USE OF ROTATIONAL THROMBOELASTOMETRY FOR OPTIMAL MANAGEMENT OF EARLY RESUSCITATION OF BLEEDING TRAUMA PATIENTS

by

Precilla Vinutha Veigas

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Institute of Medical Science
University of Toronto

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Abstract

Early trauma-induced coagulopathy (TIC) is associated with uncontrollable bleeding, high transfusion requirements and mortality. Current management strategies of coagulopathic bleeding trauma patients are either guided by untimely laboratory tests (goal-directed) or blind transfusion protocols according to pre-established formulas (damage-control, 1:1:1). Both strategies have significant limitations that may account for the poor outcome of many patients. Viscoelastometric tests such as rotational thromboelastometry (ROTEM®) are emerging technologies with potential to revolutionize resuscitation of rapidly bleeding trauma patients. This project aims to investigate the role of ROTEM® in early trauma resuscitation, including TIC diagnosis, guiding blood transfusion and predicting 24h mortality. The overarching hypothesis is that ROTEM® parameters measured immediately upon hospital admission, may predict increased transfusion requirements, including massive transfusion (MT), and 24h mortality, and thus could guide blood and blood product transfusions. In order to explore this hypothesis, a series of approaches have been performed. A systematic review and international consensus conference with a panel of renowned experts in trauma, critical care, hematology, and surgery were done. They established the ROTEM® parameters and values used for the diagnosis of TIC, guidance of blood transfusion and prediction of 24h mortality. Next, patient data from two major Canadian
trauma centers were obtained to develop and validate prediction models for assessing the risk of requiring blood and blood product transfusion (red blood cells (RBC), plasma, cryoprecipitate and platelets), massive transfusion (MT) and 24h mortality using ROTEM® and readily available clinical variables. The systematic review and the consensus conference indicated that low clot amplitude was associated with transfusion requirements, MT and 24h mortality but exact ROTEM® parameters values could not be determined due to equipment limitations (i.e. high coefficient of variability) and poor understanding of their physiologic meaning (i.e. meaning of hyper coagulability parameters). Similarly, patient data analysis demonstrated that low clot amplitude, both in extrinsic (EXTEM) and fibrinogen pathways (FIBTEM), along with clinical parameters, accurately predicted the need for plasma and cryoprecipitate transfusion, MT, and 24h mortality. ROTEM® however, did not predict RBC or platelet transfusion. The results of our studies indicate that ROTEM® may assist in the early diagnosis and guide timely management of bleeding coagulopathic injured patients. While our findings warrant future evaluations we speculate that ROTEM® can guide blood transfusions, reduce inappropriate blood utilization and improve patient outcome (reduce mortality).
Acknowledgements

I would like to extend my deepest gratitude and appreciation to my supervisor, Dr. Sandro Rizoli. Dr. Rizoli has been a wonderful role model, teacher, mentor and a friend to me. I would like to thank you for encouraging my research and for allowing me to grow as a budding research scientist. Your advice on both academic research as well as my career has been priceless.

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I admit that this research would not have been possible without the support of MITACS Accelerate program and I express my gratitude to this agency for providing me financial support.

Finally, this thesis is dedicated to my daughter, Jadyn Abigail, my parents Eugene and Urban.
Contributions

I, Precilla V. Veigas, solely prepared this thesis and am the first author of one manuscript and primary co-author of one manuscript directly resulting from this thesis work. All aspects of this work, including the planning, execution, analysis and writing of all original research in chapter 2 publications, were performed by the primary author. The following contributions by other individuals are formally acknowledged:

Dr. Sandro Rizoli, Dr. Jeannie Callum, Dr. Avery Nathens, Dr. Ori Rotstein and Dr. Bartolomeu Nascimento all provided mentorship, guidance and assistance in the planning and execution of the analyses and manuscript/thesis preparation.

Dr. Kenji Inaba was appointed as the chair of the Consensus Conference and was responsible for writing the manuscript (Chapter 3). As a doctoral student for this chapter, I, in collaboration with Dr. Rizoli and Dr. Callum organized the international conference, generated conference questions, selected scientific experts, obtained funding, conducted two round Delphi survey, collated the data from Delphi survey and created figures for the manuscript.

Luis Theodore DaLuz was the second reviewer and the senior author for the systematic review (Chapter 2). He also assisted with the editing of the resulting manuscript. Ekaterina Petkova provided expertise and guidance in conducting the literature search for the Systematic review (Chapter 2).

Jagadish Rangrej provided expertise, statistical consulting and performed advanced statistical analysis.

Amanda McFarlan, Andrea Phillips, Carolyne Pasichny and Sandy Trpcic provided assistance with data collection and verification.

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Research described in Chapters 4-5 has the potential for publication and is yet to be published.
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<th>Definition</th>
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<tbody>
<tr>
<td>AA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>ACT</td>
<td>Activated clotting time</td>
</tr>
<tr>
<td>ACoTS</td>
<td>Acute Coagulopathy of Trauma Shock</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>aOR</td>
<td>Adjusted odds ratio</td>
</tr>
<tr>
<td>aPC</td>
<td>Activated protein C</td>
</tr>
<tr>
<td>APTT</td>
<td>Activated partial thromboplastin time</td>
</tr>
<tr>
<td>ATC</td>
<td>Acute traumatic coagulopathy</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BD</td>
<td>Base deficit</td>
</tr>
<tr>
<td>CA</td>
<td>Clot amplitude</td>
</tr>
<tr>
<td>CFT</td>
<td>Clot formation time</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CLIA</td>
<td>Clinical Laboratory Improvements Act</td>
</tr>
<tr>
<td>CT</td>
<td>Clotting time</td>
</tr>
<tr>
<td>DCR</td>
<td>Damage control resuscitation</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated intravascular coagulation</td>
</tr>
<tr>
<td>ED</td>
<td>Emergency department</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
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<tr>
<td>ELT</td>
<td>Euglobulin lysis time</td>
</tr>
<tr>
<td>EPCR</td>
<td>Endothelial protein C receptor</td>
</tr>
<tr>
<td>ER</td>
<td>Emergency room</td>
</tr>
<tr>
<td>FAST</td>
<td>Focused assessment with sonography in trauma</td>
</tr>
<tr>
<td>Acronym</td>
<td>Term</td>
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<td>---------</td>
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</tr>
<tr>
<td>FC</td>
<td>Fibrinogen concentrate</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and drug administration</td>
</tr>
<tr>
<td>FFP</td>
<td>Fresh frozen plasma</td>
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<tr>
<td>FWB</td>
<td>Fresh whole blood</td>
</tr>
<tr>
<td>GCS</td>
<td>Glasgow coma scale score</td>
</tr>
<tr>
<td>GvHD</td>
<td>Graft Versus Host Disease</td>
</tr>
<tr>
<td>HBV</td>
<td>Hepatitis B Virus</td>
</tr>
<tr>
<td>HCV</td>
<td>Hepatitis C Virus</td>
</tr>
<tr>
<td>HES</td>
<td>Hydroxy ethyl starch</td>
</tr>
<tr>
<td>HF</td>
<td>Hyperfibrinolysis</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leucocyte antigen</td>
</tr>
<tr>
<td>HNA</td>
<td>Human anti-neutrophil antibodies</td>
</tr>
<tr>
<td>ICU</td>
<td>Intensive Care Unit</td>
</tr>
<tr>
<td>INR</td>
<td>International normalized ratio</td>
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<tr>
<td>IQR</td>
<td>Inter quartile range</td>
</tr>
<tr>
<td>ISI</td>
<td>International sensitivity index</td>
</tr>
<tr>
<td>ISS</td>
<td>Injury Severity Score</td>
</tr>
<tr>
<td>LI</td>
<td>Lysis index</td>
</tr>
<tr>
<td>LIA</td>
<td>Latex immunoassay</td>
</tr>
<tr>
<td>MA</td>
<td>Maximum amplitude</td>
</tr>
<tr>
<td>MCF</td>
<td>Maximum clot firmness</td>
</tr>
<tr>
<td>ML</td>
<td>Maximum lysis</td>
</tr>
<tr>
<td>MODS</td>
<td>Multiple organ dysfunction syndrome</td>
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<tr>
<td>MT</td>
<td>Massive transfusion</td>
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xvii
NA - Not applicable
NPV - Negative predictive value
NEQAS - National External Quality Assessment Scheme
OR - Operation room
PAI-1 - Plasminogen activator inhibitor-1
POC - Point of care
PPV - Positive predictive value
PRBC - Packed red blood cells
PROMMIT - Prospective Observational Multicenter Major Trauma Resuscitation
PT - Prothrombin time
PRBC - Packed red blood cells
PTI - Prothrombin time index
ROTEM - Rotational thromboelastometry
SCT - Standard coagulation tests
SHSC - Sunnybrook Health Sciences Center
SIR - Systemic inflammatory response
SIRS - Systemic inflammatory response syndrome
SMH - St. Michael’s Hospital
TACO - Transfusion associated circulatory overload
TECC - Trauma, emergency and critical care
TEG - Thromboelastography
TF - Tissue factor
TIC - Trauma induced coagulopathy
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>TM</td>
<td>Thrombomodulin</td>
</tr>
<tr>
<td>t-PA</td>
<td>Tissue plasminogen activator</td>
</tr>
<tr>
<td>TRALI</td>
<td>Transfusion associated acute lung injury</td>
</tr>
<tr>
<td>TRAP</td>
<td>Thrombin receptor activating peptide</td>
</tr>
<tr>
<td>TRIM</td>
<td>Transfusion related immune modulation</td>
</tr>
<tr>
<td>TT</td>
<td>Thrombin time</td>
</tr>
<tr>
<td>TXA</td>
<td>Tranexamic acid</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>USA</td>
<td>United State of America</td>
</tr>
<tr>
<td>vCJD</td>
<td>variant Creutzfeldt-Jacob Disease</td>
</tr>
<tr>
<td>VE</td>
<td>Visco elastic</td>
</tr>
<tr>
<td>VIF</td>
<td>Variance inflation factor</td>
</tr>
<tr>
<td>VTE</td>
<td>Venous thromboembolism</td>
</tr>
<tr>
<td>vWF</td>
<td>von-Willebrand factor</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WNV</td>
<td>West nile virus</td>
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Preface

Trauma is a major cause of death and disability worldwide, particularly among individuals under 44 years of age (Scalea et al., 2008; MacLeod, Lynn, McKenney, Cohn, & Murtha, 2003; Sauaia et al., 1995; Holcomb, 2010). Hemorrhage is the most common preventable cause of death due to trauma, responsible for 40% to 50% of all trauma-related deaths (Brohi, Singh, Heron, & Coats, 2003; Kauvar, Lefering, & Wade, 2006; Sauaia et al., 1995).

