Coagulopathy after adult and pediatric trauma

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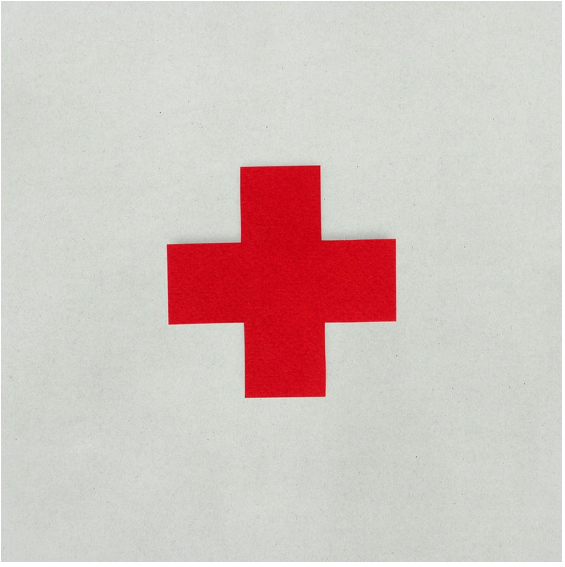

Coagulopathy after Adult and Pediatric Trauma

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Coagulopathy After

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Voor mijn lieve ouders.
Ondanks alles, maar dankzij jullie.

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Chapter 1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

BACKGROUND

Trauma is the leading cause of death between ages 1 and 46 – hemorrhage is a major cause of this mortality during the first 24-48 hours after injury.[1] One process leading to uncontrollable hemorrhage is acute traumatic coagulopathy (ATC); a disorder of the blood clotting system occurring early after trauma. Efforts to control hemorrhage and limit ATC are the cornerstones of an early therapeutic approach to traumatic injuries, as abnormalities in coagulation parameters commonly follow major injury in adults. The understanding of ATC pathogenesis has significantly changed in the past decade and continues to advance rapidly. The classical description of ATC explains it as a loss, dilution or dysfunction of the coagulation proteases. Loss is attributed to bleeding or consumption, dilution to fluid administration and massive transfusion, and protease dysfunction to hypothermia and the effect of acidemia on enzyme function.[2] But in 2003, a retrospective study of admission coagulation results of 1088 trauma patients showed that almost 25% of patients arrived to the trauma room with a clinically significant coagulopathy prior to the administration of significant volumes of fluids or other interventions.[3] Patients with ATC were four times more likely to die than those without. The occurrence of early coagulopathy has been substantiated by other study groups with similar results across 20,000 patients.[4-7] Potential mechanisms for this coagulopathy have been tested and currently the drivers of ATC appear to be multitude; instead of a dysfunction of the coagulation proteases, it appears to develop due to activation of anticoagulant and fibrinolytic pathways.

What is known about the pathogenesis of trauma induced coagulopathy? Anticoagulation is a primary component of ATC after trauma. Tissue injury results in a pro-inflammatory response and the endothelium expresses thrombomodulin which forms complexes with thrombin to divert it to an anticoagulant function. The formation of thrombin-thrombomodulin complexes activate protein C, known as the protein C pathway. This coagulation pathway serves as a major system for controlling thrombosis, limiting inflammatory responses, and potentially decreasing cell apoptosis in response to inflammatory cytokines and ischemia at the cellular level.[8] Thrombin bound to thrombomodulin is inactivated by plasma protease inhibitors, which results in increased clearance of thrombin from the circulation. However, in the early phases after trauma, during the presence of shock

and hypoperfusion, thrombomodulin levels are high and result in increased formation of thrombin-thrombomodulin complexes and widespread activation of protein C and decreases stable clot formation. When present in excess, activated protein C inhibits the extrinsic pathway through cofactors V and VIII and promotes fibrinolysis (or clot breakdown) through inhibition of plasminogen activator inhibitor-1 (PAI-1). Without the inhibition of PAI-1, tissue plasminogen activator (tPA) is free to enhance the formation from plasminogen in to plasmin and thereby enhance fibrinolysis.[9] Trauma also results in an inflammatory host response as the protein C system has anti-inflammatory properties related to both its anticoagulant activity and to cytoprotective properties independent of the coagulation cascade.[10] Recent clinical data shows that release of activated protein C after severe trauma could mitigate sterile inflammation and organ injury induced by the extracellular release of histone proteins after severe injury.[11] Similar results have been reported in experimental models of sepsis.[15] This indicates that coagulopathy is an endogenous response to injury involving both the coagulation system and the immune system. Histone proteins have been described to modulate this immune response through the formation of 'neutrophil extracellular traps (NETs) and killing bacteria.[13-17] In hemostasis, histones shift the hemostatic balance toward hypercoagulation. Histones bind to both protein C and thrombomodulin and impair protein C activation.[18] This makes histones an interesting target for potential treatment. However, few clinical studies have been performed studying the role of histones in the development of coagulopathy after trauma and they have included a limited population.

To provide optimized therapy to patients who develop coagulation abnormalities after trauma an appropriate diagnostic approach is warranted. Standard tests such as prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration and platelet count are widely used to evaluate coagulation function and guide resuscitation in trauma patients.[19-20] But, the conventional coagulation tests (CCTs) focus on selected aspects of coagulation, which may not be appropriate for diagnosis of coagulopathy after trauma.[21] Also, conventional tests are time-consuming as results have a turn-around time of 45 minutes to an hour – considerably long in the life of a trauma patient. Increasing emphasis focuses on Viscoelastic Haemostatic Assays (VHA), such as thromboelastometry (ROTEM®) and platelet aggregometry (Multiplate®). The use of VHA in the trauma setting is deemed advantageous because of their ability to evaluate the coagulation system in whole blood from

clot formation to clot breakdown. In addition, any of the available VHA's provide results within 5-10 minutes. Although promising, threshold values for the diagnosis of coagulopathy using VHA's and prediction of transfusion therapy after trauma have not been established. As the basic precondition for adequate management of trauma induced coagulopathy is timely recognition, the occurrence of coagulation abnormalities is not only of concern in the immediate aftermath of trauma. In the first days after injury the risk of endogenous coagulopathy remains and is followed by the increased risk of a hypercoagulable state. The deliberation to administer thromboprophylaxis as prevention of venous thromboembolism is often challenging as it comes with the risk of increased hemorrhage, which of specific concern in patients suffering from a traumatic brain injury (TBI). A large amount of issues regarding the safety and efficacy of thromboprophylaxis in trauma patients are still debated.

Although there is a growing body of literature investigating all aspects of coagulopathy after injury in adults, the importance of the role of ATC in pediatric trauma is still unclear. This is concerning as worldwide, injuries and violence account for an estimated 950,000 deaths annually in children less than 18 years[22]. Nearly 90% of these (about 830 000) are due to unintentional injuries – about the same number that die from measles, diphtheria, polio, whooping cough and tetanus combined. [22] Leading causes of death in pediatric trauma patients include traumatic brain injury (TBI) and haemorrhage.[23-24] The incidence of coagulation abnormalities in children after trauma is largely unknown and could differ from adults. In addition to anatomical and physiological differences between adults and children, there is a difference in mechanisms and patterns of injury.[25] The coagulation system in pediatric patients is still maturing, and our current understanding of ATC is predicated on data collected from adult samples. With close to one million pediatric trauma deaths each year globally, knowledge about the effect of ATC on outcome in children is urgently needed, as rapid correction could potentially decrease mortality in this special population.

The present thesis was initiated to answer the following questions:

What is the current knowledge on incidence and potential mechanisms of coagulopathy after pediatric trauma?

What is the role of rapid diagnostic tests for early identification of coagulopathy after pediatric

injury?

What are the different options for the treatment of coagulopathy after pediatric trauma?

What is the effect of coagulopathy on the outcome of pediatric trauma?

What is the current knowledge on the anticoagulant and cytoprotective properties of Protein C?

What is the role of histone-complexed DNA in the development of coagulation abnormalities in the pediatric trauma population?

What are the threshold values for most accurate identification of coagulopathy after trauma and prediction of massive transfusion (MT) using ROTEM® assays?

What does current literature show on efficacy and safety of the use of chemoprophylaxis for thromboembolic events in patients after sustaining traumatic brain injury

OUTLINE OF THIS THESIS

In this thesis several aspects of the development of coagulopathy after pediatric trauma will be discussed, as well as the use of viscoelastic testing in diagnosing coagulopathy and the need for massive transfusion. Furthermore, the use of chemoprophylaxis for thromboembolism prevention in traumatic brain injury patients is reviewed.

Chapter 2 provides a narrative review of the current knowledge on the incidence and potential mechanisms of coagulopathy after pediatric trauma and the role of rapid diagnostic tests for early identification of coagulopathy. Furthermore, different options for the treatment of coagulopathy after severe pediatric trauma are presented. In Chapter 3 a retrospective cohort study will be discussed including consecutive pediatric patients after severe trauma admitted to the trauma room of Children's of Alabama Hospital to analyze the incidence of coagulation abnormalities and the effects on outcome.

Chapter 4 will focus on the potential mechanisms of coagulopathy after trauma from a clinical and biological perspective on Protein C and reviews its anticoagulant and cytoprotective properties. Furthermore, we summarize the most recent preclinical and clinical literature on

the developing knowledge of the protein C system in acute inflammation to determine whether targeting the coagulation system could provide benefit to patients with severe sepsis and trauma. In Chapter 5 we present a prospective cohort study on consecutive pediatric patients after severe trauma admitted to the trauma room of Children's of Alabama Hospital to analyze the role of histone-complexed DNA fragments on the development of coagulopathy in pediatric trauma patients.

Chapter 6 focuses on the diagnostics of coagulopathy and describes a multi-center observational cohort study of patients sustaining traumatic injury admitted to one of the four participating trauma centers in three countries. This research was performed as a part of the Activation of Coagulation and Inflammation in Trauma study (ACIT) 3, led by the International Trauma Research Network (INTRN) collaboration. The INTRN is a consortium of 8 level-1 trauma centers in Europe and the US to perform research in the field of coagulopathy after trauma.[26] This study was conducted in collaboration with INTRN and by using a large database of trauma patients. Early detection of coagulopathy is important to counteract the hemostatic disturbances. Aim was to identify the threshold values that most accurately identify coagulopathy after injury and the need for massive transfusion using ROTEM®.

The use of chemoprophylaxis for thromboembolic events after traumatic brain injury has become a treatment of choice after the Brain Trauma Foundation Guidelines for the Management of Severe Traumatic Brain Injury (2007) stated that low-molecular-weight heparin (LMWH) or low dose unfractionated heparin (UFH) should be used in combination with mechanical prophylaxis to prevent venous thromboembolic complications. This guideline also suggests that there is an increased risk of expansion of intracranial hemorrhages (ICH) with venous thromboembolic prophylaxis.[27] Our specific aim was to review literature and perform a meta-analysis on the risk-benefit of the use of chemoprophylaxis in patients with traumatic brain injury on the progression of intracranial hemorrhage and the prevalence of thromboembolic events of which results are presented in Chapter 7.

Finally, in Chapters 8 and 9 the findings of preceding chapters are summarized and discussed.

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Chapter 2

COAGULOPATHY AFTER PEDIATRIC TRAUMA

SC Christiaans, AL Duhacheck, RT Russell, S Lisco, J Kerby, JF Pittet

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ABSTRACT

Trauma remains the leading cause of morbidity and mortality in the United States among children from the age 1 year to 21 years old. The most common cause of lethality in pediatric trauma is traumatic brain injury (TBI). Early posttraumatic coagulopathy has been commonly observed after severe trauma and is usually associated with severe hemorrhage and/or traumatic brain injury. In contrast to adult patients, massive bleeding is less common after pediatric trauma. The classical drivers of posttraumatic coagulopathy include hypothermia, acidosis, hemodilution and consumption of coagulation factors secondary to local activation of the coagulation system following severe traumatic injury. Furthermore, there is also recent evidence for a distinct mechanism of early posttraumatic coagulopathy that involves the activation of the anticoagulant protein C pathway. Whether this new mechanism of posttraumatic coagulopathy plays a role in children is still unknown. The goal of this review is to summarize the current knowledge on the incidence and potential mechanisms of coagulopathy after pediatric trauma and the role of rapid diagnostic tests for early identification of coagulopathy. Finally, we discuss different options for treating coagulopathy after severe pediatric trauma.

INTRODUCTION

Trauma remains the leading cause of morbidity and mortality in the United States among children from the age of 1 year to 21 years old.[1-2] Compared to adults, children appear to sustain higher rates of blunt than penetrating trauma.[3] Children may also be victims of non-accidental trauma that is often associated with TBI.

Perturbations in blood coagulation have been commonly observed in trauma, and are associated with adverse outcomes in adults as well as children.[3-9] Attempts to define the perturbations in blood coagulation after trauma haven been hindered by inadequate measures of coagulation; also there is no common laboratory parameter that defines coagulopathy appropriately. Acute traumatic coagulopathy (ATC) has been described by Davenport as an early endogenous process, driven by a combination of tissue injury and shock that is associated with increased mortality and worse outcome in the severely injured trauma patient. In adults, endothelial activation of Protein C is a central mechanism of ATC, which produces rapid anticoagulation and fibrinolysis following severe trauma.[10] Trauma-induced coagulopathy (TIC) includes not only ATC, but also other mechanisms of hypocoagulation, such as dilution, acidosis and hypothermia. It is a global failure of the coagulation system to sustain adequate hemostasis after major trauma. Derangements in coagulation screens identifying hypo- or hypercoagulation, are detectable in the hyper acute phase following severe trauma.[10] As early as 1982, Miner et al. described the presence of at least one coagulation abnormality in 71% of children with head trauma.[11] However, only a limited number of studies have been performed on the incidence of TIC after pediatric trauma. The incidence of coagulation abnormalities on admission reported in these retrospective pediatric studies range widely from 10% to 77 % (**Table 1**). Expanded knowledge on coagulation status of severely injured children is critical to further improvement of pediatric trauma care. In adults, damage control resuscitation (DCR) strategies have been developed to achieve early aggressive correction of TIC in conjunction with other interventions designed to achieve early hemostasis.[12] These strategies have been accompanied by improved outcomes.[13-15] In contrast to adults,

Study design	Number of subjects	References	Year	Definition of coagulopathy	Incidence of coagulopathy on admission	Study population	Main Results
Retrospective	87	(11)	1982	Abnormal clotting tests/ DIC: organ failure + low fibrinogen, ↑ PT and aPTT, ↑ FDP, thrombocytopenia or rapid declining PC	71% one abnormal clotting test 32% 'DIC' and fibrinolysis	Pediatric TBI patients < 2hr of injury	'DIC' is associated with ↑ mortality
Retrospective	147	(120)	1997	Moderately elevated PT >16s. ↑ PT and aPTT, or an elevated PT in conjunction with a low PC , low fibrinogen, and/or a positive FDP	37%	Pediatric TBI patients evaluated for child abuse, radiological evidence and coag testing with two days	↑PT and activated coagulation strongly related to presence of parenchymal brain damage. Non-survivors: coagulation abnormalities more frequent and severe.
Prospective	60	(121)	2001	PT, aPTT, low fibrinogen, PC, FDP	10% DIC	Pediatric TBI patients admitted to PICU with blood draws within 4 hrs of injury	Patients with longer aPTT, ↑ FDP, ↓ fibrinogen and low PC greater risk of a poor outcome/worse GOS
Retrospective	69	(18)	2001	FDP > 1000 g/mL	NA	Isolated TBI patients < 16 years	FDP > 1000 µg/mL predicts poor outcome (GOS 1-3) in children with isolated TBI. FDP's are a strong independent prognosticator of outcome in children with GCS between 7 and 12.
Retrospective	830	(122)	2001	INR ≤ 1.2 or aPTT ≥ 33 s	28%	Blunt head or torso trauma < 15 years	Minor elevations on coag studies independently associated with GCS ≤13, ↓SBP, open/multiple bone fractures and major tissue wounds
Retrospective	53	(123)	2001	PT > 14.5, INR > 1.2, aPTT > 38	67% of patient with GCS ≤ 14 and 7% with GCS 15	Pediatric patients with TBI	Patients with GCS ≤ 14 ↑ risk for intracranial injury and coagulopathy. Risk increases inversely with the GCS. A mean of 1 unit of FFP was required in patients with GCS ≤ 14.
Retrospective	122	(19)	2002	DIC: organ failure + low fibrinogen, ↑ PT and aPTT, ↑ FDP, thrombocytopenia or rapid declining PC	14.8% DIC	Pediatric patients with severe TBI admitted to PICU	Hemocoagulative disorders are predictors of GOS.
Retrospective	521	(124)	2007	PT INR ≥ 1.2 PTT ≥ 33 s PC < 100 x 10^3	51%	Blunt TBI < 15 years with ≥ 2 CT scans	Coagulopathy was associated with worsening CT findings and prognostic for poor outcome.
Retrospective	16	(125)	2007	↑PT, aPTT, fibrinogen PC, FDP	50%	Severe TBI < 12 months	Major coagulative alterations had a high positive correlation with GOS. Lesser hematocoagulative disorders did not correlate with outcome
Cross-sectional	301	(126)	2007	PA < 70 % and/or PT> 16 s and/or aPTT >10 s when compared to controls and/or PC < 150 x 10^3	77%	Moderate or severe TBI < 17 years requiring ICU admission	Coagulopathy directly associated with trauma severity, but not with a rise in mortality.
Retrospective	58	(127)	2009	PT test < 50%	29%	TBI and GCS ≤ 8 less than 6 years	Coagulation disorders independent predictor of mortality
Prospective Cohort	57	(128)	2010	Abnormal clotting tests	NA	Children with suspected TBI requiring a head CT	D-Dimer was an independent predictor of brain injury on head CT and was a stronger predictor than initial GCS
Retrospective	320	(20)	2011	PC of < 100 x 10^3 µL and/or INR > 1.2 and/or aPTT > 36 s	42.80%	Isolated TBI < 18 years	Low GCS, increasing age, ISS ≥ 16 and intraparenchymal lesions independently associated with TBI coagulopathy
Retrospective	744	(8)	2012	INR ≥ 1.5	38.30%	Trauma patients <18 years in combat facility with ISS, INR, BD and mortality data	Coagulopathy and shock independently associated with mortality
Retrospective	200	(15)	2012	PT test <70%, aPTT > 38 s Or PC < 100 x 10^3 µL	28%	Blunt isolated TBI <14 years	GCS ≤ 8 at scene in isolated TBI is associated with ↑risk for coagulopathy and mortality
Prospective	102	(9)	2012	PT < 15.9 s aPTT < 42.1 s	72%	Pediatric trauma patients receiving a MTP	No difference in mortality or improved outcome

				fibrinogen <180 mg/dL or PC < 185 x 10^3 µL			
Retrospective	803	(3)	2013	INR > 1.2	37.90%	Level 1 trauma patients < 18 years requiring ICU admission and received coag studies	Coagulopathy is an independent predictor of mortality after trauma. Significant increase in mortality in TBI patients.
Retrospective	86	(57)	2013	NA	NA	Level 1 trauma patients < 14 years admitted to an ICU.	Admission TEG correlated with conventional coag tests and predicted early Tx, early LSI and outcome.

TABLE 1 **Overview of published studies on coagulopathy after pediatric trauma**.

DIC= Disseminated Intravascular Coagulation. PT= Prothrombin Time. aPTT = Activated partial thromboplastin time. INR= International normalized ration. FDP=Fibrinogen degrading product. PC =platelet count. BD= Base Deficit. TBI= Traumatic brain injury. PICU= Pediatric Intensive Care Unit.ICU=Intensive Care Unit. GOS= Glasgow Outcome Scale. GCS= Glasgow Coma Scale. SBP= systolic blood pressure. ISS= Injury Severity Score. PA: Prothrombin Activity. Tx = Transfusion. LSI=Lifesaving Intervention. CT= Computed tomography. MTP: Massive Transfusion Protocol. TEG= Thromboelastography. NA=Not available.

massive bleeding is less common after pediatric trauma. TBI appears to be the common trigger of TIC and mortality in children.[16-17] The complex pathophysiological mechanisms of the coagulation abnormalities associated with TBI are not yet fully understood, but might differ from coagulation disturbances associated with massive systemic bleeding.

The goal of this review is to summarize the current knowledge on the incidence and potential mechanisms of coagulopathy after pediatric trauma as well as the role of rapid diagnostic testing for early identification of TIC. Finally, we discuss different options for treating coagulopathy after severe pediatric trauma.

DIFFERENCES BETWEEN THE ADULT AND PEDIATRIC HEMOSTATIC SYSTEMS

Determining the extent of TIC requires reliable testing and an understanding of the physiology of hemostasis in pediatric patients. The hemostatic system develops in utero and evolves over the first few months of life, leading up to maturational differences of many levels of coagulation factors. This inevitably also leads to differences in the normal ranges of coagulation screening tests for very young infants as compared to adults. A series of papers by Andrews et al. in the late 1980's describes the differences between the pediatric and adult hemostatic systems, and how age-related changes occur as the hemostatic system matures.[22-25] In healthy children from 1 to 16 years old, the hemostatic system has reached a higher degree of maturation. The screening tests consisting of prothrombin time (PT), activated partial

thromboplastin time aPTT, and fibrinogen are almost identical to those of adults. However, mean values of seven coagulant proteins (II, V, VII, IX, X, XI, XII) in children might still be significantly lower than adult values[22,26,27] and the PT might be slightly prolonged because plasma prothrombin concentrations during childhood can be 10% to 20% lower than for adults, along with the factor VII levels. [25-26] Plasma concentrations of anti-thrombin (AT), protein C (PC) and protein S (PS), all major inhibitors of the coagulation system shows low levels at birth. The mean values for PS and AT are similar to those in adults by 3 and 6 months of age respectively, whereas PC is still markedly lower at 6 months of age.[23-24] Lower values of tissue factor pathway inhibitor (TFPI) have been observed in newborns.[28]

Although all key components of the fibrinolytic system are present at birth, important age-dependent quantitative and qualitative differences can be observed in children. The major age-dependent differences include decreased plasma concentrations of plasminogen, tissue plasminogen activator (t-PA) and α-antiplasmin (α_2-AP), increased plasma concentrations of plasminogen activator inhibitor-1 (PAI-1), as well as a decrease in both plasmin generation and overall fibrinolytic activity.[29]

Limited studies are available on platelet count and function in children; most have been performed in neonates or young infants. Platelet counts have been studied in young healthy infants of varying ages and it appears that they are significantly higher at 2 months and lower at both 5 and 13 months.[30] Differences in platelets between healthy neonates and adults in regards to their response to platelet agonists have also been described. Initial platelet aggregation using flow cytometry, consistently demonstrated that platelets from neonatal cord blood were less responsive than adult platelets to agonists such as adenosine 5'diphosphate, epinephrine, collagen, thrombin and thromboxane analogs.[31] The mechanism(s) underlying these differences are still poorly understood, although it has been suggested that the hyporesponsiveness to epinephrine is probably due to the presence of fewer α2-adrenergic receptors.[32] In addition, the reduced response to collagen likely reflects the impairment of calcium mobilization[33], and the decreased response to thromboxane may result from differences in signaling downstream from the receptor in neonatal platelets.[34]

Studies of primary hemostasis revealed significantly shorter bleeding times on healthy neonates compared to adults.[22] Other studies using a platelet function analyzer found shorter closure time in neonates than adults.[35] This apparently paradoxical finding of enhanced

primary hemostasis in the face of platelet hypoactivity has been attributed to the higher hematocrit levels, higher mean corpuscular volumes, and higher von Willebrand factor concentrations in the blood of neonates.[22] Whether this *in vitro* platelet hyporeactivity of neonates translates into poor platelet reactivity under *in vivo* conditions is not well known.

The thrombin hemostasis system might also differ in children. It has been observed that the capacity to generate thrombin *in vitro* by a chromogenic assay is decreased by 26% in plasma from children aged 1 to 16 years compared to adults, this would justify the lower prevalence of thromboembolic complications in this period.[36] When compared to adult reference ranges, children ages 1 to 5 might display higher values of soluble thrombomodulin, thrombin-antithrombin complex and D-dimer.[37]

Taken together, the results of these studies indicate some variability in the maturation of the different coagulation proteins and of the functional activity of platelets in young children. However, the susceptibility to bleeding is based upon the contextuality of the entire hemostatic system as evaluated by coagulation monitoring devices assessing the viscoelastic properties of whole blood and platelet function testing and not just on coagulation factor and anti-coagulation factor balance changes over time.

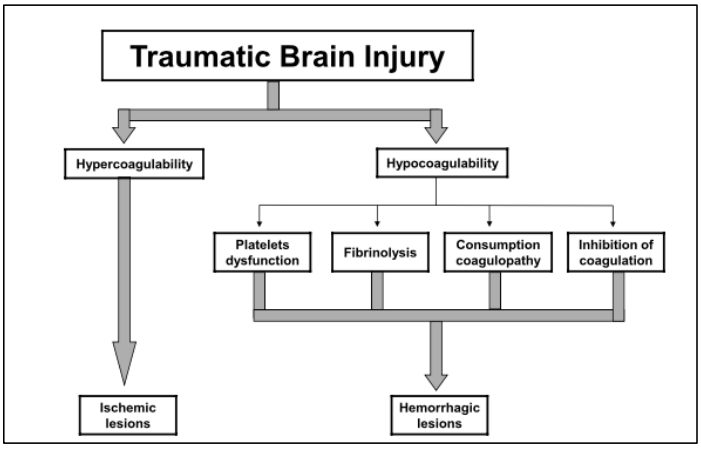

FIGURE. 1 **Current hypothesis for the development of coagulation abnormalities after blunt traumatic brain injury**. A combination of hypocoagulable and hypercoagulable states triggered by the extent of brain injury will lead to secondary injury by way of ischemic and hemorrhagic lesions. Figure modified from (134).

EARLY DETECTION OF TIC AFTER PEDIATRIC TRAUMA

The basic precondition for adequate management of a coagulation problem in the acute phase after trauma is timely recognition. A variety of different tests are available to assess coagulation in the pediatric population. Standard coagulation monitoring comprises the early and repeated determination of conventional coagulation tests (CCT) such as PT, aPTT, INR, and fibrinogen. It is frequently assumed that these CCTs monitor coagulation; however, these tests monitor only the initiation phase of blood coagulation and represent only the first 4% of thrombin production.[38] It is, therefore, possible that the conventional coagulation screen appears normal, while the overall state of blood coagulation is abnormal.[39-41] Moreover CCT, originally developed for the guidance of anticoagulation therapy or management of certain disease states, assess only plasma-based components of the coagulation system and do not account for the contribution of the endothelium and cellular components of blood. Also, the detection of hypercoagulability is limited by the use of CCT. As the majority of trauma patients becomes hypercoagulable it would be important to use coagulation monitoring devices, such as thromboelastography, that have been shown to accurately assess hypercoagulation in other conditions.[42]

Increasing emphasis focuses on the importance of coagulation monitoring devices assessing the viscoelastic properties of whole blood and platelet function testing, i.e., thromboelastography (TEG®), rotation thrombelastometry (RoTEM®), and impedance aggregometry (Multiplate®; DiaPharma, West Chester, Ohio). (**Table 2 and Figure 2**). TEG/RoTEM® measure and graphically display the changes in viscoelasticity at all stages of the developing and resolving clot, starting with fibrin formation and continuing on through clot retraction and fibrinolysis with minimal delays. Furthermore, the coagulation status of patients is assessed in whole blood, providing a functional assay that allows the plasma-based coagulation system to interact with platelets, red cells and white blood cells, thereby providing useful information on platelet function.[43] In addition, with the development of the Multiplate® device and FDA clearance for two of its tests, a rapid point of care platelet function testing will soon become available clinically and has successfully been used in research studies to identify platelet dysfunction in adult trauma patients.[44] A major benefit of these assays is their ability to evaluate the coagulation system in the whole blood, which may improve the accuracy of

monitoring hemostasis.

Early variables of clot firmness assessed by viscoelastic testing, such as thromboelastography have been shown to be good predictors for the need for massive transfusion, the incidence of thrombotic/thromboembolic events and for mortality in adult surgical and trauma patients.[41,45-54] The delay in detection of TIC can influence outcome and the turn-around time of viscoelastic devices (TEG/RoTEM®) has been shown to be significantly shorter by 30 to 60 minutes compared to conventional laboratory testing in both adult and pediatric patient populations.[41,55,56] Data on the measurement of viscoelastic properties of whole blood in children after trauma are limited. An initial study detailing the use of viscoelastic devices has recently been described in 86 children sustaining severe trauma.[57] Interestingly, rapid TEG was used in that study which produced faster results than conventional TEG measurements. Similarly, the use of interim ROTEM® values (A10) have been shown to provide an early and specific assessment of the coagulation after trauma in adult patients in order to guide resuscitation.[58] These investigators described results comparable to adult studies[49,59] with admission data correlating with CCT and predicting early transfusion and outcome. Thus, although normal values of viscoelastic properties of whole blood have been established in healthy children of all ages for thromboelastography, thromboelastometry and impedance aggregometry[60-63], carefully designed prospective trials on the use of these global measurements of hemostasis are warranted to obtain a more detailed description of the coagulation abnormalities that occur post-trauma in this special population.

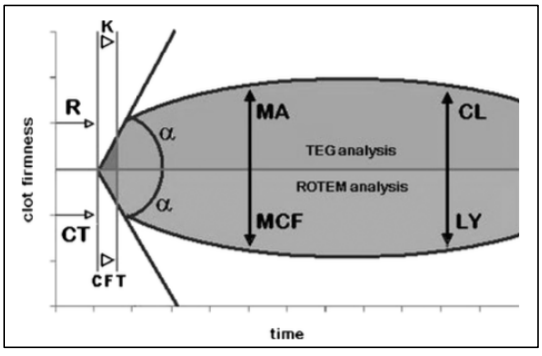

FIGURE. 2 **Typical tracings of viscoelastic coagulation devices.** A, Upper side: Thrombelastograph (TEG) tracing: r, reaction time; K, kinetics; α, slope between r and k; MA, maximum amplitude; CL, clot lysis. B, Lower side: ro- tation thrombelastography (RoTEM) tracing: CT, clotting time; CFT, clot for- mation time; α, slope of tangent at 2-mm amplitude; MCF, maximal clot firmness; LY, lysis. Figure modified from (105).

TABLE 2 **Viscoelastic tests available for the pediatric trauma population**

Test		Definition	Hemostatic phase	Cause for abnormalities	Intervention	Studies on the use of viscoelastic tests after pediatric trauma	
TEG® Assay time: 10-15 min	RoTEM® Assay time: 5-10 min					TEG®	RoTEM®
R	CT	Time from initiation of test until the beginning of the clot formation	Initiation of coagulation	Prolonged R/CT: - Factor deficiencies - Anticoagulants Short R/CT: - Plasma hypercoagulability	Plasma	Admission rapid TEG results correlate with conventional coag tests and predict early transfusion, early LSI and outcome. (57) Report on TEG guided hemostatic resuscitation (129)	Age related reference ranges established in children (60) Report on Successful RoTEM-guided Hemostatic therapy after blunt trauma. (77)
K	CFT	Time from start of the clot formation to the curves reaches amplitude of 20 mm	Amplification of coagulation	ProlongedK/CFT: - Factor deficiencies - Hypofibrinogenaemia - Thrombocytopenia - Platelet dysfunction	Cryoprecipitate		
α	α	Angle between baseline and the tangent to the curve through the starting point of coagulation	Propagation of coagulation 'Thrombin burst'	Low α - Factor deficiencies - Hypofibrinogenaemia - Thrombocytopenia - Platelet dysfunction	Cryoprecipitate		
MA	MCF	Amplitude measured at max curve width		Low MA/MCF - Hypofibrinogenaemia - Thrombocytopenia - Platelet dysfunction - FXIII deficiency	Platelets (consider FXIII concentrate if ongoing bleeding and persistently low MA/MCF)		
LY	ML	Reduction in area under curve (LY) or in amplitude (ML) from the time MA/MCF is achieved until 30 or 60 min after MA/MCF	Fibrinolysis	Increased LY/ML - Hyperfibrinolysis	Antifibrinolytics		

Table modified from (130)

POTENTIAL MECHANISMS OF TIC AFTER PEDIATRIC TRAUMA

There are several potential mechanisms that contribute to the development of TIC. Much adult trauma literature details mechanisms and drivers of TIC, but there are only limited descriptions characterizing these mechanisms in pediatric trauma. The principal mechanistic drivers are summarized in **Figure 3**. As the number of aforementioned drivers of TIC mount following injury, the probability of life-threatening coagulopathy increases exponentially. Previous studies have shown that the conditional probability of developing TIC with moderate injury without the presence of additional triggers for coagulopathy is 1%. However, with increased

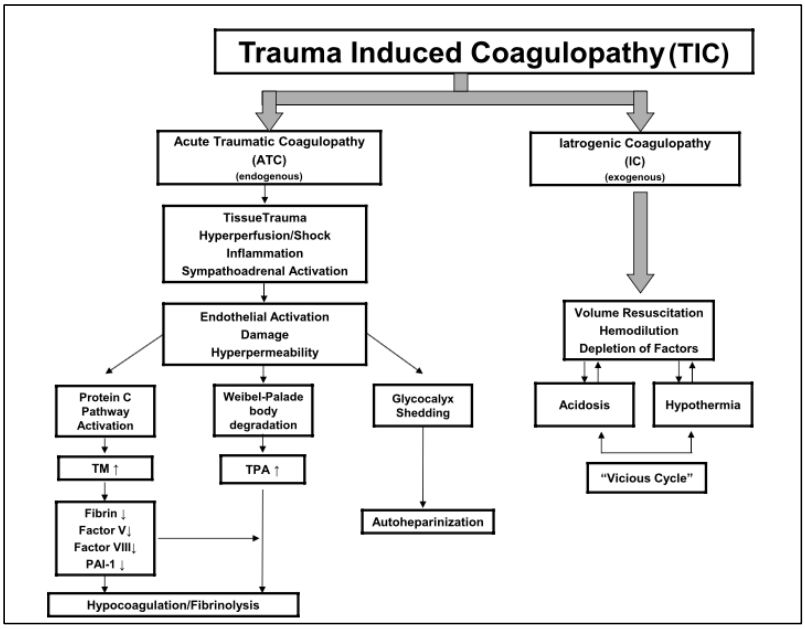

FIGURE 3 **Potential mechanisms involved in the trauma-induced coagulopathy in children.** There is much adult literature detailing mechanismsanddrivers of acute traumatic coagulopathy (ATC) and iatrogenic coagulopathy (IC). The classical physiologic drivers include hypothermia, acidosis, and dilution secondary to intravenous administration of crystalloids and consumption of coagulation factors and might be similar between children and adults, although there is a limited description of these mechanisms in pediatric trauma. There is recent evidence for a distinct mechanism for early ATC in patients who have not been exposed to the traditional coagulopathy triggers and that may involve the activation of the anticoagulant protein C pathway, the Weibel-Palade body degradation, and glycocalyx shedding. Whether these new mechanisms of ATC play a role in children is still unknown. TM, thrombomodulin; TPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor 1. Figure modified from (76) and (135).

