, come together to form a single product C at the rate k^+, and that C dissociates back into A and B at the rate k^-.

Scheme (35.2) is an irreversible reaction where a single reactant F is converted into a single product G at a rate k. The LMA states that the rate of reaction is often found to be proportional to the product of the reactant concentrations. Therefore, the rate of the forward reaction for Scheme (35.1), i.e., the rate of accumulation of the product C, is $k^+[A][B]$. The square brackets are used to denote concentration, but for simplicity, when writing rates from this point forward, we will drop the brackets so that $k^+AB = k^+[A][B]$. The rate of the backward reaction is simply the dissociation of the product, which happens at the rate k^-C. The rate of the reaction in Scheme (35.2) is $k\,F$. The proportionality constants, k^+, k^-, and k, are called *rate constants* and depend on the interaction thermodynamics between reactants and their shape and size.

We can now translate this information into mathematical equations. First we will consider tracking species C in reaction (35.1). Here the total rate of change of C is equal to the rate of the accumulation of C minus the rate of dissociation of C. Using the definition of these rates from the previous paragraph, we can express the total rate of change of C with the following mathematical equation:

$$\frac{dC}{dt} = k^+AB - k^-C. \tag{35.3}$$

Equation (35.3) is called an ODE. It is a *differential* equation because it relates the unknown, C, to its derivative, or how it changes, in time, dC/dt. It is *ordinary* because the dependent variable, C, depends on only one independent variable, t. Using the LMA, A and B have similar ODEs:

$$\frac{dA}{dt} = -k^+AB + k^-C, \tag{35.4}$$

$$\frac{dB}{dt} = -k^+AB + k^-C. \tag{35.5}$$

Together, Eqs. (35.3), (35.4), and (35.5) comprise a coupled (each equation depends on all of the others) system of three ODEs. The three coupled equations must be solved simultaneously and numerically given that the rate constants and initial concentrations for each variable are known. Since mass is conserved we can reduce the number of equations that need to be solved simultaneously from three to two. That is, A exists as either itself, A, or as a component of the product, C. So, if the initial concentration of A is denoted by A_0, then the sum of A and C remains constant in time:

$$A + C = A_0. \tag{35.6}$$

With this, C can be replaced in Eqs. (35.3), (35.4), and (35.5) by $A_0 - A$ so that one only needs to solve the two equations for A and B. Once A is known, C is calculated by Eq. (35.6).

Now consider the Scheme (35.2). The total rates of change of F and G are

$$\frac{dF}{dt} = -kF, \tag{35.7}$$

$$\frac{dG}{dt} = kF. \tag{35.8}$$

Here, the concentration of species F decays exponentially and whatever is lost is converted into G. Using conservation, $F + G = F_0$, where F_0 is the initial concentration of F so that one only needs to solve the ODE for F and then $G = F_0 - F$. Example solutions to both systems of ODEs are shown in Fig. 35.1.

Although the above examples are of elementary reactions, the same basic principles can be applied to the two-step enzyme reactions of coagulation. The most general reaction is one that includes a substrate, S, enzyme, E, complex, $E{:}S$, and product, P:

$$E + S \underset{k^-}{\overset{k^+}{\rightleftharpoons}} E:S \xrightarrow{k^{cat}} E + P.$$

Using the LMA, the ODE for the complex $E{:}S$ is

$$\frac{dE:S}{dt} = k^+ES - (k^- + k^{cat})E:S. \tag{35.9}$$

Similar equations can be formulated for the substrate, enzyme, and product.

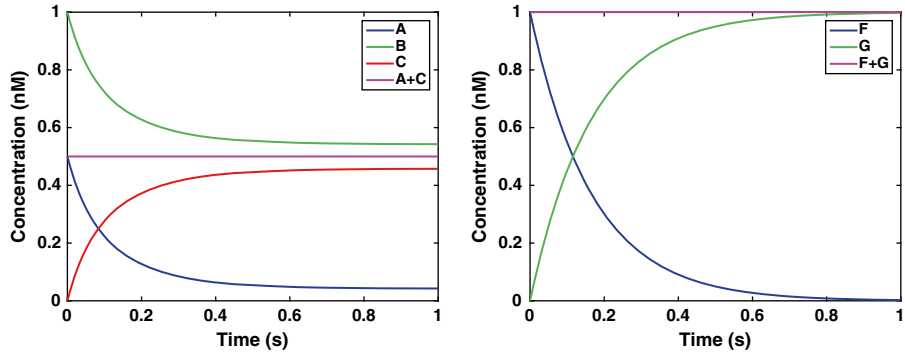

Fig. 35.1 Example solutions to systems of coupled ODE. Numerical solutions to Eqs. (35.3)–(35.5) (*left*) and (35.7)–(35.8) (*right*). Initial concentrations are $A_0 = 0.5\ nM, B_0 = 1\ nM, C_0 = 0, F_0 = 1\ nM$, and G_0=0. Rate constants set to $k^+ = 5 \diagup (nM \cdot s)$, $k^- = 0.5 \diagup s$, and $k = 6 \diagup s$. *Left*: species *A* and *B* are depleted as the product *C* is formed. The sum *A* + *C* remains constant. *Right*: species *F* is converted to species *G* and the sum *F* + *G* remains constant

Spatially Heterogeneous Partial Differential Equation Models

Tracking the reaction and motion of clotting factors in space and time is often desirable and requires the use of PDE models. These are different from ODEs in that the unknown dependent variable is now related to its derivatives taken in more than one independent variable. For example, *partial* derivatives in space (one dimension) and time look like $\partial F(x,t) \diagup \partial x$ and $\partial F(x,t) \diagup \partial t$, respectively.

So what *kind* of motion is modeled and how is it represented mathematically? The most common types of motion found in clotting models are *advection* and *diffusion*. Consider the motion of factor X (FX) in plasma. Advection is the process by which the FX molecules are carried by the bulk flow of the plasma; think of a leaf being carried in a stream. Diffusion describes changes in FX concentration due to random motion from thermal fluctuations. It causes the net flux of FX molecules to move from areas of high concentration to low concentration until no gradients exist. Here one can think of a single drop of blue food coloring in a cup of water spreading out until all the water in the cup is blue.