It is estimated that at least ¼ of all severely injured patients develop an early (immediately after the trauma) intrinsic coagulopathy known as trauma induced coagulopathy (TIC). This coagulopathy is complex, often impairs multiple stages of the haemostatic response resulting in many different clinical presentations that change over time as the coagulopathy progresses and in response to iatrogenic interventions (Brohi, Singh, Heron, & Coats, 2003; Maegele et al., 2007; Hess et al., 2008). Aberrant fibrinolytic profiles have been described as hyperfibrinolysis, occurring in approximately 3-4% of all severely injured patients and also fibrinolysis shutdown which is observed in 20-30% of major trauma patients (Moore Gonzalez 2016). Other phenotype variation occurs in clot formation with hyper or hypo coagulability being described with different outcomes (Brohi, Singh, Heron, & Coats, 2003; Maegele et al., 2007; Hess et al., 2008). The complex etiology of TIC is poorly understood and consequently its diagnosis and management in particular during the early resuscitation phase, remain simultaneously a major area of research interest and a source of heated controversy. The current diagnostic methods available to the clinician during early resuscitation include tests such as prothrombin time (PT)/international normalized ratio (PT/INR) and activated partial thromboplastin time (APTT).

They are now recognized as being insufficient to diagnose coagulation defects such as TIC in clinically meaningful time frame. This makes them ineffective to guide early resuscitation including the selection of blood and blood products. The most widely used resuscitation strategies for coagulopathic bleeding patients are either based on such limited coagulation laboratory tests or clinical judgement where blood and blood products are intuitively transfused according to established formulas such as 1:1:1 (RBC:plasma:platelets). Failure to promptly diagnose important coagulation defects and correct them with blood transfusions in a timely manner may result in the patient’s death. Rapid diagnosis and management of coagulopathic
bleeding should occur as early as possible, and is of immense interest to the scientific community striving to find ways to reduce hemorrhage-induced mortality. Experts agree that many early deaths due to coagulopathic bleeding could be prevented if the coagulopathic defects were rapidly diagnosed and managed appropriately (MacLeod, Lynn, McKenney, Jeroukhimov, & Cohn, 2004).

The ideal resuscitation strategy should be guided by immediate recognition of TIC and all its clinically-relevant coagulation defects. Viscoelastic (VE) assays (eg: ROTEM®, Pentapharm, Munich, Germany) can be used as point-of-care assays to immediately diagnose TIC and are capable of diagnosing the range of coagulation defects (Lang et al., 2005; Luddington, 2005; Davenport et al., 2011; Johansson, Stissing, Bochsen, & Ostrowski, 2009). Recently a few centres, based on their own experience, have proposed VE-based management algorithms for trauma resuscitation which however lack broad acceptance. We propose to investigate the role of ROTEM® for diagnosing TIC and guiding blood and blood product transfusion in early trauma resuscitation, based on evidence and multi center clinical experience. We speculate that transfusion guidelines based on VE tests might change current trauma resuscitation practices, optimizing the use of blood transfusion and improving patients’ outcome.

The results of this dissertation will enhance our understanding of the role of VE-based transfusion management for early resuscitation of significantly bleeding trauma patients. By understanding the variables and factors associated with increased risk of transfusion and mortality, we can propose changes to current transfusion practices in early resuscitation of trauma patients, reduce mortality and arguably reduce costs.

**A. Research objectives**

The present work aims to assess the ability of Rotational Thromboelastometry (ROTEM®) test parameters used during early resuscitation phases of bleeding trauma patients to promptly diagnose clinically relevant coagulation defects and to guide transfusion management (goal-directed hemostatic resuscitation).

The overarching hypothesis of this thesis is that ROTEM® parameters, along with readily available clinical variables, can predict the need for blood and blood product transfusions, MT
and 24h mortality. Consequently ROTEM® parameters could be used to guide blood and blood product transfusion. The specific research objectives of this thesis are to:

(i) Systematically evaluate the evidence published in the medical literature describing the utility of reported thresholds of ROTEM® parameters for the diagnosis of coagulopathy, transfusion guidance and mortality prediction.

(ii) Organize and conduct a consensus conference with panel of world experts in ROTEM® and TIC to identify the ROTEM® thresholds used by experts in diagnosing and managing early coagulopathy;

(iii) An analysis of a large cohort of injured patients from two large Canadian Trauma Centres using ROTEM® and readily available clinical variables to assess the risk factors for transfusion of blood products, MT and 24h mortality and externally validate the models.

2. Thesis Organization

This thesis has been written following a four-step approach.

Chapter one provides the rationale and context for this work, mainly serving as an introduction to traumatic injury, mechanism and pathophysiology of early TIC, its diagnosis, current treatment options and an introduction to the VE test methods such as ROTEM® and TEG (Thromboelastography) and their evolving application for both clinical patient management and research.

The three specific research aims stated above are addressed in Chapters 2, 3, 4 and 5 respectively. Aim 3 is divided in two chapters (chapter 4 and 5) for the purpose of explaining the methodology and ease of publication.

We explored the published evidence on threshold values of ROTEM® parameters used for the diagnosis of TIC, blood transfusion guidance and prediction of mortality in Aim 1. This was done by systematic review of the literature to identify the reported thresholds of VE test parameters used for the diagnosis of coagulopathy, transfusion guidance and mortality prediction. These results are presented in chapter two which is a slightly modified version of a

An international consensus conference was conducted with the aim of identifying the ROTEM® thresholds used by experts and advanced institutions to diagnose and manage early coagulopathy of trauma shock. The organization and conduct of the conference is described in Chapter three. This work has been published in Journal of Trauma Acute Care Surgery (Inaba et al., 2015).

Chapter four provides detailed information about institutional experience of two large Canadian Level I Trauma Centres with ROTEM® with a view to analyse ROTEM® and other clinical variables as risk factors and for developing and validating prediction models for the following six outcomes: transfusion of blood products (red blood cells, plasma, cryoprecipitate and platelets); massive transfusion and 24h mortality among trauma patients. This chapter also presents detailed methodology used in the development and external validation of prediction models for the transfusion of blood products.

Chapter five provides detailed information about the development and validation of prediction models for massive transfusion and 24h mortality. The contents of chapter five have potential for early publication.

Finally, Chapter six discusses the opportunities for future studies.

The formatting of these published or submitted manuscripts has been adapted to conform to this thesis. A common reference section for all chapters follows Chapter 6. Permission from the publisher was obtained prior to use of all formerly published materials included in this thesis.
CHAPTER 1  General Introduction: Traumatic Injury, Coagulation, Trauma induced Coagulopathy (TIC), laboratory diagnosis and its management

Globally, traumatic injury is the leading cause of death and disability. Even though perceived to be affecting mainly younger individuals below 44 years, it spares no age group (Krug, Sharma, & Lozano, 2000). Major hemorrhage following a trauma is the number one cause of in-hospital deaths, majority of which occur in the first 12 hours following injury and responsible for up to 50% of all trauma related deaths within the first 24h of trauma (Sauaia et al., 1995; Kauvar & Wade, 2005; Chiara, Cimbanassi, Pitidis, & Vesconi, 2006). Traumatically injured patients with hemorrhagic shock develop coagulopathy, an impaired ability of blood to clot which occurs soon after injury (Hunt, 2014; Frith et al., 2010). Coagulopathy once developed further intensify the bleeding tendency which is difficult to manage. Patients who present with coagulopathy on hospital arrival have three to four fold chances of death (Brohi, Singh, Heron, & Coats, 2003; MacLeod, Lynn, McKenney, Cohn, & Murtha, 2003; Maegele et al., 2007). Untreated coagulopathy especially in trauma patients may lead to continuing hemorrhage, higher transfusion requirements, longer in-hospital stay and complications, higher risks of multi-organ damage and ultimately three to four fold increase in death (Maegele et al., 2007; Brohi, Cohen, & Davenport, 2007; MacLeod et al., 2003; Brohi et al., 2003). Furthermore, transfusion on its own carries higher risks. The early deaths due to coagulopathy could be salvageable if the cause of hemorrhage and underlying coagulopathy is promptly identified and managed (MacLeod et al., 2004).

Traumatic injury not only affects personal and emotional aspects of the patient and the family, it also imposes a major burden in terms of health care resources as well as societal productivity. In recent years our understanding of coagulopathy of trauma has dramatically improved. Accordingly the management of coagulopathy and hemorrhage has improved with the advent of new diagnostic tests. The principles in treating the trauma patients need to be changed from traditional approach to a more tailored approach. Maintaining normal hemostasis in the event of traumatic coagulopathy requires a new approach in resuscitating these patients.
This research aims to change the current transfusion management in significantly bleeding trauma patients. This chapter will describe the basic mechanism of coagulation, acute coagulopathy of trauma shock, diagnosis of coagulation and its management.