ISS > 25 and hypotension, the probability increases to almost 40%, and in cases with ISS > 25, hypotension, hypothermia, and acidosis, the probability of developing TIC increases to 98%.[64]

Physiologic and iatrogenic dilution in trauma patients when present can act as an additional mechanistic driver of TIC. In times of hypotension, physiologic or iatrogenic dilution potentiates the osmotic activity of plasma leading to a shift of extravascular water into the intravascular space. Until equilibrium is reestablished, this osmotic activity causes a

proportional dilution of plasma proteins and coagulation factors adversely affecting their subsequent interactions. Monroe et al. modeled the action of factor VIIa in dilutional coagulopathy and demonstrated a calculated reduction in single factor concentration of 37% resulting in a 75% reduction in overall factor complex activity.[65]

The effects of iatrogenic dilution in trauma were nicely demonstrated in a study of patients from the German Trauma Society Database (TR-DGU). Investigators observed TIC upon emergency room (ER) admission in greater than 40% of patients receiving more than 2000 mL in transport, in greater than 50% of patients with more than 3,000 mL in transport, and in 70% of patients with more than 4,000 mL of fluid administered in the pre-hospital phase of care.[66] This dilution is accompanied by consumption and inactivation of coagulation factor substrates and coagulation enzymes of varying magnitudes depending upon the degree of individual injury.[67]

In addition to dilutional mechanisms of TIC, the effects of temperature and pH on coagulation factor and complex activity have also been well described. The pace of coagulation factor reactions is affected by hypothermia and acidosis. Kermode et al. and Jurkovich et al. have demonstrated that coagulation interactions are slowed down by approximately 5% with each degree Celsius drop in temperature. Similarly, the critical interactions between factors and glycoproteins that activate platelets are absent in 75% of individuals at 30 degrees Celsius.[68,69] A reduction in pH to 7.2 has been shown to reduce coagulation factor complex activities by 50% with activity falling to 20% of normal at a pH of 6.8.[70]

Fibrinolysis is another important mechanism controlled by the coagulation system, which plays a role in TIC. The coagulation system modulates fibrinolysis maintaining stable blood clots for the time necessary to control bleeding. In the normal setting, high concentrations of thrombin inhibit plasmin activation by the activation of thrombin-activated fibrinolysis inhibitor (TAFI) and PAI-1. However hypothetically, in the setting of trauma, if the thrombin burst is not robust, TAFI remains inactivated allowing thrombin to bind to thrombomodulin on endothelial cells leading to protein C activation, subsequent Factor V, VII, and PAI-1 inactivation, and increased fibrinolysis. Hyperfibrinolysis has been identified as a significant risk factor for mortality in bleeding trauma patients.[71-72]

Over a quarter of adult trauma patients demonstrate detectable coagulopathy on arrival to the emergency department before the development of the classic triad of hypothermia, dilution,

and acidosis. Brohi and colleagues, in a large prospective study of 209 patients presenting with a severe trauma (ISS equal or more than 16) and meeting the criteria for the highest trauma activation, documented the development of TIC within one hour after injury in approximately 30% of patients. In this study, patients arriving coagulopathic had significantly increased mortality of 40% (5). Other authors subsequently reported similar findings.[6]To test potential mechanisms for this TIC, In the study by Brohi et al., potential mechanisms for TIC were also evaluated. In this cohort of severely injured adult patients, plasma levels of protein C zymogen were found to be depleted on admission to the hospital.[4] More recent data from the same investigators showed that in a similar group of 200 adult trauma patients, the combination of tissue injury, elevated ISS, and shock was associated with TIC nearly immediately after their injury.[73] They found TIC was strongly correlated with the activation of the protein C pathway. Further evidence for protein C activation is demonstrated by the fact that they also found a strong inverse correlation between plasma levels of activated Protein C (aPC), factor Va and VIIIa inactivation and the derepression of fibrinolysis. Activated protein C directly inhibits PAI-1, which usually serves to limit t-PA activity. Without the limitation of PAI-1, tPA is free to enhance the conversion of plasminogen to plasmin and thereby enhance fibrinolysis. In summary, aPC exerts its profound anticoagulant activity by inhibiting coagulation and through derepression of fibrinolysis.[4]

The possible mechanistic role of the protein C pathway in the development of TIC was also demonstrated in a mouse model of trauma-hemorrhage.[74] Mice subjected to a pressure-controlled hemorrhage to a MAP of 40 mmHg for 60 min developed a severe metabolic acidosis (BD > 10), were hypocoagulable (had an increase in their aPTT) and had a significant increase in their plasma levels of aPC. The aPTT returned to normal values 12h later. When mice were pretreated with an antibody that blocks the anticoagulant domain of aPC it reversed the coagulopathy induced by severe trauma, indicating that the activation of the protein C pathway might play a mechanistic role in TIC.

One would assume that certain physiologic drivers would be similar between children and adults, including hypothermia, acidosis, dilutional effects and consumption of coagulation factors. However, on a more detailed level, minimal literature exists on a pediatric patient's response to significant traumatic tissue injury and the release of inflammatory markers and anticoagulation factors, like aPC, which may interfere with coagulation and hemostasis. A

detailed description of the mechanistic changes in the coagulation system associated with severe trauma has not been performed in the pediatric population and will require further investigation.

TREATMENT OPTIONS FOR TIC IN PEDIATRIC TRAUMA

Administration of procoagulant concentrates

In adults, DCR strategies have been developed to achieve an early aggressive correction of ACT.[12,75] This strategy has been accompanied by improved outcomes.[13-15] We hypothesize that in pediatric patients with coagulopathy that is rapidly identified and amenable to correction a goal-directed approach to resuscitation may be more appropriate than an empiric blood product approach. The most important procoagulant concentrates include fibrinogen concentrate, prothrombin complex concentrate (PCC), recombinant factor VIIa (rFVIIA) and antifibrinolytics such as the tranexamic acid. The effect of these adjuvant interventions has not been systematically studied in the pediatric population. The use of these hemostatic agents in a goal-directed fashion guided by TEG/RoTEM monitoring to assess effectiveness and avoid potential thromboembolic complications make for a compelling therapeutic strategy (**Table 3 and Figure 4**).

Intervention	Study design	Number of subjects	References	Year	Study population	Main Results
PCC	Case Report	1	(85)	2011	8 kg infant with liver trauma and severe hemorrhage	Patient with acidosis (pH 6.67) and severe anemia (Hb 4 mg/dL). Poorly controlled bleeding despite surgical intervention, FFP and platelets. Vit K and 30 IU/kg PCC administered, with rapid cessation of bleeding and INR ↓ from 2.9 to 1.5.
rFVIIa	Case Reports	3	(81)	2003	5 wks, 20 mo and 1 yr old with (T)BI	One patient received rFVIIa (bolus of 90 µg/kg) after failing of repeated FFP to correct coagulopathy; two patients received rFVIIa as initial therapy. Two of three children had good neurologic outcomes; third progressed to brain death. No thrombotic complications; intracranial devices placed w/o intracranial hemorrhage.
rFVIIa	Retrospective case series	135	(80)	2009	Pediatric patients receiving rFVIIa	Median transfusion volume decreased in the 24 hours after rFVIIa vs. prior 24 hrs. (11.7 mL/kg vs. 29.7 mL/kg). Mortality lower in surgical/trauma patients (16%) compared to medical patients (58%). Three thrombotic events resulted in two deaths.
Fibrinogen	Case Report	1	(77)	2013	20 kg, 7- year old with severe abdominal and pelvic trauma	EBL of >70 mL/kg. Goal-directed therapy with ROTEM resulted in administration of 2 g fibrinogen and 3 u of RBC with no FFP or platelets. The ratio of intra-operative fibrinogen concentrate (g) to RBC (U) was 0.7
MT	Prospective	53	(104)	2012	Pediatric trauma patients	Outcome data compared before and after institution of pediatric MTP. Median FFP: RBC ratio was higher after MTP (1:1.8 vs. 1:3.6). Time to FFP dosing decreased 4-fold with MTP. No difference in mortality.
MT	Prospective cohort Therapeutic Level IV	55	(107)	2012	Pediatric patients requiring un–cross-matched blood	Coagulopathy, aPTT >36 s associated with initiation of MTP. ISS for the MTP group was 42 vs 25 for the non–MTP group. More thromboembolic complications in the non–MTP group.
MT	Retrospective cohort	105	(108)	2013	Pediatric trauma patients < 18 yo requiring massive transfusion	No difference in mortality. Higher plasma/RBC and platelet/RBC ratios were not associated with increased survival.

TABLE 3 Published studies of treatment regimens for coagulopathy following pediatric trauma

PCC= Prothrombin Complex Concentrate. FFP=fresh frozen plasma INR= International Normalized Ratio. TBI= traumatic brain injury. rFVIIa= recombinant Factor VII. U=unit. EBL= estimated blood loss. MT= Massive Transfusion. MTP=massive transfusion protocol. RBC=red blood cell. aPTT = Activated partial thromboplastin time. ISS= Injury Severity Score.

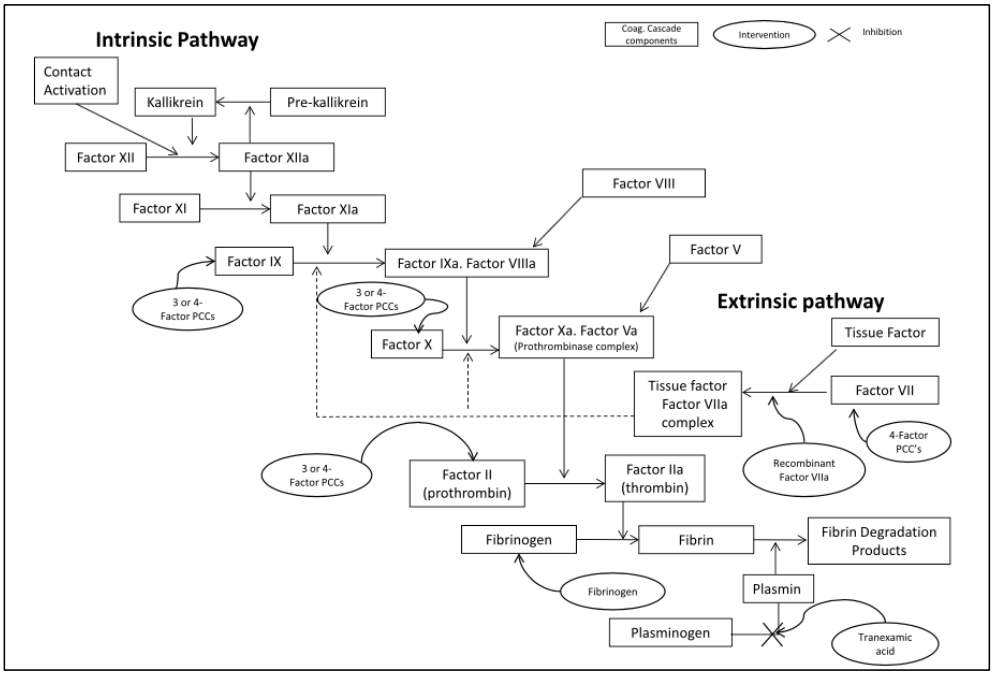

FIGURE 4 **Treatment of acute traumatic coagulopathy in children.** This cartoon represents the coagulation cascade and the effect of potential therapeutic approaches for treating acute traumatic coagulopathy in children. PCC, prothrombin complex concentrate. Figure modified from (136).

Fibrinogen concentrate

Fibrinogen concentrate (HaemocomplettanP/RiaSTAP, CSL Behring, USA) has been marketed for a number of years for the treatment of congenital hypofibrinogenemia, but has been advocated as a fibrinogen replacement therapy for patients requiring massive transfusion.[76] It is produced from pooled human plasma by fractionation and undergoes inactivation steps; it has a fibrinogen concentration of around 20 mg/ml. Despite the evidence supporting maintenance of adequate fibrinogen levels in bleeding patients, little data is available on the administration of fibrinogen concentrate to trauma patients. In pediatric trauma, the use of a fibrinogen concentrate was recently reported in a seven-year-old patient with severe abdominal and pelvic trauma.[77] On arrival to the emergency department, he

received 250 mL red blood cells (RBC), 250 mL crystalloid and 0.5 g fibrinogen concentrate, which were given pre-emptively. He then underwent goal-directed hemostatic therapy using RoTEM®. A total of 2 g fibrinogen was administered, while fresh frozen plasma (FFP) and platelets were avoided. Despite an estimated blood loss of >70 mL/kg, the patient received only 3 Units of RBC. The ratio of intra-operative fibrinogen concentrate (g) to RBC (U) was 0.7, which is similar to the ratio of 0.9 described by Schochl when looking at thromboelastometry-guided coagulation factor concentrate based therapy versus FFP in adult trauma.[78] Fibrinogen or cryoprecipitate (for fibrinogen replacement) received a grade 1C recommendation in a recent European guideline for management of traumatic bleeding in adult patients with thromboelastometric signs of fibrinogen deficiency or a fibrinogen level of less than 1.5-2.0 g/L and significant bleeding.[79]

Recombinant Factor VIIa

Recombinant Factor VIIa (rFVIIa) was initially developed for treatment of hemophilia and acquired inhibitors, but off-label use of rFVIIa has become increasingly prevalent. rFVIIa has a more developed presence in the pediatric literature than that of the other factor concentrates. Its effectiveness in neonates, infants and children with TIC and clinically significant bleeding, as well as complications following its administration in pediatric patients have been described in several reports. A retrospective case series of 135 pediatric patients receiving rFVIIa for off label use revealed its potential for clinical utility in the setting of surgery and trauma. In this case series, 15 patients received rFVIIa for trauma, 19 patients for surgical bleeding, 16 patients for procedural prophylaxis and 28 patients for bleeding resulting from disseminated intravascular coagulation/sepsis. There was a decrease in 24-hour median transfusion volume after rFVIIa administration. Surgical patients had control of life-threatening bleeding with low associated mortality. Indeed, the mortality rate was significantly lower in the surgical/trauma patients (16%) in comparison to medical patients (58%). Major thrombotic events were seen in 3 patients after rFVIIa, resulting in two deaths and one leg amputation.[80] Another case review study on pediatric patients suffering from severe TIC after cerebral injury reports a rapid correction of hemostatic abnormalities after administration of a bolus of 90 µg/kg rFVIIa in three children aged 5 weeks, 20 months and 11 years.[81]

Dosing recommendations in the pediatric-aged patient are extrapolated, in part, from the adult

literature, supplemented by the pediatric hemophiliac population. Bolus doses have ranged from 40 - 100 μg/kg in the non-hemophiliac pediatric population. With ongoing bleeding or risk for bleeding, repeat doses at intervals of two to six hours have been administered. In addition to bolus dosing, continuous infusion (20-30 μg/kg/h) following the bolus to maintain hemostatic levels of rFVIIa have been reported. Compared to adults, the pharmacokinetics in pediatric patients demonstrate a shorter half-life and an increased clearance.[82] In addition to its effects on coagulation function, recent data reports enhanced platelet function[81], suggesting a potential role in patients suffering from qualitative platelet disorders, which may include severely injured pediatric trauma patients, more specifically brain-injured children. However, some limitations in the use of rFVIIa have been observed in adults. Data from 21 institutions and 380 patients was collected from the Western Trauma Association web-based registry and revealed several indicators of poor response to rFVIIa, including acidosis (pH < 7.2), thrombocytopenia (platelets < 100,000) and hypotension (systolic </= 90). Based on these results, maximal benefit cannot be achieved with administration late in the treatment of a hemorrhaging trauma patient.[83]

Prothrombin complex concentrate

Prothrombin complex concentrate (PCC), also referred to as factor IX complex, is derived from pooled human plasma and contains 25-30 times the concentration of clotting factors as FFP. Four-factor PCCs contain factors II, VII, IX and X, while 3-factor PCCs contain little or no factor VII. Depending on the formulation, PCCs may additionally contain protein C, protein S, anti-thrombin and low dose heparin[84]. Most formulations available in the United States are 3-factor PCCs and are approved for prevention and control of bleeding in patients with hemophilia B. However, due to the availability of highly purified and recombinant factor IX products, PCCs are rarely used for this indication. There have been no controlled clinical trials evaluating the use of PCC in massive bleeding; recommendations are generally based on retrospective or observational studies, case reports, and expert opinion.[84] Literature regarding use of PCC in the pediatric trauma patient is scarce. One case report described an 8 kg infant with liver trauma and severe hemorrhage who was acidotic (pH 6.67) and severely anemic with a hemoglobin of 4 mg/dl.[85] The patient underwent two surgical procedures and transfusion of packed RBCs, platelets, and FFP. Following the second operation, the infant continued to bleed

despite the administration of FFP, platelets, and red blood cells. Vitamin K and 30 IU/kg PCC were administered due to ongoing hemorrhage, at which point there was a rapid cessation of bleeding and the INR decreased from 2.9 to 1.5.

The variability in factor concentration between formulations creates challenges in standardization of dosing. When using the package information regarding dosing recommendations for hemophilia B, an expected increase in factor IX between 20-50% would occur with a dose of 20-50 units/kg.[86] Similarly, Australasian guidelines recommend a dose of 25-50 units/kg of 3-factor PCC to reverse INR following administration of vitamin K antagonists.[87] Patanwala recommended a maximum cumulative dosage of <50 units/kg due to the risk of thromboembolism. While some studies have shown benefits of PCC, there is currently only level 2C evidence (GRADE working group) for its usage in patients with massive bleeding in concert with FFP.[84] In the European guidelines for management of traumatic bleeding, it is only recommended for the emergent reversal of vitamin-K dependent anticoagulants (grade 1B recommendation).[88] In order for stronger recommendations to be developed for use in hemorrhage secondary to trauma, there is a need for randomized studies to evaluate outcomes following administration, especially in children.

Tranexamic acid

Tranexamic acid (TXA), an anti-fibrinolytic agent, is a synthetic lysine analog that functions by competitive inhibition of the enzymatic activation of plasminogen to plasmin, responsible for the degradation of fibrin. While the CRASH-2 trial revealed a significant decrease in death secondary to bleeding when TXA is administered early following trauma, there is little data regarding the safety of TXA in children. A 2008 systematic review analyzing the use of TXA in pediatric patients undergoing spine surgery revealed six studies. TXA led to a modest decrease in volume of blood transfused, but not the number of patients requiring transfusion. No deaths or major adverse events were reported, however, the number of patients was too small and follow-up duration too brief to draw conclusions regarding safety.[89] Similar results have been found in pediatric cardiac literature.[90]

The Royal College of Paediatrics and Child Health (RCPCH) and the Neonatal and Paediatric Pharmacists Group (NPPG) Medicines Committee published an evidence statement in November 2012 addressing the use of TXA for major trauma in children in response to

CRASH-2.[91] This Evidence Statement strongly encouraged the need for on-going research into the use of TXA in the pediatric population, but offered pragmatic dosing guidelines based on extrapolation from adult literature, as published use of TXA in pediatric patients has revealed wide variability in dosing. The recommendation by this group was a 15 mg/kg loading dose (max 1 g) over 10 minutes followed by 2 mg/kg/h for at least 8 hrs or until bleeding stops. As no indication recommendations were given, the group urged caution with administration of TXA in the pediatric trauma population, as a potential risk of thrombosis exists.

TRANSFUSION OF BLOOD AND PLASMA

Massive blood transfusion

In the adult trauma setting, resuscitation strategies have evolved with a trend toward the early and liberal use of blood products, including RBC, FFP and platelets in patients with hemorrhagic shock. Several studies have supported the use of a 1:1:1 platelet to FPP to RBC ratio when transfusing severely injured patients.[13,14,92,93] However, the results of these studies may have been affected by survival bias.[94-97] Other published studies have not shown any improvement in survival utilizing this approach.[98-100] In contrast, two recent studies have still shown a benefit of using a high FFP-blood ratio after adjusting for survival bias.[101,102] Regardless of these results, a higher ratio transfusion approach has been adopted at the majority of level 1 adult trauma centers and prospective, randomized controlled trials are currently underway to determine optimal ratios for patients with severe hemorrhagic blood loss.[103]

Massive transfusion in children is uncommon, and in non-neonatal pediatric patients, transfusion guidelines are similar to those in adults. In children, because blood volume varies per age, gender and weight[104-106], it is unclear as to what constitutes a massive transfusion in a pediatric patient. Moreover, the response to massive bleeding in children is thought to differ from the adult response because of their greater physiological reserve and an improved tolerance of blood loss.[107] Data analyzing the effects of a balanced ratio of blood product component administration in massive transfusion is limited in pediatric populations. To date, only three single center studies have been reported on experience with massive transfusion protocol (MTP) in pediatric patients (**Table 3**). A prospective study on 102 pediatric trauma patients was completed following the institution of a pediatric MTP and outcomes compared

with a time period prior to protocol implementation.[104] Following MTP institution, the median FFP: RBC transfusion ratio was 1:1.8 compared with a ratio of 1:3.6 in the pre-MTP patient population. Although this study was not powered to show improvement in outcome, there were two important findings. First, the majority of patients had a least one coagulation value abnormality. Second, implementation of a pediatric MTP with early and aggressive use of plasma transfusion in children with TIC was feasible. In the same year, Chidester et al. performed a prospective cohort study of 55 children, of whom 22 patients received transfusions according to MTP while the other 33 patients received blood at physician discretion.[107] Similar to results reported by Hendrickson et al., mortality was not significantly different between the two groups. However, the MTP group received a greater overall amount of blood products and was more likely to be severely injured. Thromboembolic events were observed exclusively in the non-MTP-group, which the authors attributed to under transfusion in those patients. Importantly, despite utilizing a MTP, neither study was able to reach the protocols' goal of 1:1 ratio for FFP:RBC transfusion due to the lack of availability of thawed plasma. Recently a retrospective study on 105 pediatric trauma patients receiving massive transfusion found no association between blood product ratios and survival.[108] Interestingly, all causalities suffered from severe TBI (head AIS $\geq$ 3) and not hemorrhage. Taken together, additional prospective, randomized clinical trials are needed to fully evaluate the effectiveness of varying ratios of blood component therapies in the pediatric trauma population.

Fresh Frozen Plasma

FFP is the most common blood component transfused to treat coagulopathy. FFP is plasma produced from whole blood and frozen to - 40 degree Celsius to preserve labile coagulation factors. FFP typically contains coagulation factors close to normal blood levels as well as other plasma proteins, including immunoglobulins and albumin. Volume is still potential disadvantage of using FFP in the pediatric trauma setting where TIC may be present and rapidly progressing but no volume expansion is needed. Most guidelines suggest that plasma should be only transfused in the case of active bleeding, and not based on abnormal coagulation screens alone.[109,110]

There are inherent risks to the transfusion of FFP. These risks include, but are not limited to, exposure to pathogens, transfusion related acute lung injury (TRALI), transfusion associated

circulatory overload (TACO), and adverse immunological reactions. In a retrospective study, Karam et al. found a three-fold increase in risk of new or progressive multiple organ dysfunction syndrome in pediatric patients receiving one or more plasma transfusions.[111] Those patients receiving plasma also had an increase in nosocomial infections and intensive care unit length of stay. Another retrospective study compared trauma patients receiving FFP alone versus coagulation factor concentrates (fibrinogen and PCC) and no FFP. While mortality was similar, patients receiving FFP received more PRBCs and had an increased frequency of multi-organ failure.[112] Recently, a solvent/detergent (SD) treated plasma has been licensed in the U.S, this product has been shown to dramatically reduce the risk of adverse advents associated with single donor FFP, including reduced TRALI.[113] The application of SD plasma needs to be explored to determine if its use is safer but equally efficacious compared to the use of regular FFP in pediatric trauma patients who are both hypocoagulable and hypovolemic.

Advantages of coagulation factor concentrates include immediate availability for administration, lack of excessive volume expansion, standardization of factor concentration and dose, and lack of elevated risk of TRALI.[114] In addition, coagulation factor concentrates have minimal risk of pathogen transmission, as they undergo viral inactivation steps. However, it should be pointed out that plasma may have protective properties that are unrelated to its procoagulant activity, but are related to the restoration of the endothelial glycocalyx layer that is damaged by hypoperfusion and hypoxia.[115]

In summary, pediatric data on successful management of TIC after trauma are limited and practices are largely extrapolated from the adult trauma experience. Few studies have directly looked at hemostatic interventions in children. It is clear that TIC accompanying pediatric trauma is an area where future prospective randomized trials are needed to define ideal treatment strategies necessary to improve outcomes in this unique patient population.

CONCLUSION

Coagulation abnormalities after pediatric trauma are more common than previously thought and are associated with increased morbidity and mortality. Essential prerequisites needed to investigate coagulation abnormalities after trauma in the pediatric population are the accurate interpretation of coagulation tests, along with a thorough understanding of the normal postnatal development of the human coagulation system. The laboratory assisted diagnostic approach to several hemostatic disturbances in the newborn and the child is challenging, because collection procedures and coagulation assays must be adapted for very small amounts of blood, and the reference intervals for many assays may differ broadly from those for adults. Measurements of viscoelastic properties of whole blood provides a rapid evaluation of clot dynamics in whole blood and are of greater value than coagulation screens in diagnosing and managing TIC. A number of interventions have been undertaken in trauma patients to minimize TIC and hemorrhage, including balanced MTPs, factor concentrate administration and antifibrinolytic therapy. Despite these interventions, hemorrhage remains the second largest cause of death in adult trauma patients and is responsible for half of the deaths occurring in the first 24 hours.[16] The widespread application of adult traumatic coagulopathy management principles to pediatric traumatic coagulopathy management should not be done blindly and caution needs to be applied in the care of these patients. The mechanisms behind the development of acute traumatic coagulopathy in the pediatric population need to be elucidated and well-designed prospective clinical trials studying the efficacy of early detection and management in TIC after pediatric trauma are urgently needed.

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Chapter 3

EARLY COAGULOPATHY IS AN INDEPENDENT PREDICTOR OF MORTALITY IN CHILDREN AFTER SEVERE TRAUMA

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ABSTRACT

To determine whether early coagulopathy affects the mortality associated with severe civilian pediatric trauma, trauma patients < 18 years of age admitted to a pediatric intensive care unit from 2001 to 2010 were evaluated. Patients with burns, primary asphyxiation, preexisting bleeding diathesis, lack of coagulation studies or transferred from other hospitals > 24 hours after injury were excluded. Age, gender, race, mechanism of injury, initial systolic blood pressure (SBP), Glasgow Coma Score (GCS) score, Injury Severity Score (ISS), prothrombin time (PT), partial thromboplastin time (PTT), platelet count and International Normalized Ratio (INR) were recorded. An arterial or venous blood gas was performed, if clinically indicated. Coagulopathy was defined as an INR > 1.2. The primary outcome was in-hospital mortality. Secondary outcomes were lengths of ICU and hospital stay. Eight hundred three patients were included in the study. Overall mortality was 13.4%. The incidence of age-adjusted hypotension was 5.4%. Early coagulopathy was observed in 37.9% of patients. High ISS and/or hypotension were associated with early coagulopathy and higher mortality. Early coagulopathy was associated with a modest increase in mortality in pediatric trauma patients without traumatic brain injury (TBI). In contrast, the combination of TBI and early coagulopathy was associated with a four-fold increase in mortality in this patient population. Early coagulopathy is an independent predictor of mortality in civilian pediatric patients with severe trauma. The increase in mortality was particularly significant in patients with TBI either isolated or combined with other injuries, suggesting that a rapid correction of this coagulopathy could substantially decrease the mortality after TBI in pediatric trauma patients.

INTRODUCTION

Trauma remains the leading cause of death and disability in adults and children age 1 – 44 years.[1] Worldwide, one in seven deaths is due to injury, and this is expected to rise to one in five in the next 15 years despite continuing advances in resuscitation, trauma surgery, and critical care.[1] Hemorrhage is the major mechanism responsible for death during the first 24 to 48 hours after trauma and efforts to control hemorrhage and restore circulatory homeostasis form the core of the early therapeutic approach to traumatic injuries.[2,3]

Perturbations in blood coagulation are common following major trauma in adults, and are associated with poor outcomes.[4-6] Classically, coagulopathy associated with trauma was thought to be due to the consumption of coagulation factors, dilution from fluid therapy, and/or hypothermia.[1] While advances in trauma resuscitation protocols have focused on limiting these exposures, the traditional post-injury resuscitative protocol accelerated coagulopathy due to large volumes of crystalloids (dilution), exposure of the patient (hypothermia), and prolonged surgery (more exposure, hypothermia and continued bleeding), all of which precipitated metabolic failure (acidosis).[2] However, it has recently been recognized that a quarter of the severely traumatized adult patients have coagulopathy on presentation to the emergency department that is physiologically and mechanistically distinct from the classical iatrogenic posttraumatic coagulopathy. Two studies have described this acute traumatic coagulopathy and have shown it to be associated with higher transfusion requirements, a greater incidence of multiple organ dysfunction syndrome (MODS), longer intensive care unit (ICU) and hospital stays and a four-fold increase in mortality in coagulopathic patients compared to those with normal coagulation.[4,7] Furthermore, we have recently shown that early traumatic coagulopathy is characterized by a significant activation of the anticoagulant protein C pathway and a derepression of fibrinolysis after severe trauma in humans.[8] The mechanistic role of the protein C pathway in the development of the early coagulopathy after severe trauma was confirmed with our experimental mouse model of trauma- hemorrhage.[9]

The effect of early coagulopathy on the outcome of pediatric trauma patients is less clear. A recent retrospective study including pediatric trauma patients from combat hospitals in Iraq and Afghanistan reported that coagulopathy and shock on admission was associated with an increased mortality.[10,11] However, whether early coagulopathy plays an important role in civilian pediatric trauma patients is still unknown. We found in the present retrospective study of 803 severely traumatized children from a level 1 pediatric trauma center that the presence of early coagulopathy (INR > 1.2) was an independent predictor of mortality. Although there was a modest, but significant increase in mortality in pediatric trauma patients without brain injury, a four-fold increase in mortality was seen in patients with traumatic brain injury, either isolated or combined with injuries to other organs.

MATERIAL AND METHODS

Patient selection

After Institutional Review Board approval, we retrospectively identified all pediatric trauma

patients < 18 of age admitted from 2001 to 2010 to the pediatric intensive care unit (PICU) of the Children's Hospital of Alabama, the only level 1 pediatric trauma center in the state of Alabama. Only patients with recorded coagulation studies were included. Burn patients, patients sustaining primary asphyxiation, patients with preexisting bleeding diathesis and patients transferred from other hospitals > 24 hours were excluded from the study.

Data collection

Demographic and clinical information that was collected included age, sex, race, mechanism of injury, transfer times, and systolic blood pressure (SBP) on arrival to the hospital. In addition, Glasgow Coma Scale (GCS) score was recorded on admission, and Injury Severity Score (ISS) and Abbreviated Injury Scale (AIS) for each body region were calculated for each patient. Laboratory data collected on admission included prothrombin time (PT), partial thromboplastin time (PTT), platelet count, and INR values. Arterial or venous blood gas analysis was performed only when possible.

Outcome measures

Coagulopathy was defined retrospectively as an elevated INR greater than 1.2 in accordance with previously published adult studies.[5,12] The primary outcome was in-hospital mortality. Secondary outcomes were lengths of ICU and hospital stay (length of stay [LOS]).

The pediatric population was divided into two groups based on ISS values with a cutoff of 25 and then assessed for mortality and incidence of coagulopathy. Systolic blood pressure measured on arrival to the hospital was stratified in age- specific reference points according to the 2010 Pediatric Advanced Life Support guidelines: neonates, less than 60 mmHg; infants, less than 70 mmHg; children 1 to 10 years, less than 70 mmHg + (age in years x 2) mmHg; and children older than 10 years, less than 90 mmHg. When possible, an arterial blood gas or venous blood gas was obtained immediately after hospital admission. The base deficit (BD) was used as a marker for tissue hypoperfusion. In adult trauma patients, an admission BD greater than 6 mmol/L has previously been identified as predictive of worse outcome in these patients (12-14). The cohort was also assessed according to age and divided into four groups: infants and toddlers (≤ 2 years old), preschool age (3-7 years), school age (8-12 years), and adolescents (13-17 years). The mechanisms of injury within our trauma cohort were reviewed

and stratified into four main categories: motor vehicle crash, fall, gunshot wound, and nonaccidental trauma (NAT). A subanalysis on mortality and coagulopathy was performed on patients with and without severe TBI. Severe TBI was subdivided into isolated TBI (AIS head ≥ 3 and AIS extracranial >3) and nonisolated TBI (AIS head ≥ 3 and AIS extracranial ≥ 3).

Statistical analysis

Data analysis was performed using SAS software (SAS, Cary, NC). Parametric data are presented as means ± SD. Nonparametric data are presented as median (interquartile range [IQR] and mean). Student *t* test was used to compare two samples of parametric continuous data and Mann-Whitney *U* test for non- parametric continuous data. For three-group comparison of continuous data, the Kruskal-Wallis one-way analysis of variance (if nonparametric data) or analysis of variance (for parametric data) was used. Fisher protected least significant differences method was used to control for multiple comparisons. That is, only in cases of significance ($P < 0.05$), the Mann-Whitney *U* test (for non-parametric data) or the Student *t* test (for parametric data) was used to analyze specific sample pairs for significant differences. Categorical data were compared using $/^2$ test or Fisher exact test as appropriate. Multivariate logistic regression was used to determine which variables were independently associated with mortality and coagulopathy by initially including all variables with a $P < 0.1$. The Hosmer- Lemeshow goodness-of-fit test for the logistic regression model showed a P 9 0.2 for both analyses, indicating that the fit was adequate. Receiver operating characteristic curves were calculated for mortality and coagulopathy. All tests are 2-sided, and the significance for all comparisons was set at $P \leq 0.05$.