Let us return to species *F* from Scheme (35.2). Imagine a thin cylindrical tube with length *L* and constant cross-sectional area. Assume that the number of molecules of F inside this tube is changing due to (1) being carried along the length of the tube with velocity u by advection, (2) being spread out along the length of the tube by diffusion characterized by a coefficient D that depends on temperature, size and shape of the molecules, and (3) decay according to Scheme (35.2). For simplicity the motion is restricted to one spatial dimension. Letting $F(x,t)$ represent the concentration of *F* at location *x* along the length of the tube and time *t*, the rate of change of *F* is

$$\underbrace{\frac{\partial F}{\partial T}}_{\text{rate of change}} = -\underbrace{u\frac{\partial F}{\partial X}}_{\text{transport by advection}} + \underbrace{\frac{\partial}{\partial X}\left(D\frac{\partial F}{\partial X}\right)}_{\text{transport by diffusion}} - \underbrace{kF}_{\text{decay}}. \tag{35.10}$$

As with an ODE, obtaining the solution requires an initial condition, i.e., the initial distribution of molecules. Further, since a PDE involves space, conditions at the boundaries must also be specified. For example, one might want to specify a fixed concentration of molecules at a boundary, allow

molecules to enter/leave a boundary at a specified rate, or require that they remain inside the domain.

This advection–diffusion–reaction PDE can be derived from physical principles using conservation laws. With initial and boundary conditions, it can be solved analytically using standard mathematical techniques. Derivations and basic solution techniques are found in most PDE textbooks [27, 59]. Excellent references on mathematical modeling in biology and differential equations in the biological setting are [15, 55].

In higher dimensions, the PDE takes the form:

$$\underbrace{\frac{\partial F}{\partial t}}_{\text{rate of change}} = -\underbrace{u \cdot \nabla F}_{\text{transport by advection}} + \underbrace{\nabla \cdot (D \nabla F)}_{\text{transport by diffusion}} - \underbrace{kF}_{\text{rate of decay}} \tag{35.11}$$

where some short-hand notations are used; the bold **u** represents the velocity vector (a vector has information about both magnitude and direction), the ∇ represents the derivative of the function it operates on (F) in each spatial dimension.

Figure 35.2 shows a sample solution to Eq. (35.11) that represents the time evolution of a bolus of a chemical species in a fluid-filled channel. The chemical is advected with the fluid, diffuses in the fluid and is decaying at a rate determined by k. The velocity, **u**, flows from left to right and is set to be parabolic, i.e., highest velocity in the center of the channel and zero on the top and bottom walls. This is the shape of pressure driven flow through a tube as in a blood vessel. The plot on the top shows the initial bolus and the plot on the bottom shows the concentration after a short period of time. What the reader should notice is that (1) the flow carries the species from left to right (at a higher velocity in center of the tube), (2) the species spreads due to diffusion, and (3) the species decays by means of Scheme (35.2). These are precisely the three terms on the right-hand side of Eq. (35.11).

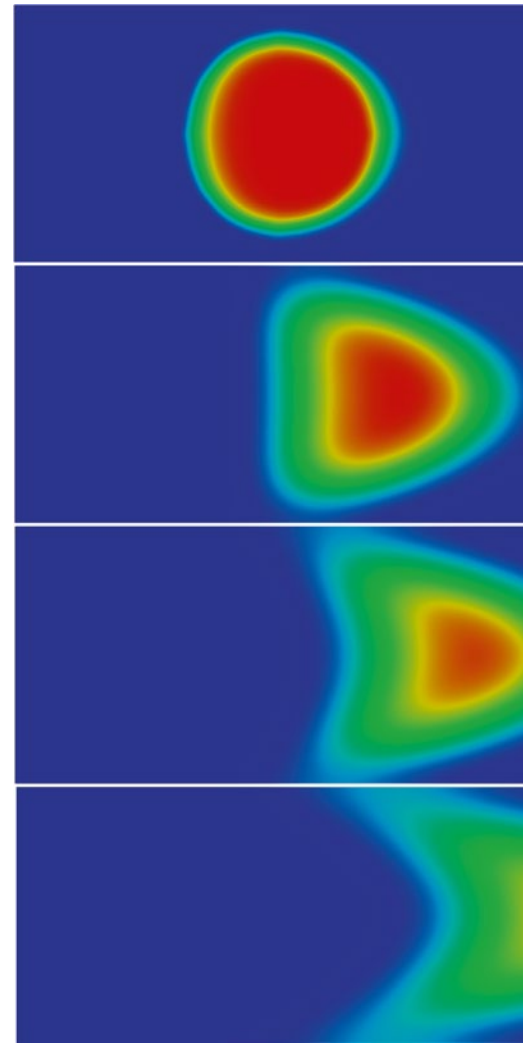

Fig. 35.2 Example solution of Eq. (35.11). Time evolution (*top to bottom*) of a bolus of a chemical species in a fluid-filled channel undergoing an irreversible reactions. The chemical is dispersed by the flow that runs from *left to right* and diffusion, which causes the species to spread out in all directions. The species decays by an irreversible reaction described by Scheme (35.2)

Treatment of Blood Flow

When considering blood flow in coagulation models it is necessary to either solve for the velocity vector **u** explicitly, or make some simplifying assumptions about the flow field and how it affects the transport of chemical species. The simplest approach is to treat transport as simply another rate process in the kinetic ODE that describes the consumption and generation of a component by biochemical reactions. This is done by calculating a mass transfer coefficient, which combines the influence of advection and diffusion on solute transport to a solid boundary

Table 35.1 ODE models of coagulation

Model	Initiation pathway	Static/flow	Phospholipids
Beltrami–Jesty [3]	Extrinsic	Static	Saturating
Hockin et al. [29]	Extrinsic	Static	Saturating
Chatterjee et al. [11]	Extrinsic, Intrinsic	Static	Saturating
Bungay et al. [6, 7]	Extrinsic	Static	Nonspecific lipid binding sites
Fogelson and co-workers [21, 22, 36]	Extrinsic	Flow	Explicit platelet binding sites

into a single rate constant. The mass transfer coefficient approach is valid for relatively small injuries where the well-mixed assumption can still be used and for cases where the velocity near the boundary does not change much during the formation of a clot. A slightly more complicated approach is to assume a certain flow profile, as we have done in Fig. 35.2, and insert the velocity vector into the PDE solute conservation equation. This approach is valid when the velocity vector either does not change with time or changes in a known manner. The most rigorous, but difficult, approach is to couple the conservation of solute equation (Eq. (35.11)) to the conservation of momentum equation. The conservation of momentum equation is a PDE that describes how fluid forces evolve in time and space. This approach is useful when the formation of the thrombus affects the blood flow, as is the case for arterial thrombosis.