1.1 Coagulation system/basic mechanism of coagulation in vivo

Coagulation is a process by which the body prevents further blood loss from the injured vessel by forming a stable clot (thrombus) to maintain normal hemostasis (Greek “to stop bleeding”). In normal coagulation there is a fine balance between hemostatic and fibrinolytic system with bleeding control following mild trauma and minimal intravascular thrombosis. However coagulopathy in traumatic injury is multifactorial which involves various components of haemostatic, humoral, cell based and hormonal system. Derangement in this system leads to either uncontrolled bleeding or premature and abnormal formation of clot in blood vessels. The classical model of coagulation which was proposed in the early 1960s, involves a series of reactions in which each protease (clotting factor) systematically led to the activation or breakdown of subsequent protease finally leading to thrombin generation (Davie & Ratnoff, 1964; Macfarlane, 1964). The classical model has two components: extrinsic and intrinsic pathways, both pathways finally end in the activation of Factor X resulting in a thrombin burst. However, according to the most recent cell based model of coagulation, coagulation is a complex process which involves complex interplay of multiple components of entire hemostatic system such as blood vessel wall, platelets, enzymes, hormones and coagulation factors to form a stable clot consisting of fibrin and platelets (Kembell-Cook et al., 2005). Hemostasis involves the following four important steps:

1.1.1 Primary hemostasis:

Platelet activation and aggregation is an important step in primary hemostasis. Traumatic insult to the blood vessel endothelium exposes sub-endothelial collagen, von-Willebrand factor (vWF) and tissue factor (TF) into blood circulation triggering a local reflex of vasoconstriction to limit the blood flow(Furie & Furie, 2008). Platelets within the blood stream navigate to the injury site to form a platelet plug. The vWF plays a key role in platelet function. It aids in platelet adhesion to the exposed sub endothelial collagen with the help of glycoprotein lb, a platelet receptor (Furie & Furie, 2008). More circulating platelets aggregate with each other through the interaction of
fibrinogen with activated glycoprotein IIb-IIIa receptors to form a platelet plug (Furie & Furie, 2008). However, the platelet plug alone is not sufficient to maintain hemostasis as it only helps to seal small endothelial lesions (Guyton AD 2006).

1.1.2 Secondary hemostasis:

Tissue factor released following vessel wall injury, or present in circulation also activates platelets and triggers a cascade of pro-coagulant factors finally culminating in thrombin generation to convert fibrinogen into a stable fibrin monomers (Mackman, 2004). This fibrin mesh covers the underlying platelet plug and the process constitutes secondary hemostasis, commonly represented as “Waterfall” model or “Cascade” of hemostasis. Coagulation factors involved in the coagulation process are represented in roman numerals followed by “a” indicating an activated state of that particular factor. The simplified form of this classical “cascade” which involves interaction and function of each procoagulants involved in secondary hemostasis is represented in Figure 1.1 below (Davie & Ratnoff, 1964; Macfarlane, 1964):

![Image of coagulation cascade]

**Figure 1.1: Secondary hemostasis: The cascade model of coagulation.**
Adapted from Hoffman and Monroe III with the kind permission from the publisher (Hoffman & Monroe, III, 2001)
HMK - high molecular weight kininogen; PK- prekallikrein; PL- phospholipid; Ca^{2+} - ionised calcium.

According to the classical model of coagulation, disruption of endothelium exposes subendothelium which in turn exposes TF on cell surface. The exposed TF then binds with circulating plasma factor VII in the blood stream and forms a TF –factor VII complex which in
turn auto activates factor VII to form VIIa. The TF-VIIa complex in the presence of calcium and membrane proteins activates factor X which constitutes the extrinsic pathway. The intrinsic pathway is promptly activated following injury to blood vessel. Exposure of blood vessel causes activation of factor XII, also known as “contact activation”. Activated factor XII sequentially activates factor XI and factor IX. Activated factor IX and factor VIII activated by small amount of thrombin in the presence of calcium ions forms tenase complex and activates factor X producing thrombin and fibrin.

The classic coagulation cascade did not consider the role of cells and endothelium in the coagulation process. In 2001, Hoffman and colleagues emphasised the contribution of cells to the coagulation process and proposed a cell based model of coagulation in vivo which replaced the traditional “cascade” model (Hoffman & Monroe, III, 2001). The cell based model of hemostasis consists of three distinct overlapping phases: initiation of coagulation, amplification and propagation. Each of these phases is associated with a specific cell surface to prevent systemic coagulation, which is widely accepted now and is described below.

1.1.2.1 Initiation Phase

Tissue factor, a membrane protein, is the major initiator of coagulation (Martin, Boys, & Ruf, 1995). Under normal physiological condition, tissue factor is restricted to the cell membrane. In the initiation phase, the tissue factor is released following injury to vessel wall and/or activation of vascular endothelium. Experimental studies have shown that platelet activation in this step is independent of vWF and Glycoprotein VI (Dubois, Panicot-Dubois, Merrill-Skoloff, Furie, & Furie, 2006; Dubois, Panicot-Dubois, Gainor, Furie, & Furie, 2007). Apart from platelet activation, tissue factor accelerates conversion of Factor VII to Factor VIIa, an enzymatically active form of Factor VII (Guyton AD 2006). The tissue factor and Factor VIIa complexes to form Tissue factor/ Factor VIIa complex, which activates small amount of Factors IX and X (Figure 1.2). The activated factor Xa in turn activates factor V to form Va to generate small amount of thrombin (Factor IIa) from prothrombin (Factor II) (Morrissey, Macik, Neuenschwander, & Comp, 1993; Monroe, Hoffman, & Roberts, 1996). Factor XII along with other contact factors play a minor role in the activation of factor XI.
1.1.2.2 Amplification phase

Injury to vascular system also brings platelets and plasma to get exposed to extra vascular tissues. Platelets attach to extravascular matrix components at the site of damage. This binding of platelets to matrix proteins activates platelets and localizes them close to TF exposure site (Hoffman & Monroe, III, 2001). In the amplification phase (Figure 1.3), small amount of thrombin that was generated on TF bearing cells also promotes the activation of Factor V to Factor Va; Factor VIII to VIIIa, Factor XIII to XIIIa and also activates more platelets (Alberio & Dale, 1999).

Figure 1.2: Cell based model of coagulation: Initiation phase
Adapted from Hoffman 2003 with the kind permission from the publisher (Hoffman 2003).
Abbreviation: TF- tissue factor

Figure 1.3: Cell based model of coagulation: Amplification phase
Adapted from Hoffman 2003 with the kind permission from the publisher (Hoffman 2003).
Thrombin also activates factor XI to XIa. The activated platelets activates factors V and VIII attached to their surface, large amount of thrombin generations starts along with the accumulation of procoagulant cofactors (Hoffman & Monroe, III, 2001).

1.1.2.3 Propagation phase

In the propagation phase, additional factor Xa is produced when Tissue factor/Factor VIIa complex activates Factor IX to form IXa. Factor IXa combines with Factor VIIIa to form “tenase” complex which activates Factor X to Xa (Monroe et al., 1996). Factor Xa which is generated by Tissue Factor/VIIa complex or the Factor IXa/VIII complex binds to Factor V on calcium and phospholipid surface of cells. This complex converts prothrombin (Factor II) to thrombin (Factor IIa). A “thrombin burst” is generated to form a stable fibrin clot.

![Figure 1.4: Cell based model of coagulation: Propagation phase](image)

Adapted from Hoffman 2003 with the kind permission from the publisher (Hoffman 2003)

The cell based “Cascade” model is used in the choice of conventional laboratory coagulation tests such as prothrombin time (PT) which detects the defects in extrinsic pathway and activated partial thromboplastin time (aPTT) which detects defects in intrinsic pathway and routinely used.
1.1.3 Formation of fibrin clot

In this phase, thrombin hydrolyses fibrinogen to fibrin (Factor Ia) monomers which polymerizes to form a strong insoluble mass covering the platelet plug. Thrombin then activates Factor XIII to XIIIa which cross-links the fibrin monomers and stabilizes the clot (Lorand, 2001).

1.1.4 Inhibition of coagulation and fibrinolysis

As the clot is formed, body’s physiologic mechanism also limits further formation of obstructive thrombus and prevent blockage of arteries. This is achieved by two mechanisms. In the first mechanism, plasma protease inhibitors (Tissue factor pathway inhibitor and antithrombin) stop the initiation phase and prevent clot propagation phase. Second mechanism is activation of protein C anticoagulant pathway which inhibits activity of factor Va and VIIIa to reduce thrombus formation (Brohi et al., 2008; Rezaie, 2001).

Figure 1.5: Schematic diagram of formation of the Thrombin/Thrombomodulin (TM) complex and its subsequent activation of Protein C. The TM complex promotes the EPCR (Endothelial Protein C Receptor) which presents the Protein C. This stimulates Tissue Plasminogen Activator Inhibitor (t-PAI) which inhibits fibrinolysis. Modified from (Schochl, Voelckel, Maeggele, & Solomon, 2012; Schochl et al., 2012; Schochl et al., 2012)
A small amount of thrombin generated during the coagulation process is found free in circulation. Thrombin comes in contact with an intact endothelial cell bearing Thrombomodulin (TM), complexes with it to form TM-thrombin complex and triggers protein C pathway (Figure 1.5). Following injury, endothelial cells also release large amounts of tissue plasminogen activator (t-PA) in response to shock and hypoperfusion (Brohi et al., 2007). The activated protein C (aPC), along with its co-factor protein-S suppresses the activity of activated Factor V (Va) and VIII (VIIIa), by proteolytic activity thereby shutting down the thrombin generation. The TM-thrombin complex is no longer available to activate procoagulant factors or platelets to the normal coagulation cascade. Large quantity of protein C is also responsible for consumption of tissue plasminogen activator inhibitor-I (PAI-1) which is a major antagonist of tissue plasminogen activator. A large quantity of t-PA are accumulated creating hypofibrinolytic state (Brohi et al., 2007). In addition, accelerated production of plasmin leads to the destruction of fibrinogen. A large quantity of fibrin/fibrinogen degradation products (eg: D-dimer) inhibits fibrin polymerization resulting into a weak clot (Hunt & Segal, 1996).