RESULTS

Patient selection and demographics

During the study period (2001-2010), 1062 patients were admitted to the Children's Hospital of Alabama pediatric ICU after severe trauma. The majority of patients excluded did not have coagulation tests performed upon arrival to the hospital (n = 221). In addition, patients were excluded for the following reasons: late transfer (n = 2), age limit (n = 2), death on arrival (n

= 10), and burns or asphyxiation (n = 17). Table 1 shows the characteristics of the patients enrolled in the study. In all, 803 severely injured trauma patients were included in the study over a 10-year period. Sixty-two percent of patients were male, and blunt trauma was the cause of injury in 92% of patients. Overall mortality was 13.4%. The incidence of age-adjusted hypotension was 5.4%. Finally, 37.9% of the patients presented with coagulopathy (defined as an INR 91.2) measured on arrival to the hospital. In this patient group, there was a direct relationship between mortality rate and the initial INR value on arrival to the hospital (Fig. 1).

TABLE 1. Patient demographics

Variables	N=803
Age (yr), mean ±SD	8.7 ±5.0
Gender male	62%
Race	
% Black	33.7%
% White	60.6%
% Hispanic	4.9%
% Other	0.7%
Mechanism blunt	92%
Mortality	13.4%
ISS median	22 (0-75)
% Arterial hypotension [a]	5.4 %
PT median/IQR	15.7 (14.6-17.5)
APTT median/IQR	29.7 (26.7-34.0)
INR median/IQR	1.2 (1.1-1.4)
Coagulopathy	37. 9%
Platelets	319 ± 114

ISS: Injury Severity Score (ISS); PT: Prothrombin Time; aPtt: Activated Partial Thromboplastin Time
[a] Corrected for age

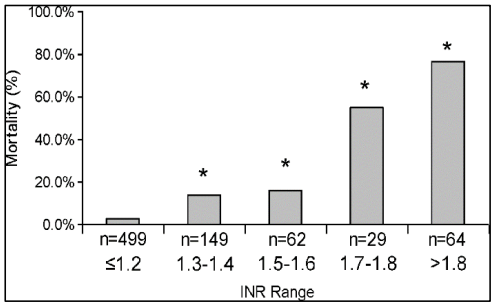

FIGURE 1 **High initial INR values are associated with increased mortality rate in pediatric trauma patients**. Initial INR values were directly correlated with mortality rate in pediatric trauma patients; P < 0.05 from patients with normal INR values (0.9-1.2) on admission to the hospital.

Effect of injury severity and shock on early posttraumatic coagulopathy and mortality

High ISS or presence of hypotension on arrival to the hospital was associated with an increased incidence of early coagu- lopathy and higher mortality rate in pediatric trauma patients (Fig. 2, A-D). Moreover, the combination of high ISS and hypotension was associated with the highest rate of coagulopathy and mortality in this patient population (Fig. 2, E and F). These results were confirmed by measurement of the initial BD that indicated that a BD greater than 6, a marker of tissue hypoperfusion, is associated with an increased incidence of early posttraumatic coagulopathy and higher mortality rate in pediatric trauma patients (Fig. 3).

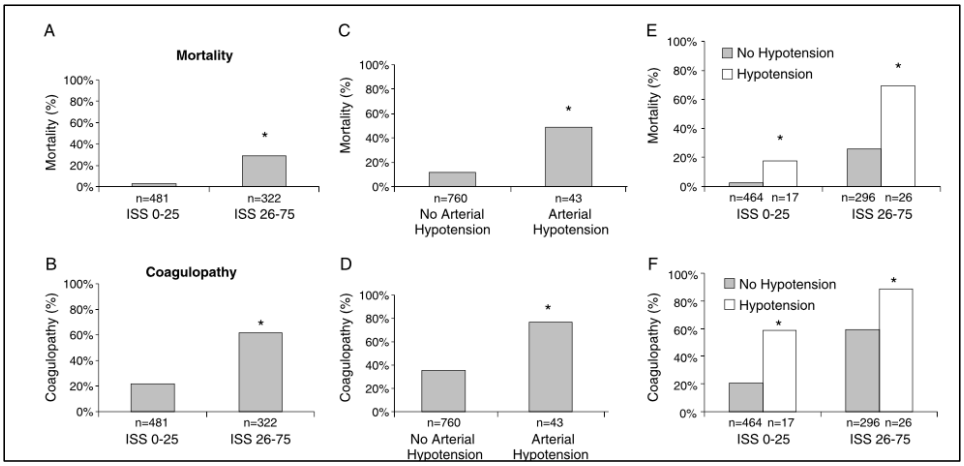

FIGURE 2 **A combination of tissue injury and systemic arterial hypotension is associated with an increased incidence of early coagulopathy and higher mortality rate in pediatric trauma patients.** A-D, Patients were divided into groups using previously described definitions of injury severity based on ISS and arterial hypotension corrected for age. High ISS or presence of systemic arterial hypotension on admission to the hospital was associated with an increased incidence of early coagulopathy and higher mortality rate in pediatric trauma patients. E and F, The combination of high ISS and arterial hypotension was associated with the highest rate of coagulopathy and mortality in this patient population. A-D, **P*< 0.05 from patients with low ISS or without arterial hypotension on admission to the hospital. E and F, **P* < 0.05 from patients without arterial hypotension on admission to the hospital.

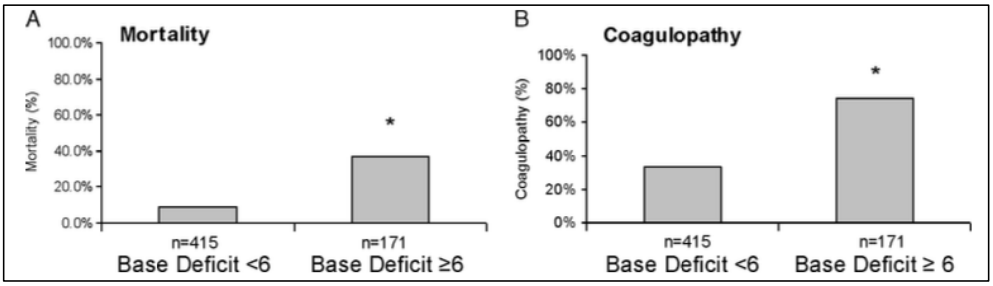

FIGURE 3 **High BD (greater than -6), a marker of tissue hypoperfusion, is associated with an increased incidence of early post-traumatic coagulopathy and higher mortality rate in pediatric trauma patients.** A and B, Patients were divided into two groups based on the initial BD (greater than -6) measured from the arterial or venous blood gas samples taken after admission to the hospital. A and B, $*P < 0.05$ from patients with low BD (less than -6).

Effect of age and mechanism of injury on the incidence of early coagulopathy and mortality

Mortality rate was higher in the patients who were younger than 3 years compared with the entire patient population (Fig. 4A). Falls were associated with a lower incidence of early coagu- lopathy and lower mortality rate (Fig. 4B). In contrast, patients who sustained a NAT were significantly more coagulopathic on arrival to the hospital and had a higher mortality rate (Fig. 4B).

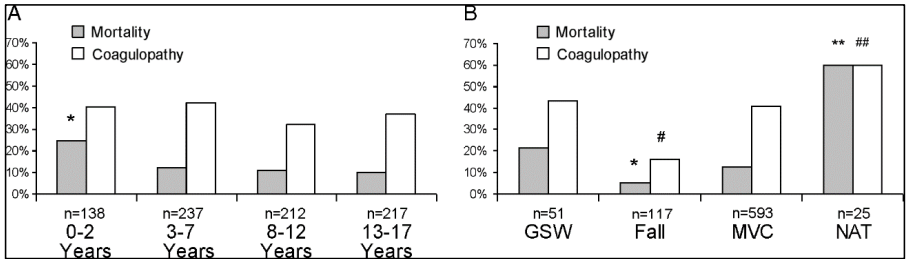

FIGURE 4 **Effect of age and mechanism of injury on the incidence of early coagulopathy and mortality in pediatric trauma patients**. A, Patients were divided in four groups based on the age range. The patients who are younger than 2 years had a significantly higher mortality than the other age groups. B, Patients were divided into four groups based on based on the mechanisms of injury. Patients with fall had a lower incidence of post-traumatic coagulopathy and mortality than the rest of the patient population. In contrast, patients with NAT had a significantly higher incidence of post-traumatic coagulopathy and mortality than the rest of the patient population; $*P < 0.05$ for comparison of mortality rate between falls and the other causes of injury; $^{\#}P < 0.05$ for comparison of the incidence of coagulopathy between falls and the other causes of injury; $**P < 0.05$ for comparison of mortality rate between NAT and the other causes of injury; $^{\#\#}P < 0.05$ for comparison of the incidence of coagulopathy between NAT and the other causes of injury.

The combination of TBI and early coagulopathy is associated with a statistically significant increase in mortality in pediatric trauma patients

Overall mortality was significantly higher in patients with TBI (either isolated or combined with other injuries) than in patients without TBI (Table 2). Furthermore, patients with combined injuries had a higher incidence of tissue hypo- perfusion, as shown by an initial BD of greater than 6, and of early posttraumatic coagulopathy than the two other groups of patients (Table 2). The presence of early coagulopathy was associated with a small increase in mortality in patients without TBI. However, in presence of TBI, there was a more than fourfold increase in mortality in patients with early coagulopathy who had an initial INR of more than 1.2 (Fig. 5). Finally, the data summarized in Table 3 indicate that TBI patients with an INR between 1.2 and 1.4 on admission to the hospital have a statistically significant increase in mortality, although their INR value was below the usual threshold for diagnosis of coagulopathy (>1.5).

Characteristics	No TBI (n = 267)	Isolated TBI (n = 296)	Nonisolated TBI (n = 240)	*P*
Age, mean ± SD, y	8.9 ± 4.9	8.6 ± 5.1	8.6 ± 4.9	NS
Sex male, %	62	62	62	NS
Mechanism blunt, %	87.3	91	95	<0.05*
Mortality, %	2	14	25	<0.05†
Arterial hypotension, %	4.9	3.4	8.3	NS
Base deficit −6 or less, %	14.6	17.6	33.3	<0.05†
aPTT ≥36, %	14.2	16	30	<0.05†
INR >1.2, %	29.6	27	60	<0.05*†‡
Platelets <150, %	2.6	4	7	NS

*Nonisolated TBI vs. all patients, statistically significant.
†No TBI vs. all patients, statistically significant.
‡Isolated TBI vs. all, patients statistically significant.
aPTT indicates activated PTT; NS, not statistically significant.

TABLE 2 **Demographics, incidence of shock, and coagulopathy including abnormal coagulation parameters according to different injury groups**

INR	Non-TBI (n = 267)	Isolated TBI (n = 296)	Nonisolated TBI (n = 240)
<1.3	0% (0/188)	2.3% (5/215)	8.3% (8/96)*
1.3–1.4	2.5% (1/40)	20.9% (9/43)*	16.7% (11/66)*
1.5–1.6	4.8% (1/21)	27.3% (3/11)	20% (6/30)
1.7–1.8	33.3% (3/9)	100% (4/4)	56.3% (9/16)
>1.8	11.1%(1/9)	87% (20/23)*	84.4% (27/32)*

*$P < 0.05$ compared with non-TBI group.

TABLE 3 **Mortality is related to INR and presence of TBI**

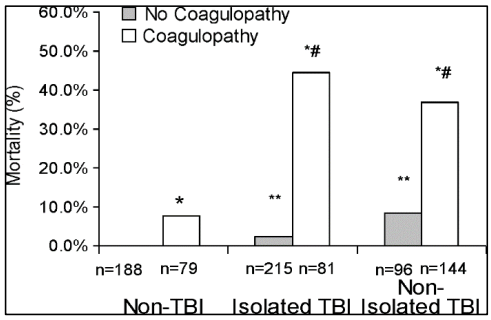

FIGURE 5 **The combination of TBI and early coagulopathy is associated with a large increase in mortality in pediatric trauma patients.** Patients were divided into three groups: severe TBI was subdivided into isolated TBI (AIS head of ≥ 3 and AIS extra-cranial <3) and non-isolated TBI (AIS head ≥ 3 and AIS extracranial >2). The absence of TBI was defined as AIS head of 2 or less. The association of an early post-traumatic coagulopathy and TBI increased the mortality by threefold to fourfold compared with the patients without TBI; *$P < 0.05$ from patients without coagulopathy; #$P < 0.05$ from patients with coagulopathy but without TBI; **$P < 0.05$ from patients without coagulopathy and without TBI.

Outcome

Age younger than 3 years and presence of early coagulopathy were associated with an increased odds ratio for mortality. Furthermore, higher ISS and lower GCS were also independent predictors for mortality in this pediatric patient population (Table 4). In addition, higher ISS and the presence of arterial hypotension were both independent predictors of the development of early coagulopathy after severe trauma in pediatric patients (Table 4). Receiver operating characteristic curve analysis showed areas under the curve of 0.94 for mortality and 0.80 for coagulopathy (Fig. 6). Finally, the presence of early coagulopathy was associated with a significantly longer ICU LOS (INR >1.2: median ICU LOS 4 days [range, 0-28 days] vs. INR <1.2: median ICU LOS 2 days [range, 0-42 days]; $P < 0.0001$) and hospital LOS (INR >1.2: median hospital LOS 13 days [range, 1-276 days] vs. INR <1.2: median ICU LOS 5 days [range, 1-132 days]; $P < 0.0001$) in survivors from severe trauma in pediatric patients.

	Odds ratio	*P*
Mortality		
Age <3 y	4.2	<0.0001
Coagulopathy	4.0	0.0008
ISS	1.1	<0.0001
GCS	0.7	<0.0001
Coagulopathy		
ISS	1.1	<0.0001
Arterial hypotension	3.3	0.0057

TABLE 4. **Multivariate comparison for all pediatric trauma patients**

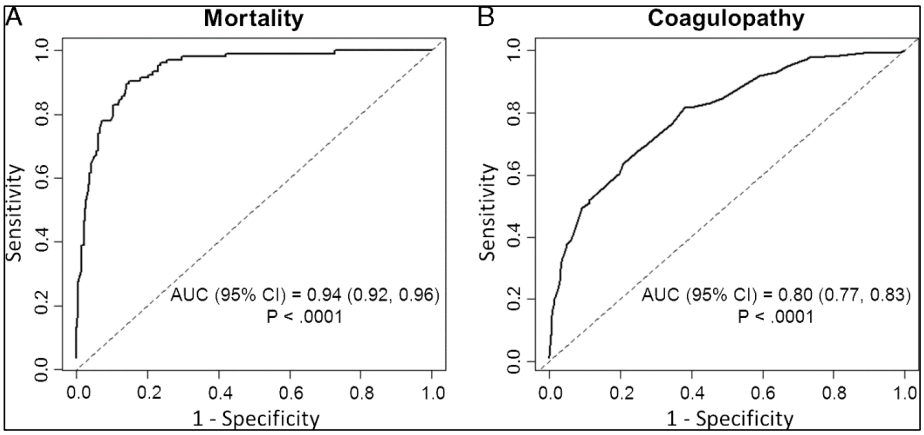

FIGURE 6 **Receiver operator characteristic curves for predictive models of mortality or coagulopathy in pediatric trauma patients.** A and B, the model includes all four clinical predictors for mortality and two clinical predictors for coagulopathy. AUC indicates area under the curve.

DISCUSSION

The results of the present study indicate that early coagulopathy is an independent predictor of mortality in civilian pediatric trauma patients. Comparable findings have previously been reported in children admitted to combat support hospitals in Iraq and Afghanistan.[10,11] Although major differences exist between the injured children in war zones and our civilian pe- diatric trauma patients with regard to mechanism of injury (penetrating vs. blunt) and definition of coagulopathy (INR 1.5 vs. 1.2), early coagulopathy was an independent predictor of mortality in both groups of pediatric patients. This important finding was confirmed by the results of a recently published smaller study that included transfused civilian pediatric trauma patients.[16] In the present study, early posttraumatic coagulopathy was defined as an INR greater than 1.2. This is based on recent work showing that a PT ratio of greater than 1.2 is associated with an increased mortality in adult trauma patients[5]. Previous trauma studies have defined posttraumatic coagulopathy as an INR greater than 1.5 that was originally adopted from international guidelines for initiating administration of fresh frozen plasma. This threshold was derived from correlations between clotting times and the incidence of microvascular bleeding in patients undergoing massive blood transfusions.[17-19] The second important finding of this study is that the combination of injury severity and tissue hypoperfusion (hypotension and/or

increased BD on arrival to the hospital) were associated with a higher incidence of coagulopathy and mortality. A similar association between hypovolemic shock, severity of injury, and development of coagulopathy has previously been reported in children suffering from penetrating trauma from Middle East war zones.[10,11] Furthermore, we have previously reported comparable results in adult trauma patients and have shown that the severity of shock and traumatic injury directly correlated with the activation of the anticoagulant protein C pathway that may play an important role in the early post-traumatic coagulopathy.[8,20] In contrast to the results reported for children injured in the war zones[10], age and mechanism of injury did not significantly affect the incidence of early coagulopathy and mortality in our civilian pediatric trauma population with the exception of children younger than 3 years who had an in- creased mortality rate compared with the entire pediatric trauma population. The likely explanation for the increased mortality in this very young age group is that the mechanism of trauma was mostly related to NAT that has a higher mortality rate than accidental trauma.[21]

Finally, in pediatric patients with isolated TBI or TBI combined with other injuries, an INR greater than 1.2 was associated with a significant increase in mortality compared with patients without coagulopathy. Although 30% of patients without TBI were coagulopathic on admission to the hospital, the overall mortality was very low (2.2%), and none of the patients without coagulopathy died. In contrast, patients with isolated TBI or TBI combined with other organ injuries had a sharp increase in the mortality when the initial INR was more than 1.2. Previously published studies have reported variable results about the value of coagulopathy as an independent predictor of mortality. A recently published European study reported that early coagulopathy that was defined as a PT of twice the normal value was predictive of mortality in pediatric trauma patients with combined injuries that include TBI.[22] Interestingly, coagulopathy was also present in patients who did not suffer from heavy bleeding because of their injuries. Comparable results were reported in a small study including pediatric patients with iso- lated TBI.[23] Using the database of the German Trauma Registry, another study reported that GCS is a predictor of coagulopathy after blunt pediatric TBI.[24] In contrast, a study from the University of Southern California that included children with isolated TBI did not show that coagulopathy (defined as an INR >1.2) was an independent predictor of mortality, al- though a large percentage of patients were coagulopathic (40%).[25] The difference between

that study and those previously cited (including our study) could be related to the fact that the reported overall mortality was lower in the University of Southern California study. In addition, there are other factors that are also independent predictors of mortality in children with severe TBI, such as young age (<3 years old), accidental hypothermia, and hyperglycemia[23] that may not have been present with the same prevalence in all cited studies. The results of the present study clearly demonstrate that a small increase in the initial INR value is associated with a large in- crease in mortality in children with TBI, but not in patients without TBI. However, despite the large number of patients included in our study, there are several limitations, the most important being the retrospective nature of data analysis. Second, the effect of therapeutic interventions on outcomes, such as transfusion of blood products, could not be determined. Finally, data regarding long-term outcome were not available for analysis including the exact cause of death in each of the patients. Well-performed prospective clinical trials should be conducted to determine the potential beneficial effect of early treatment of acute TBI-associated coagulation abnormalities in children with severe trauma.

In summary, the results of the present retrospective study that includes 803 patients admitted over a 10-year period at the level I trauma center demonstrate that early coagulopathy defined as an INR greater than 1.2 is an independent predictor of mortality in pediatric patients with severe trauma. The increase in mortality was particularly significant in patients with TBI either isolated or combined with other injuries, suggesting that a rapid correction of this coagulopathy could substantially decrease the mortality after TBI in pediatric trauma patients.

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Chapter 4

PROTEIN C AND ACUTE INFLAMMATION: A CLINICAL AND BIOLOGICAL PERSPECTIVE

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ABSTRACT

The protein C system plays an active role in modulating severe systemic inflammatory processes such as sepsis, trauma, and acute respiratory distress syndrome (ARDS) via its anticoagulant and anti-inflammatory properties. Plasma levels of activated protein C (aPC) are lower than normal in acute inflammation in humans, except early after severe trauma when high plasma levels of aPC may play a mechanistic role in the development of posttraumatic coagulopathy. Thus, following positive results of preclinical studies, a clinical trial (PROWESS) with high continuous doses of recombinant human aPC given for 4 days demonstrated a survival benefit in patients with severe sepsis. This result was not confirmed by subsequent clinical trials, including the recently published PROWESS-SHOCK trial in patients with septic shock and a phase II trial with patients with nonseptic ARDS. A possible explanation for the major difference in outcome between PROWESS and PROWESS-SHOCK trials is that lung-protective ventilation was used for the patients included in the recent PROWESS-SHOCK, but not in the original PROWESS trial. Since up to 75% of sepsis originates from the lung, aPC treatment may not have added enough to the beneficial effect of lung-protective ventilation to show lower mortality. Thus, whether aPC will continue to be used to modulate the acute inflammatory response in humans remains uncertain. Because recombinant human aPC has been withdrawn from the market, a better understanding of the complex interactions between coagulation and inflammation is needed before considering the development of new drugs that modulate both coagulation and acute inflammation in humans.

INTRODUCTION

The protein c system is best known for its anticoagulant activity seen most clearly in patients with total protein C deficiency that develop lethal thrombosis if not properly treated.[32] The protein C system also has anti-inflammatory properties related both to its anticoagulant activity and to cytoprotective properties independent of the coagulation cascade. Because of these properties and following the purification of the activated form of protein C (aPC) from plasma,[96] it was demonstrated that administration of aPC protected baboons from *Escherichia coli* sepsis.[98] Alternatively, reducing protein C levels in mice or blocking its activation in baboons increased a sublethal to a lethal challenge with *E. coli* endotoxin or bacteria.[38,58,98] In addition, because results from clinical studies support the concept that disseminated intravascular coagulation (DIC) is an independent predictor of organ failure and mortality, there was strong evidence that strategies aimed at improving the coagulation status of patients with severe sepsis should be beneficial. The initial double-blind, randomized, placebo-controlled multicenter clinical trial (PROWESS) with a recombinant form of aPC (drotrecogin alfa activated) demonstrated a survival benefit in patients with severe sepsis.[9] Following publication of these results, recombinant aPC was licensed for treatment of severe sepsis by the U.S. Food and Drug Administration in 2001 and by the European Medicines Agency in 2002. Nevertheless, these initial positive results could not be replicated in septic patients with lower risk of death (ADDRESS study) or in children with severe sepsis (RESOLVE study).[1,68] In 2011, drotrecogin alfa was withdrawn from the market worldwide after a new double-blind, randomized, placebo-controlled trial (PROWESS-SHOCK) failed to meet its primary end point, i.e., a significant decrease in the 28-day mortality of patients with severe sepsis.[72]

So, what is next regarding the role of the protein C system in acute inflammatory processes, such as severe sepsis, trauma, or acute respiratory distress syndrome (ARDS)? Could the administration of pharmacological doses of aPC given to select groups of patients and/or earlier in the course of the disease affect the outcome of these patients? The goal of this clinical perspective is to review the anticoagulant and cytoprotective properties of protein C and to summarize the most recent preclinical and clinical literature on the developing role of the protein C system in acute inflammation to determine whether targeting the coagulation system could provide benefit to patients with severe sepsis and trauma.

Anticoagulant and Cytoprotective Properties of Protein C

Protein C zymogen is a vitamin K-dependent protein secreted by the liver that has a plasma concentration of 40 nM with a half-life of 8 h. Upon binding with the endothelial protein C receptor (EPCR), thrombin-thrombomodulin complexes activate protein C by removing 14 amino acids (106). Generation of aPC from protein C depends on the presence of thrombin in plasma. Plasma concentration of aPC is low (less than 40 pM). However, the fact that the plasmatic clearance of aPC depends on serine protease inhibitors contributes to a long half-life of 20–25 min in humans. Activated protein C has known inhibitors in plasma that include protein C inhibitor, α1-antitrypsin, α2-macroglobulin, and α2-antiplasmin (Fig. 1).[42]

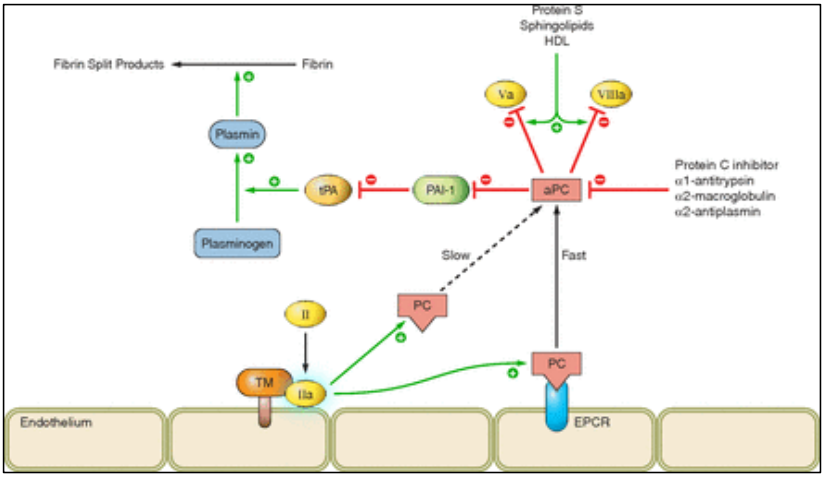

FIGURE 1 **Anticoagulant properties of activated protein C (aPC).**
Protein C (PC) is activated after binding to endothelial protein C receptor (EPCR)at the endothelial surface via cleavage by thrombin. This cleavage happens in the presence of thrombin-thrombomodulin (TM) complexes. Activated protein C (aPC)'s major anticoagulant effect is inhibition of factors Va and VIIIa, which is accelerated by protein S, sphingolipids, and high-density lipoproteins (HDL). Its other anticoagulant effect is via inhibition of plasminogen activator inhibitor 1 (PAI-1), thereby promoting fibrinolysis. aPC activity is inhibited by protein C inhibitor, α1-antitrypsin, α2-macroglobulin and α2-antiplasmin, tPA (tissue plasminogen activator).

One of the main functions of the protein C system is its anticoagulant activity. Upon its dissociation from EPCR, the anticoagulant activity of aPC involves proteolytic inactivation of coagulation factors Va and VIIIa,[37] a mechanism that is enhanced by lipid and protein cofactors, such as protein S, sphingolipids, and high-density lipoproteins. [42] It should be noted

that, independent of its role as a cofactor for aPC, protein S also has a direct anticoagulant effect via its direct binding to factors Va and VIIIa and Xa.[46] In addition, aPC also inactivates plasminogen activator inhibitor 1 (PAI-1), which results in increased fibrinolysis.[69]

The protein C system also has cytoprotective properties related both to its anticoagulant activity and to inhibitory properties independent of the coagulation cascade. Thrombin generation can induce the expression of several proinflammatory events: for example, the expression of P-selectin or the activation of the NF-κB pathway.[21] The initial PROWESS trial stimulated research on nonanticoagulant properties of aPC that lead to the elucidation of multiple cytoprotective properties of aPC that independent of its anticoagulant activity. The multiple cytoprotective effects of aPC include protection of the endothelial barrier function, antiapoptotic activity, anti-inflammatory properties, and modification of gene expression (Fig. 2). These cytoprotective effects of aPC are mediated by several receptors. The best known pathway is the EPCR-aPC-protease-activated receptor 1 (PAR1) pathway, which plays an important role in the barrier stabilization of endothelial cells via sphingosine-1-phosphate (S1P) and RAC1-dependent mechanisms as aPC causes EPCR-dependent transactivation of the S1P receptor in membrane lipid rafts.[34] Interestingly, thrombin and aPC both bind to PAR1. However, to explain this paradox, recent work has shown that the localization of PAR1 in the caveolin-1 lipid rafts is required for aPC signaling, but not for thrombin activation of the receptor.[82] In addition, thrombin-cleaved PAR1 is internalized whereas aPC-activated PAR1 stays at the cell membrane, which can prolong the aPC signaling via this receptor.[92] The anti-inflammatory effect of aPC is also mediated on other cells by additional receptors other than PAR1. Integrins are important receptors for aPC on lymphocytes, neutrophils, and macrophages.[42] PAR3 plays an important role in the cytoprotective effect of aPC in the brain and kidney.[44,50] Finally, the low-density lipoprotein-related protein 8 (ApoER2) binds aPC with great affinity and mediates aPC-induced activation of anti-inflammatory signaling pathways in U937 cells via a phosphoinositide-3-kinase-dependent mechanism.[110] Taken together, these facts make it appear less and less likely that there is one unifying mechanism that explains all the cytoprotective properties of aPC. In contrast, the cytoprotective effects of aPC on different cells appear to be associated with cell-type-specific expression of aPC receptor complexes. This discovery had led to the development of aPC mutants that are only activating receptors on specific cell types, allowing a better understanding of the multiple

anticoagulant and cytoprotective functions of aPC.

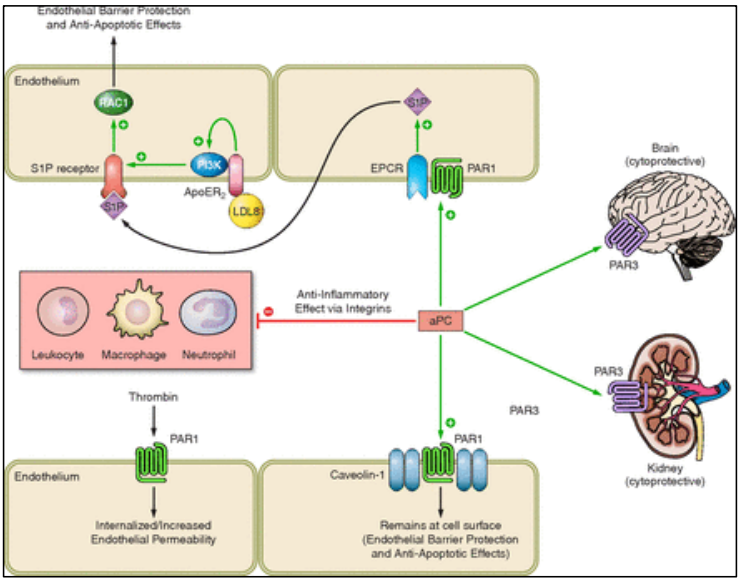

FIGURE 2 **Cytoprotective properties of aPC**
aPC induces cytoprotective effects for the brain and kidney via the PAR3 receptor. Anti-inflammatory effects of aPC in immune cells are mediated by integrins. aPC activates the PAR-1-EPCR complex to produce sphingosine-1-phosphate (S1P), which binds its cognate receptor, causing activation of RAC1 to mediate endothelial barrier protection and anti-apoptotic effects. These effects are further induced by activation of phosphoinositol-3-kinase (PI3K) by ApoER2-LDL8, which positively influences the S1P receptor. Finally, aPC can activate the PAR1 receptor when it is present in lipid rafts with caveolin-1. This interaction leads to receptor signaling remaining at the cell surface and promoting endothelial barrier protection and antiapoptotic effects. Conversely, when PAR1 is not in lipid rafts it binds thrombin, which causes internalization and inactivation of the receptor and increase in endothelial permeability.

PROTEIN C AND SEPSIS

Severe sepsis is defined as sepsis complicated by acute organ dysfunction. In severe sepsis caused by bacteria, fungus, or virus, dysregulation of the hemostatic system may lead to DIC associated with microvascular thrombosis, tissue hypoperfusion, multiorgan failure, and death.[45] In animal models of severe sepsis, this characteristic sequence usually causes a procoagulant and antifibrinolytic state within a few hours after onset of the disease.[52] In

humans, the progression to a full DIC may be rapid or slow depending on the bacterial virulence and the patient's underlying condition. If the patient survives, the restoration of the hemostatic balance will depend on individual protein synthesis capacity, stability of the synthesized coagulation enzymes and substrates, and efficacy of natural coagulation inhibitors. There are three principal natural anticoagulant pathways: tissue factor pathway inhibitor, which, when bound to factor Xa, inhibits the tissue factor-factor VIIa complex, thus suppressing the initial steps of thrombin generation[17]; antithrombin, which is a direct thrombin inhibitor forming complexes with thrombin[80]; and protein C that is converted to its activated form (aPC) by proteolysis by the thrombin-thrombomodulin complex. After binding to its own receptor, EPCR, aPC acts as a potent inhibitor of thrombin via the inactivation of factors Va and VIIIa and also as a profibrinolytic agent by inhibiting plasminogen activator inhibitor 1 and thrombin-activatable fibrinolysis inhibitor.[73]

Activation of the coagulation system may represent a potent mechanism of innate defense against bacterial invasion. The formation of fibrin network allows microbial trapping and limits bacterial growth. In addition, it facilitates the engagement of neutrophils, particularly via the recent description of the prothrombotic and antibacterial properties of neutrophil extracellular traps (NETs).[12,59] The presence of histone proteins in these NETs also enhances platelet aggregation and impairs thrombomodulin (TM)-dependent activation of protein C, thus further activating the coagulation system.[2] Although all procoagulant changes in severe human sepsis have been considered as pathological, this concept might have been erroneous. In septic DIC, some mediators such as fibrinogen or factor V are upregulated, whereas the hepatic synthesis of some anticoagulants such as antithrombin and protein C is significantly decreased.[4] Thus, not only may the decrease in plasma levels of protein C in severe sepsis represent consumption coagulopathy, but also the decrease in plasma levels of thrombin inhibitors and the fact that protein C is a negative acute-phase protein may be interpreted as participating in the host defense mechanisms. However, there are numerous clinical reports showing that excessive deregulation of hemostasis, including severely decreased plasma levels of protein C, occurs by 24 h after onset of the syndrome and is associated with multi-organ failure and death.[28,87] These observations suggest that administration of natural anticoagulants such as aPC may be of therapeutic value.