Mathematical Models of Coagulation and Fibrin Formation

Mathematical models of coagulation and anti-coagulation pathways, fibrin polymerization, fibrinolysis, platelet adhesion, activation and aggregation have been developed that range from length scales of individual receptors to entire thrombi. These models can be categorized in terms of the biochemical phenomena they model and their coupling to biophysical processes. Here, we focus on ODE and PDE models of coagulation, including those that contain features of blood flow and platelet-mediated coagulation. Models of platelet adhesion, activation and aggregation in the absence of coagulation can be found in recent review articles [18, 20, 61].

ODE Models of Coagulation

Table 35.1 summarizes five of the seminal ODE models of coagulation developed over the last 20 years. These models are categorized based on extrinsic (tissue factor; TF) and intrinsic pathways (factor XII; FXII), treatment of binding reactions to lipid surfaces and the inclusion of blood flow. Thrombin generation models of the extrinsic pathway under static conditions are the most numerous and mature models. These models mimic ex vivo thrombin generation in a test tube or well plate initiated by TF in the presence of lipid membranes (synthetic or platelet derived).

ODE models have been central to elucidating the mechanisms of the threshold behavior of coagulation. Coagulation shows an activation threshold whereby a sub-threshold stimuli results in thrombin levels that are quickly neutralized by endogenous inhibitors and a threshold or higher stimulus results in a burst in thrombin generation that overcomes the inhibitors. This behavior serves a physiological function in that coagulation is not propagated by weak stimuli such as small changes in circulating procoagulant molecules (e.g., TF), and thus preventing systemic clotting. But, when sufficient stimuli is present such as at the site of a vascular injury or by elevated levels of procoagulant molecules following trauma, thrombin is produced quickly and at

levels that can activate platelets and catalyze fibrin polymerization.

Beltrami and Jesty show that threshold activation in coagulation is a characteristic of enzyme systems with feedback loops and inhibitors [3]. In the case of coagulation, thrombin provides positive feedback on its own production by catalyzing the formation of FVa and FVIIIa, which must overcome inhibition of thrombin and FXa by ATIII and FVa and FVIIIa by activated protein C (APC). Enzyme networks are initiated by low levels of enzymes such as small amounts of FVIIIa:FIXa or FXa. Their analysis on generic enzyme system predicts that sub-threshold stimuli can be greatly amplified by feedback, for example, of thrombin on FVIIIa, and in the case of threshold stimuli, feedback greatly increases total thrombin generation. A nice review of the structures of the various feedback mechanisms of coagulation, the mechanisms by which they can produce threshold behavior, and the possible role of thresholds in clotting system regulation can be found in [30].

The earliest ODE models of coagulation considered individual reactions or a small number of reactions, for example, to investigate the functional properties of the prothrombinase complex and the effects of hirudin and APC on thrombin generation [45, 65]. Jones and Mann were the first to describe the extrinsic pathway in significant detail including activation of FIX, FX, FV, and FVIII and the assembly of the FVIIIa:FIXa and FVa:FXa complexes [31]. This model includes 18 ODEs and is based on the reactions in a purified system of zymogens and enzymes [38]. Their model results are in good agreement with their own experimental measurements of thrombin generation in a purified system. The Jones–Mann model was extended by Hockin et al. [29] to include the inhibitors TFPI, ATIII, and a more detailed description of certain enzymatic and binding reactions. The extended model includes 34 ODEs and thrombin generation curves showed good agreement with those in a purified system with TFPI and ATIII for varying initial levels of prothrombin [8]. Variances in other initial concentrations were not compared. In both Jones–Mann and Hockin–Mann, coagulation is triggered with initial levels of TF and FVII/FVIIa where in the purified systems, TF:VIIa complexes were preformed. The activation of FX by FIXa and APC was later incorporated into the model [5, 9]. The Hockin–Mann model and its derivatives have served as the basis for other numerical analyses of coagulation including points of robustness and fragility [41] and sensitivity analyses of kinetic rate constants and plasma composition [13] (Fig. 35.3). A limitation of these models is the assumption that lipid surfaces are in excess, and therefore, does not address the regulatory role of the lipid surface. Moreover, validation of the models against empirical results has only been shown under very specific conditions and/or by fitting parameters of the model to the results.

Chatterjee, Diamond, and colleagues built a "platelet-plasma" ODE model to describe the initiation of coagulation in the absence of initial levels of TF in diluted whole blood (Fig. 35.3) [11]. The model, made up of 76 ODEs, includes the extrinsic pathway based on the Hockin–Mann model, with the addition of FXIa and FXIIa dependent thrombin generation, activation-dependent platelet phosphatidylserine, and fibrin formation. It also describes the kinetics of the fluorogenic thrombin substrate boc-VPR-AMC, which can act as a competitive substrate from thrombin, slowing down positive feedback. This explicit treatment of the thrombin substrate also provides a more faithful comparison to experiments that use boc-VPR-AMC as a thrombin reporter. The inclusion of the intrinsic pathway was necessary to predict experimental observations of coagulation in the absence of TF, even when treated with the FXIIa inhibitor corn trypsin inhibitor (CTI). Importantly, the model predicts, and thrombin generation experiments confirm, that FXIIa can leak past high concentrations of CTI and initiate coagulation. Phospholipids are provided by platelets in this model and it was shown that platelet activation by the GPVI agonist convulxin dramatically reduces the initiation time for coagulation compared to unstimulated platelets.