1.2 Trauma induced coagulopathy (TIC)

The past decade has experienced burst of research interest in understanding coagulopathy following traumatic injury. Traditionally, trauma associated coagulopathy was viewed to be the result of dilution, consumption and malfunction of procoagulant serine proteases (Armand & Hess, 2003; Schreiber, 2005). However in a major milestone study in 2003, Brohi and colleagues reported that nearly a quarter of trauma patients who arrived to the hospital were coagulopathic as defined by PT >1.5 or APTT of 1.5 times normal (Brohi et al., 2003). They observed coagulopathy in these patients even with minimum resuscitative fluid administration prior to hospital arrival. The mortality rate in this group of patients was four fold compared to those who were not coagulopathic on arrival (46% vs. 11%). More research in this area has further supported their findings about early coagulopathy in trauma patients with similar findings with rates of arrival coagulopathy ranging from 25% to 60% and higher mortality rates (Brohi et al., 2007; MacLeod et al., 2003; Maegle et al., 2007; Rugeri et al., 2007). Even though the classical belief that trauma associated coagulopathy was due to dilution, consumption and acidosis causing dysfunction of coagulation factors, the same group of investigators have
emphasized on an endogenous component involving tissue trauma, characterised by higher ISS and shock irrespective of dilution or hypothermia (Brohi et al., 2007). Tissue injury is positively associated with the degree of coagulopathy and shock is the key driver of disseminated intravascular coagulopathy (Brohi et al., 2003; Brohi et al., 2007; Brohi et al., 2007; Maegele et al., 2007). All these six initiators of coagulopathy are divided into two groups depending on the role they play in the initiation of coagulopathy: initiators (tissue trauma and shock) and propagators (dilution/consumption, hypothermia, acidosis, and inflammation). These are described below:

1.2.1 Tissue trauma

Tissue trauma triggers both coagulation and hyperfibrinolysis (Hess et al., 2008). In patients without vascular injury, endothelial cells prevent systemic coagulation by producing nitric oxide and tissue plasminogen activator which prevent platelet activation and fibrin degradation. Traumatised patients mainly experience blunt, penetrating or crush injuries with varying degrees of tissue damage and coagulopathy. The extent of tissue damage is clinically associated with degree of coagulopathy in patients presenting with higher injury severity scores (>45) (Brohi et al., 2007; MacLeod et al., 2003). Traumatic injury causes tissue and vascular damage resulting in exposure of subendothelial type III collagen and tissue factor in circulation (Mann, 1999). Tissue factor binds with von-Willebrand factor, platelets and activated factor VII initiating coagulation cascade. The TF/VIIa complex activates serine proteases, finally resulting in thrombin generation and fibrin formation (Roberts, Hoffman, & Monroe, 2006). A stable platelet plug is formed to prevent further blood loss. However, the platelet plug is insufficient to maintain hemostasis.

Hyperfibrinolysis (HF) is a common event following trauma which is a direct effect of tissue trauma and shock (Brohi et al., 2008). According to Brohi and colleagues, hemorrhagic shock and hypoperfusion leads to enhanced release of tissue plasminogen activator (t-PA) derived from endothelial cells initiating hyperfibrinolytic pathway (Brohi et al., 2003). Hypoperfusion also leads to expression of thrombomodulin (TM) on endothelial surfaces which combines with thrombin and activates the protein C pathway (Brohi et al., 2007). Activated protein C along with its cofactor protein S slows down acceleration of coagulation by deactivating activated factors V
and VIII. As a result large amounts of protein C leads to consumption of plasminogen activator inhibitor-I (PAI-I) which is a major opponent of t-PA. As a result exceedingly large amount of t-PA are liberated causing a hyperfibrinolytic state, leading to breakdown of fibrin mesh and a weak clot (Fig 1.5) (Brohi et al., 2007).

HF is detected in 7-20% of trauma patients and is associated with higher mortality rates (Levrat et al., 2008; Schochl, Frietsch, Pavelka, & Jambor, 2009; Ives et al., 2012; Kutcher et al., 2012a). Early HF detected within one hour of hospital arrival, characterized by lysis of >15% as detected by ROTEM® is associated with massive transfusion needs, impaired hemostasis, and mortality (Schochl et al., 2009).

Injuries to particular organs/tissues are also associated with coagulopathy. Long bones fractures have been associated with coagulopathy with limited evidence in literature (Kunz, Hortnagl, Kroesen, & Rumpl, 1978). Patients experiencing long bone, pelvic and tibia fractures also develop circulating fat macroglobules causing fat embolism ultimately leading to multi system dysfunction predominantly the triad of lung, skin and brain (Gurd & Wilson, 1974; Georgopoulos & Bouros, 2003). However, the incidence of fat embolism following trauma varies from <1 to 29% (Bulger, Smith, Maier, & Jurkovich, 1997; Fabian, Hoots, Stanford, Patterson, & Mangiante, 1990). Incidence of fat embolism syndrome and association of coagulopathy is rare immediately following injury, rather it develops at a later stage. The development of coagulopathy is mainly due to tissue injury, shock and inflammation rather than due to bone marrow associated pathogenesis (Hauser et al., 1997). Severe traumatic brain injury is also associated with coagulopathy (Hulka, Mullins, & Frank, 1996). Injured brain tissue releases tissue factor (thromboplastins) which triggers coagulation cascade leading to consumption of coagulation factors in the process. (Stein & Smith, 2004).

### 1.2.2 Shock

Base deficit (BD) is a marker of extent and duration of shock and is associated with higher transfusion requirements and poor outcomes (Davis, Parks, Kaups, Gladen, & O'Donnell-Nicol, 1996). Shock in combination with tissue injury is an important driver of coagulopathy and is associated with higher transfusion requirement, inhospital complications, organ injury and death (Siegel, Rivkind, Dalal, & Goodarzi, 1990; Rutherford & Meyer, 1992; Davis et al., 1996).
There is a dose dependent association between severity of shock and the extent of admission coagulopathy characterized by prothrombin time (PT) and activated partial thromboplastin time (aPTT) independent of injury severity scores (ISS) (Brohi et al., 2007; Niles et al., 2008). Brohi and colleagues also reported a higher laboratory BD values among 25% of patients who arrived coagulopathic (Brohi et al., 2007). This group observed normal coagulation parameters and normal BD values in patients presenting with severe injury without hypotension suggesting that coagulopathy is independent of factor consumption but is due to hypoperfusion. They also demonstrated an association between increased BD, thrombomodulin and decreased Protein C levels with excessive activation of protein C pathway.

1.2.3 Dilution and consumption

Dilution and consumption of clotting factors is important contributing factors of TIC leading to impaired thrombin generation, platelet activation and fibrin formation (Brummel-Ziedins, Whelihan, Ziedins, & Mann, 2006; Schreiber, 2005). Consumption coagulopathy is generally followed by dilutional coagulopathy. Hemorrhage itself contributes to direct loss of coagulation factors and reduce fibrinogen level and platelet counts (Martini, Pusateri, Uscilowicz, Delgado, & Holcomb, 2005).

Hypoperfusion following trauma leads to decreased intravascular hydrostatic pressure leading to migration of fluid deficient in coagulation factors both from cellular and interstitial spaces into circulation. Prehospital administration of intravenous resuscitation fluids aimed at compensating the massive blood loss further exacerbates this condition (Ng, Lam, & Chan, 2002). During the initial resuscitation phase, the practice of empiric administration of colloids, crystalloids, and packed red blood cells often results in dilutional coagulopathy and reduced clotting ability (Armand & Hess, 2003; Malone, Hess, & Fingerhut, 2006). Thus consumptive coagulopathy and dilutional coagulopathy often occur concurrently. Crystalloids exacerbate the components of “death triad” namely acidosis, hypothermia and coagulopathy. Effects of crystalloids and colloids in invitro studies have demonstrated reduced activity of coagulation factors leading to dilutional coagulopathy (Petroianu, Maleck, Koetter, Liu, & Schmitt, 2003; Ruttmann, James, & Viljoen, 1996). Furthermore, crystalloids contain higher amount of chloride, which cause hyperchloremic acidosis leading to further worsening of existing acidosis (Tieu, Holcomb, &
Schreiber, 2007). Increased fluid can lead to oedema in lungs, gastrointestinal tract, and increased intra-abdominal pressure, infections, and impaired wound healing (Attuwaybi et al., 2004).

A number of factors also influence dilution coagulopathy. For example, gelatin preparations cause impaired fibrin polymerization and disrupt fibrin network resulting in reduced clot quality and strength apart from contributing to dilutional effects (Mardel et al., 1998; Engvall, Ruoslahti, & Miller, 1978). Use of large volumes of hydroxyethyl starch (HES) is associated with impaired fibrin polymerization and increased bleeding (Treib, Baron, Grauer, & Strauss, 1999; Strauss, 1981). Packed red blood cell transfusion (PRBC) leads to dilution of clotting factors prolonging coagulation time and reduced clot quality. A clinical study further established prolonged coagulation time in patients receiving prehospital administration of large volume of packed blood cells versus those who did not (Faringer, Mullins, Johnson, & Trunkey, 1993). To overcome this effect, there has been an increased awareness towards the use of ratio based transfusion of red cell: plasma: platelets. However an optimum ratio has yet to be confirmed and large scale clinical trials are required to support this view (Borgman et al., 2007).