Because of the lack of causal therapy for severe sepsis in humans, and the positive results of a

major study in septic primates with high dose of aPC, the first multi-center trial with a high dosage of aPC was organized (PROWESS trial). Importantly, the design of the trial stipulated that, irrespective of the time period of the sepsis, the type of bacteria, or the degree of activation of the coagulation system, every patient would receive the same high dose of aPC for a limited time (4 days). Administration of protein C zymogen was excluded because it was considered that its activation by thrombomodulin was dysfunctional in sepsis and thus unpredictable. Despite these shortcomings, PROWESS was stopped early because of a 6.1% absolute and a 19.4% relative reduction in mortality with a logical relation noted between severity of the disease and survival advantage.[9] Soon after the publication of this study, new trials reported a lack of effect in patients with less severe sepsis or in children.[1,68] All subsequent clinical trials did not demonstrate that aPC would cause a significant decrease in mortality in patients with sepsis (Table 1). Because of these negative results and the cost of the drug, the European health authorities decided to request another randomized multi-center placebo-controlled trial to confirm the benefits reported in PROWESS. The results of PROWESS-SHOCK showed no difference in mortality between patients with septic shock treated with recombinant human aPC or placebo.[72] This trial reported a mortality that was much lower (25%) than in other recent trials that also included patients with severe sepsis (35–50%)[23,70] and raised the question of whether this trial was underpowered to detect a difference in mortality between the two groups of patients. Second, another possible explanation for the major difference in outcome between PROWESS and PROWESS-SHOCK trials is that lung-protective ventilation was used for the patients included in the recent PROWESS-SHOCK, but not in the original PROWESS trial. Since a large degree of sepsis originates from the lung, aPC treatment may not have added enough to the beneficial effect of lung-protective ventilation to show lower mortality. Finally, it also possible that defense mechanisms that locally attenuate the inflammatory response might be detrimental in the presence of systemic inflammation.[85] The immediate consequence resulting from the lack of protection of aPC in patients included in the PROWESS-SHOCK trial was that the manufacturer of recombinant human aPC withdrew the drug from the market immediately after the publication of the results of this clinical trial.

TABLE 1 An overview of the study characteristics of the most important studies performed in humans on activated protein C and protein C zymogen

APC	Intervention	References	Study name	Study year	Study Phase	Study population	Main Results	Remarks
	RCT APC	Bernard GR, Vincent JL	PROWESS	2001	Phase III	Sepsis adults (n=1690)	Decrease in mortality, higher incidence of serious bleeding events	
	RCT APC	Bernard GR, Ely EW	rhAPC Sepsis study	2001	Phase II	Sepsis adults (n=131)	No difference in mortality, no difference in bleeding or adverse events	
	RCT APC	Yan SB, Nelson DR	NA	2004	phase III	Sepsis adults factor V Leiden mutation (n=150)	No difference in mortality, no serious adverse events or bleeding, similar benefit/risk profile	
	Non-randomized open label APC	Barton P, Kalil AC	NA	2004	phase III	Sepsis pediatrics (n=83)	No efficacy conclusion possible	
	RCT APC	Abraham E, Laterre PF	ADDRESS	2005	Phase III	Sepsis adults (n=2640)	No difference in mortality, higher rate of serious bleeding	trial terminated early due to low likelihood of meeting objective
	Retrospective single group APC	Higgins TL, Steingrub JS	NA	2005	NA	Sepsis adults (n=44)	Mortality higher than in Prowess	
	Prospective single group APC	Maurice A, Seguin P	NA	2005	NA	Sepsis adults -23	Reduction in mortality, no increase in bleeding episodes	
	Non-randomized open label APC	Vincent JL, Bernard GR	ENHANCE	2005	phase III	Sepsis adults (n=2378)	Favorable benefit/risk ratio with early treatment (<24 hrs)	
	Non-randomized open label APC	Goldstein B, Nadel S,	ENHANCE	2006	phase III	Sepsis pediatrics (n=187)	No efficacy conclusion possible	
	Retrospective single group APC	Spriet I, Meersseman W	NA	2006	NA	Sepsis adults (n=23)	Hospital mortality similar as Prowess.	
	Prospective unmatched controlled cohort APC	Bertolini G, Rossi C	NA	2007	NA	Sepsis adults -1849	Crude ICU mortality lower in DAA group, higher mortality in DAA group in scheduled surgery patients	
	RCT APC	Nadel S, Goldstein B	RESOLVE	2007	Phase III	Sepsis pediatrics (n=477)	No difference in mortality, more instances in CNS bleeding	
	Retrospective cohort APC	Kanji S, Perreault MM	NA	2007	phase II	Sepsis adults (n=261)	higher mortality rate, higher rate bleeding events	
	Prospective single group	Ernst FR, Johnston JA	NA	2007	NA	Adult sepsis (n=1179)	Different timing of DAA administration is associated with difference in outcome	
	Retrospective single group APC	Ridley S, Lwin A	NA	2008	NA	Sepsis adults (n=351)	Reduction in mortality suggested. Bleeding events could not be linked to DAA	
	Retrospective single group APC	Wheeler A, Steingrub J	NA	2008	NA	Sepsis adults (n=274)	Similar result to Prowess. High percentage bleeding events	
	RCT APC	Liu KD, Levitt J	NA	2008	Phase II	ALI adults (n=75)	No improved outcome	trial stopped early by DSMB
	Prospective unmatched controlled cohort APC	Rowan KM, Welch CA	NA	2008	NA	Sepsis adult (n=1097)	Reduced mortality, no effect in less severe illness	
	Prospective unmatched controlled cohort APC	Vincent JL, Laterre PF	PROGRESS registry	2008	NA	Sepsis adults (n=697)	Reduction in mortality	
	RCT APC	Dhainaut JF, Antonelli M	NA	2009	Phase III	Sepsis adults (n=193)	No difference in mortality, no serious adverse events	
	Prospective unmatched controlled cohort APC	Martin G, Brunkhorst FM	PROGRESS registry	2009	NA	Sepsis adults (n=11344)	Reduction in odds of mortality	
	Prospective observational	Ferrer R, Artigas A	NA	2009	NA	Sepsis adults (n=2796)	Reduction in mortality when treated with DAA and antibiotics	
	Retrospective single group APC	Gentry CA, Gross KB	NA	2009	NA	Sepsis adults (n=73)	Higher rates of bleeding events and death	
	Non-randomized open label APC	Decruyenaere J, De Backer D	NA	2009	phase IV	Sepsis adults (n=97)	Consistent results with previous studies	

	Prospective single group APC	Steingrub JS, Cheatham ML	NA	2010	NA	Sepsis adults (n=548)	DAA group more ill, higher mortality compared to Prowess. Similar bleeding events	
	Retrospective matched controlled cohort APC	Lindenauer PK, Rothberg MB	NA	2010	NA	Sepsis adults (n=3152)	Reduction in mortality	
	RCT APC	Shorr AF, Janes JM	RESPOND	2010	Phase II	Sepsis adults (n=433)	Increased protein C levels in treatment group	
	Non-randomized open label APC	Barie PS, Hydo LJ	NA	2011	phase II	Sepsis adults (n=108)	improved survival in surgical patients with septic shock and organ dysfunction	
	RCT APC	Ranieri VM, Thompson BT	PROWESS-SHOCK	2012	Phase III	Sepsis adults (n=1697)	No difference in mortality	
	RCT APC	Annane D, Timsit JF	NA	2013	Phase III	Sepsis adults (n= 411)	No difference in mortality, no serious adverse events	trial suspended before end due to drug withdrawal
PC zymogen	PC case series	Rivard GE, David M,	NA	1995	NA	Menigococcal sepsis, purpura fulminans (n=4)	Favorable outcome, normalization of protein C levels, no adverse effects	
	PC case series	Rintala E, Seppälä OP	NA	1998	NA	Meningococcal sepsis (n=3)	Favorable outcome, no adverse effects	
	PC case series	Smith OP, White B	NA	1999	NA	Purpura fulminans (n=12)	Favorable outcome, no adverse effects	
	PC case series	Rintala E, Kauppila M	NA	2000	NA	Sepsis associated purpura fulminans(n=12)	No conclusion on mortality, no drug related adverse effects	
	PC open label prospective non-randomized	White B, Livingstone W	NA	2000	phase III	Pupura fulminans, organ failure, menigococcemia adults and peds (n=36)	Reduction in morbidity and mortality	
	PC RCT	De Kleijn ED, de Groot R	NA	2003	phase II	Sepsis, purpura fulminans pediatrics (n=40)	Safe and effective to restore physiological plasma PC activity resolution of coagulation imbalance	
	PC retrospective	Fourrier F, Leclerc F	NA	2003	NA	Meningococcal purpura fulminans (n=15)	No efficacy conclusion	Anti-thrombin given together with PC concentrate
	PC double blind placebo endotoxemia model	Spiel AO, Firbas C	NA	2005	NA	Endotoxemia model in healthy adults (n=11)	No major anti-inflammatory, anti-coagulant or pro-fibrinolytic effetcs, no adverse effects	
	PC retrospective	Silvani P, Camporesi A	NA	2005	NA	Sepsis pediatrics (n=29)	No difference in mortality, increase in PC activity, restore some coagulation abnormalities	
	PC case series, pilot study	Schellongowski P, Bauer E	NA	2006	NA	Sepsis/purpura fulminans adults (n=8)	Clinical improvement, no major side effects	
	PC case series	Baratto F, Michielan F	NA	2008	NA	Adult sepsis (n=20)	safe and effective to restore physiological plasma PC activity	
	PC case series, pilot study	Crivellari M, Della Valle P	NA	2009	NA	Sepsis adults post cardiac surgery (n=9)	clinical improvement, improvement in inflammation and coagulation parameters, no bleeding	
	PC case series, pilot study	Decembrino L, D'Angelo A	NA	2010	NA	Sepsis coagulopathy neonates (n=18)	favorable changes in coagulation and inflammation parameters, clinical improvement, no adverse effects	
	PC retrospective	Veldman A, Fisher D	NA	2010	n/a	Purpura fulminans, pediatrics (n=94)	clinical improvement, no major bleeding	

Was this decision too abrupt? Are we sure that aPC does not work in sepsis? Since the publication of the first PROWESS trial, we have learned much more about the multiple

functions of the protein C system including its nonanticoagulant properties. Some preclinical trials have shown that these nonanticoagulant and anti-inflammatory properties may have an important protective effect in sepsis (reviewed in Ref. 67). Furthermore, several propensity-matched cohort studies have suggested that early use of aPC in patients with septic shock was associated with reduced mortality.[60,75,83] Another multicenter trial showed that the application of a bundle of measures could decrease mortality in severe sepsis and that the administration of aPC was by far the most significant of these measures.[33] Finally, a recent analysis reviewed the published clinical work related to the effectiveness and safety of recombinant human aPC for the past 10 years and compared these results to the ones of the PROWESS and PROWESS-SHOCK trials. The results of this review that includes more than 45,000 patients with severe sepsis indicate that the relative risk of hospital mortality was reduced by 18% by treatment with aPC compared with controls, a reduction close to what was reported in the original PROWESS trial. Importantly, the overall benefit associated aPC treatment shows a 1.3% reduction in the relative risk of death for every 1% increase in control mortality, despite the fact that the risk of serious bleeding was higher than in the PROWESS trial (5.6 vs. 3.5%).[53] It should be pointed out that a major limitation of this meta-analysis is that only 10% of the patients included in that study were from randomized controlled trials. Taken together, it is possible that additional clinical studies might identify selected patients who could benefit from aPC or refine the therapeutic window to optimize its administration. However, the negative clinical trials done in follow-up of PROWESS may weaken enthusiasm for allocation of further funding and resources allocations to accomplish such trials. Additional preclinical studies may help identify clinically tractable strategies.

PROTEIN C AND TRAUMA

There is new interest to understand the involvement of the protein C system in coagulation abnormalities after severe trauma in humans. Previous clinical studies have reported that a quarter of severely traumatized patients present with acute impairment of coagulation on arrival in the emergency department before any surgical treatment or administration of blood transfusion.[14,64] This early posttraumatic coagulopathy is physiologically and mechanistically distinct from classical iatrogenic posttraumatic coagulopathy associated with massive hemodilution, metabolic acidosis, and hypothermia. Importantly, this early posttraumatic

coagulopathy is associated with higher blood transfusion requirements, a greater incidence of multiple organ dysfunction syndrome, longer intensive care unit and hospital stays, and a fourfold increased risk of mortality compared with trauma patients without coagulation abnormalities at admission to the hospital.[13,18] These studies demonstrate that when traumatic injury is combined with tissue hypoperfusion (shock) the resultant early traumatic coagulopathy is characterized by elevated plasma levels of aPC and corresponding low levels of protein C zymogen, a decrease in factor V activity, and an activation of the fibrinolysis. These results raise two critical questions. First, what is the importance of the protein C system in the development of this early posttraumatic coagulopathy? The answer came from results of a mouse study demonstrating that severe hemorrhage and trauma caused severe coagulopathy associated with a fourfold increase in the plasma level of aPC. Inhibition of the anticoagulant domain of aPC by a blocking antibody to murine aPC completely corrected this early coagulopathy.[15] However, aPC cytoprotective/anti-inflammatory functions play a key role in preventing death in this model, in part apparently by preventing excessive thrombosis that might result from tissue necrosis or apoptosis.[15]

The second question is whether protein C activated by the binding of thrombin to thrombomodulin in the microcirculation during the early phase after trauma could have a protective effect against microvascular thrombosis in these patients. Recent clinical data from Cohen's laboratory showed that release of aPC after severe trauma could mitigate sterile inflammation and organ injury induced by the extracellular release of histone proteins after severe injury.[57] Similar results have been reported in an experimental model of sepsis. Indeed, a preclinical study from Dr. Esmon's laboratory has demonstrated that extracellular histones released in response to inflammatory challenge contribute to endothelial dysfunction, organ failure, and death in a primate model of sepsis. Furthermore, coinfusion of aPC with *E. coli* prevented lethality in baboons by causing the cleavage of histones.[108] Plasma levels of histone proteins either derived from apoptotic cells or secreted are known to have a strong prothrombotic effect by activating the procoagulant function of platelets associated with NETs[31] and by inducing plasma thrombin generation secondary to the impairment of TM-dependent aPC activation.[2] Furthermore, the procoagulant effects of extracellular histones have recently been linked to signaling via platelet Toll-like receptors 2 (TLR2) and 4.[86] Plasma levels of histones were elevated in response to traumatic injury and correlated with fibrinolysis

and activation of protein C. Elevated plasma levels of histones during the first 6 h after trauma were a multivariate predictor of mortality in this trauma patient population. However, when plasma levels of aPC were included in this multivariate analysis, the impact of histone level increase on mortality was abrogated.[57] This data strongly suggests that elevated aPC levels may have a protective effect by cleaving histones. However, a large increase in plasma levels of aPC early after severe trauma may represent a maladaptive response to an important protective mechanism against microvascular thrombosis after severe trauma in humans (Fig. 3).

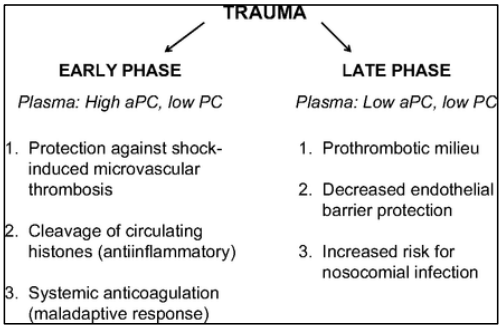

FIGURE 3 **Roles of the protein C pathway after severe trauma**

There is a massive activation of the protein C pathway in 20–25% of the patients early after severe trauma that is secondary to the intravascular formation of thrombin within the microcirculation. aPC activation is important to maintain blood flow within the microcirculation and to cleave circulating histones. However, the massive activation of aPC is also associated with an early coagulopathy that is seen as a maladaptive response to a physiological mechanism that protects against microvascular thrombosis. Severe trauma is associated with the development of a prothrombotic milieu within a few days after injury. Some patients do not recover a physiological plasma level of aPC after the early activation of this pathway. These patients are at higher risk for microvascular thrombosis and development of nosocomial pneumonia.

The massive activation of protein C that causes early coagulopathy after severe trauma is followed in some patients with depletion of this system characterized by low plasma levels of both protein C zymogen and aPC.[18,19] These patients are known to later develop a hypercoagulable state and have a higher risk for thrombosis and nosocomial infections including bacterial pneumonia.[55] In one study, trauma patients who subsequently developed infections had normal white blood cell count and functional coagulation profile 24 h after admission but showed depletion of protein C and increased levels of plasmin-antiplasmin

complexes. These coagulation system derangements characterize a patient group with normal hemostatic profile by thromboelastometry, but potentially abnormal immunocompetence at increased risk for nosocomial pneumonia. Indeed, lower plasma levels of protein C zymogen were correlated with higher rates of both gram-positive and gram-negative infections and longer hospital stay.[19] Whether there is a causal relationship between low plasma levels of aPC observed hours after trauma-induced activation of this pathway and the later development of nosocomial pneumonia is not fully understood. In addition, it is unclear whether it is the anticoagulant and/or the anti-inflammatory and cytoprotective activity of aPC that is responsible for a possible protective effect of aPC against bacterial infection. A previous study from Dr. Esmon's laboratory has shown that the cytoprotective but not the anticoagulant activity of endogenous aPC plays an important role in sepsis, as blocking the cytoprotective domain of aPC caused lethality after injection of a sublethal does of *E. coli* endotoxin.[107] Furthermore, a study from our laboratory has shown that treatment with aPC or its nonanticoagulant mutant significantly attenuated lung injury caused by *Pseudomonas aeruginosa* in mice.[11] In that study, we found that this wild-type aPC or its mutant protected the lung endothelial barrier against injury caused by *P. aeruginosa* by preventing the activation of the small GTPase RhoA by this bacterium. In another study, we have also shown that RhoA activation plays an important role in the *P. aeruginosa*-mediated increase in PAI-1 that itself also increases RhoA activation[41] and is a marker of poor outcome in patients with *P. aeruginosa* pneumonia.[93] These results indicate that the anti-inflammatory and cytoprotective activity of aPC might play a predominant role in the protection provided by aPC against injury caused by bacterial products, although additional work is needed to fully understand the causal relationship between posttraumatic coagulation derangements and development of nosocomial lung infection (Fig. 3).

The parallels between mechanisms of pathogen-induced sepsis and those underlying the sterile inflammatory response to trauma are becoming more evident, suggesting that insights and therapies from the critical care and sepsis literature may be applicable earlier in the hospital course of the acutely injured trauma patient. Future studies are warranted to identify drivers of both early coagulopathy induced by protein C and later depletion of the protein C system. From this knowledge, putative future clinical intervention could involve blocking the anticoagulant domain of aPC early after trauma, which would correct the early posttraumatic coagulopathy,

while maintaining its cytoprotective effect that is critical for the homeostasis of the vascular endothelium. Furthermore, there is a possibility that administration of aPC, possibly a mutant that does not have the anticoagulant effect of the wild-type protein, to correct trauma-induced depletion of protein C may protect against development of later lung infection and represent a putative biological link between the coagulation cascade and later infectious complications in these patients.

PROTEIN C AND ACUTE RESPIRATORY DISTRESS SYNDROME

Bronchoalveolar lavage of patients with ARDS reveals increasing activation of the coagulation system with intra-alveolar generation of thrombin and inhibition of fibrinolysis that correlates with the severity of the inflammation.[36,43] These coagulation disturbances are largely mediated by the tissue factor-factor VIIa pathway, since inhibition of this pathway prevents intra-alveolar fibrin deposition.[66] There is also reduction of the natural anticoagulant activity within the alveolar space characterized by decreased levels of protein C and increased levels of soluble thrombomodulin secondary to shedding and oxidation of this protein that correlate with poor outcome in ARDS patients.[102.] Furthermore, despite enhanced production of intra-alveolar fibrin, fibrinolytic activity is depressed in the bronchoalveolar lavage fluid of patients with ARDS because of high levels of PAI-1, the main inhibitor of fibrinolysis. Elevated levels of PAI-1 in the pulmonary fluid of patients with ARDS are also associated with higher mortality in this patient population.[71] Extensive cross talk between coagulation and inflammation may further damage the lungs because activated coagulation factors may cause impairment of the alveolar aeration and perfusion and promote lung fibrosis. Despite these results, clinical trials, except for one small recently published study,[20] have not shown beneficial effects with systemic administration of aPC in patients with ARDS. Results from the original PROWESS trial suggested that patients with a pulmonary origin for sepsis benefited more from the systemic administration of aPC than other groups of patients included in his trial.[9] Because of these results, Dr. Matthay's group[61] performed a new clinical trial with ARDS patients from non-septic origin. The results showed that recombinant human aPC decreased pulmonary dead-space fraction in these patients, but without improvement in the clinical outcome. Although the reasons for this lack of effect of aPC in this recent trial are not clear, it is possible that in some ARDS patients, for example those with bacterial pneumonia, activation of the

coagulation within the air spaces may provide protection against a widespread dissemination of the bacteria. Indeed, two experimental studies have shown that the systemic administration of high doses of aPC aggravated the lung injury associated by *P. aeruginosa* pneumonia in rodents.[16,79] Furthermore, recent clinical trials with other anticoagulants did not provide survival benefit for ARDS patients,[51,105] raising the hypothesis that in some ARDS patients for whom anticoagulation could be beneficial, high pulmonary concentrations of aPC may not be achieved with systemic treatment without causing serious systemic bleeding.

Local administration of anticoagulants via nebulization may be an alternative to deliver higher pulmonary concentrations of these drugs while possibly preventing systemic bleeding. Small clinical trials tested the effect of nebulized heparin in patients with ARDS.[29,65] In one trial, the combination of heparin, *N*-acetylcysteine, and albuterol improved survival and lung injury score.[65] This was a trial including patients with smoke inhalation-induced injury, which is characterized by large airway clots as a prominent feature of the model. Thus, the beneficial effects may have largely related to the inhibition of the precipitation of fibrin in the bronchial tree rather than alveolar injury. Although there is no published clinical trial that includes the administration of aPC via nebulization in patients with ARDS, preclinical studies with nebulized recombinant aPC reported that this mediator attenuated the activation of coagulation and inhibition of fibrinolysis in experimental models of ARDS while improving oxygenation without systemic bleeding.[48,49,56,90] However, nebulization of aPC inconsistently reduced lung inflammation in these preclinical models of ARDS, although the dose of nebulized aPC used in these preclinical studies was in the same range as the dose of aPC used in clinical studies, if we take into account the inefficiency of the nebulization process. In summary, these preclinical studies suggest that the local administration of recombinant aPC is safe. However, several important questions need to be answered before aPC could be considered as a potential local treatment for ARDS. First, the correct aPC dosage for nebulization is not really known because the maximal dose of aPC used in humans was established by toxicology and substantive refinements to optimize lung bioavailability may be problematic; second, it is not clear whether the potential protective effect of aPC is related to its anticoagulant or anti-inflammatory properties. Additional studies performed with the proper aPC mutants may help to respond to this important question.

CONCLUSION

In summary, the protein C system appears to play a major role in modulating severe acute inflammation via its anticoagulant and anti-inflammatory properties. However, the mechanisms of action of aPC in modulating the acute inflammatory response are only partially understood. Thus, more basic science work will be required to have a full understanding of how the protein C system modulates the acute inflammation associated with sepsis, trauma, or ARDS. Plasma levels of aPC are lower than normal in acute inflammation, with the exception of the early time period after severe trauma when high plasma levels of aPC may play a mechanistic role in the development of early posttraumatic coagulopathy. Following positive results of preclinical studies, a clinical trial (PROWESS) with high continuous doses of recombinant aPC given for a limited period of time demonstrated a significant decrease in mortality compared with placebo in patients with severe sepsis. This survival benefit from aPC treatment was not confirmed by subsequent clinical trials. The results of recently published PROWESS-SHOCK trial with patients that included septic shock were also negative. A lack of survival benefit was also observed in a large clinical trial that included patients with nonseptic ARDS. In contrast, a recent meta-analysis of 45,000 patients treated for severe sepsis with aPC during the last 10 years (including PROWESS and PROWESS-SHOCK patients) suggests an overall survival benefit for aPC treatment in severe sepsis. Despite the results of this meta-analysis, whether aPC will continue to be used to modulate the acute inflammatory response in humans remains uncertain. With the withdrawal of aPC (also called drotrecogin alfa activated) from the market immediately after the publication of the negative results of the PROWESS-SHOCK trial, there is currently no recombinant aPC available for clinical use in humans. Because of the technical difficulty to produce this drug and the fact that European and American health authorities would require at least two large phase III clinical trials before a new form of aPC could be licensed for marketing, it would be difficult and costly for any manufacturer to bring a new form of aPC on the market.

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Chapter 5

HISTONE-COMPLEXED DNA FRAGMENTS ARE ASSOCIATED WITH COAGULOPATHY, ENDOTHELIAL CELL DAMAGE, AND INCREASED MORTALITY AFTER SEVER PEDIATRIC TRAUMA

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ABSTRACT

Background: The release of damage-associated molecular pattern molecules (DAMPs) in the extracellular space secondary to injury has been shown to cause systemic activation of the coagulation system and endothelial cell damage. We hypothesized that pediatric trauma patients with increased levels of histone-complexed DNA fragments (hcDNA) would have evidence of coagulopathy and endothelial damage that would be associated with poor outcomes.

Methods: We conducted a prospective observational study of 149 pediatric trauma patients and 62 control patients at two level 1 pediatric trauma centers from 2013-2016. Blood samples were collected upon arrival and at 24 hours, analyzed for hcDNA, coagulation abnormalities, endothelial damage, and clinical outcome. Platelet aggregation was assessed with impedance aggregometry (Multiplate®) and coagulation parameters were assessed by measuring PT ratio in plasma and the use of viscoelastic techniques (ROTEM®) in whole blood.

Results: The median age was 8.3 years, the median injury severity score (ISS) was 20, and overall mortality was 10%. Significantly higher levels of hcDNA were found on admission in patients with severe injury (ISS > 25), coagulopathy, and/or abnormal platelet aggregation. Patients with high hcDNA levels also had significant elevations in plasma levels of syndecan-1, suggesting damage to the endothelial glycocalyx. Finally, significantly higher hcDNA levels were found in non-survivors.

Conclusion: hcDNA is released following injury and correlates with coagulopathy, endothelial glycocalyx damage, and poor clinical outcome early after severe pediatric trauma. These results indicate that hcDNA may play an important role in development of coagulation abnormalities and endothelial glycocalyx damage in children following trauma.

INTRODUCTION

Trauma is the leading cause of pediatric mortality, potential years of life lost, and accounts for significant medical cost in the developed world.[1,2] While it is understood that coagulopathy is common following major trauma and is associated with poor outcomes in adults[3], the effects of early coagulopathy and physiologic changes in the endothelial microenvironment in the pediatric population are less clear. Coagulopathy associated with trauma has traditionally been thought to be due to consumption of coagulation factors, dilution from intravenous fluids, and/or hypothermia. However, it was recognized in adults, and more recently in children, that at least a quarter of severely traumatized patients have coagulopathy on presentation to the emergency department that is physiologically and mechanistically distinct from classical iatrogenic posttraumatic coagulopathy.[4-6] Minimal literature exists on the effect of significant traumatic tissue injury on the endothelial glycocalyx, the release of inflammatory markers, anticoagulation factors and their subsequent interaction following severe pediatric trauma.

Previous research has shown that tissue injury accompanying massive trauma results in an immediate increase in circulating levels of damage associated molecular patterns (DAMPs) such as high-mobility group box-1 (HMGB1) and nucleic acids including histone-complexed DNA (hcDNA).[7-11] Although exact mechanisms of coagulation derangements remain elusive, there is emerging consensus that coagulation abnormalities in the adult population are an endogenous response to injury involving the neurohumoral, inflammatory, and hemostatic systems.[12-18] Other correlations have been shown to exist between increased extracellular histone levels and injury severity, platelet activation, endothelial damage, and mortality after adult trauma.[19-20] These findings suggest that histone release may be involved in the development of coagulation abnormalities in children after trauma.[16] To our knowledge, prospective studies on the proposed mechanisms of posttraumatic coagulopathy in children are limited. Our current understanding of these mechanisms is predicated on data collected from adult samples. However, in addition to the anatomical and physiological differences between adults and children, there is a variance in mechanisms and patterns of injury.[5] In the present study, we tested the hypothesis that the release of hcDNA could be detected early after pediatric trauma, and that this would be associated with coagulopathy, platelet dysfunction, endothelial cell damage, and poor outcome in this population.

MATERIAL AND METHODS

The Internal Review Boards of the University of Alabama at Birmingham and of University of Nebraska Medical Center approved this prospective observational cohort study performed between March 2013 and March 2016. Written consent was obtained from the patients or their legally authorized representative once they arrived at the hospital following the traumatic event.

Participants

Consecutive pediatric trauma patients admitted to the Children's Hospital of Alabama, the only level 1 pediatric trauma center in the state of Alabama, and University of Nebraska Medical Center were studied. Pediatric trauma patients under the age of 18 who met level 1 trauma criteria (Table 1) were eligible for enrollment. Exclusion criteria included: patients admitted > 6 hours after their injury; patients with burns >20% of the total body surface area; patients admitted for primary asphyxiation; patients with known or expected pregnancy, known liver disease, and/or known coagulation disorders. The control cohort was represented by 62 pediatric volunteers (mean age, 6.24 ± 6.2 years). The trauma and control cohort numbers were determined by a set period of time from March 2013 through March 2016.

Level 1 Criteria for Two Study Institutions
• Intubation prior to arrival
• Airway compromise/need of emergent airway
• Hemodynamic Instability, Cardiopulmonary Resuscitation (CPR) at scene or in progress
• Glasgow Coma Score (GCS) less than 10
• Deteriorating level of consciousness
• Focal neurologic findings, paralysis, or suspected cord injury
• Deep penetrating wounds to torso, head, or neck
• Amputations, partial amputations, and/or crush injuries proximal to ankle/wrist
• Pelvic ring fracture with associated long bone fractures
• Multi-System injury of 2 or more organ systems*
• Emergency Medicine or Attending Trauma Surgeon's discretion
• Flail Chest*
• Open or Depressed Skull Fracture*

TABLE 1: *Only Level 1 Criteria at one trauma center

Sample collection and measurements

Blood samples were collected within 20 minutes of arrival to the trauma room and at 24 hours following admission. The sample was placed in sodium citrated tubes (one part 0.106 mol L-1 sodium citrate and nine parts venous blood) and transported to the laboratory for immediate processing. The plasma from each sample was extracted and stored at -80°C until analysis. Prothrombin time (PT), platelet count (PC) and base deficit (BD) determination were analyzed as part of the standard clinical tests by the hospital laboratory. Control samples were collected from healthy children with no history of coagulation abnormalities who were undergoing elective surgery. Blood was collected at the time of intravenous catheter insertion prior to induction of general anesthesia. The blood was processed in an identical fashion as described above for trauma patients.

Platelet Function

Platelet function was assessed at point of care using the Multiplate® multiple electrode aggregometer (Verum Diagnostica GmbH; Munich, Germany) immediately after sample collection following admission and at 24 hours. Analyses were conducted by diluting 0.175mL of whole blood in warmed normal saline containing 3mM CaCl2 and incubating for 3 minutes at 37°C within a Multiplate® mini test cell. Each test cell contains two sets of 3mm silver-coated copper wires, across which electrical resistance is measured at 0.57 second intervals. Platelet activation was induced by adenosine diphosphate (ADP, final concentration 6.5µM; via P2 receptors) and thrombin receptor activating peptide-6 (TRAP, final concentration 32µM; via PAR receptors). Platelet adhesion to the electrodes was detected as increasing electrical impedance, measured by duplicate sets of sensor wires in each test cell. Agonist responses are reported as area under the aggregation curve (AUC) over a 6-minute measurement period. Since there is no normative data in the literature guiding us as to a normal range in pediatric patients, we utilized our control samples to create a normal range. Those patients that fell outside of the range that 95% of our controls fell into were considered abnormal.

Rotational Thromboelastometry

Rotational Thromboelastometry (ROTEM®) is a point of care testing device measuring the viscoelastic properties on multiple aspects of blood coagulation in a sample of citrated whole blood. Unlike conventional coagulation assays, ROTEM® assesses the coagulation system as a dynamic process by determining not only the clotting time, but also dynamics of clot formation, mechanical clot stability, and clot lysis over time. Activators are added to whole blood to evaluate the coagulation pathway. The clotting time (CT) is the latency time from addition of the activating reagent until the blood clot starts to form. Mini-cup cells were utilized with 0.150 ml of whole blood according to manufacturer's recommendations. Prolongation of the CT may be a result of coagulation deficiencies or altered levels of coagulation factors. Since there is no normative data in the literature guiding us as to a normal range in pediatric patients, we utilized our control samples to create a normal range. The range of 90-232 seconds was the range that 95% of our controls fell into allowing this to be our normal range. Patient samples were collected and analyzed immediately after admission and at 24 hours.

Enzyme Linked Immunosorbent Assay (ELISA) measurements

Samples were analyzed at the conclusion of the study by researchers blinded to all patient data. Plasma samples were measured in duplicate for the following: Histone-Complexed DNA fragment (hcDNA, Cell Death Detection ELISA plus, Roche, CT, USA), and Syndecan-1 (Cell Sciences, MA, USA). All measurements were performed in accordance with the manufacturer's instructions.

Data collection, outcome measures

Data were prospectively collected on patient demographics, time of injury, mechanism of injury (blunt or penetrating), Injury Severity Score (ISS), baseline vital signs, transfusion requirements, and pre-hospital fluid administration. Head Abbreviated Injury Severity (AIS) > 3 was used as a surrogate for the presence of significant traumatic brain injury (TBI). A blood gas was obtained on patient arrival per protocol for the management of high-level pediatric trauma patients. Base Deficit (BD) was used as a measure of the degree of tissue hypoperfusion. Patients were followed until discharge or death. Secondary outcome measures were recorded for acute kidney injury (defined by the pediatric RIFLE criteria),[21] acute lung injury (defined by the Pediatric Acute Lung Injury Consensus as a PaIO2/FIO2 ratio ≤ 300)[22], and blood transfusion requirements in the first 6 hours.

Statistical Analysis

Data analysis was performed by the investigators using Statistical Analysis System (SAS version 9.4, Cary, NC). Data are expressed as median (interquartile range) for continuous variables and count (percent) for categorical variables. Fisher's exact test and the Wilcoxon rank sum test were used, as appropriate, to investigate relationships between covariates. Relationships between quartiles of hcDNA and continuous variables were tested with the Kruskal-Wallis test. For any relationships found to be statistically significant, multiple comparison tests were conducted using a non-parametric version of the Tukey-Kramer test. A *P* value of $< .05$ was chosen to represent statistical significance. Finally, logistic regression was used to assess whether admission levels of hcDNA and syndecan-1 were predictive of patient mortality.