The initiation, amplification, and propagation of coagulation require phospholipid surfaces that support the assembly of the enzyme complexes

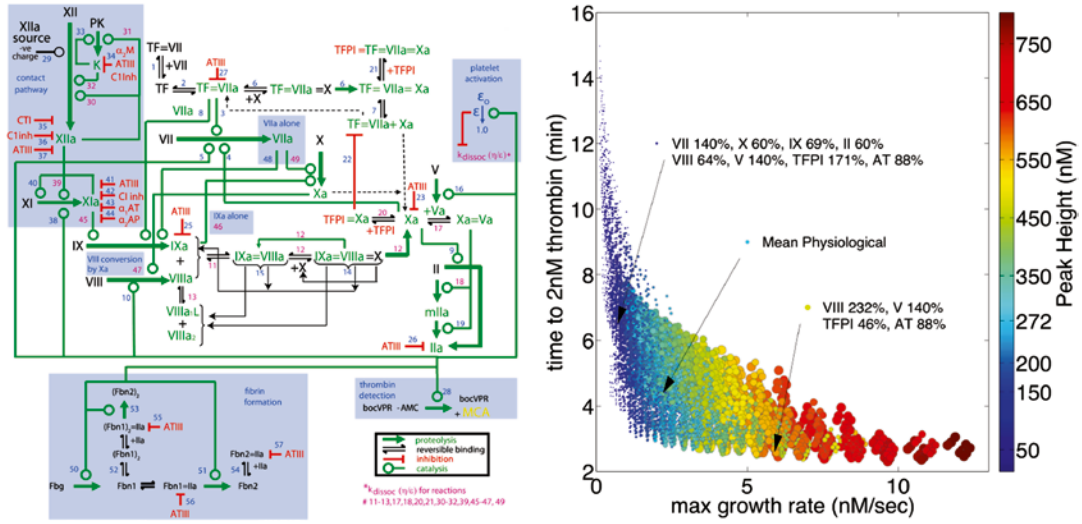

Fig. 35.3 ODE models of coagulation. *Left*: wiring diagram of the Chatterjee–Diamond platelet-plasma model [10]. *Right*: thrombin generation phenotypes in an hypothetical population defined by normal range variation in factor levels based on the Hockin–Mann model [13]

TF:FVIIa, FVIIIa:FIXa, and FVa:FXa. The models discussed above assume lipids are in excess and thus do not explicitly account for the binding reactions and/or finite number of binding sites. Bungay and Gentry developed a model in which association and dissociation to a finite concentration of lipids are explicitly modeled in a system of 31 reactions and 73 ODEs [7]. Although more than a few reactions in the Bungay–Gentry models differ from those in the Hockin–Mann model, results from both models seem to be in qualitative agreement with results from the same empirical study [8].

Simulations of the Bungay–Gentry model showed that the lag time, peak thrombin concentration, and duration of thrombin generation are strongly influenced by lipid concentration up to a saturating concentration of 300 nM. No thrombin is produced for less than 10 nM lipids, thus providing another threshold that is necessary for thrombin generation. While it is not surprising that thrombin generation is dependent on lipid concentration, the model does make nonintuitive predictions with respect to the effectiveness of different inhibitors. For example, their results show that TFPI almost completely inhibits TF at an intermediate lipid concentration of 150 nM, but is limited at low lipid concentrations by low levels of FVIIa and FXa production (the enzymes responsible for TFPI activation) and at high lipid concentrations because most FXa is bound to lipids. The effectiveness of APC in inhibiting FVa and FVIIIa also shows a strong dependence on lipid concentration. The Bungay–Gentry model was later extended to study thrombin production in human ovarian follicular fluid [6]. Results show that the amount of thrombin produced in ovarian follicular fluid is much lower than that in plasma, supporting the hypothesis that in follicular fluid thrombin functions to initiate cellular activities via intracellular signaling receptors.

The models discussed thus far all simulate coagulation under static conditions. These are appropriate models for specific clinical clotting assays and perhaps even clotting under conditions of stasis such as extravascular bleeding or deep vein thrombosis. However, most hemostatic and thrombotic clots form under conditions of blood flow and where TF is localized at the vessel wall. Furthermore, only the Chatterjee–Diamond model accounts for the role of platelets in mediating coagulation. In the cell-based model of thrombus formation [43], activated platelets with exposed phosphatidylserine (PS) are the primary

surface for the propagation of coagulation. Thus, a model without platelets is unlikely to capture the dynamics of coagulation in vivo.

Kuharsky and Fogelson developed an ODE model that combined coagulation, blood flow, and platelet function [36]. The model considers blood flowing over a 10 μm patch of TF where, because of the small size of the injury, the well-mixed assumption is justified. The transport of zymogens, co-factors, inhibitors, enzymes, and platelets to and from the injury site are modeled with mass transfer coefficients, as discussed in section "Treatment of Blood Flow." Platelets are treated like chemical solutes and are allowed to accumulate with kinetics based on experimental measurements of platelet adhesion and are activated by both surface bound (e.g., collagen) and soluble agonists (ADP or thrombin). Platelets can physically block access to surface bound enzyme complexes such as TF:FVIIa as they adhere and aggregate. The model shows that thrombin generation has a threshold-dependence on surface concentration of TF. Below the threshold concentration of TF, little to no thrombin is generated, above it thrombin generation becomes almost saturated. Interestingly, the model also shows that dilution by flow and blocking access to wall-bound TF by adherent platelets are the *primary mechanisms* that inhibit coagulation and are *more potent* than TFPI and APC pathways, at least for small injuries [21]. The model has been extended to include activation of FXI by thrombin and FIX by FXI [22]. Simulation results predict that the effect of a severe FXI deficiency depends on the platelet count, and that FXI becomes more important in coagulation at high platelet counts.