1.2.4 Hypothermia

Hypothermia is characterized by drop in normal core body temperature, below 35°C (Cosgriff et al., 1997). The reported incidence of hypothermia upon hospital arrival vary from 10% to 60% (Tsuei & Kearney, 2004). Hypothermia is a key component of lethal triad and is associated with poor outcomes (Slotman, Jed, & Burchard, 1985; Wang, Callaway, Peitzman, & Tisherman, 2005). Mild hypothermia defined as core temperature between 35° and 32° C is common in trauma patients and has minimum impact on coagulopathy (Hess et al., 2008; Luna et al., 1987). Hypothermia results from multiple etiologies such as convection and radiation from exposure of damaged tissue, exposure of body cavities during surgery as well as administration of cold resuscitation fluids (Farkash et al., 2002; Steinemann, Shackford, & Davis, 1990). Traumatic insult itself alters normal central thermoregulation and blocks shivering response (Stoner, 1972). Simultaneously, rate of metabolic activities of tissues slows down resulting in reduced heat generation (Tsuei & Kearney, 2004). Hypovolemic shock also results in reduced delivery of
oxygen to the tissues, exceeding maximum oxygen carrying capacity and results in reduced heat generation (Weg, 1991).

Hypothermia causes and worsens bleeding abnormalities in a variety of mechanisms making it difficult to manage using normal resuscitative techniques. Temperature below 34°C significantly affects coagulation protease activity, platelet activation and adhesion, as well as bleeding (Dirkmann, Hanke, Gorlinger, & Peters, 2008; Tsuei & Kearney, 2004). Hypothermia in severely injured patients is associated with poor outcomes, supported by retrospective studies (Jurkovich, Greiser, Luterman, & Curreri, 1987; Luna et al., 1987; Steinemann et al., 1990; Martini et al., 2005). Low temperature also alters fibrinolysis and decreases thromboxane B2 production (Tsuei & Kearney, 2004). Effects of hypothermia on coagulopathy are difficult to correct. Rewarming the patient with warm blanket, administering warm resuscitation fluids may help in rapid correction of hypothermia.

A prospective randomized study investigated the association of hypothermia with mortality by controlling injury severity (Gentilello, Jurkovich, Stark, Hassantash, & O'Keefe, 1997). One study reported 100% mortality in patients with core temperature below 32°C when controlled for other co morbidities (Jurkovich et al., 1987). However it was unclear whether hypothermia was the cause of coagulopathic dysfunction leading to early mortality (Gentilello et al., 1997). Hypothermia is also associated with higher transfusion requirements, fluid administration and longer in hospital stay compared to normothermic patients (Jurkovich et al., 1987; Gunning, Sugrue, Sloane, & Deane, 1995; Leben, Tryba, Bading, & Heuer, 1996).

1.2.5 Acidosis

Metabolic acidosis which is common following traumatic injury, develops due to ionic chloride during fluid resuscitation and adversely affects activities of enzyme complexes on lipid surfaces as well as coagulation proteases (Siegel et al., 1990). Lactate is produced as a result of anaerobic metabolism due to prolonged and insufficient tissue perfusion if untreated also leads to metabolic acidosis (El & Noureddine, 2014). Normal saline used to resuscitate patients contains higher chloride concentration which causes hyperchloremic acidosis further worsening lactic acidosis (Brill, Stewart, Brundage, & Schreiber, 2002). Transfusion of large volume of blood products also exacerbates acidosis due to the presence anticoagulant citrate in blood products. Acidosis
with hypothermia can modify coagulation cascade as both these factors alter normal pH and subsequent enzyme activity ultimately affecting normal coagulation (Cosgriff et al., 1997). Persistent hypoperfusion causes metabolic acidosis which has detrimental effect on compromised cardiovascular system. One study reported a reduction in functional ability of factor VIIa was by 90%; factor VIIa/TF complex by 55%; and rate of prothrombin activation by factor Xa/ Va complex by 70% when the pH was lowered from 7.4 to 7.0 (Meng, Wolberg, Monroe, III, & Hoffman, 2003). Acidosis caused by administration of infusion fluids containing hydrochloric acid increased clotting times, reduction in clot amplitude as well as depletion of fibrinogen levels in animal models (Martini, Dubick, Wade, & Holcomb, 2007; Engstrom, Schott, Romner, & Reinstrup, 2006). Acidosis at or below pH of 7.2 is associated with decreased contractility and cardiac output, central nervous system dysfunctions, vasodilation, hypotension, bradycardia, increased dysrhythmias, reduced blood flow to the vital organs and multiple organ failure ultimately leading to higher mortality (Mikhail, 1999; Ferrara, MacArthur, Wright, Modlin, & McMillen, 1990; Cosgriff et al., 1997).

1.2.6 Inflammatory response

It is long known that severe tissue trauma induces inflammation leading to the systemic inflammatory response syndrome (SIRS). This involves the interaction of hormonal, metabolic and immunological mediators and occurs immediately upon injury and endothelial activation (Zedler & Faist, 2006; Ganter et al., 2008). There is a link between activation of coagulation and complement system (Huber-Lang et al., 2006). Immediate activation of immune system following a trauma triggers complement system that recruits neutrophils at the site of tissue injury. The complement system has been implicated in the development of systemic inflammation (Zedler & Faist, 2006). The systemic inflammatory response is necessary for tissue repair, defence against pathogens and assist in the healing process. In uncomplicated trauma the SIR is self-limiting and there is a balance between pro and anti-inflammatory mediators. However in severe trauma there is an accelerated pro inflammatory response causing imbalance between the pro and anti-inflammatory mediators resulting in SIRS, MODS (multiple organ dysfunction syndrome) (Bone, 1996). Imbalance in systemic pro inflammatory reaction results in shock, flow of fluids into organs, and coagulation abnormalities. Similarly a disrupted systemic anti-inflammatory response may lead to allergy and immune suppression (Bone, 1996).
1.3 Mechanism/pathogenesis of early trauma induced coagulopathy

Coagulopathy in trauma has long been believed to occur mainly due to consumption and dilution of coagulation factors from the administration of resuscitative fluids, further compounded by hypothermia and acidosis (Armand & Hess, 2003; Hess et al., 2008). Based on this belief most trauma centers manage coagulopathy by transfusing large volume of plasma and cryoprecipitate to replenish the deprived coagulation factors (College of American Pathologists, 2017). In the absence of hypoperfusion, injury triggers extrinsic pathway leading to formation of fibrin as described earlier; however coagulopathy occurs only in the presence of shock.

Initial studies from Vietnam war reported incidence of arrival coagulopathy in 8% of patients which was closely associated with the extent of shock (Simmons et al., 1969). In the recent years, It has been shown that approximately 25% of patients arrive to the hospital with coagulopathy even before fluid administration; and before the development of hypothermia and acidosis (Brohi et al., 2003; MacLeod et al., 2003). Coagulopathy of trauma is endogenous and occurs only in combination of tissue injury and hypoperfusion leading to systemic anticoagulation and hyperfibrinolysis through the activation of anticoagulant protein C pathway now termed as Acute Coagulopathy of Trauma or trauma induced coagulopathy (Brohi et al., 2007). As per the new investigations mechanism of TIC now includes activation of protein C pathway, endothelial glycocalyx disruption, exhaustion of fibrinogen and platelet dysfunction.

1.3.1 The Protein-C (PC) pathway

Protein C is an integral part of anticoagulant system. It is a vitamin K dependent serine protease which circulates as an inactive zymogen and is activated by thrombin-thrombomodulin complex expressed on the endothelial cell surface (Weiler, 2010). Activated protein C displays powerful anticoagulant and anti inflammatory properties. Tissue injury and increased hypoperfusion leads to rise in thrombomodulin, activated protein C (aPC) and depletion of non-activated protein C levels (Brohi et al., 2007). Accordingly activation of thrombomodulin – protein C system is the main pathway mediating TIC, which is different from originally presumed consumption and dysfunction of factors. Furthermore, injury and hypoperfusion exposes the endothelial cells that lines the vessel walls. This rupture, in the presence of hypoperfusion and shock leads to an increased expression of thrombomodulin, a transmembrane glycoprotein on the endothelial cell
wall and endothelial protein C Receptor (EPCR) (Brohi et al., 2007). In case of severe injury, thrombin flows systemically and this systemic release enables it to perform anticoagulant function in combination with thrombomodulin (Brohi et al., 2007). Activated protein C, along with its non-enzymatic cofactor S degrades procoagulant factor V and VIII, thus reducing thrombin availability to convert fibrinogen to fibrin, leading to deficiency of fibrinogen and enhanced breakdown of clot. This results in continued bleeding warranting more blood transfusion where other drivers of coagulopathy such as dilution, hypothermia, and acidosis come into effect worsening hemostasis. Activated protein C also consumes plasminogen activator inhibitor I (PAI-1), reducing tissue plasminogen activator (tPA) inhibitor and enhances the conversion of plasminogen to plasmin subsequently facilitating enhanced fibrinolysis (Brohi et al., 2008). The ultimate result is increased D-dimer and tPA levels in the plasma (fig 1.5). A most recent study, Prospective Observational Multicenter Major Trauma Transusion (PROMMIT) involving 1,198 severely injured trauma patients reported that patients with higher ISS or severe hypoperfusion were found to be coagulopathic. This study also found that APC was a strong predictor of coagulopathy (adjusted odds ratio: 1.038, p=0.03) (Rahbar et al., 2012). Further research also demonstrated that higher concentration of circulating aPC correlated with higher mortality, transfusion requirements and in hospital complications (Cohen et al., 2012). This study also demonstrated that coagulopathy occurs immediately after injury in severely injured patients arriving at the hospital with shock and higher ISS.