RESULTS

Patient population

During the study period, 186 patients requiring full trauma team activation were evaluated. A total of 37 patients were deemed ineligible by study exclusion criteria. Patients were excluded for the following reasons: age (n=2), downgrade to level 2 trauma (less severe) (n=2), unable to obtain blood (n=5), dead on arrival (n=5), missed trauma activation (n=6), arrival >6 hours after injury (n=8), burns or asphyxiation (n=9). In all, 149 consecutive pediatric trauma patients were enrolled in the study. Median age was 8.3 years (IQR: 4.6, 12.3) with patients falling into the following age categories: 0-2 (n=25), 3-7 (n=48), 8-12 (n=45), 13-17 (n=31). Median ISS was 20 (IQR: 11, 29), 71% sustained blunt trauma and 36% had severe TBI, defined by a head AIS score > 3. Clinical characteristics of the children included are shown in Table 2. Coagulopathy (defined as PT ratio > 1.2) was present in 29% of patients on arrival to the hospital, and 26% had a base deficit ≥ 6 mmol/L. Overall mortality was 10%. The median time from injury to blood sample collection was 90 minutes (IQR: 60, 160).

Clinical Characteristics of Trauma Patients	
Demographic data	
Age	
Median	8.3 (4.6, 12.3)
Gender	
Male	105 (71%)
Female	44 (29%)
Characteristics of injury	
ISS	
Median	20.0 (11.0, 29.0)
ISS ≥15	53(36%)
ISS ≥25	88 (59%)
Mechanism Type	
Blunt	124 (83%)
Penetrating	25 (17%)
Severe head injury (AIS head ≥ 3)	54 (36%)
Physiology	
Base deficit > 6 mmol/L	39 (26%)
PT ratio > 1.2	43 (29%
Platelet Count 103/μL	283 (234, 341)

TABLE 2 Total number of patients included is 149. Data are presented as median (interquartile range) and numbers (%). ISS: Injury Severity Score, AIS: Abbreviated Injury Scale. PT ratio: Prothrombin Time ratio

HcDNA plasma level, injury severity and hypoperfusion

In our injured pediatric population, we observed a median hcDNA plasma level of 3.47 AU (IQR: 1.23, 7.13) compared to 1.67 AU (IQR: 1.26, 3.19) (P =.024) in healthy pediatric controls. We performed two analysis evaluating separate cutoffs for "severe injury" with different ISS scores (ISS < or ≥ 15 and ISS < or ≥ 25). Recent literature has demonstrated that an ISS of 25 denotes the most appropriate cutoff for "severe injury" in children and

approximates similar mortality to an ISS of 15 in severe adult trauma (23). The release of hcDNA occurred early after trauma and was significantly higher in children with higher ISS scores and higher base deficit versus those with less severe injury, or healthy control patients ($P < .001$, Figure 1A). All groups of trauma patients have significantly higher hcDNA levels than controls, except the group with ISS < 25 and BD ≥ 6 ($P = .098$). To evaluate the differences between the multiple groups at time of admission, we performed multiple comparisons with a Bonferroni correction. Group D (ISS ≥ 25 and BD ≥ 6) had a significantly higher hcDNA at admission than Groups A (ISS < 25 and BD < 6; $p < 0.001$) and C (ISS ≥ 25 and BD < 6; $P = .002$) No other groups significantly differed from each other with multiple comparisons. The difference in hcDNA levels between pediatric trauma patients and controls persisted at 24 hours after admission ($P = .002$, Figure 1B). At 24 hours, all groups were different from controls, except the group with ISS < 25 and BD ≥ 6 ($P = .924$). However, the median hcDNA levels of severely injured patients were markedly decreased at 24 hours, compared with admission levels, suggesting that the hcDNA levels were beginning to return back to the levels of the control patients. Similar results were seen when utilizing a lower ISS cutoff of 15 with similar BD groups on admission. All groups of trauma patients hcDNA levels were significantly different from control patients ($p<0.001$), except the group with ISS <15 and BD <6 ($P = .469$). However, at 24 hours, fewer trauma groups hcDNA levels were different from controls—those with ISS <15 and BD <6 ($P = .014$) and those with ISS ≥ 15 and BD < 6 ($P = .001$) were significantly different from controls.

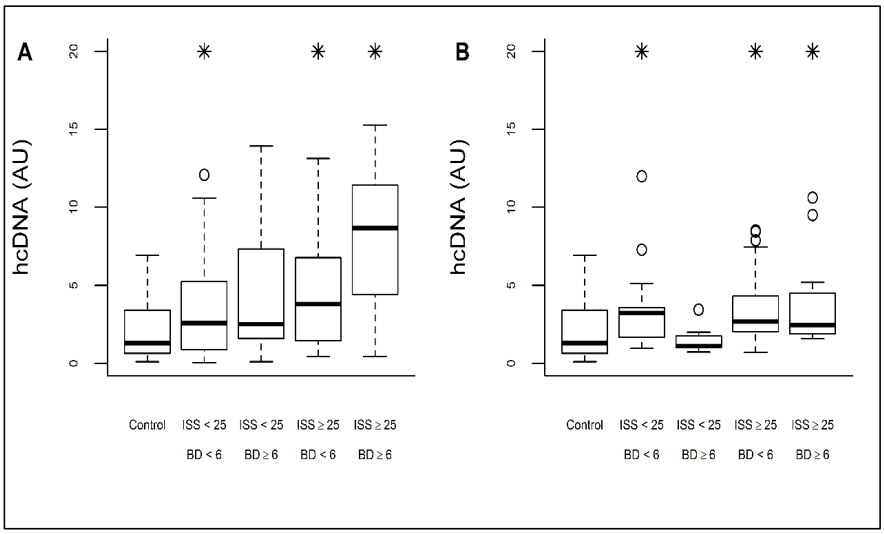


FIGURE 1

A. **Effects of injury and base deficit on levels of histone-complexed DNA (hcDNA) early after pediatric trauma.** Plasma levels of histone-complexed DNA (hcDNA) were significantly increased in all groups except those with Injury Severity Score (ISS) <25 and Base Deficit ≥ 6. $P < .001$ from Kruskal-Wallis test comparing the five groups. Groups A ($P = .037$), C ($P = .001$), and D ($P < .001$) all have significantly higher hcDNA than controls. No significant difference between Group B and controls ($P = .113$). Number of patients for each group are: Controls=42, A=62, B=11, C=32, D=24.

B. Effects of injury and base deficit on levels of histone-complexed DNA (hcDNA) 24 hours after pediatric trauma. Plasma levels of histone-complexed DNA (hcDNA) were significantly increased in all groups except those with Injury Severity Score (ISS) <25 and Base Deficit ≥ 6 (Group B). $P = 0.001$ from Kruskal-Wallis test comparing the five groups. Groups A ($P = .007$), C ($P = .002$), and D ($P = .009$) have significantly higher hcDNA than controls. Group B did not significantly differ from controls ($P = .944$). Number of patients for each group are: Controls=42, A=27, B=7, C=35, D=13.

Plasma levels of hcDNA and early coagulation derangements in pediatric trauma patients

Trauma patients with clinically significant coagulation abnormalities upon admission (PT ratio >1.2) had significantly higher levels of hcDNA ($P < .001$, Figure 2A) compared with those patients with a PT ratio of ≤ 1.2. The difference between these groups was not detected 24 hours after admission ($P = .209$, Figure 2B). The median level of hcDNA in coagulopathic patients showed less variability and returned to the level of non-coagulopathic patients. Figure 2C further demonstrates the change in hcDNA level based on different PT ratios after

admission. In pairwise comparisons, all of the groups with elevated PT ratio have significantly higher hcDNA levels than the group with a normal PT ratio ($P< 0.05$, for each pairwise comparison). However, there was no significant difference between severity of PT elevation and absolute hcDNA levels once a threshold had been reached.

In addition to conventional coagulation studies, we utilized rotational thromboelastometry (ROTEM®) to evaluate the relationship between coagulopathy and hcDNA levels. The INTEM test evaluates the intrinsic pathway by addition of an activator to the sample and measuring, among other parameters, the speed of the clotting process with clotting time (CT) and the strength of the clot (amplitude) after 5 minutes of testing. In our cohort prolonged CT was associated with increase in plasma levels of hcDNA ($P = .002$, Figure 3A). Furthermore, we demonstrated that those patients with elevated hcDNA levels had a significantly lower clot strength (amplitude, A5) at 5 minutes ($P = .049$, Figure 3B). Together these findings demonstrate a correlation between increasing levels of hcDNA, severe traumatic mechanism, and coagulopathy as measured with viscoelastic techniques.

Finally, we evaluated circulating platelet number and function to determine the potential effect of injury and hcDNA release on platelets. In our population, the mean platelet count on admission was normal (288 ± 93.1 x 103/μL). Patients were categorized based on a normal or abnormal area under the curve (AUC) for each test, ADP and TRAP based on the values measured in control patients. At admission, patients with abnormal AUC for both ADP and TRAP, compared to controls, had significantly higher median hcDNA levels (Figure 4A, $P =.003$; Figure 4B, $P < .001$). However, the difference in hcDNA levels at 24 hours among these same groups was no different. (Figure 4C, $P=0.185$; Figure 4D, $P = .883$)

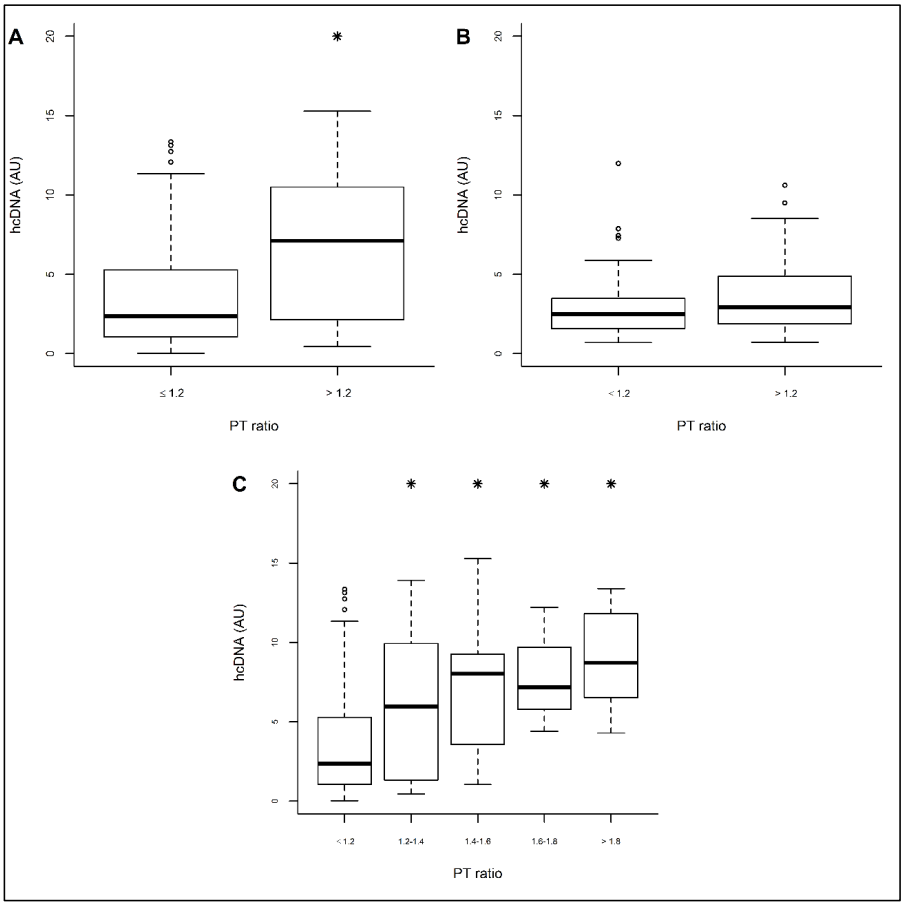


FIGURE 2 **High plasma levels of hcDNA are associated with coagulation abnormalities early after pediatric trauma**

A. Pediatric patients with coagulation abnormalities early after trauma (prothrombin time (PT) ratio ≥ 1.2) had significantly higher levels of hcDNA (*P = .0001).

B. Twenty-four hours after admission, pediatric patients with coagulation abnormalities after trauma (prothrombin time (PT) ratio ≥ 1.2) had equivalent levels of hcDNA (P = .209).

C. Early after trauma, those pediatric patients with coagulopathy (prothrombin time (PT) ratio ≥ 1.2) had significantly higher levels of hcDNA than those without coagulopathy. $p < 0.001$, Group A had significantly lower hcDNA than Groups B, C, D and E ($P < 0.05$ for each pairwise comparison). Groups B, C, D, and E did not significantly differ from each other. Number of patients for each group are: A=96, B=23, C=7, D=3, E=7.

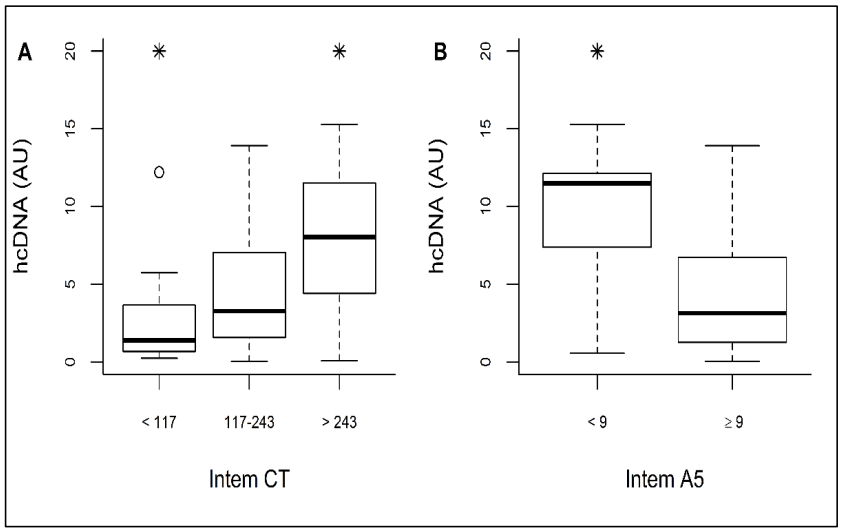


FIGURE 3 **Relationships between whole blood thromboelastometry and hcDNA levels**

A. On admission, pediatric trauma patients with longer INTEM clotting times (CT), as measured by ROTEM, had significantly higher hcDNA levels. *P* = .002, All pairwise comparisons were statistically significant: A vs B, *P* = .011; A vs C, *P* = 005; B vs C, *P* = .013.

B. On admission, pediatric trauma patients with a shorter amplitude at 5 minutes (A5, measure of clot strength) had significantly higher hcDNA levels. *P* =.049

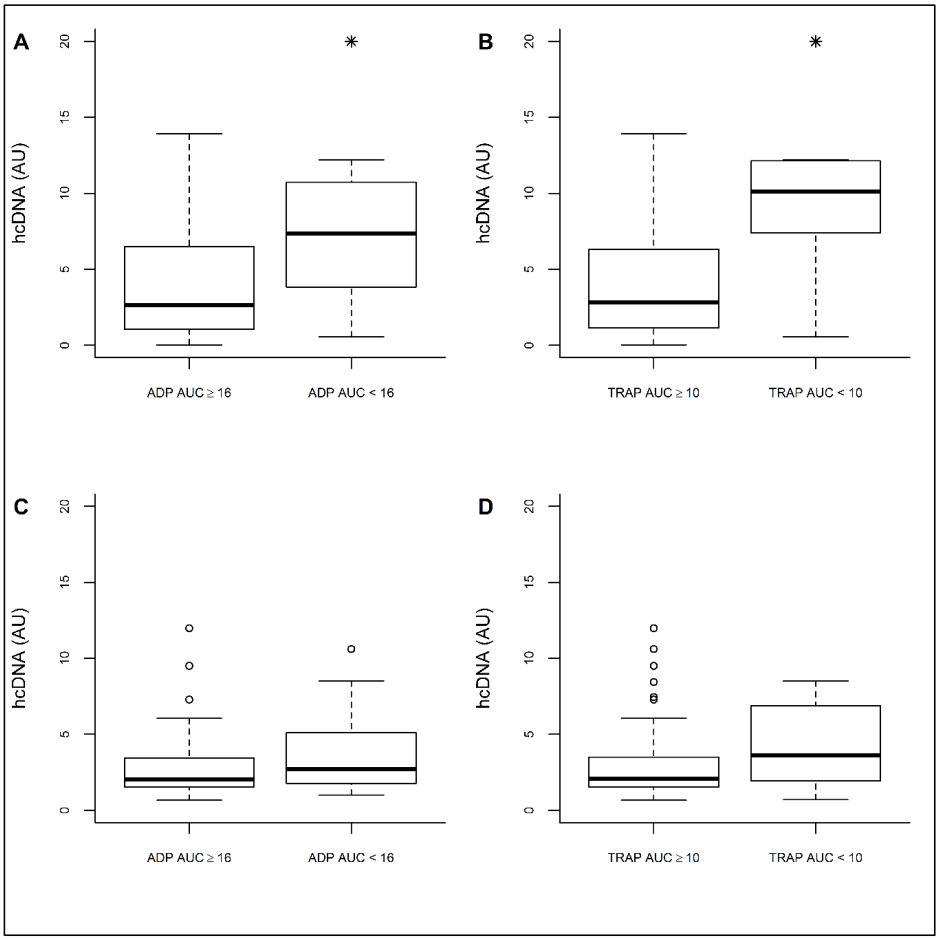


FIGURE 4 **High plasma levels of hcDNA are associated with platelet function abnormalities in pediatric trauma patients.**

A. Pediatric trauma patients with lower responses to adenosine diphosphate (ADP) induced platelet aggregation had significantly higher levels of hcDNA (**P* = .022).

B. Pediatric trauma patients with lower responses to thrombin receptor activating peptide-6 (TRAP) induced platelet aggregation had significantly higher levels of hcDNA (**P* = .025).

C. At 24 hours following trauma admission, the differences in hcDNA levels in those patients with lower responses to adenosine diphosphate (ADP) induced platelet aggregation did not persist. *P* = .078

D. At 24 hours following trauma admission, the differences in hcDNA levels in those patients with lower responses to thrombin receptor activating peptide-6 (TRAP) did not persist. *P* =.18

Plasma levels of hcDNA and markers of endothelial cell damage in pediatric trauma patients

To investigate the role of endothelial glycocalyx damage and its association with hcDNA release early after pediatric injury, shedding of the glycocalyx was evaluated by measuring the plasma levels of circulating syndecan-1. We evaluated patients' syndecan-1 levels in relation to hcDNA levels at admission and 24 hours after admission (Figure 5A and B). Control patients had low levels of both circulating syndecan-1 levels and hcDNA levels. Pediatric trauma patients were divided in quartiles for their hcDNA levels. Trauma patients in each quartile had significantly higher levels of circulating syndecan-1 when compared to controls (Figure 5A). Multiple comparisons with a Bonferroni correction were performed for those groups at the time of admission. Group D (hcDNA $\geq$ 7.12 AU) had significantly higher syndecan-1 levels at admission when compared to Groups A (hcDNA < 1.39 AU; $P < .001$) and B 1.39 $\leq$ hcDNA < 3.51 AU; $P < .001$). Also, Group C (3.51 $\leq$ hcDNA < 7.12) had significantly higher syndecan-1 levels at admission than Group A (hcDNA < 1.39 AU; $P = .001$). No other groups showed significant differences in multiple comparisons. These findings persisted at 24 hours after admission (Figure 5B).

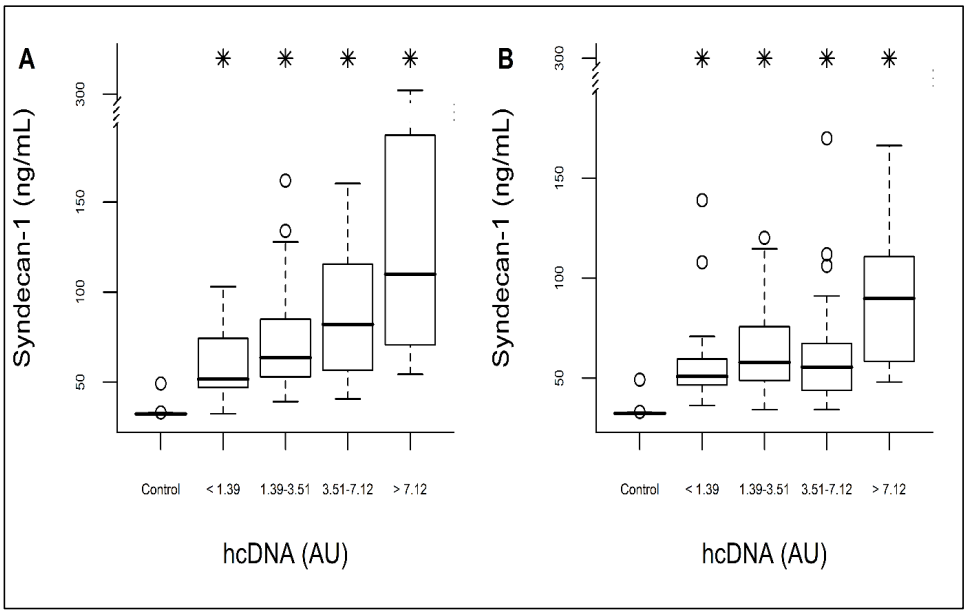


FIGURE 5 **High plasma levels of hcDNA are associated with the release of markers of endothelial damage in pediatric trauma patients.**

A. Plasma levels of hcDNA are associated with shedding of the glycocalyx shedding early after pediatric trauma, as shown by the plasma levels syndecan-1. As the levels of hcDNA levels increase in each quartile, syndecan-1 levels are significantly higher. $P < .001$ from Kruskal-Wallis test comparing the five groups. All groups significantly differ from controls ($P < .001$ for each).

B. Plasma levels of hcDNA are associated with shedding of the glycocalyx shedding 24 hours after pediatric trauma, as shown by the plasma levels syndecan-1. As the levels of hcDNA levels increase in each quartile, syndecan-1 levels are significantly higher. $P < .001$ from Kruskal-Wallis test comparing the five groups. All groups significantly differ from controls ($P < .001$ for each).

Plasma levels of hcDNA and clinical outcome pediatric trauma patients

In evaluating clinical outcomes in regard to coagulopathy and pediatric trauma, consideration of transfusion and head injury in relation to hcDNA levels were evaluated. Patients who required blood transfusion < 6 hours after admission had higher plasma levels of hcDNA (7.38 AU (3.33, 11.41)) compared with those patients that did not require transfusion (2.51 AU (1.08,

3.33); $P < .001$). In addition, those patients who required transfusion of blood transfusion < 6 hours after admission still had significantly higher hcDNA levels at 24 hours following admission (3.38 AU (2.10, 4.85) than patients who did not require transfusion (2.09 AU (1.41, 3.37); $P = .007$). In evaluating hcDNA levels in relation to head injury, we split the population into those with GCS ≤ 8 and > 8. Those patients with GCS ≤ 8 had significantly higher hcDNA levels (4.11 AU, (1.59. 7.39) than those with GCS > 8 (2.51, (0.86, 5.40); $P = .047$) (Figure 6). In regard to other clinical outcomes, the median hospital stay for the entire cohort was 7 days[3,18]. 79% of patients were admitted to the intensive care unit (ICU) and median ICU stay was 4 days (IQR: 2, 8). We found no significant correlation between hcDNA plasma levels, hospital and ICU length of stay. Of all patients, 5 children later developed organ dysfunction not due to direct trauma. Only one patient developed acute kidney injury. Those patients (n=4) who developed acute lung injury (ALI) had a significantly higher admission hcDNA levels than those without ALI (9.35 AU (IQR: 7.18, 10.90) versus 3.47 AU (IQR: 1.26, 7.00) ($P = .046$)).

Finally, to evaluate the direct relationship with mortality, a logistic regression analysis was performed to evaluate if hcDNA and syndecan-1 levels on admission predict mortality. hcDNA is significantly associated with mortality ($P = .036$) (Figure 7A). The odds ratio (95% CI) associated with a 1-unit increase in hcDNA is 1.14 (1.01, 1.30). Syndecan-1 is also significantly associated with mortality by logistic regression ($P = .026$) (Figure 7B). The odds ratio (95% CI) associated with a 100-unit increase in syndecan-1 is 1.37 (1.04, 1.81.

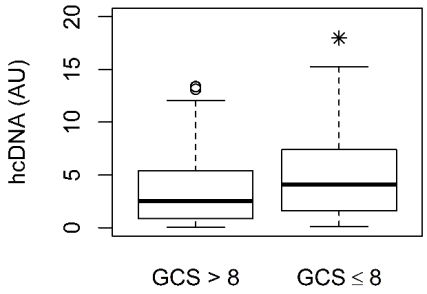
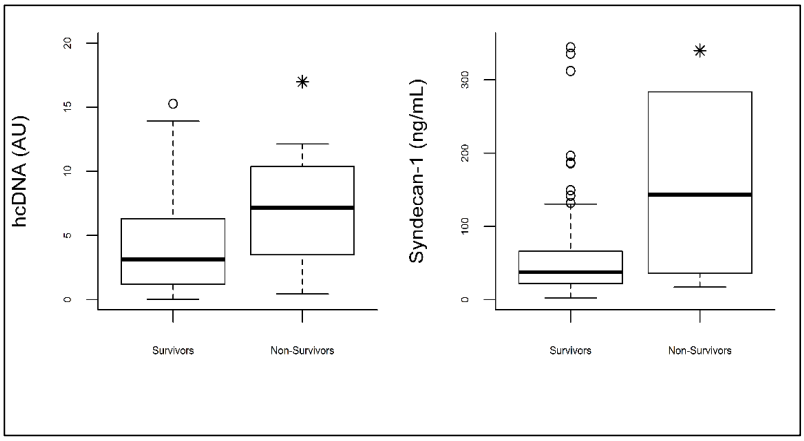


FIGURE 6

Patients with significant head injury (GCS ≤ 8) had significantly higher hcDNA levels on admission than those without significant head injury. *P* = .047



A **B**

FIGURE 7

A. High plasma levels of hcDNA on admission are associated with increased mortality following severe pediatric trauma. *P* = .036

B. Higher syndecan-1 levels on admission are also associated with increased mortality. *P* =.026

DISCUSSION

The main results of this prospective study showed that plasma levels of hcDNA were significantly higher on admission to the hospital in pediatric trauma patients with severe injury (ISS > 25), coagulopathy, and abnormal platelet aggregation. Patients with high hcDNA levels had also significant elevations in plasma levels of syndecan-1, suggesting a significant damage to the endothelial glycocalyx. Finally, significantly higher plasma hcDNA levels were found in children who did not survive their severe trauma. These results demonstrate that hcDNA release may play an important role in the development of coagulopathy, endothelial gylcocalyx damage, and ultimate outcomes in children with severe trauma.

The first result of the present study shows that pediatric trauma patients, like adult trauma patients,[19,20] have an early exaggerated extracellular release of histone-complexed DNA

fragments in response to injury. The pediatric patients with higher hcDNA levels also have a higher incidence of early coagulation abnormalities. Previous experimental studies have reported that hcDNA fragments may cause a procoagulant phenotype via a systemic activation of the coagulation cascade by several mechanisms that include binding to platelets and recruiting plasma adhesion proteins, such as fibrinogen, to cause platelet aggregation,[24] inhibiting the anticoagulant protein C pathway,[25] increasing plasma levels of von Willebrand factor antigen that contributes to platelet activation,[26] increasing the extracellular release of HMGB1, a known pro-coagulant DAMP,[27] and binding to negatively charged endothelial heparan sulfate thus disrupting the anticoagulant property of this protein layer.[28] The relationship between elevated plasma levels of hcDNA and acute traumatic coagulation abnormalities have been described in adult patients with severe trauma. Interestingly, these studies reported that patients with higher plasma hcDNA levels had significantly higher ISS scores, a higher proportion of coagulopathy, and hyperfibrinolysis that was associated with an enhanced inflammatory response.[19,20] These clinical results indicate that high levels of circulating hcDNA fragments are associated with a coagulopathy phenotype, not a procoagulant one, after severe trauma. The explanation for the difference between the results of experimental and clinical studies could be related to the fact that patients with high plasma levels of hcDNA also have high plasma levels of anticoagulants such as tissue plasminogen activator, tissue factor pathway inhibitor and activated protein C, suggesting a secondary modulation of the coagulation cascade by these anticoagulant mediators released to prevent diffuse microvascular thrombosis.[19]

Although several studies have reported an incidence of acute traumatic coagulopathy in pediatric trauma patients comparable to the one observed in adult trauma patients,[5,16] these studies have not examined whether the extracellular release of hcDNA fragments is associated to similar coagulation abnormalities in traumatized children and whether there are potential differences in the response of children and adults to severe trauma. Our data suggests a relationship between elevated plasma hcDNA levels and worsening coagulopathy on admission to the hospital, as demonstrated by traditional coagulation studies and data generated by thromboelastometry (clotting time and clot strength A5). Indeed, patients with significantly increased hcDNA levels on admission demonstrated significant alterations in time to clotting and strength of the formed clot. In summary, our study demonstrates that pediatric trauma

patients, like adult trauma patients, have an early exaggerated extracellular release of histone-complexed DNA fragments in response to severe injury. The pediatric patients with higher hcDNA levels also have a higher incidence of early coagulopathy that correlates with outcome. This study represents the first prospective description of how traumatic injury may affect circulating levels of hcDNA fragments and their relationship with trauma-induced coagulopathy in children with severe trauma.

The second result of this study shows that there is poor aggregation of circulating platelets in response to exposure to ADP or TRAP in severely injured pediatric trauma patients that correlates with high plasma levels of hcDNA. Wohlauer et al. prospectively assessed platelet function in whole-blood samples from 51 adult trauma patients versus controls with thromboelastography-based platelet functional analysis.[29] There were significant differences in platelet response between trauma patients and controls. This study indicated the significant platelet dysfunction manifested after severe trauma and before substantial fluid or blood administration. Furthermore, Kutcher and colleagues prospectively evaluated platelet function by impedance aggregometry in 101 patients sustaining severe trauma. Likewise, they showed that 45% of these patients had below-normal platelet response at admission and 91% had platelet hypofunction at some point during their ICU stay.[19] Although experimental and clinical studies mentioned above showed that hcDNA may activate platelets (indicated increased concentration of soluble CD40 ligand), circulating platelets of adult and pediatric patients with severe trauma show a clear hyporesponsiveness to further stimulation. This circulating platelet dysfunction is likely mediated by the exhausted platelet syndrome that corresponds to an initial platelet activation and depletion of intracellular mediators resulting in platelet hyporesponsiveness to further stimulation.[29] Interestingly, pretreatment with valproic acid, an inhibitor of histone deacetylation, attenuated this abnormal responsiveness of circulating platelets in a swine model of traumatic brain injury and hemorrhagic shock.[30]

The third result of this study shows a relationship between high levels of circulating hcDNA and endothelial glycocalyx degradation characterized by high plasma levels of syndecan-1 after severe pediatric trauma. This result is of importance because of the critical role of the endothelial glycocalyx layer in controlling the permeability of the vascular endothelium.[31] Prior studies of adult trauma patients by Johansson et al. reported that patients with higher circulating markers of endothelial degradation, syndecan-1, had increased evidence of

inflammation, coagulation abnormalities and mortality. Furthermore, they also demonstrated that there was a direct correlation between high circulating syndecan-1 and hcDNA levels in these patients.[4] In addition, the same investigators reported in another study that high shedding of syndecan-1 correlated with endogenous heparinization measured by thromboelastography and secondary coagulopathy that is likely secondary to the release of heparan sulfate.[31] These results suggest that this coagulopathy may reflect an adaptive response to the trauma-induced release of procoagulant DAMPs in order to maintain the perfusion of the microcirculation.

The results presented here are subject to the limitations related to the fact that it is a dual center observational study. Although there were a large number of trauma patients and controls in most groups included in the study, care must be taken in interpreting the data as there were limited numbers in some stratified groups utilized in the analysis. The correlations that we observed are not necessarily mechanistic. However, this study represents a severely injured pediatric trauma population in which these observations have not been described to date. This will require further confirmatory studies on larger pediatric trauma populations. A second limitation is the fact that diagnostic tools often used to measure circulating components of the chromatin do not necessarily distinguish free histones and nucleosomes, complexes formed of DNA and DAMPs, such as histones.[32] Experimental studies found that free extracellular histones induce endothelial damage, organ failure, and death in sepsis and in animal models confirmed the administration of histones induced endothelial damage, hemorrhage, and thrombosis.[33] Free histones cause direct damage to the cell membrane and induce a major calcium flux resulting in cell lysis. In addition, free histones activate Toll-like receptors (TLR) 2 and 4. In contrast, cell free DNA or nucleosomes follow different routes of immunostimulation that include TLR9.[32] In this study, we measured the level of nucleosomes present in the study plasma, not the levels of free histones. While ELISA developed to quantify histone-subtypes are unlikely to solely detect free histones because most of the circulating histones are bound to DNA, we used in the present study an ELISA that combined a monoclonal anti-histone antibody with a monoclonal anti-DNA antibody. This assay that is widely used, allows for a reliable measurement of circulating chromatin fragments.[32] Interestingly, in a recently published study, Abrams et al. measured both histones by immunoblot and nucleosomes by ELISA in severely traumatized adult patients. The authors found that both plasma levels were elevated during the first hours after trauma. However,

plasma histone, but not nucleosome, levels were still elevated 72h after admission to the hospita These results are in accordance with our own data that showed that although different from controls, the difference between the median levels of severely injured patients at admission and 24 hours later is markedly different and suggests that the hcDNA levels are beginning to return back to the levels of the control patients.

In summary, this study demonstrates that severe pediatric trauma induces significant extracellular release of histone-complexed DNA fragments that is associated with early coagulation abnormalities, abnormal circulating platelet aggregation and endothelial glycocalyx damage. This study also shows that high plasma levels of hcDNA are associated with poor outcome in pediatric patients with severe trauma. As it has previously been reported that extracellular hcDNA causes a systemic activation of the coagulation cascade and may induced a severe inflammatory response via the activation of TLRs 2, 4 and 9, the present clinical results suggest that the release of hcDNA into the blood stream might play an important mechanistic role in the development of coagulation abnormalities and endothelial glycocalyx damage in children with severe trauma.