In summary, existing ODE models of thrombin generation have shown qualitative agreement with empirical results under limited conditions in purified, closed systems. If phospholipid surfaces are in excess, then a qualitative description of thrombin generation is described by ODE rate equations of the extrinsic pathway, intrinsic pathway, and inhibitors as described in the Hockin–Mann and Chatterjee–Diamond models. If the amount of phospholipid surface is limited and can be saturated, then additional ODEs are required that describe the binding kinetics to these surfaces as described by the Bungay–Gentry model. An ODE model of coagulation under flow requires additional terms to describe the rate of transport of solutes and platelets to and from an injury site as described by the Kuharsky–Fogelson model. A unifying theme and outcome from all of these models is the concept a of threshold stimuli needed to induce robust thrombin generation. These models all use the "well-mixed" assumption such that time is the only independent variable. This is a good assumption when a system is homogenous, as in a well-plate assay, or when an injury is small. However, when this assumption begins to break down, for example, in large injuries, PDEs are necessary to account for variations in space.

PDE Models of Coagulation

Table 35.2 summarizes four PDE models of coagulation that investigate the spatial dependence of thrombin generation, fibrin formation, and thrombus growth. All models consider thrombin generation initiated by TF immobilized on a surface. However, they each couple the biochemical reactions pathways of coagulation with transport mechanisms (Eq. (35.10)) including surface diffusion within a phospholipid membrane [4], diffusion away from a TF-rich wall [14, 34, 52], and thrombus formation on a TF-rich wall under flow [39, 40, 68, 70]. These models reveal important biophysical mechanisms that regulate coagulation.

Beltrami and Jesty extended their solution phase ODE model [3] described in section "ODE Models of Coagulation" to investigate model enzyme feedback networks on the surface of membranes [4]. Here, a PDE is used to describe the evolution of enzyme generation on a phospholipid membrane where enzymes and zymogens are transported by flow in the solution phase and by diffusion in the membrane phase. Similar to the predictions from the Kuharsky–Fogelson ODE model, flow is a profound inhibitor of coagulation, and at sufficiently high flow rates, more potent than the biochemical inhibition. This model also provided important predictions about

Table 35.2 PDE models of coagulation

Model	Pathway	Type of PDE	Spatial dimensions, biophysical phenomena
Beltrami–Jesty [4]	TF	Diffusion–reaction	1, surface diffusion
Ataullakhanov and co-workers [14, 34, 52]	TF	Diffusion–reaction	1, excitable wave
Leiderman–Fogelson [39, 40]	TF	Diffusion–reaction–advection	2, flow in a vessel
Xu et al. [68, 70]	TF	Diffusion–reaction–advection	2, flow in a vessel

what is the minimum injury size that can induce significant enzyme production. For the base case parameters in this model, the membrane patch must be greater than $5\,\mu$m, but that size depends on the kinetics of the enzyme and inhibition reactions, as well as the flow rate and binding site density. A simplified analytical model predicts that a minimum threshold patch size is needed when the diffusivity of bound proteins is considered. This prediction was later confirmed experimentally for both static and flow conditions [33, 57].

Ataullakhanov and colleagues developed a series of mathematical and experimental models of the spatial-temporal dynamics of thrombin generation and fibrin formation initiated on a procoagulant wall in the absence of flow [25]. Here, coagulation acts like an excitable media, where once initiated by TF bearing cells or lipid on a surface, travels like a wave away from the surface [49, 51]. Different factors play unique roles at different times and distances from the surface. Initiation is controlled primarily by the concentration and distribution of TF, and is insensitive to FVIII, FIX, or FXI levels [2, 49]. However, propagation away from the TF-rich surface is controlled by thrombin feedback mechanisms that produce FVa, FVIIIa, and FXIa [14, 50]. Interestingly, thrombin generation will continue to grow for hours over several centimeters even in the presence of ATIII and TFPI. Only by the addition of thrombomodulin (TM) does the thrombin wave arrest [14, 52]. These reaction–diffusion models may be particularly apt for describing extravascular clot formation where blood flow is low or absent.

Leiderman and Fogelson developed a two-dimensional model of thrombus formation under flow that accounts for coagulation biochemistry, including surface-dependent reactions, as well as platelet activation and deposition [39]. The model extends the Kuharsky–Fogelson model to treat spatial variations in concentrations discussed in section "ODE Models of Coagulation," heterogeneities within the thrombus, and flow disturbances caused by the growing thrombus. Platelets are treated as solutes, rather than as discrete particles. The thrombus is treated as a porous material which allows for analysis of intra-thrombus fluid and solute transport, and reveals that diffusive solute transport aids upstream thrombus growth. Results also show that thrombus growth is strongly influenced by wall shear rate and the near-wall excess of platelets and that thrombus permeability strongly impacts growth and structure. Motivated by experiments that suggest an important role for intra-thrombus transport [58], the Leiderman–Fogelson model was extended to further explore the effect of hindered transport of proteins within the thrombus [40]. Results show that early growth is promoted because platelets shelter enzymes from the flow, and that later growth is attenuated because enzymes are confined to the interior core of the thrombus and the supply of substrates to them is reduced (Fig. 35.4). With the transport hindrance, the final thrombus that emerges from the model is smaller, has a dense core and a less dense shell similar to thrombi described in the laser injury model [58].

Xu and colleagues developed a model of thrombus formation that includes several submodels that exchange information with each

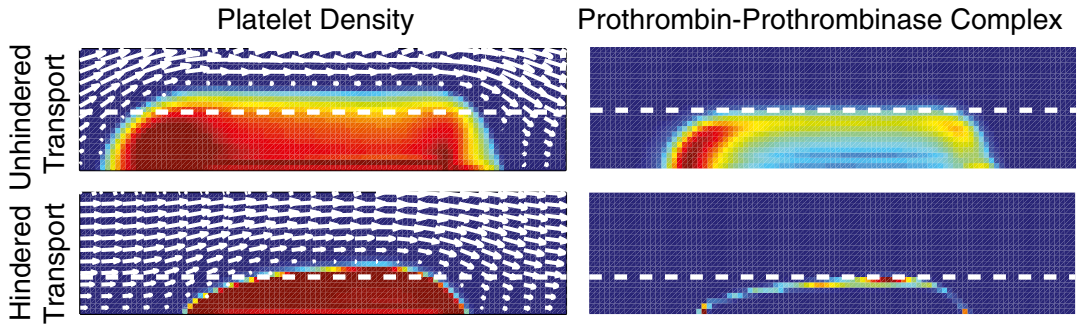

Fig. 35.4 Simulations of thrombus growth in a model of platelet deposition and coagulation chemistry under flow in the Leiderman–Fogelson PDE model [39, 40]. A thrombus is modeled as a porous medium that allow for interstitial fluid and solute transport. The rate of interstitial fluid flow decreases as the density of bound platelets increases. For "hindered transport" simulations, the diffusion and advection of proteins was further hindered to reflect their macromolecular size. *Left*: hindered protein transport produced smaller thrombi (*bottom*) with a uniformly highly dense (*dark red*) core of platelets compared to the unhindered case. *Right*: hindering protein transport severely limits the ability of fluid-phase prothrombin to penetrate thrombus to reach platelet-bound prothrombinase (*bottom*) and so greatly reduces thrombin production