**1.3.2 Hypofibrinogenemia**

Low levels of admission fibrinogen values are now considered to be significantly associated with early TIC. Fibrinogen is also the first coagulation factor to drop to low levels during severe bleeding (Hiippala, Myllyla, & Vahtera, 1995; Stainsby, MacLennan, & Hamilton, 2000).

Fibrinogen plays a vital role in maintaining normal hemostasis by acting as an antecedent for formation of insoluble fibrin clot and as a mediator of platelet aggregation (Fries & Martini, 2010; Lang et al., 2009; Rahe-Meyer & Sorensen, 2011; Weisel & Nagaswami, 1992).

Even though there is lack of clinical evidence on optimum cut off value of fibrinogen, current European guidelines recommend a plasma fibrinogen cut off of <1.5 to 2.0g/L to administer fibrinogen concentrate (Rossaint et al., 2010). Low fibrinogen levels have been found to be
associated with increased bleeding and/or transfusion requirements in a variety of settings such as trauma (Tauber et al., 2011; Schochl et al., 2011a), cardiac surgery (Karlsson et al., 2009; Rahe-Meyer et al., 2013) and postpartum hemorrhage (Charbit et al., 2007). Two of the most prominent studies in trauma reported correlation between reduced levels of admission fibrinogen with hypotension (SBP<90mmHg), shock (low base deficit), dilution due to administration of resuscitative fluids and injury severity (ISS>25) (Rourke et al., 2012; Schlimp et al., 2013). Rourke et al., reported 33% reduction of fibrinogen levels among patients with TIC among 517 trauma patients upon hospital arrival. Schlimp study reported 81% patients had reduced levels of fibrinogen (<2 g/L) in the presence of base deficit (<-6 mmol/L) and 63% patients had levels below 1.5 g/L. Low fibrinogen levels are also found to be associated with higher mortality among trauma patients requiring MT (Inaba et al., 2013; Rourke et al., 2012; Danes, Cuenca, Bueno, Mendarte, & Ronsano, 2008). All these studies indicate low fibrinogen reserve in early TIC among significantly traumatised and bleeding patients.

Platelets play a fundamental role in maintaining vascular integrity and hemostasis after traumatic injury. Role of platelet dysfunction is not clearly elucidated and few studies have assessed platelet dysfunction in TIC (Nekludov, Bellander, Blomback, & Wallen, 2007; Davis et al., 2013). According to the cell based model of Hoffman and Monroe, coagulation consists of three distinct phases such as initiation, amplification and propagation which explains the contribution of platelet, endothelium and coagulation factors to achieve hemostasis (Hoffman & Monroe, III, 2001). Low platelet counts are associated with mortality in trauma (Stansbury et al., 2013). Likewise, in trauma, admission platelet counts within the normal range are common findings, but fall after admission which implies that their consumption or dilution may not be an important contributing factor in the pathogenesis of early TIC (Stansbury et al., 2013). Conventional coagulation tests cannot detect platelet dysfunction as they are performed on cell free plasma. Current gold standard, aggregometry, which is used to characterize platelet dysfunction is time consuming, labor intensive and not available in most trauma centers. Multiple electrode impedance aggregometry is a new and advanced point of care device used to analyse platelet function (Toth, Calatzis, Penz, Losonczy, & Siess, 2006). Stimulation by adenosine diphosphate (ADP) and arachidonic acid (AA) are two of the pathways among many other pathways involved in the activation of platelets (Woulfe, Yang, & Brass, 2001). Kutcher et al., used blood samples from 101 critically injured trauma patients and assayed for responsiveness to ADP, thrombin
receptor-activating peptide (TRAP), AA and collagen using multiple electrode impedance aggregometry (Kutcher et al., 2012b). In this study 45.5% patients had platelet dysfunction in response to ADP, TRAP, arachidonic acid and/or collagen. This study found ten-fold increase in mortality in patients presenting with one or more platelet aggregation defects. Admission arachidonic acid and collagen responsiveness were predictors of mortality. Subsequent investigation by Solomon et al reported similar findings in 163 trauma patients suggesting even minor abnormalities in platelet aggregation was associated with mortality (mortality:12.3%)(Solomon et al., 2011). These studies support the role of platelet aggregation in TIC.

1.4. Diagnosis of TIC

Rapid diagnosis of coagulopathy is critical to guide early resuscitation to the trauma patient. Traditionally, coagulopathy has been diagnosed based on the results of standard coagulation tests (SCTs) such as Prothrombin time (PT), International Normalized Ratio (INR), activated Partial Thromboplastin Time (APTT), platelet count, fibrinogen assay, fibrinogen degradation products etc. are described below.

1.4.1 Prothrombin time (PT)

PT is a measure of the time taken to form a fibrin clot and is used to evaluate the defects or deficiency of factors involved in the extrinsic pathway (Factor VII) and common pathway (Factors X, V, II and Fibrinogen). Citrated platelet free plasma is warmed at 37°C to which PT reagent containing tissue factor, phospholipid and calcium is added. PT, time taken to form a fibrin clot, is measured in seconds. The normal value is 9-15 seconds, which can vary slightly depending on the laboratories. In the laboratory, the automated tests are used and the time is electronically measured in the analyser.

1.4.2 International Normalized Ratio (INR)

PT in seconds is used to calculate INR. The PT reagent has an International Sensitivity Index (ISI) assigned to it. The ISI is the determinant of reagent’s sensitivity to deficiencies in the Vitamin K dependent factors compared to the World Health Organization (WHO) reference standard. The INR is calculated using the following formula.
INR = (Patient PT/Mean Normal PT) \(^{\text{ISI}}\)

ISI is the International Sensitivity Index for the thromboplastin used to conduct the PT assay at any given laboratory.

### 1.4.3 Activated Partial Thromboplastin Time (aPTT)

The aPTT measures the defects of factors involved in the intrinsic pathway (Factors XII, XI, IX, VIII,) and the common pathway (Factors X, V, II and Fibrinogen). The aPTT reagent contains a contact activator (e.g. Ellagic acid or kaolin, a phospholipid surface activator) and phospholipid. The citrated plasma is mixed with aPTT reagent and calcium chloride and incubated at 37°C. The time to clot formation is measured in seconds. The normal value is 25-35 seconds.

### 1.4.4 Thrombin Time (TT)

The TT is used to detect deficiencies or functional aspects of fibrinogen or the presence of inhibitor of thrombin (Factor IIa). The reference range is 15-19 seconds. As the functional fibrinogen assay is now commonly used in majority of laboratories, its routine use is limited.

### 1.4.5 Fibrinogen assay

Fibrinogen is a major plasma protein coagulation factor synthesised by hepatocytes with normal concentration ranging from 1.5 to 4.0 g/L. Fibrinogen activity is assessed by the popularly used Clauss method, which is based on the time taken to form fibrin clot. In this assay, a high concentration of thrombin (ranging from 35 - 200 U/ml, but usually about 100 U/ml) is added to dilute plasma. The thrombin breaks down fibrinogen into fibrin monomers which polymerize resulting in clot formation. The time taken for the diluted plasma to form a clot is measured in seconds (Clauss, 1957) and is inversely proportional to fibrinogen activity which is derived from a calibration curve obtained by clotting a series of dilutions of a reference plasma sample of known fibrinogen concentration. The lower the fibrinogen concentration, longer is the clotting time. Due to time consuming nature of this test, most modern laboratories now use automated coagulation analysers which measure fibrinogen levels using photo-optical detection principle to monitor the change in optical density and the degree of change is proportional to the fibrinogen concentration (Becker, Bartl, & Wahlefeld, 1984).
1.4.6 D-dimer assay

D-dimers are the degradation products of specific cross-linked fibrin derivatives which are produced when fibrin is degraded by fibrinolytic system. D-dimers are widely used markers to diagnosing and/or monitoring of wide range of thrombosis associated conditions such as venous thromboembolism (VTE), pregnancy, and disseminated intravascular coagulation (DIC).

D-dimer levels are elevated where fibrin is generated and degraded in conditions such as trauma, venous thromboembolism, infection, pregnancy, stroke and acute myocardial infarction (van Beek et al., 1996). There are several techniques used to assess D-dimers such as Enzyme Linked Immunosorbent Assay (ELISA), Latex Immunoassay (LIA), and whole blood hemeagglutination assay (SimpliRED) and immunochromatography test (Bounaumes, de, Perrier, & Reber, 1994; Bounaumes, Schneider, Reber, de, & Krahenbuhl, 1989; John et al., 1990).

1.4.7 Point of care viscoelastic assays

With the paradigm of classic coagulation theory shifting to cell based coagulation models, viscoelastic point of care (POC) assays such as Thromboelastography (TEG, Haemonetics, Braintree, MA) and Rotational thromboelastometry (ROTEM®, TEM international, Munich, Germany) are rapidly emerging as efficient technologies to assess viscoelastic properties of blood during the clotting process (Johansson et al., 2009).

Thromboelastography originally developed by Hartert in Germany is a point of care viscoelastic test which assesses global hemostatic and fibrinolytic activity and has been used for over 60 years. Initially it has been extensively used in cardiac and transplant surgery but now increasingly used in trauma in North America. It has two channels. The test is performed using a small sample of whole blood placed in a cuvette into which a wire is suspended. The cuvette rotates at an angle of 4°75’. As the clot begins to develop, the change in viscosity and subsequent shear is transmitted through the pin. This change is captured electronically in the form of a graph.