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Chapter 6

DETECTION OF ACUTE TRAUMATIC COAGULOPATHY AND MASSIVE TRANSFUSION REQUIREMENTS BY MEANS OF ROTEM: AN INTERNATIONAL PROSPECTIVE VALIDATION STUDY

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CRITICAL CARE. 2015 Mar 23;19:97

ABSTRACT

Objective: To re-evaluate findings of a smaller cohort study on the functional definition and characteristics of acute traumatic coagulopathy (ATC). To identify the threshold values for most accurate identification of ATC and prediction of massive transfusion (MT) using ROTEM® assays.

Design: International multi-center prospective cohort study

Setting: Level 1 Trauma Centers

Methods: Adult trauma patients who met the local criteria for full trauma team activation from four major trauma centers were included. Blood was collected on arrival to the ED and analyzed with laboratory international normalized ratio (INR), fibrinogen concentration and two rotational thromboelastometry (ROTEM®) assays (EXTEM and FIBTEM). ATC was defined as laboratory INR > 1.2. Transfusion requirements of ≥10 units of packed red blood cells within 24 hours were defined as MT. Performance of the tests were evaluated by receiver operating characteristic curves, and calculation of area under curve (AUC). Optimal cut-off points were estimated based on Youden index.

Results: 808 patients were included in the study. Among the ROTEM® parameters, the largest AUCs were found for the CA5 value in both the EXTEM and FIBTEM assays. EXTEM CA5 threshold value of ≤37 mm had a detection rate of 66.3% for ATC. An EXTEM CA5 threshold value of ≤40 mm predicted MT in 72.7%. FIBTEM CA5 threshold value of ≤8 mm detected ATC in 67.5%, and a FIBTEM CA5 threshold value ≤9mm predicted MT in 77.5%. Fibrinogen concentration ≤ 1.6 g/L detected ATC in 73.6% and a fibrinogen concentration ≤1.90 g/L predicted MT in 77.8%. Patients with either an EXTEM or FIBTEM CA5 below the optimum detection threshold for ATC received significantly more PRBCs and plasma.

Conclusion: This study confirms previous findings of ROTEM® CA5 as a valid marker for ATC and predictor for MT. With optimum threshold for EXTEM CA5 ≤ 40 mm and FIBTEM CA5 ≤ 9 mm, sensitivity is 72.7% and 77.5% respectively. Future investigation should evaluate the role of repeated viscoelastic testing in guiding haemostatic resuscitation in trauma.

BACKGROUND

Haemorrhagic shock following injury has been shown to induce coagulopathy.[1-3] Acute traumatic coagulopathy (ATC) may potentiate bleeding and is associated with multiple organ failure and increased mortality.[2,4,5] Early detection of coagulopathy is important in order to counteract the haemostatic disturbances. Standard tests such as prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration and platelet count are widely used to guide resuscitation in trauma patients.[6-7] However the conventional coagulation tests (CCTs) focus on selected aspects of coagulation, which may not be appropriate for ATC.[8] Full blood viscoelastic haemostatic assays (VHA), such as ROTEM® and TEG®, may provide a more complete assessment of haemostasis and as point of care devices should be able to provide results in a more clinically useful timeframe for targeted therapy.[9-11]

In a previous prospective cohort study, the tissue factor (TF) activated ROTEM® assay (EXTEM) was used to characterize ATC and the need for transfusions.[12] This study suggested that coagulopathy could be identified using the clot amplitude five minutes after the initiation of clot build-up (CA5). Thus, the CA5 value potentially may be used as a diagnostic tool for detecting ATC and the need for massive transfusion.

The objective of our study was to re-evaluate the previous findings in a larger international multi-center setting. Specifically, we aimed to identify the threshold values that most accurately identify ATC and the need for massive transfusion, using the EXTEM assay, as well as the platelet-inhibited FIBTEM assay.

METHODS

Design and patient selection

This multi-centre observational cohort study was conducted as a part of the Activation of Coagulation and Inflammation in Trauma study (ACIT) 3, led by the International Trauma Research Network (INTRN) collaboration. Patients were non-consecutively recruited at four major trauma centers in three different countries: UK, Denmark and Norway. The inclusion period was from January 2009 to November 2011, thereby also including the cohort in the previous study.[12] Patients 18 years or older requiring full trauma team activation were eligible

for inclusion. Patients who received more than 2000 ml of fluids before arrival or who arrived in the emergency department (ED) more than 2 hours from time of injury were excluded. Additional exclusion criteria comprised patients who were pregnant, had known liver failure, bleeding disorders or were taking oral anticoagulant medications other than acetyl salicylic acid.

Informed consent was obtained from participating patients or their next of kin where appropriate. The study was performed in accordance with local ethical regulations and approved by local ethical authorities.

Sampling techniques and measurements

Blood samples were collected within 20 minutes of arrival in hospital. Samples for ROTEM® and CCTs were collected in citrated tubes, whereas samples for blood gas analyses were collected in heparinized syringes in accordance with local routines. ROTEM® assays were performed within one hour by dedicated study personnel using the ROTEM® Delta (TEM; TEM international, Germany). The assays used were the EXTEM assay, where the citrated sample is recalcified before it is activated by TF, and the FIBTEM assay where the platelet inhibitor cytochalasin D was added for platelet inhibition, to isolate the fibrin component of the clot.

The clotting time (CT) of the ROTEM® trace is the time from initiation of the test to first detectable rotational resistance. Clot formation time (CFT) is the time from first detectable resistance to trace amplitude of 20 mm. The alpha angle is the angle of increase at the point where 20 mm amplitude is reached. Maximum clot firmness (MCF) is the maximum clot amplitude detected. The clot amplitude (CA) after 5 (CA5) and 10 (CA10) minutes were also recorded. Due to the fact that the FIBTEM trace rarely reaches amplitude of 20 mm, the CFT and alpha angle was omitted for the FIBTEM assays in this study.

CCTs and blood gas analyses were performed with the shortest possible delay. The CCTs included in this study were PT, fibrinogen concentration and platelet count. PT was converted to international normalized ratio (INR) in accordance with the specific reagents and device characteristics in the respective laboratories. Fibrinogen was measured by the Clauss method.[13]

Data collection and statistical analyses

Patient data on demographics, time of injury, pre-hospital fluid administration and vital signs were collected prospectively. The total amount of packed red blood cell (PRBC) and plasma units required within the first 24 hours were recorded. Mechanism of injury and Injury Severity Score (ISS) were retrieved from the respective institutional trauma registries. ATC was defined as an INR value >1.2, consistent with the previous study.[12] We defined massive transfusion (MT) as the administration of 10 or more units of PRBC within 24 hours.

Groups with ATC and need for MT were compared to normal groups by Student´s t-test or Mann-Whitney U-test as appropriate. Receiver operating characteristic (ROC) curves and area under the curve (AUC) were used to compare test accuracy. Optimal threshold for best sensitivity and specificity was defined using the Youden index. One-way ANOVA was used for detection of differences in transfusion requirements between groups. Statistical calculations were made using SPSS 21.0 (IBM Cooperation NY, USA) and MedCalc 3.0 (MedCalc Software, Ostende, Belgium). A p-value <0.05 was considered statistically significant. Values are given as mean (standard deviation) unless stated otherwise.

RESULTS

A total of 808 patients were included in this study. The patient cohort is described in Table 1. Massive transfusion was required for 49 patients (6.1%) and 89 patients (11.0%) had ATC. All ROTEM® parameters and CCTs differed significantly between ATC and non-ATC groups, as well as between MT and non-MT groups (p<0.001).

Test characteristics based on previously suggested threshold values for INR (>1.2), CA5 (<35 mm), CT (>94 seconds) and alpha angle (< 65°) are presented in Table 2. The detection rate for massive transfusion requirement was found to be highest for INR and EXTEM CA5 with 51.1% and 45.5%, respectively.

Table 3 summarizes test performance measured by AUC for ROTEM® parameters and CCTs. All included ROTEM® parameters, fibrinogen concentration, INR and platelet counts significantly predicted MT. The highest ROTEM® AUC values were found for EXTEM CA5 and FIBTEM CA5, both in detecting ATC and predicting massive transfusion requirements. These AUC values did however not differ significantly from the AUC of the other ROTEM

parameters. AUC for fibrinogen concentration, on the other hand, was significantly higher than any other ROTEM parameter in detecting ATC.

The optimal threshold value for specificity and sensitivity for EXTEM CA5 in detecting ATC was found to be ≤ 37 mm, and in predicting MT ≤ 40 mm (Table 4). The corresponding values for FIBTEM were ≤ 8 mm and ≤ 9 mm, respectively. The optimal threshold for fibrinogen concentration in detecting ATC was ≤ 1.61 g/L and ≤ 1.90 g/L in predicting MT.

With the calculated optimal thresholds for massive transfusion, detection rate with EXTEM CA5 was 72.7%, for FIBTEM CA5 77.5%, for fibrinogen concentration 77.8% and for INR 70.2%.

The number of units PRBC and plasma transfused was significantly higher in the groups with either EXTEM CA5 or FIBTEM CA5 below the optimum threshold for ATC detection as depicted in Figure 1.

TABLE 1 Descriptive statistics for the study population (N=808)

	All (N=808)	INR > 1.2 (n=89)	MT (n=49)
Age	38 (28)	38 (29)	41 (33)
Male gender (%)	77.4	71.9	65.3
ISS	16 (20)	33 (22)	29 (16)
Penetrating injury (%)	17.5	17.1	12.24
Base excess mEq/ml	-1.90 (4.90)	-8.0 (8.7)	-9.9 (7.7)
ISS > 15 (%)	52.5	89.2	93.6
Base excess < -5 (%)	19.5	63.5	78.7
INR > 1.2 (%)	11	100	51.1
Any PRBC administered (%)	31.7	76.7	100
PRBC ≥10 administered (%)	6.1	27.9	100

ISS: Injury Severity Score, BP: blood pressure, INR: international normalized ratio, PRBC: packed red blood cells. Age, ISS and Base excess are given as median (inter quartile range). MT: massively transfused (≥ 10 PRBC)

TABLE 2 Test characteristics in predicting massive transfusion (≥10 units of packed red blood cells) based on previously suggested threshold values[9].

	Detection rate		*False positive rate*		*PPV*		*NPV*	
INR > 1.2	51.1	(36.1-65.9)	8.8	(6.8-11.0)	27.3	(18.3-37.9)	96.7	(95.0-97.9)
CT > 94 sec	28.9	(16.4-44.3)	8.8	(6.9-11.2)	16.5	(9.1-26.5)	95.5	(93.7-96.9)
CA5 ≤35 mm	45.5	(30.4-61.2)	16.1	(13.5-19.0)	14.4	(9.0-21.3)	96.3	(94.5-97.6)
Alpha angle< 65 °	37.2	(23.0-53.3)	12.2	(9.9-14.8)	15.1	(8.9-23.4)	96	(94.2-97.3)

TABLE 3 ROC analyses of parameters predicting acute traumatic coagulopathy (ATC) and massive transfusion.

	ATC			**Massive transfusion**		
	AUC	*(95% CI)*	*p-value*	*AUC*	*(95% CI)*	*p-value*
EXTEM CT (s)	0.73	(0.70-0.76)	< 0.001	0.68	(0.65-0.71)	< 0.001
EXTEM CA5 (mm)	0.79	(0.76-0.81)	< 0.001	0.75	(0.72-0.78)	< 0.001
EXTEM CA10 (mm)	0.78	(0.75-0.81)	< 0.001	0.75	(0.72-0.78)	< 0.001
EXTEM CFT (s)	0.77	(0.74-0.80)	< 0.001	0.73	(0.70-0.76)	< 0.001
EXTEM Alpha (°)	0.78	(0.75-0.81)	< 0.001	0.73	(0.69-0-76)	< 0.001
EXTEM MCF (mm)	0.73	(0.70-0.76)	< 0.001	0.7	(0.67-0.73)	< 0.001
FIBTEM CT (s)	0.72	(0.68-0.75)	< 0.001	0.65	(0.62-0.69)	0.001
FIBTEM CA5 (mm)	0.8	(0.77-0.83)	< 0.001	0.78	(0.74-0.81)	< 0.001
FIBTEM CA10 (mm)	0.79	(0.76-0.82)	< 0.001	0.76	(0.73-0.79)	< 0.001
FIBTEM MCF (mm)	0.77	(0.74-0.80)	< 0.001	0.76	(0.73-0.79)	< 0.001
Fibrinogen concentration	0.87*	(0.84-0.89)	< 0.001	0.81	(0.78-0.83)	< 0.001
INR	N/A	N/A	< 0.001	0.82	(0.79-0.84)	< 0.001
Platelet count	0.74	(0.70-0.77)	< 0.001	0.7	(0.66-0.73)	< 0.001

AUC: area under curve CT: clotting time. CFT: clot formation time CA5: clot amplitude after 5 minutes CA10: clot amplitude after 10 minutes. MCF: maximum clot firmness. INR: international normalized ratio. ROC: receiver operating characteristics. ATC: acute traumatic coagulopathy defined as INR>1.2. Massive transfusion defined as 10 or more packed red blood cells.

* AUC is significantly larger than the AUC of the ROTEM parameters (P = .002 for difference to FIBTEM CA5)

PPV: positive predictive value, NPV: negative predictive value, INR: international normalized ratio, CT: clotting time, CA5: clotting amplitude after 5 minutes.

TABLE 4: Optimum thresholds and respective test accuracy parameters for predicting a) acute traumatic coagulopathy (ATC) defined as INR > 1.2 and b) massive transfusion (defined as ≥ 10 units of PRBC)

a)

Test Parameter	*Optimum Threshold*		*Detection Rate*		*False Positive Rate*		*PPV*		*NPV*	
EXTEM CA5	≤37	(34-39)	66.3	(55.1-76.3)	18.8	(15.9-21.9)	29.9	(23.4-37.1)	95.2	(93.2-96.8)
FIBTEM CA5	≤8	(5-8)	67.5	(55.9-77.8)	20.7	(17.7-23.9)	26.9	(20.8-33.8)	95.6	(93.5-97.1)
Fibrinogen	≤1.61	(1.36-1.9)	73.6	(63.0-82.4)	11.5	(9.2-14.1)	45.1	(36.7-53.6)	96.3	(94.5-97.7)
Platelet count	≤199	(128-199)	*61.7*	(46.4-75.5)	29.9	(26.6-33.4)	11.9	(8.1-16.7)	96.5	(94.6-97.9)

b)

Test Parameter	*Optimum Threshold*		*Detection Rate*		*False positive rate*		*PPV*		*NPV*	
EXTEM CA5	≤40	(32-40)	72.7	(57.2-85.0)	31.3	(28.0-34.8)	12.2	(8.5-16.8)	97.7	(96.0-98.8)
FIBTEM CA5	≤9	(6-9)	77.5	(61.5-89.2)	32.8	(29.4-36.4)	11.4	(7.9-15.8)	98.2	(96.6-99.2)
Fibrinogen	≤1.90	(1.39-2.18)	77.8	(62.9-88.8)	29.7	(26.4-30.1)	14	(9.9-18.9)	98.1	(96.5-99.1)
INR	≥1.13	(1.0-1.16)	70.2	(55.1-82.7)	19	(16.2-22.1)	19.2	(13.6-25.9)	97.7	(96.2-98.7)
Platelet count	≤ 174	(159-182)	52.8	(41.9-63.5)	14.8	(12.2-17.7)	32.2	(24.7-40.4)	93.1	(90.8-95.0)

PRBC: packed red blood cells. CA5: clot amplitude after 5 minutes, INR: international normalized ratio, PPV: positive predictive value, NPV: negative predictive value.

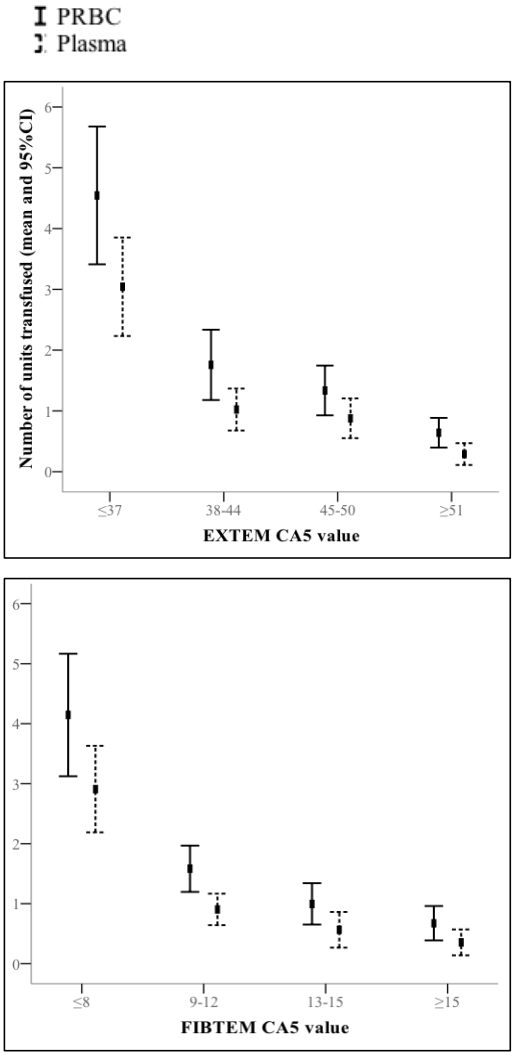

FIGURE 1 Units transfused grouped by EXTEM CA5 (left panel) and FIBTEM CA5 (right panel). The difference between the number of transfusions in the group with CA5 below optimum threshold (≤ 37 mm and ≤ 8 mm respectively) and other groups is statistically significant. PRBC: Packed red blood cells.

DISCUSSION

This study shows that the amplitude of the ROTEM® assay after five minutes (EXTEM CA5) detects ATC and predicts the need for massive transfusion. The detection rate for MT of 45.5% was however lower in the current study compared to the predictive values previously reported (71.4%) when a threshold value of ≤ 35 mm was used.[12] When false positive and false negative test results were weighted equally, the threshold for best sensitivity and specificity (≤ 40 mm) was slightly higher in our dataset than the threshold suggested by Davenport et al. With a threshold of ≤ 40 mm we found the detection rate for EXTEM CA5 to be 72.7%, comparable to the previous findings. This was however associated with an increased false positive rate in our data set (31.3% versus 15.3%). The reasons for the differences between our results and the results of the single centre study by Davenport et al may be due to the differences in number of massively transfused patients (11 vs 49).

From the ROC curve analyses it appears that the platelet-inhibited assay (FIBTEM) may increase the test accuracy with respect to the need for massive transfusion. This is in accordance with the findings reported by Schöchl et al.[14] In a retrospective single centre study of 323 trauma patients they found that the FIBTEM assay had a better overall test accuracy than the EXTEM assay. They identified the FIBTEM MCF as the parameter with the largest AUC, with detection rate similar to that of FIBTEM CA5 in our study. They identified an optimum threshold of FIBTEM MCF of ≤ 7 mm, with a sensitivity of 77.5%. In their study, fibrinogen concentration also had a large AUC, comparable to the best ROTEM parameter, and a sensitivity of 84.2%, with a threshold of 1.48 g/L.

Excellent correlation has previously been demonstrated between clot amplitude in platelet inhibited ROTEM® assays and fibrinogen measured by Clauss method.[15-17] Low fibrinogen concentration has been closely linked to mortality and need for massive transfusion in a number of studies.[4,18,19] In a study by Harr and colleagues, fibrinogen concentration was closely correlated to the clot strength (R2 =0.87) in an assay similar to the FIBTEM assay (TEG Functional Fibrinogen assay).[16] Adding fibrinogen in vitro increased both clot strength and the relative contribution to clot strength of fibrinogen compared to platelets. This finding is supported by animal studies, case reports and observational studies in humans demonstrating a reversal of ATC by fibrinogen concentrate.[20-23] The crucial role of fibrinogen in traumatic

coagulopathy, supported by these findings, may to some extent explain why the FIBTEM assay present better test characteristics than the EXTEM assay in our study.

Since the introduction of VHAs in trauma management, some researchers propose that the role of CCTs in guiding transfusion therapy is marginalized.[24,25] The turnaround time for CCTs may be considered too long, and these in vitro tests assess only isolated components of the coagulation system, However, it should be noted that based on the results of several studies, INR,[17,26] fibrinogen concentration,[17] and haemoglobin concentration (or haematocrit)[17,27] appears to be non-inferior to VHAs when it comes to predicting MT from a single blood sample on arrival. Readily available Point of Care-testing devices may in the case of haemoglobin concentration and INR overcome the time delay usually associated with the conventional laboratory analyses. The precision and feasibility of such diagnostics should be a target for further studies.

Limitations of our study include the fact that few patients required MT, and the confidence intervals of the test characteristics are correspondingly wide. The test results from ROTEM® analyses were not blinded to clinicians in all centers and may to some extent have biased the results. In the case of such a bias this would have favored the test performance of VHAs since they usually are available to clinicians faster than the CCTs. A survivor bias in this study cannot be excluded as some patients may have died before receiving the required amount of transfusions. This potential bias may have resulted in an underestimation of the accuracy in predicting MT in our study. Our analyses are based only on the first sample obtained shortly after arrival in the ED. The value of repeated VHA analyses to guide transfusion during the course of resuscitation was not evaluated in this study. Finally, our study is not addressing the impact of ROTEM® on clinical outcomes.

In conclusion, our study confirms the previous finding that the ROTEM® CA5 value measured on arrival is a valid marker for ATC and predicts massive transfusion requirements. An EXTEM CA5 threshold value of ≤ 40 mm has a detection rate of 72.7%, whereas a FIBTEM CA5 threshold value of ≤ 9 mm detects massive transfusion requirements in 77.5% of cases. Fibrinogen concentration was significantly better than ROTEM ®assays in predicting ATC, and a fibrinogen concentration ≤ 1.90 g/L had a detection rate 77.8% for massive transfusion requirement. Future studies should be directed at identifying the role of repeated VHA measurements in guiding haemostatic resuscitation in trauma.

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Chapter 7

THE RISK-BENEFIT OF THE USE OF CHEMOPROPHYLAXIS FOR THROMBOEMBOLIC EVENTS IN PATIENTS AFTER SUSTAINING TRAUMATIC BRAIN INJURY: SYSTEMIC REVIEW AND META-ANALYSIS

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In preparation

ABSTRACT

Traumatic brain injury (TBI) patients are at high risk for venous thromboembolism (VTE). The Brain Trauma Foundation Guidelines state that low-molecular-weight heparin (LMWH) or low dose unfractionated heparin (UFH) should be used in combination with mechanical prophylaxis to prevent VTE complications, but it also states that there might be an increased risk of progression of intracranial hemorrhages (ICH) with VTE prophylaxis.

Objective: To study the risk benefit of the use of LMWH/UFH for thromboembolic prophylaxis after TBI.

Systemic review and meta-analysis on studies including TBI patients ≥16 years reporting on progression of intracranial hemorrhage and/or prevalence of thromboembolic events with the use of VTE prophylaxis were eligible for inclusion. Results of the included studies were evaluated with meta-analyses of pooled data. Primary end-points were VTE rates and ICH progression and were pooled using a random-effects model.

In total, 14 studies were included with a sample size of 7699 patients. Ten studies were used to analyze the safety and 8 to analyze the effectiveness of chemoprophylaxis versus no prophylaxis. Four studies, with a sample size of 1871 patients, were single-arm studies and only assesed for occurrence of VTE. High heterogeneity prevented pooling of data.

Current available literature is insufficient to formulate conclusions with regard to the safety and efficacy of the use of LMWH/UFH prophylaxis for thromboembolic events in patients after sustaining traumatic brain injury. The results of this review emphasize the urgent need for further research.

INTRODUCTION

Traumatic brain injury (TBI) is a major cause of mortality in the United States, contributing to approximately 30% of all injury-related deaths.[1]

Prevention of clot formation in extremities and clot migration to the lungs is an important, yet complicated aspect in the care of TBI patients. TBI itself may confer as much as a 4-fold increased risk of deep vein thrombosis (DVT) in trauma patients,[2] which probably relates to the development of endogenous hypercoagulability after trauma in a population that is

immobilized. Without any intervention more than half of TBI patients will develop venous thromboembolism (VTE) such as deep venous thrombosis (DVT) and/or pulmonary embolism (PE).[3] However, the use of chemoprophylaxis in TBI patients may increase the risk of intracranial hemorrhage (ICH) expansion.

Currently, the exact risk-benefits of administering thrombosis prophylaxis in patients suffering from traumatic brain injury remain unclear. The Brain Trauma Foundation Guidelines for the Management of Severe Traumatic Brain Injury (2016) state that low-molecular-weight heparin (LMWH) or low dose unfractionated heparin should be used in combination with mechanical prophylaxis to prevent VTE complications, but suggests there is an increased risk of expansion of ICH with VTE prophylaxis. Our specific aim was to review literature and perform a meta-analysis on the risk-benefit of the use of LMWH in patients with traumatic brain injury on the progression of intracranial hemorrhage and the prevention of thromboembolic events.

METHODS

The present study was reported according to the PRISMA guidelines (preferred reporting for systemic reviews and meta-analyses).[4]

Search Strategy

An electronic search was conducted in PubMed, Scopus, Embase and Cochrane Collaborations for English-language articles published from January 1993 - December 2018.

The following subject headings and free text words were used: ("Heparin, Low-Molecular-Weight"[mh] OR "LMWH"[tiab] OR "Low Molecular Weight Heparin"[tiab] OR "Enoxaparin"[tiab] OR "Dalteparin"[tiab] OR "Tinzaparin"[tiab] OR "Bemiparin"[tiab] OR "Certoparin"[tiab] OR "Nadroparin"[tiab] OR "Parnaparin"[tiab] OR "Reviparin"[tiab] OR "Ardeparin"[tiab]) AND ("prevention and control"[Subheading] OR Pre-Exposure Prophylaxis[mh] OR Post-Exposure Prophylaxis[mh] OR "Antithrombotic"[tiab] OR "Prophylactic"[tiab] OR "Prophylaxis"[tiab] OR Anticoagulants[mh] OR "Anticoagulation"[tiab] AND Venous Thromboembolism[mh] OR "VTE"[tiab] OR "Venous Thromboembolism"[tiab] OR "Thromboprophylaxis"[tiab] OR "Thromboembolic"[tiab] OR "Chemoprofylaxis"[tiab]) AND (((Wounds and Injuries[mh] OR Hemorrhage[mh] OR

"Trauma"[tiab] OR "Traumatic"[tiab] OR Traumas[tiab] OR "Injury"[tiab] OR Injuries[tiab] OR "Hemorrhage"[tiab] OR Lesion[tiab] OR Posttraumatic[tiab] OR Post-traumatic[tiab])AND (Brain[mh] OR "Intracranial"[tiab] OR "Head"[tiab] OR "Brain"[tiab] OR "Neurocritical"[tiab] OR Hematoma[mh] OR "Hematoma"[tiab] OR Subdural Space[mh] OR "Subdural"[tiab] OR "Epidural"[tiab] OR "Subarachnoid"[tiab] OR cerebral[tiab] OR cerebrovascular[tiab] OR encephalopathy[tiab] OR encephalopathies[tiab])) OR (Brain Injuries, Traumatic[mh] OR TBI[tiab] OR TBIs[tiab] OR Intracranial Hemorrhage, Traumatic[mh] OR cerebrovascular trauma[mh]). In addition, we searched for ongoing trials on www.controlled-trials.com and www.clinicaltrials.gov.

Study Selection

The inclusion criteria that were used to select eligible studies were trauma patients who suffered blunt or penetrating traumatic brain injury and age ≥ 18 years. Randomized controlled trials (RCTs) and observational studies investigating the use of a LMWH/UFH or placebo as a prophylaxis for thromboembolic events were included. Studies were eligible when 1 of the study outcomes was reported, including progression of intracranial hemorrhage, thromboembolic events (as assessed by ultrasound, computed tomography [CT] of pulmonary arteries, or by ventilation-perfusion gammagraphy, and/or ICH progression (defined as larger or new areas of hemorrhage demonstrated by a second cranial CT). Two independent reviewers (SC and DO) screened the titles and abstracts of primary identified studies for eligibility. Full-text articles were read for further assessment if the eligibility was unclear by screening the abstracts. Any discrepancies were resolved through discussion between the reviewers. Both authors extracted data from all studies independently, fulfilling the inclusion criteria. Both prospective and retrospective studies were included. Reviews, correspondences, case reports, expert opinions, and editorials were excluded. Non-relevant studies were excluded based on title and abstract alone. Language was limited to English, Dutch, or German. We reviewed the bibliographies of the eligible studies for citations of additional suitable studies.

Quality Assessment

We performed a quality assessment according to the JADAD scale for randomized controlled trials[5] and according to the Newcastle-Ottawa Scale.[6] Characteristics of the studies examined

and scored included comparability of the study groups, methods used to select study participants and determination of outcome variables. The quality of selection of patients in the included studies was rated at maximum points if they included severely injured trauma patients and the control group was drawn from the same community as the exposed cohort. The assessment of comparability of the studies was based on the design and/or analysis used in the studies. Quality of outcome variables was determined by follow-up period and <10% of patients lost-to-follow-up.

Data Synthesis

Review Manager (RevMan 5, The Nordic Cochrane Centre) was used to combine findings of studies in a meta-analysis. Studies were pooled if homogeneity was deemed acceptable by assessing study population, intervention, and outcome. In addition, RevMan was used to determine homogeneity by the inverse variance method for a random effects model. Studies were excluded from meta-analysis when homogeneity was not obtained, defined as a I^2 of >75%. Meta-analysis was performed on observational studies and RCTs, comprising both single and double arm studies. Results of meta-analyses were expressed by odds-ratio and 95% confidence intervals.

RESULTS

In total, 1431 studies were screened and assessed for eligibility on abstract and title. Of these, 975 records were removed because of duplicates, leaving 456 records. Of these, 47 studies were reviewed for full text review based on inclusion criteria. After full text review, 33 studies were excluded for the following reasons: case reports/letters to editor/surveys (n=8), studies lacking TBI patients (n=8), non-human studies (n=5), reviews (n=4), non-english studies (n=3), no report on chemoprophylaxis (n=2), irrelevant outcome (n=2) and pediatric study (n=1). Reviewing of the bibliographies resulted in 3 additional articles, bringing the total on 14 included articles with a total sample size of 7699 patients. Figure 1 illustrates the flowchart of the inclusion process and Table 1 the characteristics of studies included. Of the 14 included studies, 13 studies were observational cohort studies (11 retrospective and 2 prospective) and 1 randomized controlled trial (Fig 2). Of those included, 10 studies had a two-arm study design

and 4 were single-arm studies. Risk for bias was moderate as scores of all included studies on the Newcastle-Ottawa scale ranged from 5-8 with a median of 6.