other to simulate the interplay between platelet function, coagulation, and flow [68–70]. The coagulation submodel is based on the Hockin–Mann ODE model of coagulation, and binding reactions and binding site density are based on the treatment by Kuharsky–Fogelson. In contrast to the Leiderman–Fogelson models, platelets are modeled as discrete particles, rather than as a solute. Such particle-based simulations provide a direct coupling of platelet motion, adhesion, and aggregation to the forces imparted on them by the flow. It also allows the model to track the history and activation state of each platelet. This feature allows for identification of potential structures and conditions that result in embolism. Porosity and permeability properties of thrombi were based on intravital imaging data in a laser injury model. The model predicted that low levels of FVII in blood result in a significant delay in early stage thrombin production in thrombi formed under venous conditions in agreement with in vivo data in mice expressing low levels of FVII [70]. This type of model is considered "multiscale" because the different submodels operate at different length scales. Microscale phenomena like coagulation reactions and platelet adhesion are tied to macroscale phenomena such as blood flow.

Fibrin Polymerization and Deposition

ODE and PDE models of fibrin polymerization have been reported under both static and flow conditions, but have yet to be integrated into full coagulation models as described above. An ODE model of fibrin polymerization kinetics includes cleavage of fibrinopeptide A by thrombin, formation of protofibrils by fibrinogen monomers and oligomers, and lateral aggregation of protofibrils into fibers [63]. The model is in good agreement with turbidity and electron microscopy data. The effects of fibrinopeptide B cleavage, thrombospondin, and ionic strength can be simulated by varying kinetic rate constants. This is a good example of how a simple model can capture a wide range of dynamics even if the details of all known mechanisms are not included in the model.

There is a well-established relationship between thrombin concentration and gel structure, where higher thrombin concentration yields gels with thinner fibers and higher degrees of branching that are less susceptible to fibrinolysis [62]. The branch point density determines, in part, the mechanical properties of a fibrin clot, but the branching process itself is poorly understood. Fogelson and Keener developed a kinetic fibrin gelation model that considers how two types of

reactions—bimolecular linear protofibril extension and trimolecular branch formation—can form different gel structure based on the supply rate of fibrin monomers [19]. The kinetic gelation model tracks the molecular weight of polymer chains with a single PDE and gelation is defined by when the average cluster size approaches infinity. Higher supply rates of fibrin monomers, which would correlate to higher thrombin concentration, lead to faster gelation, higher branching, and shorter fibers. These results agree qualitatively with experimental results.

The morphology and density of fibrin formed under flow conditions is dramatically different than fibrin gels formed under static conditions. Instead of a voluminous hydrogel that is mostly water, fibers form a dense mat of fibers emanating from activated platelets or procoagulant particles [48, 54]. There is an inverse relationship between shear rate and fibrin deposition in vitro flow assays [64]. Neeves et al. described the transport limitations of fibrin formation in a purified system where thrombin emanates from a wall at a known rate into a flowing stream of fibrinogen [44]. A PDE model of fibrinogen and thrombin concentration profiles shows that at shear rates exceeding $\sim 10\,s^{-1}$, fibrin deposition is limited to very thin boundary layer near wall and that at shear rates of $>100\,s^{-1}$ dilution of fibrin monomers is faster than the rate of gelation. Guy et al. used a kinetic gelation model, in combination with PDEs to describe the transport of prothrombin, thrombin, and fibrinogen, to predict the height of fibrin gels formed under flow on a wall capable of converting prothrombin to thrombin [26]. At low shear rates, thrombin concentration limits gel growth such that with increasing shear rate the dilution of thrombin reduces fibrin deposition. At high shear rates, fibrin deposition is limited by the ability of prothrombin to penetrate through the existing fibrin gel to the surface. The penetration of solutes into and out of the gel is a function of a physical property called the permeability, which is essentially a measure of the pore size of the gel. Gels with low permeability have small pores that hinder the transport of solutes by both convection and diffusion. The permeability of fibrin gels varies over several orders-of-magnitude depending on the fiber density [66]. The predicted gel heights from this model are in agreement with fibrin gels formed under flow on TF-coated microparticles [48].

Predictions and Applications of Mathematical Models

Many of the models in section "Mathematical Models of Coagulation and Fibrin Formation" have been partially validated against experimental data. However, validation is just the first step in testing the utility of a model. The ultimate test of these models is whether they can make predictions, assess risk, and guide therapeutic decisions. A few examples are highlighted in this section that lead to either mechanistic insight or clinically relevant predictions.