ROTEM® is developed from the TEG technology, which was originally developed by H. Hartert in 1948 which was available even before the introduction of aPTT (White, 2003) and is the focus
of this research. Using a small sample of patient’s whole blood, ROTEM® provides comprehensive overview of coagulation starting from initiation, propagation and subsequent lysis (Johansson et al., 2009). In contrast to conventional laboratory coagulation tests, it captures the real time physiological interaction of cellular components such as platelets with the coagulation factors, platelet interaction and aggregation, stability of the clot due to fibrin cross linking culminating in lysis of the clot (Ganter & Hofer, 2008). The test is initiated in a stationary plastic cup containing 340 microliter of citrated blood in which is suspended a mobile pin (Figure 1.6).

**Figure 1.6**: A pictorial representation of working mechanism of ROTEM®. A cup containing whole blood has a pin immersed in it which in turn is attached to a shaft. The pin oscillates through an angle (4° to 75°). Formation of clot between the cup and the pin develops a torque which is captured in the form of a graph with numerical values (Reproduced with permission from Tem Innovations GmbH, Germany).

The pin is attached to the device through a shaft. The pin moves back and forth at an angle of 4° 45’. As the blood begins to clot, initial thrombin is generated, platelets are activated to express glycoprotein IIb and IIIa receptors and fibrin is generated and polymerized to form a fibrin network. This phase consisting of interplay of glycoprotein receptors and polymerized fibrin causes a torque between the cup and the pin. As a result, the free movement of the pin is restricted due to the change in visco-elasticity of blood during the clotting process. After a stable clot is formed, the breakdown of fibrin strands by fibrinolysis diminishes the torque. The change in the torque is optically detected via an optical beam of light and processed by a microprocessor. The results are displayed both graphically and numerically on a touch screen.
computer monitor. The results can also be reviewed remotely using remote viewing software (Ganter & Hofer, 2008; Luddington, 2005) (Figure 1.6 A-B ROTEM® analyser, ROTEM® trace).

There are four measurement channels (assays) each providing information about specific coagulation defects such as factor deficiency, thrombocytopenia, hyperfibrinolysis, and effects of heparin. The test assay is initiated by adding assay specific activator or inhibitors to the cup.

The following four assays are used in routine practice:

**Tissue factor activated extrinsic pathway (EXTEM):** In EXTEM assay, tissue factor or calcium is added to initiate clot formation. EXTEM provides information similar to the extrinsic pathway of coagulation cascade and provides information about plasma factors, fibrin polymerization, platelet contribution and fibrinolysis.

**Ellagic acid activated intrinsic pathway (INTEM):** In this assay, ellagic acid is used as an activator. INTEM mimics the intrinsic pathway of coagulation cascade and provides information on plasma factors, fibrin polymerization and platelet function.

**Platelet inhibited test for assessing fibrinogen component (FIBTEM):** FIBTEM is a measure of functional level of fibrinogen to the clot strength. It is a basic EXTEM assay where the contribution of platelets is inhibited using Cytochalasin D, thus providing the measure of contribution of fibrinogen and/or fibrin to the clot strength (Innerhofer, Streif, Kuhbacher, & Fries, 2004). A low EXTEM and FIBTEM MCF are indicative of defects in functional aspects of platelets and fibrinogen. So a relative contribution of platelets should be assessed using platelet count. A reduced EXTEM MCF but normal FIBTEM MCF indicates either absence or malfunction of platelets. If the laboratory tests show normal platelet number, platelet dysfunction can be considered. A differential diagnosis of thrombocytopenia or hypofibrinogenemia is made using these two assays.

**APTEM:** Is a basic EXTEM assay where addition of aprotinin, a plasmin inhibitor is used to confirm hyperfibrinolysis (Schochl et al., 2009; Ganter & Hofer, 2008).
**HEPTEM:** In HEPTEM assay, coagulation is initiated via INTEM assay with the addition of lyophilized heparinise which inactivates heparin and is used to assess heparin effect.

A typical ROTEM® trace in each assay provides the following information (Figure 1.7):

**Clotting time (CT, sec)** – is measured as the time from initiation of test until the blood starts to clot. CT detects initiation of clotting by extrinsic and intrinsic activation (Ganter & Hofer, 2008). It is the measurement from the commencement of clot activation until the new clot attains two millimeter thickness. An extended CT in EXTEM and INTEM assay reflects the deficiency in clotting factors (II, VII and X) and fibrinogen (Weiss, Lison, Spannagl, & Heindl, 2010). Clotting time exceeding 80 sec has been shown to be associated with reduced levels of clotting factor II, VII and X by 36 % (Weiss et al., 2010). Extended CT is used to guide administration of fresh frozen plasma (FFP), prothrombin complex concentrate (PCC) and fibrinogen to maintain CT within the normal range (42-79 sec).

**Clot formation time (CFT, sec)** – is measured as the time from clot initiation to reach a thickness of 20 mm which is dependent on thrombin formation, fibrin formation and contribution of platelets to form a clot.

**Maximum clot firmness (MCF, mm)** - is a measure of the highest recorded amplitude or thickness of the TEMogram, and reflects the strength of the clot which is a result of the interaction of thrombin activated platelets number and function and fibrinogen levels. A decrease in MCF is due to either deficiency in platelet number or function, deficit in fibrinogen levels and/or defects in fibrin polymerization or defects in activity of factor XIII. A weak clot is associated with higher risk for bleeding, massive transfusion and mortality (Davenport et al., 2011; Hagemo et al., 2015; Leemann et al., 2010; Tauber et al., 2011; Schochl et al., 2011a). EXTEM and FIBTEM MCF are used for the differential diagnosis of platelet deficiency and fibrinogen deficiency. A reduced MCF in EXTEM should be confirmed with MCF in FIBTEM trace. A normal FIBTEM-MCF (>8-10mm) trace and a reduced EXTEM-MCF (<45mm) are indicative of either malfunction or deficiency of platelets (Larsen, Fenger-Eriksen, Christiansen, Ingerslev, & Sorensen, 2011). EXTEM MCF <35 mm warrants the administration of fibrinogen and platelets (Lang et al., 2009). A parallel laboratory test to confirm platelet count is suggested.
to confirm thrombocytopenia. Studies have reported a correlation between low levels of FIBTEM MCF and laboratory fibrinogen (Rugeri et al., 2007; Tauber et al., 2011).

**Clot amplitude (CA, mm)** - is the amplitude of the clot measured at different time intervals such as 5, 10, 15, 20, 25 and 30 minutes respectively. A normal TEM trace takes approximately 30 minutes to reach the highest clot firmness (Woolley et al., 2013). However Clot amplitude (CA) at five or ten minutes have been shown to predict an upcoming MCF and can be used to assess the clot strength and predict coagulopathy within minutes (Schochl, Maegele, Solomon, Gorlinger, & Voelckel, 2012; Gorlinger, Dirkmann, Solomon, & Hanke, 2013; Woolley et al., 2013).

**Maximum lysis (ML, %)** - is a measure of the difference between MCF and the clot firmness during the entire test duration. ML > 15% reflects hyperfibrinolysis. A complete breakdown of the clot within 20 min (ML=100%) is considered as fulminant hyperfibrinolysis and carries 100% mortality. (Levrat et al., 2008; Schochl et al., 2009; Theusinger et al., 2011) ML > 15% within 30-60 minutes is intermediate HF whereas ML after 60min is considered as late HF (Levrat et al., 2008; Schochl et al., 2009).

**Lysis index (LI, %)** - is the quantity or percentage of a stable clot remaining in relation to MCF following CT and is measured at 30 and 60 minutes. A patient with normal hemostasis should have a normal value at 94%. LI 60 < 85% represents hyperfibrinolysis.

![Graphical representation of a ROTEM® trace](image)

Figure 1.7: Graphical representation of a ROTEM® trace (TEM international GmbH) and parameters definitions (Reproduced with permission from Tem Innovations GmbH, Germany).
1.5 Treatment strategies in trauma resuscitation

Hemorrhage following major trauma is the leading cause of preventable deaths which occurs as early as 6-12h (Cothren, Moore, Hedegaard, & Meng, 2007; Sauaia et al., 1995). Timely recognition of coagulopathy in bleeding patients may help reduce further blood loss, restore blood volume and attain hemostasis.

1.5.1 Damage control resuscitation (DCR)

Damage control resuscitation, a term originally coined by US Navy, was a technique used to rapidly stop hemorrhage and contamination of open wounds with immediate surgery aiming to reinstate normal physiology. It is the medical technique used in the resuscitation of critically injured trauma patients which includes very early delivery of blood and blood products as initial resuscitation fluids, early control of ongoing bleeding, and restoration of blood volume and maintenance of hematologic stability using temporary and most reliable means (Holcomb et al., 2007; Brohi et al., 2003; Hess, Holcomb, & Hoyt, 2006). As the DCR techniques have shown to decrease mortality in critically injured trauma patients, it has become the norm in early treatment of trauma patients. DCR aims to manage early coagulopathy and avoids dilutional coagulopathy by limiting large volume of crystalloid administration. DCR is performed simultaneously with damage control surgery.

1.5.2 Haemostatic resuscitation

Hemostatic resuscitation is the early use of blood and blood products to manage acute coagulopathy and to prevent development of dilutional coagulopathy (Holcomb et al., 2007). This strategy improves hemostasis, reduces bleeding, minimizes clinical complications and improves outcomes (Spinella & Holcomb, 2009). Transfusion practices in trauma have been constantly evolving from delivery of whole blood to component therapies which is most commonly used in many trauma centers (Hess & Thomas, 2003; Hoyt et al., 2008).