FIGURE 1 **The process of selecting studies suitable for inclusion**

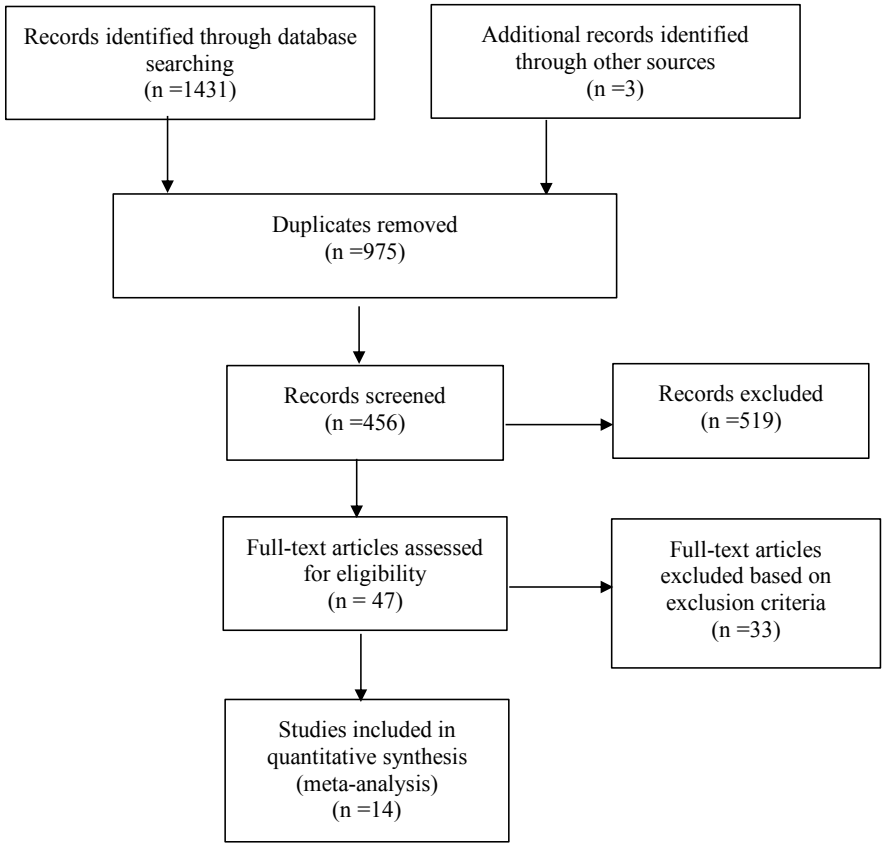

TABLE 1 **Study Characteristics**

Author/Year	Design/ N	Participants	Comparison	LMWH	Progression ICH	VTE	Conclusion	NOS
Levy[1] 2010	Retrospective/ 340	TBI with skull fracture, cerebral contusion or laceration, ICH, diffuse axonal injury	TP (early ≤72 hours and late > 72 hours) vs No TP	Enoxaparin 30 mg 2dd after 26 hours (n=213) Heparin 5000 units 2dd (n=8)	TP 73 (34 %) No TP 37 (31%)	TP 13 DVT, 3 PE No TP 2 DVT, 1 PE	TP is associated with a 13-fold increase in progression of ICH in patients with ICH progression on CT within 1 day of admission	8/10
Dudley[2] 2010	Retrospective/ 287	TBI with GCS between 3-12	Enoxaparin vs Dalteparin	Enoxaparin 30 mg 2dd (n=128) Dalteparin 5000 IU 1dd (n=159)	Enoxaparin 1 (8%) Dalteparin 0 (0%)	Enoxaparin 9 (7%) VTE Dalteparin 12 (7.5 %) VTE	TP decreases the risk of VTE's without increased risk of ICH progression when started after 48-72 hrs and two stable head CT's	6/10
Scudday[3] 2011	Retrospective/ 812	TBI with ICH, hematoma, contusion, diffuse axonal injury with AIS head >2	TP vs No TP	Heparin (n=364) Enoxaparin (n=38)	No TB 25 (6%) TB 11 (3%)	No TB 11 (3%) VTE TB 3 (1%) VTE	TP reduces incidence of VTE without increased risk of ICH progression	8/10

Saadeh[4] 2012	Retrospective/ 205	TBI that includes ICH	TP vs No TP	LMWH Unfractioned heparin	No TB 0 (%) TB 0 (0%)	No TB 0 (0%) TB 0 (0%)	Early use of TP does not result in progression of ICH and reduces the rate of VTE	5/10
Salottolo[5] 2011	Retrospective/ 480	TBI with skull fracture, cerebral contusion or laceration, ICH, diffuse axonal injury	TP (early ≤72 hours and late > 72 hours) vs No TP	Levonox 30 mg 2dd Heparin 5000 IU 2dd	-	Early TP 6 (6%) Late TP 4 (3%) No TP 5 (2%)	The use or timing of TP is not associated with increased risk of VTE. Interrupted TP increases risk for VTE.	8/10
Reiff[6] 2009	Retrospective/ 2000	Patients with blunt or penetrating injuries TBI subgroup: Skull fracture, cerebral contusion or laceration, subarachnoid/subdural/extradural haemorrhage, other unspecified ICH, diffuse axonal injury	No TP vs 0-24 hr TP 24-48 hr TP ≥ 48 hr TP	LMWH Unfractioned heparin No prophylaxis (n=100)	-	No TP 6 (0.4 %) TP 56 (10 %)	3 to 4-fold increased risk of VTE with TP started after 48 hrs	5/10
Kim[7] 2009	Retrospective/ 500	TBI patients (not further defined)	TBI vs ICH (no trauma) vs SAB (no trauma)	Unfractioned heparin 5000 units 2dd Enoxaparin 30 mg 2dd	TP 0 (0%)	TP 15 (3%) DVT TP 2 (0.4 %) PE	TP was not associated with increased risk of VTE	5/10

Koehler[8] 2011	Retrospective/ 669	TBI determined by presence of ICH on CT	Early TP (0-72 hours) vs Late TP (>72 hours)	Enoxaparin 30 mg 2dd	Early TP 7 (1.5%) Late TP 12 (1.5%)	Early TP 4 (1.5 %) PE, 14 (5.2 %) DVT Late TP 9 (2.2) PE, 41 (10.2 %) DVT	No evidence for increased ICH progression after early TP Incidence VTE possibly increased in late TP group	6/10
Norwood[9] 2008	Prospective/ 525	All blunt TBI patients documented by CT	-	Enoxaparin sodium 30 mg 2dd	TP 62 (12%)	TP 6 (1.1 %) DVT TP 0 (0%) PE	No increased risk of progression of ICH after TP	7/10
Norwood[10] 2002	Prospective/ 150	All blunt TBI patients documented by CT	-	Enoxaparin sodium 30 mg 2dd	TP 34 (23%)	TP 2 (2%) DVT TP 0 (0%) PE	No increased risk of ICH expansion when TP started 24 hours after injury	6/10
Phelan[11] 2012	RCT/ 62	TBI Patients	Enoxaparin vs Placebo	Standard Care Enoxparin	TP 2 (5.9%)	TP 0 (0%) No TP 1 (3.6 %)	TBI progression after TP in low risk TBI patients 24 hours after injury is similar to those in placebo and subclinical.	JADAD 5

Depew[12] 2008	Retrospective/ 82	Trauma patients with ICH	Enoxaparin	Enoxaparin 30 mg 2dd Heparin 5,000 IU 2dd	TP 3 (2.5%)	10 (8%)	Early TP in low-risk TBI patients with stable head CT shows no increase in ICH progression. Higher number in VTE with late TP but no change in ICH progression	7/10
Kwiatt[13] 2012	Retrospective/ 1215	Trauma patients with ICH	LMWH vs none	LMWH	No TP 239 (24%) TB 93 (42%) TP 32 (14.5%) after TB start	NA	TP increased the risk of ICH progression	7/10
Minshall[14] 2011	Retrospective/ 386	TBI patients with HAIS>3 and ICU stay >48 hrs	LMWH vs UFH	Enoxaparin 30mg 2dd UFH 5,000U 3dd	UFH 34 (20%) LMWH 20 (13%) No TB 14 (25%)	UFH DVT 2(1 %) PE 7 (4) LM WH DVT 1(1 %) PE 0(0 %) NO TB DVT 1(1 %) PE 1 (1%)	TP (LMWH>UFH) is effective in preventing VTE with low rates of ICH progression	5/10

*TP=Thromboprophylaxis, VTE= Venous thromboembolic events, NOS=Newcastle Ottawa Scale, ICH=Intracranial hemorrhage, TBI= Traumatic Brain Injury, UFH= Unfractionated Heparin, LMWH= Low molecular weight heparin, RCT=

Randomized Controlled Trail, SAB=Subarachnoid bleeding, GCS= Glasgow Coma Scale, HAIS=Head Adjusted Injury Severity Score, ICU=Intensive Care Unit, CT= computer Tomography, DVT=Deep Venous Thrombosis.

Methods and definitions of included studies
1 Progression CT defined as: hemorrhage that worsened or any newly developed bleeding on CT certified by radiologist. Clinically significant progression of hemorrhage was defined as ICH progression associated with change in either patient management or a change in patient`s clinical condition. VTE: DVT documented on ultrasound, PE on chest CT, chest CT angiogram or ventilation perfusion scan
2 DVTs diagnosed by duplex ultrasonography, PE by spiral chest CT
3 Progression TBI defined as evolution of intracranial hemorrhage as documented on faculty read of a follow-up radiologic scan, VTE directed by clinical suspicion
4 VTE assessed by venous Doppler, chest CT angiogram for PE
5 DVT was suspected clinically and documented by ultrasound. PE on chest CT, chest CT angiogram or ventilation perfusion scan.
6 DVT with ultrasonography with spectral and color Doppler imaging
7 DVT was diagnosed by duplex ultrasonography, PE by spiral chest CT scanning.
8 Progression intracranial hemorrhage was documented as expansion of hemorrhagic lesion by radiologist or development of new intracranial hemorrhage on follow-up CT. VTE by Doppler duplex color flow ultrasound, chest CT or CT angiogram for PE.
9 Doppler performed in patients with delayed ≥5 days TB prophylaxis. Progression of TBI was determined by Marshall CT classification or radiologist report.
10 Progression of TBI was determined by Marshall CT classification or radiologist report, PE by pulmonary angiography and DVTs by venous color flow duplex ultrasound scans
11 Progression of TBI was determined by follow up CT scanning and read by study investigators. DVT was determined by Duplex ultrasound ordered at the discretion of treating physician. PE was diagnosed by CT angiogram, pulmonary angiogram or high-probability ventilation/perfusion scan at the discretion of treating physician.
12 Surveillance of DVT was done by duplex ultrasound, PE by CT pulmonary angiography or ventilation percussion scan. Progression of ICH was determined by CT scan.
13 Progression of intracranial hemorrhage was documented on CT scan
14 DVT diagnosed by clinical examination and confirmed with duplex ultrasound. CT pulmonary angiogram was used to confirm PE on clinical indication. Worsening of intracranial hemorrhage was documented on CT scan, if repeat scan demonstrated larger of new areas of intracranial hemorrhage, these were considered to be worse.

Safety and efficacy of two-arm studies

There were 10 studies with two-arms, so called comparator studies, with a drug investigation arm and a placebo-arm or no treatment arm. We included one randomized controlled trial, one significant multicenter retrospective cohort study and 8 remaining observational studies. Overall pooled odds ratio for these 10 studies on efficacy of VTE chemoprophylaxis was 1.67 (0.80 to 3.48) with an overall heterogeneity of I^2 70.6% (Fig 3). Furthermore, 8 studies on safety of VTE prophylaxis were pooled, this resulted in a non-significant odds ratio 0.79 (0.31 to 1.69). Heterogeneity of the 8 pooled studies was I^2 90.9% (Fig 4). Due to high heterogeneity, results are reported narratively rather than systemically.

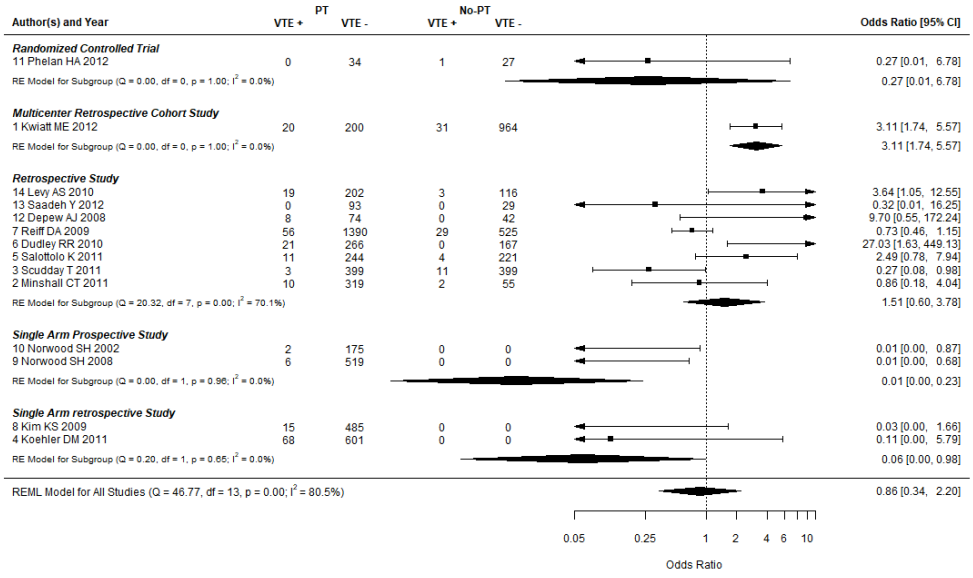

FIGURE 2 **Meta-analysis: the effect of chemical thromboprofylaxis versus no thromboprofylaxis on the development of venous thrombo-embolisms in TBI patients in all studies**. VTE= venous thrombo embolism, PT= thromboprophylaxis TBI= traumatic brain injury

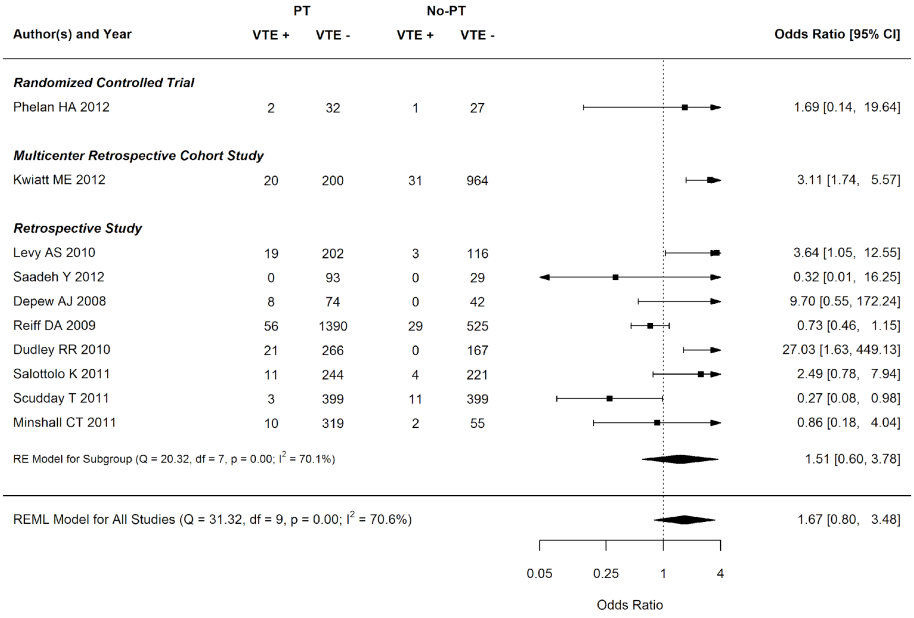

FIGURE 3 **Meta-analysis: the effect of chemical thromboprofylaxis versus no thromboprofylaxis on the development of venous thrombo-embolisms in TBI patients in two arm studies.** VTE= venous thrombo embolism, PT= thromboprophylaxis

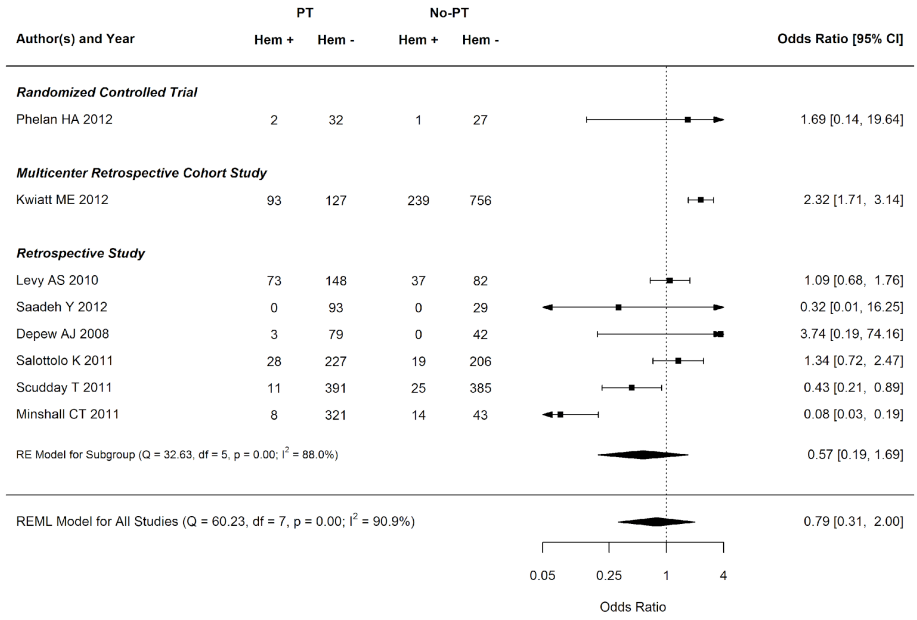

FIGURE 4 **Meta-analysis: the effect of chemical thromboprofylaxis versus no thromboprofylaxis on the progression of intracranial hemorrhage in TBI patients**. PT= Thromboprophylaxis Hem= Hemorrhage

Phelan et al. conducted a randomized, double-blinded, placebo-controlled pilot trial including 62 patients.[7] Patients presenting within 6 hours of injury with pre-specified small TBI patterns and stable scans at 24 hours after injury were randomized to receive enoxaparin 30 mg bid (n=34) or placebo (N=28) from 24 to 96 hours after injury. The primary end point was the radiographic worsening of TBI after initiation of enoxaparin or placebo, by dichotomous measurement (worse/not worse) on CT scan. Subclinical, radiographic TBI progression rates on the scans performed 48 hours after injury and 24 hours after start of treatment were 5.9% (95% confidence interval [CI], 0.7-19.7%) for enoxaparin and 3.6% (95% CI, 0.1-18.3%) for placebo, a treatment effect difference of 2.3% (95% CI, 14.42-16.5%). No clinical ICH progressions occurred. One deep vein thrombosis occurred in the placebo arm. Thereby, this trial reported an odds ratio of -0.27 (95% CI 0.01 to 6.78) for the development of VTE and an OR of 1.69 (95% CI 0.14 to 19.64) for the progression of ICH in the group randomized to enoxaparin. Of note, the determination of progression was made qualitatively rather than

quantitatively, which could potentially have led to under-estimation of progression. It is also important to note that this study was conducted amongst low-risk TBI patients.

The multi-center retrospective cohort study performed by Kwiatt on the use of LMWH included 1215 patients with intracranial hemorrhage caused by blunt trauma[8]. Patients were divided into two groups; those who received LMWH (n=220) and those who did not (n=995). The primary outcome was progression on intra-cranial hemorrhage on repeated head CT. Hemorrhage progression occurred in 239 of 995 (24%) control subjects and 93 of 220 (42%) LMWH patients, resulting in an OR of 2.32 (95% CI 1.71-3.14) in the group receiving prophylaxis. The LMWH group had 20 episodes of VTE while the control group had 31 episodes of VTE (9.1% vs. 3.1%, *p* 0.001); OR 3.11 (95% CI 1.74 to 5.75). Although the authors report that the study was adequately powered, the significant disproportionate numbers in the two study groups offers limitations.

The study by Scudday et al. was initiated after an institutional thromboprophylaxis protocol change for patients with a traumatic head injury.[9] Records of 812 patients admitted with a TBI to a level I trauma center from 2006 to 2008 were reviewed. Primary outcome was progression of hemorrhage and VTE events. Almost 50% of included patients received chemical VTE prophylaxis (n=402). Patients receiving prophylaxis had a lower rate of progression of bleeding (3% versus 6%), although this was not statistically significant. These patients also had a lower incidence of VTE (1% versus 3% *P*= .019). Thereby, this study reports and odds ratio of 0.27 (95% CI 0.08 to 0.98) for the development of VTE and 0.43 (95% CI 0.21 to 0.89) for the progression of ICH. Authors concluded that the use of chemical VTE prophylaxis in TBI patients with a stable or improved head CT after 24 hours substantially reduced the incidence of VTE and does not increase the risk of progression of intracranial hemorrhage. The retrospective nature of the study offers limitations. DVT and PE were directed by clinical suspicion, which may have led to underreporting of subclinical VTEs. Authors also reported there was only 55% compliance to the thromboprophylaxis protocol.

A retrospective single center cohort study by Minshall et al. was conducted in 386 trauma patients admitted to the ICU with a Head and Neck Abbreviated Injury Severity Score (HAIS) > 2.[10] In this group,158 patients were treated with LMWH and 171 were treated with UFH. Chemical VTE prophylaxis was initiated once patients no longer needed ongoing blood

product transfusion, had a stable neurologic examination, or had a repeat head CT that demonstrated no evidence of active or increased intracranial hemorrhage. The remaining patients (n= 57) had no VTE prophylaxis. All patients received mechanoprophylaxis. In the group of patients who were treated with LMWH, the rate of progression of their intracranial hemorrhage was 13%. This was significantly lower than the rate in the non-chemoprophylaxis group (25%). However, the rate of progression of intracranial hemorrhage in the UFH group (20%) was not significantly different to the rate in either the LMWH or the non-chemoprophylaxis group (25%). The overall DVT and PE rate for all patients was 0.9% and 1.9%. The UFH group had a significantly higher rate of DVT 1% and PE 3.7% compared to the group of patients treated with LMWH. The DVT and PE rate in patients that did not receive chemical VTE prophylaxis was 2% and 2% respectively and not significantly different from the other groups. Thereby, the study reported an odds ratio of 0.86 (95% CI 0.18 to4.04) for the development of VTE and an OR of 0.08 (95% CI 0.03 to 0.19) for the progression of ICH. The patients in the untreated group had a high mortality rate (47%) when compared with either group treated with chemical VTE prophylaxis, 5% in the LMWH group and 16% in the UFH group respectively.

A retrospective study was conducted by Saadeh et al. in 122 patients with TBI. All patients received mechanical prophylaxis.[11] In total, 93 patients (76.2%) received chemical VTE prophylaxis at some point in their hospital stay and 29 patients (23.8%) received no chemical VTE prophylaxis. In the chemical prophylaxis group, none had progression of ICH or VTE complications. In the group of patients who did not receive chemical VTE prophylaxis, there were also no VTE complications or progression of ICH detected. A limitation of this study is the small sample size. Also, authors relied on physical examination as the initial screen for DVT and pulmonary embolism and did not use routine ultrasound or CT to screen for VTE. Thereby, it is possible that subclinical VTE was missed.

A retrospective study amongst 2000 TBI patients was performed by Reiff et al. In total 1446 patients received chemical VTE prophylaxis and 554 patients did not.[2] In the group who received chemical VTE prophylaxis there were 56 VTEs (3.9%) compared to 29 VTEs (5.2%) in the group that did not. Thereby, the study reported an odds ratio of 0.73 (95% CI 0.46 to 1.15) for the development of VTE. There was no report on the incidence of ICH progression.

Levy et al. retrospectively studied 340 patients with TBI (12). There were 221 patients (65%) who received chemical VTE prophylaxis during their hospital stay and 119 patients (35%) who did not. Among patients who received chemical VTE prophylaxis there were 73 patients (33%) who had hemorrhage progression versus 37 patients (31%) in the group without chemical VTE prophylaxis. In the chemical VTE prophylaxis group, there were 13 DVTs (5.9%), 3 PE's (1.4%) versus 2 DVTs (1.7%) and 1 PE (0.8%) in the non VTE prophylaxis group. Thereby, the study reported an odds ratio of 3.64 (95% CI 1.05 to 2.55) for the development of VTE and 1.09 (95% CI 0.68 to 1.76) for the progression of ICH. This study had several limitations. There were 73 patients who started and stopped VTE prophylaxis at least once.

Depew et al. performed a retrospective study in 124 patients with intra-cranial hemorrhage from blunt head injury.[13] In total, 29 patients (23%) received early chemical VTE prophylaxis (<72 hrs from admission) and 53 patients (43%) received late chemical VTE prophylaxis (>72 hours). The remaining 42 patients in this study received no chemical VTE prophylaxis, they did however receive mechanical prophylaxis. Of the patients who received chemical VTE prophylaxis three patients developed ICH progression (3.7%) and 10 patients (8%) developed a VTE (9 DVT and 1 PE). In the group that did not receive VTE prophylaxis, no ICH progression or VTE events occurred. Thereby, the study reported an odds ratio of 3.74 (95% CI 0.19 to 74.16) for the development of VTE in patients receiving prophylaxis. Authors note that the reason no patients developed a VTE in this group might have been due to a selection bias, as the average length of stay for this group of patients was 7 days, compared to 23 days and 30 days in the group receiving early and late chemical thromboprophylaxis respectively. It was also reported that many patients in the non-chemical thromboprophylaxis group started ambulating early.

Dudley et al. performed a retrospective study among 287 patients after sustaining a moderate to severe TBI.[14] One patient developed ICH expansion while on chemical VTE prophylaxis. VTEs occurred in 7.3% of treated patients, mostly within 2 weeks after injury. This study looked specifically at differences in VTE rates between enoxaparin and dalteparin, however, no significant differences in rates were seen (7.0% vs 7.5% respectively). Thereby, the study reported an odds ratio of 27.03 (95% CI 1.63 to 449.13) for the development of VTE in patients. It is important to note that in this study, CT scans of the brain to detect hemorrhage expansion, or diagnostic exams to detect VTE, were not performed systematically for all

patients, but rather only as clinically indicated.

Salottolo et al. performed a retrospective multicenter study in 480 patients sustaining a TBI.[15] In total, 255 patients received chemical VTE prophylaxis and 225 patients did not. The entire study group consisted of patients in whom head injury was judged to be stable on follow-up scans of the brain. There were 47 patients who later had progression of hemorrhage on follow up CT (9.8%). Rates of hemorrhage progression did not differ significantly between patients who received chemical VTE prophylaxis early (6.5%), late (14.3%) or not at all (8.4%). In the group of patients that received chemical VTE prophylaxis 10 patients (3.9%) developed a VTE. Of the patients that did not receive chemical VTE prophylaxis 5 patients (2.2%) developed a VTE. Thereby, the study reported an odds ratio of 2.49 (95% CI 0.78 to 7.94) for the development of VTE and 1.34 (95% CI 0.72 to 2.47) for the progression of ICH. Authors note that the study was adequately powered to detect a clinically significant difference in VTE for the interrupted versus continuous VTE prophylaxis groups (power 0.88), but power was insufficient to detect a significant difference in VTE for the VTE prophylaxis versus non-VTE prophylaxis groups (power 0.19). Despite a large sample size, the findings that VTE prophylaxis administration and timing of VTE prophylaxis were not predictors of VTE may be the result of a type II error. Patients were categorized as receiving continuous VTE prophylaxis even when VTE prophylaxis was received for 1 day. Finally, admission orders specified weekly ultrasounds for DVT surveillance, yet high risk patients who did not receive VTE prophylaxis were screened more frequently, and patients who were receiving VTE prophylaxis without suspicion of VTE were screened less frequently. Previous investigators have noted that screening is an independent predictor of DVT because of surveillance bias.

Single arm studies

Of the 14 studies in this analysis, 4 studies were single-arm studies. Analysis of 4 these studies with a sample size of 1871 patients reported the occurrence of total of 91 VTEs (4.9%) under chemical VTE prophylaxis. These consisted of two prospective and two retrospective observational studies. Pooling for the development of ICH was not feasible due to lack of sufficient data. Overall pooled odds ratio for the development of VTE in these studies was 0.03 (95% CI 0.01 to 0.09) with a heterogeneity I^2 of 92.9%. Due to high heterogeneity, results are

reported narratively.

A single center prospective observational cohort study by Norwood (2002) included 150 patients receiving enoxaparin within 24 hours of admission[16]. Thirty-four (23%) patients showed progression of ICH on CT imaging. DVT was identified in 2 (2%) of the 106 patients that underwent duplex scans. There were no documented PEs in the study group.

Another prospective study (Norwood 2008) from the same research group was a prospective observational cohort study conducted in 525 TBI patients who received enoxaparin 48 hours after admission.[17] CT scans were obtained at time of admission, 48 hours and during clinical course. Eighteen patients (3.4%) showed progressive hemorrhagic CT changes after receiving enoxaparin. Six patients (1.14%) were diagnosed with DVT. Only 26% of eligible patients were enrolled into this study, which could potentially have led to a selection bias and misrepresentation.

A retrospective study by Kim included 500 TBI receiving LMWH or enoxaparin.[18] There was no objective evidence of intracranial bleeding associated with chemical VTE prophylaxis in VTE patients. Of the 500 TBI patients included, 19 (3.8%) developed a VTE. However, this study did not use a case–control design to analyze the risk factors for VTE based on the type of injury due to small sample size. Furthermore, the severity of brain injury was not considered in analysis and only patients with a high clinical suspicion for DVT or PE were evaluated using duplex ultrasound or spiral chest CT, to confirm the diagnosis.

A retrospective study from Koehler amongst 669 hemodynamically stable TBI patients compared the use of administrating early (0-72 hrs) versus late (>72 hrs) chemical VTE prophylaxis.[19] Progression of ICH before prophylaxis was 9.38% versus 17.41% (P = .001) and after prophylaxis was 1.46% versus 1.54% (P = .9) for the early and late group respectively. Proportions of proximal DVT were 1.5% versus 3.5% (P = .117) and pulmonary embolism were 1.5% versus 2.2% (P= .49) in the early and late group respectively. Although early chemical VTE prophylaxis was not associated with increased progression of intracranial hemorrhage, a selection bias could not be excluded. Moreover, 186 patients with TBIs were excluded from this study because they did not receive chemical VTE prophylaxis.

DISCUSSION

This systemic review studied the risk-benefit of the administration of chemical VTE prophylaxis in patients sustaining a TBI. This is an important and controversial topic, as current guidelines state that LMWH or UFH should be used in combination with mechanical prophylaxis to prevent VTE complications, but it also states that there might be an increased risk of progression of ICH with VTE prophylaxis.

The results found in his review are hampered by high heterogeneity and prevented us from pooling the studies. In our review, incidence rate of VTEs ranged from 0% to 9.1%. The pooled incidence of VTE in patients taking prophylaxis is 4.5% and in those not taking prophylaxis the incidence is 3.1%. This may suggest that prophylaxis is not efficacious. However, this is in contrast with the finding that prophylaxis reduces the risk of VTE in ICU patient populations.[20,21] Explanations may be that most studies in our review were not powered to detect a significant difference in VTE for the VTE prophylaxis versus non-VTE prophylaxis groups, selection bias may have occurred and several studies performed VTE screening only as clinically indicated which may have led to underreporting of events.

Out of concern for progression of intracranial hemorrhage and uncertainties with regard to optimal timing, the administration of chemical VTE prophylaxis in TBI patients is often delayed. In our review we found an incidence rate of ICH progression ranging from 0% to 61.5%. Pooled incidence of ICH progression in patients taking prophylaxis is 13.3% and in those not taking prophylaxis is 17.6%. These results may suggest, not taking timing into consideration, that the administration of chemical VTE prophylaxis is safe. Although based on small observational studies, this is in line with reports among trauma patients with solid organ injury which showed that chemical prophylaxis did not increase the rate of bleeding.[22-23] A significant group of patients will develop progression of ICH regardless of chemical VTE prophylaxis administration. Conform our data, an incidence rate of 14.8% of spontaneous ICH progression at 24 hours was found in patients receiving chemical VTE prophylaxis studies versus 29.9% in patients in non-VTE prophylaxis studies.[24]

The majority of studies in this review considered ICH to be a binary phenomenon (present or absent). However, Norwood et al. described a set of intracranial injury patterns deemed safe to receive enoxaparin 30 mg subcutaneously beginning 24 h after injury if a repeat CT scan of the

head was stable[17]. Although not extensively studied, we could suggest to take specific TBI patterns into consideration when weighing the risk-benefit for VTE prophylaxis administration.

Several other meta-analyses have reported specifically on timing of administration of VTE prophylaxis among low-risk TBI patients.[24-27] From these reports it appears that early administration of chemical VTE prophylaxis (<72 hrs) may be safe in the low-risk patient population, where there is no hemorrhage progression at 24 hours after admission or 48 hours after stable head CT. In moderate to high-risk TBI patients, available data is limited. This makes it impossible to draw definite conclusions. In the meantime, while VTE risk can be further reduced with administration of chemical prophylaxis, this has to be weighed against the potential risk for progression of hemorrhage, which is greatest in the first 24-48 hours. The use and timing of VTE prophylaxis in patients with TBI must therefore be individualized according to the degree of intracranial bleeding and the perceived risk of VTE development.

Interpretation of results of this review were hampered by variability in study designs and high heterogeneity in patient populations. There were a limited number of RCT's available, most studies were observational and retrospective in design and we included four single arm studies. Furthermore, some of the included studies have a considerable risk for bias.

There are still gaps of knowledge in the pathophysiology of thromboembolic disease, as well as in the efficacy and safety of chemical VTE prophylaxis. In all, this underlines the need for more investigation of chemical VTE prophylaxis in TBI patients in order to further develop evidence-based guidelines for clinical practice.

CONCLUSION

Presently, the safety and efficacy of chemical prophylaxis as prevention for VTE in patients suffering from traumatic brain injury remains unclear and current evidence is weak. The small number of available studies may suggest that it is safe to administer chemical VTE prophylaxis within 24 hours after injury in TBI patients with stable injury patterns. Several studies showed a reduction in VTE rates, suggesting efficacy of chemoprophylaxis. Pooling data was hampered by variability in study designs and heterogeneity in patient populations. There is an urgent need for prospective clinical trials studying the safety, efficacy and timing of the administration of chemical VTE prophylaxis in patients with traumatic brain injury.

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Chapter 8

DISCUSSION AND FUTURE PERSPECTIVES

DISCUSSION AND FUTURE PERSPECTIVES

Throughout the last years, an increasing number of studies have investigated coagulopathy after trauma. However, investigations including children are scarce, fewer resources are directed and limited attention is given to researching the care of the pediatric trauma patient compared to the adult trauma patient. This is incredibly concerning, as the morbidity, mortality and potential years of life (PYLL) lost amongst the pediatric trauma population is high, specifically in patients suffering from traumatic brain injury as shown in this thesis. Besides early treatment and prevention of hypoxemia and hypotension to counter secondary brain injury in children, early management of coagulopathy potentially needs to be included in this effort.

It is well known that children respond differently to severe injury when compared to adults, they have large cardiorespiratory reserves, different thermoregulation and airway anatomy. Children also respond differently to blood loss, this begs the question of what happens with their coagulation and how we should approach therapeutically. In adults, damage control strategies have been developed to achieve an early aggressive correction of trauma induced coagulopathy, this strategy has been accompanied by improved outcomes. As discussed in this thesis, in pediatric patients with rapidly identified coagulopathy which is amenable to correction, a goal-directed approach to resuscitation may be more appropriate than an empiric blood product approach. This hypothesis has recently been substantiated by a military study including 364 combat injured children. In children undergoing a massive transfusion, a high ratio of PLAS/PRBC was not associated with improved survival,[9] however no data is currently available in the civilian pediatric population. The most important procoagulant concentrates include fibrinogen concentrate, prothrombin complex concentrate (PCC), recombinant factor VIIa (rFVIIA) and antifibrinolytics such as the tranexamic acid. The question is what the effects of these adjuvant interventions are in the pediatric trauma population, this will have to be investigated in prospective clinical trials. This thesis also reported on the thrombin hemostasis system, as this system might differ in children. It has been observed that the capacity to generate thrombin *in vitro* by a chromogenic assay is decreased by 26% in plasma from children aged 1 to 16 years compared to adults; this would justify the lower prevalence of thromboembolic complications in this period. The use of above mentioned

hemostatic agents in a goal-directed fashion guided by TEG/RoTEM monitoring to assess effectiveness and avoid potential thromboembolic complications make for a compelling therapeutic strategy and will need to be investigated. This thesis has has re-validated cut-off points for early identification of coagulopathy and the need for massive transfusion. It has shown that visco-elastic methods such as rotational thromboelastometry (ROTEM) are very attractive for monitoring coagulation, the small sample volume required and the expeditious availability of results makes these methods ideal for the use in small children. Although available to pediatric trauma providers, VHA is very infrequently used in the management of the pediatric trauma patient.[10] Moreover, there are no validated cut-off points available for coagulopathy in children using VHA, these will need to be established.