The computational power of a desktop computer or standard laptop can solve hundreds of ODEs in a matter of minutes and, in some cases, seconds. As such, ODE models are ideal for exploring how varying ranges of parameters affect model results. In other words, thousands of computational experiments can be conducted in a very short amount of time. One such experiment is a sensitivity analysis, which is an extensive study of the sensitivity of model outputs to model inputs. Analyses of this type would be either prohibitively expensive or impossible in the laboratory. For example, Danforth et al. examined the sensitivity of thrombin generation to the input values of the rate constants in a modified version of the Hockin–Mann model [12]. In this study, each of the 44 rate constants were varied by one order-of-magnitude up and down from their reported literature value. Results showed thrombin generation to be most sensitive to reactions involving the regulation of the formation and function of the TF:FVIIa complex. This identification points to where improvements in measurement accuracy might be most important if the model is to have reliable predictive capabilities. The same group used their ODE model to predict how changes in plasma composition could affect thrombin generation [13]; this means they varied initial concentrations (initial conditions to the

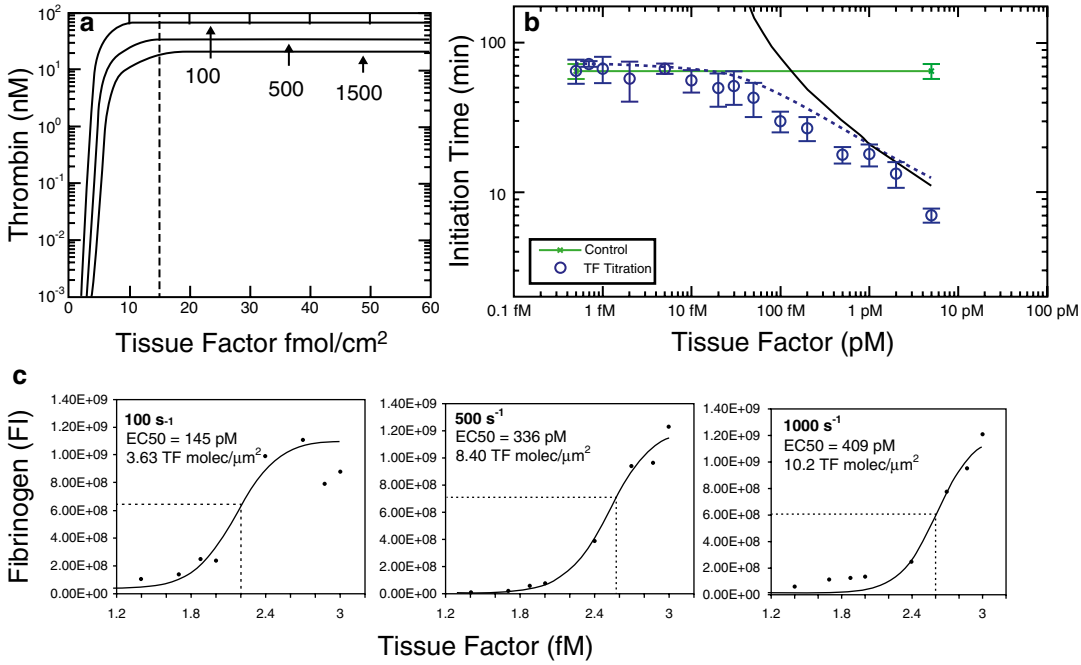

Fig. 35.5 Importance of biophysical mechanism. Kuharsky–Fogelson [36] predicted that thrombin production showed a threshold dependence (**a**) that was confirmed in experiments performed with platelets and flow [46] (**c**) but not without [10] (**b**)

ODEs) rather than the kinetic rate constants. As expected, most variation in the normal ranges did not significantly perturb thrombin dynamics, but a few factors such as TFPI and prothrombin levels had a significant influence. This analysis also identified pairs of factors with significant influence over thrombin generation. TFPI and antithrombin together were shown to be particularly potent at changing clot time. Using patient plasma levels as inputs into these models could be a way to identify thrombotic risk. In a cohort of 65 patients on warfarin, the three patients with highest predicted thrombin generation rate had thrombotic events. The model was also applied to cohort of patients anticoagulated with warfarin following atrial fibrillation and identified which patients may be at potential risk for a thrombotic event [23].

With their ODE model, Kuharsky and Fogelson predicted that thrombin production will show threshold dependence on the amount of exposed TF. This prediction was later confirmed in experiments with whole blood perfused of micropatterned TF [46]. In addition, the model was able to provide kinetic explanations for reduced thrombin production with hemophilia A and B and proposed the idea that platelets 'pave over' the subendothelium, covering enzyme complexes and physically inhibiting their activity. This mechanism, also confirmed experimentally [28] implies and highlights the idea that platelets have an important anticoagulant role, not just their well-recognized procoagulant ones. This balance is the foundation of many of the models behaviors including the aforementioned TF threshold. Even more importantly, the TF threshold was seen in experiments performed with platelets and flow [46], but not without them [11] (see Fig. 35.5). These data emphasize the importance of biophysical factors on the coagulation system and further promote the inclusion of them in mathematical models.

The Leiderman–Fogelson model was used in combination with a microfluidic vascular injury model in a cohort of FVIII deficient patients [47]. Here, the model was critical in interpreting results, specifically the relative FXa generation by intrinsic and extrinsic Xase, that could not be

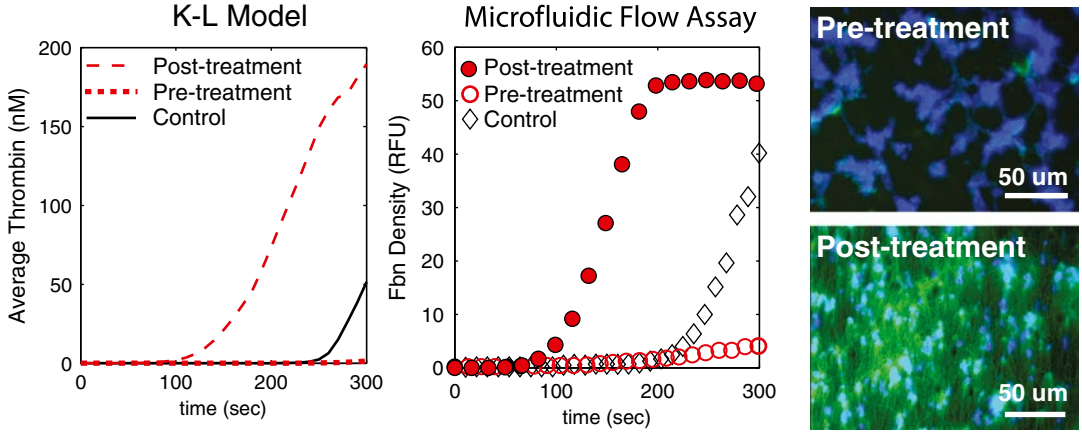

Fig. 35.6 Modeling bypass therapy in hemophilia. A patient with severe FVIII deficiency that had developed inhibitors was treated with 90 μg/kg recombinant FVIIa (rFVIIa). *Left*: predicted average thrombin concentration in the growing thrombus as a function of rFVIIa and FVIII levels in the Leiderman–Fogelson model. *Center*: fibrin deposition pre- and post-treatment in comparison to a healthy control in a microfluidic vascular injury model. *Right*: overlay of platelet (*blue*) and fibrin (*green*) accumulation pre- and post-treatment on a collagen-TF surface following perfusion for 5 min at a wall shear rate of 150 s^{-1}