Next, as it has become more evident that activation of the protein C pathway resulting in delayed clot formation and increased fibrinolysis due to hypoperfusion and tissue injury is likely just one of multiple complex pathways leading to coagulopathy after trauma. Future studies should focus on unraveling the complexity of the pathophysiological pathways. We have now learned about the involvement of not only the hemostatic system but the inflammatory, endocrine- and neurohumoral systems as part of the systemic response after trauma. These findings have led to the identification of several new markers, mediators and potential novel therapeutic targets in adults. This thesis identified several potential mechanisms for the development of coagulation abnormalities after pediatric trauma. The release and clearance of histones are a promising area of research in trauma biology and should be further pursued. aPC has been shown to proteolytically cleave extracellular histones, mitigating the lethality of histone-induced systemic inflammatory response in animal models.[11] It has been previously reported that extracellular hcDNA causes a systemic activation of the coagulation cascade and may induce a severe inflammatory response via the activation of TLRs 2, 4 and 9. Extra cellular histones could be targeted pharmacologically by a histone antibody, as studies in mice have shown that antibody to histone reduces the mortality in lipopolysaccharide (LPS), tumor necrosis factor (TNF) or cecal ligation and puncture models of sepsis[11].

Extra cellular histones and DNA are also important for pro-thrombotic functions because they stimulate the aggregation of platelets and coagulation and potentially contribute to thrombosis[12]. This thesis showed that pediatric patients had poor aggregation of circulating platelets after injury despite normal platelet counts and this was correlated with high levels of

hcDNA when compared to healthy children. These results are similar to results from studies amongst adults[13,14] and highlight the importance of the role of intact platelet aggregation, however the cellular mechanisms underlying trauma induced platelet dysfunction remain poorly understood. A recent study in an *in vivo* model of infection demonstrated a dynamic NET-platelet-thrombin axis that promotes intravascular coagulation and microvascular dysfunction in sepsis. NET-induced intravascular coagulation was dependent on a collaborative interaction between histone H4 in NETs, platelets, and the release of inorganic polyphosphate. Inhibition of NET's reduced intravascular coagulation, improved microvascular perfusion and reduced organ damage in mice.[15] It needs to be examined if such axis exists in response to traumatic injury and hemorrhage, and if so, how to therapeutically approach.

We have also reported on potential involvement of the vascular barrier or endothelial glycocalyx after trauma in children resulting in so called 'endotheliopathy', a term recently coined in studies amongst adult trauma patients. In a recently published narrative review, an accumulating body of evidence was presented describing immediate post-traumatic plasma concentrations of glycocalyx constituents as a reasonable measure of injury severity and clinical outcome and potential contributors to trauma-induced coagulopathy.[16] Fresh Frozen Plasma (FFP) resuscitation has been proposed as a way of endothelial rescue. Recently, it was shown that the administration of FFP restored the vascular barrier function in a mouse model hemorrhagic shock.[17] Furthermore, another recent study in a rat model of sepsis showed an increased 48-hour survival after plasma resuscitation when compared to crystalloid administration, as well as improved pulmonary function, decreased pulmonary edema and attenuated markers for inflammation, endothelial injury and catecholamines.[18] Increasing knowledge on the interactions of the inflammatory processes and coagulation cascade following severe trauma offers an opportunities and capabilities for new treatment approaches and drug development alongside advancing resuscitation practices. More research needs to be directed to the above-mentioned biological mechanisms in both adults AND children to identify the most promising targets that can lead to effective treatment for severely injured trauma patients.

Finally, the present studies provided a potential interesting reference for other pediatric trauma centers to evaluate their trauma care. A general recommendation is to develop collaborations

between centers treating pediatric trauma patients nationally and internationally to implement databases and registries to collect large amounts of data, which could be used for research and improve pediatric trauma care.

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Chapter 9

SUMMARY/SAMENVATTING

SUMMARY

Over the past decades, our understanding of the development, diagnosis and treatment of coagulopathy after trauma has dramatically increased. Perturbations in coagulation after trauma have been commonly observed in adults and substantial research on coagulopathy following severe injury has been conducted over the past years, yet limited research has focused on the pediatric trauma population. This is concerning as trauma is the leading cause of death amongst children between 1-18 years.[1] Exsanguination is the most important contributing factor of acute-phase mortality in trauma patients and the development of coagulation abnormalities exacerbates the bleeding. Acute traumatic coagulopathy (ATC) has been described as an early endogenous process, driven by a combination of tissue injury and shock that is associated with increased mortality and worse outcome in the severely injured trauma patient. However, we currently have a very limited understanding of the role of coagulopathy after severe pediatric trauma and how this affects their outcome.

In adults, endothelial activation of protein C is a central mechanism of ATC, which produces rapid anticoagulation and fibrinolysis following severe trauma.[2] Trauma-induced coagulopathy (TIC) includes not only ATC, but also exogenous mechanisms leading to hypocoagulation, such as dilution, acidosis and hypothermia, primarily due to the effects of resuscitation. TIC is a global failure of the coagulation system to sustain adequate hemostasis after major trauma. In children however, the exact mechanisms behind coagulopathy remain unknown. Therefore, more basic science studying the development of coagulopathy after pediatric trauma is required to improve outcome.

In order to investigate coagulation abnormalities after trauma accurate interpretation of coagulation tests are a prerequisite. For years, physicians have relied on conventional coagulation tests (CCT) to asses the clotting status of trauma patients. These tests, such as PT, aPTT, INR, and fibrinogen have been proven time-consuming and insufficient as they monitor only the initiation of blood phase coagulation[3-6]. Increasing emphasis focuses on the importance of coagulation monitoring devices assessing the viscoelastic properties of whole blood and platelet function testing. In adults, these viscoelastic hemostatic assays (VHA) show promising results in their ability to identify coagulation abnormalities after trauma and guide treatment.

While acute traumatic coagulopathy requires prompt treatment, the risk for coagulation abnormalities remains in the days after the injury, primarily the risk of developing hypercoagulability. The rate of preventable deaths due to hemorrhage has significantly decreased in the past years, which has led to more patients dying in the ICU because of inflammatory syndromes and hypercoagulability. In the ICU, physicians are often challenged with the decision to administer thromboprophylaxis as prevention of venous thromboembolism, as it comes with the risk of increased hemorrhage. This is of specific concern in patients suffering from traumatic brain injury (TBI), because of the potential progression of intracranial hemorrhage. Issues related to safety and efficacy of thromboprophylaxis after TBI are still being debated.
This thesis in the field of trauma biology originated from the gap of knowledge in the development, diagnosis and treatment of coagulopathy after trauma, primarily in the pediatric population. More knowledge about coagulopathy and bleeding in adult AND pediatric trauma patients is required to improve outcome.

SUMMARY OF RESULTS

The narrative review in **Chapter 1** described the present understanding of coagulopathy after pediatric trauma. We performed a literature review on current knowledge and research conducted to date on the development, incidence, diagnosis, mechanisms and treatment options of coagulopathy early after pediatric trauma. We concluded that coagulopathy after pediatric trauma is more common than previously thought and associated with increased morbidity and mortality. Diagnosing coagulopathy in children poses a challenge because of the availability of smaller amounts of blood. Also, reference intervals for different viscoelastic assays may differ broadly from those in adults. The mechanisms behind the development of acute traumatic coagulopathy in the pediatric population still need to be elucidated. Different treatment options for coagulopathy in children are available. However, there still is a lack of evidence for these options from prospective clinical trials. We therefore urge caution about applying adult traumatic coagulopathy management principles to the pediatric population.
Chapter 2 aimed to further determine the incidence and the effects of coagulopathy in the pediatric trauma population. A retrospective study was performed including 803 severely

injured children under the age of 18 over a period of 10 years. Coagulopathy was defined as an INR > 1.2. In this population it was observed that 38% of patients were coagulopathic on admission to the level 1 pediatric trauma center. High injury severity score (ISS) and/or hypotension were associated with this early coagulopathy. As in adults, the presence of early coagulopathy after pediatric trauma was an independent predictor of mortality. Although there was a modest, but significant increase in mortality in pediatric patients without brain injury, a four-fold increase in mortality was seen in patients with traumatic brain injury, either isolated or combined with injuries to other organs.

Elaborating on the findings of Chapters 1 and 2, in which the role of coagulopathy in pediatric trauma has been identified, the protein C pathway, an important mechanism that modulates severe acute inflammation via its anticoagulant and anti-inflammatory properties was assessed in **Chapter 3.** The parallels between mechanisms of pathogen-induced sepsis and those underlying the sterile inflammatory response to trauma are becoming more evident, suggesting that insights and therapies from the critical care and sepsis literature may be applicable earlier in the hospital course of the acutely injured trauma patient. In this chapter we provided a clinical perspective of the cytoprotective and anticoagulant properties of protein C to determine whether targeting the coagulation system would prove beneficial to patients with severe sepsis and trauma. The protein C system appears to play a major role in modulating severe acute inflammation via these properties. The mechanisms of action of aPC, however, in modulating the acute inflammatory response are only partially understood. A recombinant form of aPC, drotrecogin alfa, was introduced to the market in 2001/2002 for the treatment of sepsis. However, drotrecogin alfa failed to show survival benefit in patients with septic shock in subsequent clinical trials. After publication of the negative results from a large clinical trial, the drug was withdrawn from the market, and no form of recombinant aPC is currently available for human use. With the withdrawal of drotrecogin alfa, it has become very unlikely that a new anti recombinant aPC drug will enter the market soon and focus has shifted to other potential targets. More basic science work will be required to have a full understanding of how the protein C system modulates the acute inflammation associated with sepsis or trauma. Recent studies have shown that the release of activated protein C after severe trauma could mitigate sterile inflammation and organ injury induced by the release damage-associated molecular patterns (DAMPs) like nucleic acids including histone-complexed DNA (hc-DNA).

Extracellular histones can also be derived from dying cells or secreted by activated inflammatory cells in the form of neutrophil extracellular traps (NETs). Histones have been shown to promote blood coagulation and the formation of thrombin by activating platelets. aPC may protect against excessive microvascular thrombosis by cleaving the pro-coagulant extracellular histones associated with endothelial dysfunction, organ failure, and death. This indicates that coagulopathy is an endogenous response to injury involving both the coagulation system and the immune system. Until now no studies have reported on the role of histones proteins after pediatric trauma.

Therefore, the aim of **Chapter 4** was to investigate the release of histone complexed DNA (hcDNA) on the development of coagulopathy in pediatric trauma patients. As hypothesized, significantly higher levels of hcDNA were found on admission in children with severe injury, coagulopathy, and/or abnormal platelet aggregation. Additionally, children with high hcDNA levels also had significant elevations in plasma levels of syndecan-1, suggesting damage to the endothelial glycocalyx. Significantly higher hcDNA levels were found in non-survivors. It has previously been reported that extracellular hcDNA causes a systemic activation of the coagulation cascade and may induce a severe inflammatory response via the activation of toll like receptors (TLRs) 2, 4 and 9.[7] The present clinical results suggest that the release of hcDNA into the blood stream might play an important mechanistic role in the development of coagulation abnormalities and endothelial glycocalyx damage in children with severe trauma. Recent animal studies have shown that histone-neutralizing antibody administration protects mice from histone-mediated lethality[8]. This raises the question as to whether extracellular histones could serve as a novel therapeutic target in children after sustaining severe injury.

In order to provide optimal treatment, early detection of coagulopathy after trauma is important in order to counteract the haemostatic disturbances. Full blood viscoelastic haemostatic assays (VHA), such as ROTEM®, provide a more complete assessment of haemostasis compared to standard coagulation tests, such as INR, PT, aPTT and fibrinogen. Furthermore, as point of care devices, they are able to deliver results in a more clinically useful timeframe for targeted therapy. However, precise values for identification of coagulopathy after trauma using ROTEM® are largely unknown. The international multi-center prospective cohort study discussed in **Chapter 5** aimed to identify threshold values for most accurate identification of acute traumatic coagulopathy and the need for massive transfusion, using EXTEM assay, as

well as platelet- inhibited FIBTEM assay. We confirmed previous findings of a smaller cohort study. A ROTEM® EXTEM CA5 threshold value of ≤ 40 mm had a coagulopathy detection rate of 72.7%, whereas a FIBTEM CA5 threshold value of ≤ 9 mm detected massive transfusion requirements in 77.5% of cases.

The final chapter, **Chapter 6,** systematically reviewed papers that have examined the use of chemoprophylaxis in traumatic brain injury patients as prevention for thromboembolic events. Findings of this review suggest that additional research is required as studies in this field are limited and show contradicting results. The majority of published studies are single center, retrospective or single arm in design. Some studies favor prophylaxis by showing a reduction in VTE's, other studies have documented an increased risk in progression of intracranial hemorrhage after administration of thromboprophylaxis. Furthermore, there is a large variation in thromboprophylaxis protocols across the globe. Currently the results of only one randomized controlled trial are published, the DEEP-1 study, this study only included low-risk TBI patients. Thereby, more research is needed to estimate the risk-benefit of thrombosis prophylaxis in TBI as the efficacy of chemoprophylactic VTE prevention in TBI patients remains unknown.

SAMENVATTING

Kennis op het gebied van de ontwikkeling, diagnostiek en behandeling van stollingsafwijkingen na ernstig trauma is in de laatste decennia enorm toegenomen. Er heeft een betrekkelijke hoeveelheid onderzoek plaatsgevonden op dit gebied, we weten dat bij volwassenen stollingsafwijkingen frequent voorkomen na een ernstig ongeval. Echter, beperkt onderzoek heeft plaats gevonden bij kinderen na een ernstig trauma. Dit is zorgwekkend, aangezien trauma doodsoorzaak nummer 1 is bij kinderen in de leeftijd van 1-18 jaar.[1] Verbloeding is in de acute fase de belangrijkste factor bijdragend aan de sterfte bij traumapatiënten en het optreden van stollingsafwijkingen verergert de bloeding. Acute traumatische coagulopathie (ATC) is beschreven als een vroeg endogeen proces, gedreven door een combinatie van weefsel letsel en shock en is geassocieerd met een verhoogde mortaliteit en slechtere uitkomstmaat in de ernstige gewonde trauma patiënt. Desalnietemin hebben we zeer gelimiteerde kennis wat betreft de rol van coagulopathie na ernstig trauma bij kinderen en hoe deze stollingsafwijkingen de uitkomstmaat beïnvloed.
Bij volwassen patiënten is de endotheliale activatie van proteïne C het centrale mechanisme van ATC, deze activatie produceert een snelle antistolling en fibrinolyse na ernstig traumatisch letsel.[2] Trauma geïnduceerde coagulopathie (TIC) is niet alleen ATC, maar betreft ook exogene mechanismen die leiden tot hypocoagulatie, zoals verdunning, acidose en hypothermie, hoofdzakelijk door effecten van resuscitatie. TIC is het algeheel falen van het stollingssysteem om een adequate hemostase te behouden na een ernstig trauma. De exacte mechanismen van het optreden van stollingsafwijkingen bij kinderen zijn onbekend. Om de overleving van een ernstig trauma bij kinderen te verbeteren is er dus meer onderzoek nodig naar de ontwikkeling van stollingsafwijkingen in deze populatie.

Om stollingsafwijkingen na ernstig trauma te onderzoeken is een juiste interpretatie van stollingstesten een vereiste. Sinds jaar en dag hebben artsen vertrouwd op conventionele coagulatietesten (CCT). Deze testen, waaronder PT, aPTT, INR, en fibrinogeen zijn bewezen tijdrovend in het leven van een trauma patiënt en daarnaast insufficiënt, aangezien zij slechts de initiatie van de stollingsfase monitoren.[3-6] In toenemende mate ligt de focus op het belang van het gebruik van stollingsapparatuur waarmee de viscoelastische capaciteit van volbloed en thrombocyten kan worden getest. Bij volwassenen lijken deze viscoelastische homeostatische

assays (VHA) veelbelovend in de mogelijkheid stollingsafwijkingen na trauma te identificeren en de effecten van transfusiebeleid te monitoren. Echter, afkapwaarden voor exacte identificatie van coagulopathie na ernstig trauma door middel van het gebruik van VHA blijven discutabel. Verder is het nog onduidelijk of VHA gebruikt kunnen worden bij het voorspellen van de toediening van massa transfusie.
Hoewel acute traumatische coagulopathie een snelle behandeling verreist, blijft het risico voor stollingsafwijkingen in de dagen na het trauma bestaan, hoofdzakelijk in verband met het risico op het ontwikkelen van hypercoagulabiliteit. Op de Intensive Care is het vaak lastig voor artsen te beslissen om thromboprofylaxe toe te dienen ter preventie van veneuze thromboembolieën, aangezien dit gepaard gaat met een verhoogde kans op verergering van de bloeding. Dit is in het bijzonder een zorg bij traumapatiënten met traumatisch hersenletsel, in verband met het risico op progressie van de intracraniële bloeding. Over de problematiek met betrekking tot de veiligheid en werkzaamheid van thromboprofylaxe na traumatisch hersenletsel wordt dan ook nog gedebateerd.

Dit proefschrift op het gebied van trauma biologie is ontstaan vanuit een kenniskloof in de ontwikkeling, diagnostiek en behandeling van stollingsafwijkingen na trauma, hoofdzakelijk bij de kinderpopulatie. Meer kennis op het gebied van stollingsafwijkingen en bloeding bij volwassenen EN kinderen is noodzakelijk om uitkomsten te verbeteren.

SAMENVATTING VAN DE RESULTATEN

Het overzichtsartikel in **Hoofdstuk 1** beschreef de hedendaagse kennis van stollingsafwijkingen na ernstig trauma bij kinderen. Dit hoofdstuk is het resultaat van een literatuuronderzoek naar de huidige stand van zaken wat betreft de ontwikkeling, incidentie, diagnostiek, mechanismen en behandelingsopties van stollingsafwijkingen na ernstig trauma letsel bij kinderen. Er werd vastgesteld dat stollingsafwijkingen bij kinderen na een ernstig ongeval frequenter voorkomen dan voorheen werd gedacht en geassocieerd zijn met een verhoogde morbiditeit en mortaliteit. De diagnostiek naar stollingsafwijkingen bij kinderen vormt een uitdaging, hoofdzakelijk in verband met de beschikbaarheid van kleinere hoeveelheden bloed. Daarnaast verschillen de referentiewaarden van de diverse viscoelastische

testen in grote mate met de waarden in volwassen patiënten. De mechanismen ten grondslag liggend aan de ontwikkeling van ACT in de kinderpopulatie dienen nog onderzocht te worden. Er zijn verschillende behandelingsmogelijkheden beschikbaar voor kinderen, echter er is geen ondersteunend bewijs vanuit klinische trials voor deze behandelmethoden. Terughoudendheid wat betreft het blind toepassen van behandelingen bekend vanuit de volwassen trauma praktijk bij de kindertrauma populatie is dan ook geboden.

Hoofdstuk 2 had als doel de incidentie en de effecten van stollingsafwijkingen in de kindertrauma populatie verder vast te stellen. Dit hoofdstuk beschreef een retrospectieve studie bij 803 ernstige gewonde kinderen jonger dan 18 jaar over een periode van 10 jaar. Coagulopathie werd gedefinieerd als een INR > 1.2. In deze populatie werd vastgesteld dat 38% van de patiënten coagulopathisch was bij aankomst in een level 1 kindertraumacentrum. Een hoge injury severity score (ISS) en/of hypotensie waren geassocieerd met het optreden van deze vroege coagulopathie. De aanwezigheid van vroege coagulopathie na trauma bij kinderen was een onafhankelijke voorspeller van mortaliteit. Hoewel er een bescheiden maar significante stijging te zien was in mortaliteit bij patiënten zonder traumatisch hersenen letsel, was er een viervoudige stijging in sterfte bij patiënten met traumatisch hersenletsel, geïsoleerd danwel gecombineerd met ander orgaan letsel.

Voortbouwend op de bevindingen uit Hoofdstuk 1 en 2, waarin de rol van coagulopathie na kinder trauma werd geïndentificeerd, werd in **Hoofdstuk 3** het proteïne C systeem beschreven, een belangrijk mechanisme dat ernstige acute inflammatie moduleert via antistollings- en anti-inflammatoire eigenschappen. De paralellen tussen de mechanismen van pathogeen geïnduceerde sepsis en de steriele inflammatoire respons na traumatische letsel raken meer evident, hetgeen suggereert dat inzichten en therapieën vanuit intensive care en sepsis literatuur mogelijk toepasbaar zijn vroeg in de ziekenhuisopname van de ernstig gewonde traumapatiënt. In dit hoofdstuk werd een klinische perspectief geboden gericht op de cytoprotectieve- en antistollings eigenschappen van proteïne C gegeven om vast te stellen of een therapeutische benadering van het stollingssysteem potentieel zinvol kan zijn bij patiënten met ernstige sepsis en trauma. Het proteïne C systeem lijkt een belangrijke rol te spelen in het moduleren van acute inflammatie via deze eigenschappen, maar hoe geactiveerd proteïne C de acute

inflammatoire response exact moduleert is slechts deels bekend. In 2001/2002 werd een recombinante vorm van aPC, drotrecogin alfa, op de markt gebracht voor de behandeling van sepsis. Helaas lieten opeenvolgende klinische trails geen verbetering in overleving zien bij patiënten met septische shock. Na publicatie van de negatieve resultaten van een grote klinische trial werd het medicijn van de markt gehaald, momenteel is er geen recombinante vorm van aPC beschikbaar voor menselijke gebruik. Na het falen van drotrecogin alfa is het zeer onwaarschijnlijk geworden dat er een nieuwe vorm van recombinant aPC op de markt zal komen en de aandacht is verplaatst naar andere potentiële targets. Meer basaal wetenschappelijk onderzoek is noodzakelijk om een volledig begrip te krijgen van de manier waarop het proteïne C systeem acute inflammatie geassocieerd met sepsis en trauma moduleert. Recente studies hebben aangetoond dat activatie van proteïne C na trauma een steriele inflammatie en orgaan letsel geïnduceerd veroorzaakt door het vrijkomen van damage-associated molecular patterns (DAMPs), zoals het nucleïnezuur histoneiwit-complex DNA (hc-DNA). Extracellulaire histon eiwitten kunnen vrijkomen vanuit apoptotische cellen of uitgescheiden worden door inflammatoire cellen in de vorm van neutrophil extracellular traps (NETs). Het is aangetoond dat histon eiwitten bijdragen aan de bloedstolling en de aan vorming van trombine door de activatie van bloedplaatjes. Dit geeft aan dat coagulopathie een endogene reactie is op traumatisch letsel waarbij zowel het stollingsysteem als het imuumsysteem betrokken zijn. Tot nu toe zijn er geen studies verricht naar de rol van histon eiwitten na trauma bij kinderen.

Daarom was het doel van **Hoofdstuk 4** om het verband tussen het vrijkomen van histon eiwit complexed DNA (hcDNA) en de ontwikkeling van coagulopathie in kinderen na trauma te onderzoeken. Zoals gehypothetiseerd, werden bij opname significant hogere hcDNA waarden vastgesteld bij kinderen met zeer ernstig letsel, coagulopathie, en/of abnormale trombocyten aggregatie. Bovendien hadden kinderen met hoge hcDNA waarden significant verhoogde syndecan-1 plasma waarden, hetgeen suggereert dat er schade ontstaat aan de endotheliale glycocalyx. Significante hogere hcDNA waarden werden ook gemeten in niet-overlevers. Het is reeds aangetoond dat extracellulair hcDNA een systemische activatie van de stollingscascade veroorzaakt en mogelijk een ernstige inflammatoire respons teweegbrengt door de activatie van toll like receptors (TLRs) 2, 4 en 9.[7] De gepresenteerde klinische data suggereren dat het vrijkomen van hcDNA in het bloed mogelijk een belangrijke

mechanistische rol speelt in de ontwikkeling van stollingsafwijkingen en schade veroorzaakt aan de endotheliale glycocalyx bij kinderen na ernstig trauma. Recent onderzoek bij dieren heeft aangetoond dat histon-neutralizerende antistof toediening muizen beschermt tegen histon-gemedieerde lethaliteit. Dit roept de vraag op of extracellulaire histonen mogelijk zouden kunnen dienen als een nieuw therapeutisch target bij kinderen na een ernstig traumatisch letsel.

Voor optimale behandeling is vroegtijdige detectie van stollingsafwijkingen na trauma van belang om de hemostatische disbalans te verhelpen. Viscoelastische haemostatische assays (VHA) die volbloed gebruiken, zoals ROTEM®, bieden een completer overzicht van de hemostase in vergelijk met standaard stollingstesten, waaronder INR, PT, aPTT en fibrinogeen. Verder, als point of care apparatuur zijn zij in staat resultaten te produceren in een klinisch relevant tijdsbestek voor doelgerichte therapie. Echter, exacte afkapwaarden voor het diagnosticeren van coagulopathie na trauma door middel van ROTEM® zijn grotendeels onbekend. De internationale multicenter prospectieve cohortstudie besproken in **Hoofdstuk 5** had als doel afkapwaarden te onderzoeken voor de meest accurate identificatie van acute traumatische coagulopathie en de noodzaak voor massale transfusie door gebruik te maken van EXTEM-assay, zowel als platelet-inhibited FIBTEM-assay. De bevindingen van een voortgaand kleinere cohortstudie werden in dit hoofdstuk bevestigd. Een ROTEM® EXTEM CA5 afkapwaarde van ≤ 40 mm had een coagulopathie detectie percentage van 72.7%, en een FIBTEM CA5 afkapwaarde van ≤ 9 mm bevestigden de noodzaak voor massa transfusie in 77.5% van de gevallen.

In het laatste hoofdstuk, **Hoofdstuk 6**, werd door middel van een systematisch review het gebruik van laagmoleculair gewicht heparine onderzocht ter preventie van thromboembolische complicaties in patiënten met traumatisch hersenen letsel. De resultaten van de studie suggereren dat verder onderzoek op dit gebied noodzakelijk is, aangezien het aantal onderzoeken beperkt is en studies tegenstrijdige resultaten laten zien. De doeltreffendheid van chemoprofylactische VTE preventie bij traumatisch hersenletsel patiënten blijft hiermee onbekend.

Chapter 10

APPENDICES

List of Abbreviations

ACT	acute traumatic coagulopathy
aPC	activated protein C
aPTT	activated partial thromboplastin time
CCT	conventional coagulation test
FFP	fresh frozen plasma
ICH	intracranial hemorrhage
ISS	injury severity score
LMWH	low-molecular-weight heparin
MT	massive transfusion
MTP	massive transfusion protocol
NET	neutrophil extracellular trap
PAI-1	plasminogen activator inhibitor-1
PT	prothrombin time
RBC	red blood cell
TBI	traumatic brain injury
TIC	trauma induced coagulopathy
tPA	tissue plasma activator
VHA	viscoelastic haemostatic assay
UFH	unfractionated heparin

PhD Portfolio

Summary of PhD training, teaching and parameters of esteem

Name PhD student: Sarah Christiaans
Name PhD supervisor: Prof. Dr. N.P Juffermans

PhD TRAINING

	Year	ECTS
General Courses		
Using Animals for Teaching, Testing and Research	2012	0.3
Working with Mice in Research	2012	0.3
Working with Rats in Research	2012	0.3
Radiation Initial Safety Training	2012	0.3
Rodent Surgery	2012	0.3
Basic Laboratory Safety Training	2012	0.3
Blood Borne Pathogens Initial Training	2012	0.3
RB Initial Investigator Training 101	2012	0.3
Medical Ethics in research	2012	0.3
Financial Conflict of Interest in Research	2013	0.3
Translational Medicine Course	2015	0.3
Specific Courses		
UAB Clinical and Translation Science Training program	2014	2
Seminars, Workshops and Master Classes		
Weekly Departmental seminars	2012-2015	4
Masterclass by Dr Tung-Tien, NYU: 'Survival skills for junior investigator'	2014	0.2
Job Skills	2014	1.5
Academic Writing	2014	1.5

PRESENTATIONS

	Year	ECTS
Poster presentation: 'Abnormalities in Admission Platelet Count and Function Correlate with Survival following Severe Trauma in Children' 2014, American College of Surgeons, annual meeting	2014	0.5
Poster presentation: 'The role of activated protein C in the development of coagulopathy after pediatric trauma', 2014 American College of Surgeons Alabama and Mississippi Chapter, annual meeting	2014	0.5

Poster presentation: 'The role of activated protein C in the development of coagulopathy after pediatric trauma' 2014, American Association for the Surgery of Trauma, annual meeting	2014	0.5
Oral Presentation: 'Histone-Complexed DNA levels are associated with coagulopathy, endothelial cell damage and complement activity early after pediatric trauma'. 2015 American College of Surgeons Alabama and Mississippi Chapter, annual meeting	2015	0.5
Oral Presentation: 'Histone-Complexed DNA levels are associated with coagulopathy, endothelial cell damage and complement activity early after pediatric trauma', 2015 American Association for the Surgery of Trauma, annual meeting Las Vegas	2015	0.5
Poster Presentation: 'Histone-Complexed DNA levels are associated with coagulopathy, endothelial cell damage and compliment activation early after pediatric trauma'. 2015 Association Woman Surgeons, annual meeting Chicago	2015	0.5
International Conferences		
ROC Toronto 2012, Marriot, Toronto, CAN	2012	0.5
AAST 2013 Hilton Union, San Francisco, USA	2013	1
Bleeding Management 2014, Fairmont Toronto, CAN	2014	1
AAST 2014 Marriot Downtown, Philadelphia, USA	2014	1
AAST 2015, Wynn, Las Vegas, USA	2015	1
Other		
Journal Club	2012-2015	3

TEACHING

	Year	ECTS
Supervising students		
Graduate student:		
Rashidra Walker, Forensic Science Master's program, University of Alabama at Birmingham, class of 2014	2013-2014	2
Medical students:		
Joseph Kundukulam, University of Alabama at Birmingham, class of 2013	2013	1
Adam Threet, University of Alabama at Birmingham, class of 2015	2014	1
Clay Parker, University of Alabama at Birmingham, class of 2015	2014	1
Kayla Isbell, University of Alabama at Birmingham, class of 2016	2013	1
John M. Evans, University of Alabama at Birmingham, class of 2016	2013	1
Nursing student:		
Morgan Banks, School of Nursing, University of Alabama at Birmingham, class of 2016	2013-2014	2
Other		
Guest Lecture, 'The role of activated protein C in coagulopathy after pediatric trauma', Surgical Grand Rounds University of Nebraska Medical Center, USA	2014	0.1

PARAMETERS OF ESTEEM	
	Year
Awards and Prizes	
2013 AAST Surgery Resident Scholarship	2013

List of Publications

Publications for this thesis

2019 Christiaans SC, Otuada D, Juffermans NP, Binnekade J, Goslings JC. The use of chemoprofylaxis for thromboembolic events in patients after sustaining traumatic brain injury. Systematic review and meta-analysis on risk-benefit. Manuscript in preparation

2017 Christiaans SC, Russell RT, Nice TR, et al. Histone-Complexed DNA Fragments Levels are Associated with Coagulopathy, Endothelial Cell Damage, and Increased Mortality after Severe Pediatric Trauma. *Shock*. 2018;49(1):44-52.

2015 Hagemo JS, Christiaans SC, Stanworth SJ, et al. Detection of acute traumatic coagulopathy and massive transfusion requirements by means of rotational thromboelastometry: an international prospective validation study. *Crit Care*. 2015;19(1):97. Published 2015 Mar 23.

2014 Christiaans SC, Duhachek-Stapelman AL, Russell RT, Lisco SJ, Kerby JD, Pittet JF. Coagulopathy after severe pediatric trauma. *Shock*. 2014;41(6):476-490.

2013 Christiaans SC, Wagener BM, Esmon CT, Pittet JF. Protein C and acute inflammation: a clinical and biological perspective. *Am J Physiol Lung Cell Mol Physiol*. 2013;305(7):L455-L466. doi:10.1152/ajplung.00093.2013

2013 Christiaans SC, Whittaker B, Altice JL, et al. Early coagulopathy is an independent predictor of mortality in children after severe trauma. *Shock*. 2013;39(5):421-426.

Other publications

2020 Wagener BM, Anjum N, Christiaans SC, et al. Exoenzyme Y Contributes to End-Organ Dysfunction Caused by *Pseudomonas aeruginosa* Pneumonia in Critically Ill Patients: An Exploratory Study. *Toxins (Basel)*. 2020;12(6):E369. Published 2020 Jun 4.

2016 Chignalia AZ, Yetimakman F, Christiaans SC, et al. THE GLYCOCALYX AND TRAUMA: A REVIEW. *Shock*. 2016;45(4):338-348.

2014 de Castro SM, Christiaans SC, van den Berg R, Schep NW. Minimal invasive management of traumatic transection of the vertebral artery. *Springerplus*. 2014;3:206. Published 2014 Apr 28.

2013 Pittet JF, Koh H, Fang X, et al. HMGB1 accelerates alveolar epithelial repair via an IL-1β- and αvβ6 integrin-dependent activation of TGF-β1 [published correction appears in PLoS One. 2013;8(10). doi:10.1371/annotation/88f820f2-18dd-4d3b-8989-68f170b26b04]. *PLoS One*. 2013;8(5):e63907. Published 2013 May 16.

2013 Howard M, Roux J, Iles KE, et al. Activation of the heat shock response attenuates the interleukin 1β-mediated inhibition of the amiloride-sensitive alveolar epithelial ion transport. *Shock*. 2013;39(2):189-196.

2012 Christiaans SC, Goslings JC. Limit transfusions in patients without anemia post hip fracture surgery. Ned Tijdschr Geneeskd. 2012; 156 no 11: 490. Editorial in Dutch

2011 Christiaans SC, Goslings JC. Tranexaminic acid for every bleeding trauma patient? Ned Tijdschr Geneeskd. 2011; 155 no 29: 1317. Editorial in Dutch

2011 Saltzherr TP, Christiaans SC, Henny CP, Levi MM, Goslings JC. Transfusiebeleid bij trauma met massaal bloedverlies [Transfusion policy in trauma involving massive blood loss]. *Ned Tijdschr Geneeskd*. 2011;155:A2306.

2009 Cohen MJ, Brohi K, Calfee CS, et al. Early release of high mobility group box nuclear protein 1 after severe trauma in humans: role of injury severity and tissue hypoperfusion. *Crit Care*. 2009;13(6):R174.

2009 Ganter MT, Roux J, Su G, et al. Role of small GTPases and alphavbeta5 integrin In Pseudomonas aeruginosa-induced increase in lung endothelial permeability. *Am J Respir Cell Mol Biol*. 2009;40(1):108-118.

2008 Ganter MT, Cohen MJ, Brohi K, et al. Angiopoietin-2, marker and mediator of endothelial activation with prognostic significance early after trauma? *Ann Surg*. 2008;247(2):320-326.