measured experimentally. The model was also able to explain why platelet aggregation, but not fibrin deposition, was different between patients with severe and moderate FVIII deficiencies based on predicted thrombin concentrations. A decrease in platelet aggregate size corresponds with the predicted drop in local thrombin concentrations that approach sub-threshold concentrations of thrombin ($\sim$1 nM) for activating PAR1. Two patients received bypass therapy (rFVIIa), which resulted in a decreased lag time and increased cumulative fibrin deposition in the microfluidic model. The computational model predicts faster assembly of the TF:FVIIa complex owing to a higher flux of FVIIa being delivered to the surface compared to endogenous FVIIa levels (Fig. 35.6). Both microfluidic and computational models suggest that treatment of FVIII deficiency with rFVIIa could lead to prothrombotic risks in agreement with clinical observations [1].

Future Challenges

There are two major research areas of mathematical models of hemostasis: static coagulation as in thrombin generation assays and in vivo events involving blood flow in the context of bleeding, hemostasis and thrombosis. Models in both areas have potential to answer different but important questions. Static models with lipids could be used to probe the coagulation network and look for synergy of factors when considering therapeutics. An area of future research could be the addition of a more sophisticated treatment of platelet function into a static model which would improve our understanding of the role and function of platelet binding sites. Dynamic models show us that the biochemical, cellular, and fluid mechanical processes are truly and intricately intertwined. Because these models are more closely related to the in vivo situation, they could potentially be used to improve the development of intravascular devices, therapeutic strategies related to both the growth and dissolution of thrombi, and patient-specific therapies in conjunction with imaging data and clinical phenotyping.

There are challenges and room for improvement with both static and flow models. As mentioned above, the ODE models of static coagulation that mimic thrombin generation assays have only been validated under limited conditions. For example, in the Hockin–Mann study, their model was shown to agree with empirical results where prothrombin levels were

varied and the Bungay–Gentry model was shown to agree qualitatively with data from the same empirical study. However, the two models differ greatly. For example, the Bungay–Gentry model includes (1) the positive feedback reaction where Xa activates TF:VII and generates more Xa and (2) FIX activation by FXIa and FXI activation by thrombin. The Hockin–Mann model does not include any of these reactions, yet provides similar results. A useful and predictive model should be robust; it should be tested and validated under many differing conditions such as varying all initial coagulation factor concentrations including those that mimic hemophilic conditions, and also can predict the effect of anticoagulant drugs.

A major challenge in developing in vivo models of coagulation and thrombus formation is integrating phenomena that occur over multiple length and time scales. Such "multiscale" problems are common in biological systems and pose their own unique computational challenges. For example, a detailed model of thrombus formation would include receptor-ligands kinetics, outside-in and inside-out signaling pathways of platelets, coagulation reactions, platelet shape change and retraction, fibrin polymerization, fibrinolysis, and the influence of forces and flows on each of these processes. These phenomena span length scales of molecules to tissue, and times scales of nanoseconds to days. No one model can possibly capture the dynamics of all these processes, so one challenge is the passing of information between various submodels. In coagulation modeling, this has been done by Xu and colleagues as described in section "Mathematical Models of Coagulation and Fibrin Formation" using submodels for coagulation reactions, platelet function, blood flow, and the interface between platelets and flow [68]. Here, dynamics of blood flow are solved at the vessel scale and the interactions of platelets with the vessel wall are solved at the cell scale. In platelet modeling, Flamm et al. used a neural network to train a patient-specific intracellular calcium model coupled to stochastic and deterministic models of fluid flow and solute transport [17]. Interestingly, the model identified a blood donor with indomethacin resistance that turned out to have an unreported thromboxane receptor mutation. These are two examples of how multiscale models may have a future in identifying phenotypes and response to therapies.

The geometry and fluid mechanics of most hemostasis models consider only the simplest cases, specifically thrombus formation on the wall of a straight, flat, and rigid channel under a constant, laminar flow. The in vivo situations are significantly more complicated since vessels have complex geometries, are elastic, and flow can be pulsatile or even turbulent [35]. There are extensive modeling efforts of blood flow in large arteries, heart valves, stenoses, and other complex geometries [42, 56, 60], however, these models have yet to be coupled to detailed coagulation or platelet models. Models presented here are two-dimensional, and only recently have numerical methods and computational power allowed for simulation of vessel scale, three-dimensional blood flow [16, 24].

The models reviewed in this chapter all describe a thrombus as a rigid body, that is, they do not deform with force. However, the primary components of thrombi (fibrin, platelets, red blood cells) are elastic materials that have complex stress–strain behavior [37, 67]. Moreover, platelets feel and response to their surrounds [53], but there are no models that explicitly treat platelet retraction. The temporal evolution of thrombus composition and mechanics over time scales relevant for fibrin formation and fibrinolysis has been reported [32], but mechanical properties have yet to be incorporated into models with detailed treatment of coagulation.

In summary, mathematical models of coagulation have already made important contributions to the field of hemostasis in terms of revealing new mechanisms that regulate this complex, nonlinear process. Some of these models are beginning to show promise in making clinical relevant predictions, but there is much work to be done in terms of validating existing models against larger data sets and experimental conditions. Moreover it is unclear which mechanisms, and at what level of detail, must be included in these models to capture the essential dynamics of a system, be it ex vivo or in vivo coagulation. Nevertheless, multidisciplinary teams that include inputs from

mathematicians, engineers, physical scientists, biologists, and clinicians are likely to generate models that yield outputs that can guide the development of therapeutic interventions and cater these interventions to individual patients.

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