1.5.2.1 Avoidance of Hemodilution

Early administration of large volume of crystalloids and colloids has shown to be associated with worsening coagulopathy in trauma patients (Maegele et al., 2007) and development of adverse
clinical outcomes in critically ill patients (Moore & Shires, 1968; Cotton, Guy, Morris, Jr., & Abumrad, 2006). Limiting the use of these colloids is necessary to avoid hemodilution. Starch based colloids bind to fibrinogen and von Willeband factor thus reducing their functional ability leading to abnormal fibrin polymerization and poor clot quality (Fenger-Eriksen, Tonnesen, Ingerslev, & Sorensen, 2009). The colloid plasma expanders have also shown to produce erroneous fibrinogen values by interfering with fibrinogen analysis using Clauss method (Fenger-Eriksen, Moore, Rangarajan, Ingerslev, & Sorensen, 2010). To avoid dilution coagulopathy, minimizing use of crystalloids and use of RBCs, plasma and platelets in a 1:1:1 ratio for significantly bleeding trauma patients is advocated according to the DCR principles.

1.5.2.2 Component therapy – Red Blood Cells (RBC)

Administration of RBC to bleeding patients is necessary to restore oxygen delivery, circulating volume and maintenance of hemostasis. Studies have shown survival benefits of using early administration of RBCs in trauma patients (Rossaint et al., 2010; Brakenridge et al., 2011; Brown et al., 2011; Holcomb et al., 2011; Cotton et al., 2009). The optimum hematocrit levels or hemoglobin concentrations to achieve hemostasis are unknown. However, hemoglobin levels of 7-9g/dL was recommended by recent European trauma guidelines (Rossaint et al., 2010); and a hematocrit value greater than 30% (Hardy, de, & Samama, 2004; Reiss, 2000).

1.5.2.3 Component therapy – Fresh Frozen Plasma

Fresh frozen plasma is an integral component of massive transfusion protocol and is used to treat various coagulation defects for many decades (Sorensen & Fries, 2012). United States and British military practice recommends the delivery of FFP and RBC in 1:1 ratio for patients predicted to require massive transfusion (Jansen, Thomas, Loudon, & Brooks, 2009; Holcomb et al., 2007; Borgman et al., 2007). It contains both coagulation factors and inhibitors and hence used as the first line therapy in major trauma centers (Spinella & Holcomb, 2009). One retrospective study in military trauma patients reported a 46% reduction in mortality when FFP:RBC was used in the ratio of 1:1 to manage massive transfusion (Borgman et al., 2007). Following this observation, more studies reported the utility of higher ratios of FFP for massive transfusion with conflicting results in survival (Duchesne et al., 2008; Maegele et al., 2008; Holcomb et al., 2008; Gunter, Jr. et al., 2008; Scalea et al., 2008; Teixeira et al., 2009b). The
delay due to thawing often causes delay in administration in case pre thawed plasma is not available.

1.5.2.4 Component therapy – Platelets

In critically bleeding patients a platelet count <50 \times 10^9/l should be avoided (Contreras, 1998), which is also recommended by British Committee for Standards in Haematology Blood Transfusion Task Force, 2003 (British Committee for Standards in Haematology & Blood Transfusion Task Force (Chairman P.Kelsey), 2003). Administration of platelets in a 1:1 ratio with RBC is recommended by military guidelines for hemorrhagic shock though substantial research data is lacking to back this recommendation.

1.5.2.5 Fresh Whole Blood (FWB)

Fresh whole blood was used decades ago in combat patients requiring massive transfusion since World War I as it has all blood components, platelets including clotting factors. However its use declined due to transfusion associated side effects as well implementation of component therapy in late 1980s (Kreimeier, Lackner, Pruckner, Ruppert, & Peter, 2002). A military study recently reported association between FWB use and improved survival when component therapy is not readily available and pre-screened blood donors are readily available within short notice (Spinella, 2008). However safety concerns pertaining to infectious disease should be considered for its use outside military trauma. This finding has motivated the implementation of component therapy using RBC, FFP and platelets to mimic “whole fresh blood” in civilian trauma.

1.5.2.6 Fibrinogen replacement

Fibrinogen is a key clotting factor for clot formation which provides a milieu and mesh network important for clot stability. In massive bleeding, fibrinogen is the first coagulation factor to deplete to critical level even before other coagulation factors or platelets indicating consumption, dilution and fibrinolysis (Davenport et al., 2011; Hagemo et al., 2014; Hiippala, 1998; Martini et al., 2005). Fibrinolysis is secondary to coagulation cascade; there are other factors that are responsible for low levels of fibrinogen in plasma. They include increased fibrinolytic activity due to acidosis (Martini & Holcomb, 2007), dilution due to fluid administration (Schlimp, Cadamuro, Solomon, Redl, & Schochl, 2013), loss due to bleeding, decreased synthesis due to
hypothermia (Martini, 2007) as well as alteration in fibrinogen/fibrin polymerization due to colloid infusions (Schlimp et al., 2013). There are three sources of fibrinogen that are currently available: fibrinogen concentrate (FC), FFP and cryoprecipitate. Cryoprecipitate is the most common source of fibrinogen and routinely used in the United Kingdom and North America. It is prepared by thawing FFP at 1°C followed by centrifugation and re suspending the precipitate in 5-15 ml of plasma which is then frozen within one hour and finally stored it at -18°C (Callum, Karkouti, & Lin, 2009). It is indicated for fibrinogen deficiency due to bleeding. Human FC derived from human plasma is the only source of fibrinogen with known concentration. It does not require ABO compatibility, can be administered at bedside or outside the hospital and does not pose transfusion-associated risks (Spahn et al., 2013; Vlaar, Schultz, & Juffermans, 2009; Malone et al., 2003).

European guidelines on blood component transfusion recommend fibrinogen administration at plasma fibrinogen concentration of 1.0g/L-2.0g/L (Rossaint et al., 2010) and as per the recent updated guidelines, < 2g/L (Spahn et al., 2013). Major guidelines on blood component transfusion in the United Kingdom (UK), United States (US) and Canada recommends the administration of cryoprecipitate when plasma fibrinogen level falls below 1.0g/L and when FC is not available (O'Shaughnessy et al., 2004; Levi, Toh, Thachil, & Watson, 2009; 1996; 2006). An adult dose of 8-12 units is recommended (Ketchum, Hess, & Hiippala, 2006).

There have been few studies on the use of fibrinogen concentrate in traumatic hemorrhage. Four case studies have reported successful management of coagulopathy using FC in conjunction with other blood products, guided by thromboelastometry findings (Schochl, Forster, Woidke, Solomon, & Voelckel, 2010; Schochl, Posch, Hanke, Voelckel, & Solomon, 2010; Grassetto et al., 2012; Brenni, Worn, Bruesch, Spahn, & Ganter, 2010). These studies used goal directed management with or without antifibrinolytics and other coagulation factors however, based on these case reports a strong conclusion about reduction of blood product usage or mortality cannot be drawn. Three studies reported association between higher ratio of FC: RBC and improved survival in significantly bleeding patients (Shaz et al., 2010; Dente et al., 2009; Stinger et al., 2008). One study reported 14% reduction in mortality using FC as first line therapy followed by administration of PCC to manage trauma coagulopathy (Schochl et al., 2010). A recent case
matched study reported reduction in six-hour mortality and increased time to death (Wafaisade et al., 2013). However improvement in overall hospital mortality was not observed in this study.

Two studies did not show any survival benefits using FC in the management of coagulopathy (Nienaber et al., 2011; Schochl et al., 2011b) and two studies reported reduction in transfusion (RBC and platelets) need (Nienaber et al., 2011; Schochl et al., 2011b). More studies are required investigating safety of this product.

1.5.2.7 Prothrombin Complex Concentrate (PCC)

Risks (eg: Trauma associated acute lung injury (TRALI) associated with FFP transfusion (Watson, Hoffman, & Peitzman, 2015) has necessitated the need of alternative methods to prevent or reverse coagulopathy. Prothrombin complex concentrate is used as first line trauma resuscitation therapy in European countries. It is a plasma derived vitamin K-dependent coagulation factor product consisting of factors II, VII, IX and X and is used for rapid replenishment of factors. This product is available as 3-factor and 4-factor PCC. Three factor PCC contains Vitamin K-dependent factors II, IX and X while 4-factor PCC contains factors II, IX, X and factor VII (Bershad & Suarez, 2010; Bruce & Nokes, 2008). Schochl (Schochl et al., 2011b) reported that use of PCC with fibrinogen reduced transfusion requirements in trauma patients. However the mortality benefit between the treatment groups was not statistically significant (7.5% in fibrinogen-pcc group and 10% in the FFP group, p=0.69). Nienbar observed a reduction in RBC transfusion when patients were treated with PCC vs. FFP transfusion. The group however failed to show an improvement in survival (Nienaber et al., 2011). Even though large studies with high quality of data are lacking, few small studies have shown the reversal of coagulopathy using PCC in trauma patients with or without pre injury warfarin use (Safaoui, Aazami, Hotz, Wilson, & Margulies, 2009; Joseph et al., 2012). The use of PCC as an adjunct to FFP was also found to be associated with reduction in blood product use and overall costs (Joseph et al., 2014). Due to limitations of small studies in terms of data, use of PCC to correct coagulopathy in trauma needs to be studied in a larger cohort of patients to provide statistically significant results.

1.5.3 Other haemostatic agents

1.5.3.1 Tranexamic acid (TXA)
Tranexamic acid is a powerful inhibitor of fibrinolysis leading to reduced bleeding (Perel et al., 2012). A large randomized study explored the effect of TXA on survival benefits and transfusion requirements in 20,000 trauma patients (Shakur et al., 2010). The TXA administration in these patients showed reduction in all-cause mortality (RR: 0.85; 95% CI 0.76–0.96). The subsequent analysis in the same study reported that the benefits were higher when TXA was delivered within the first three hours of injury (Roberts et al., 2011).

1.5.3.2 Recombinant Factor VIIa (rVIIa)

Recombinant factor VIIa, is manufactured using recombinant DNA technology. It is, currently approved by United States Food and Drug Administration (FDA) for the treatment of hemophilia, in patients with congenital Factor VII deficiency; to suppress surgical or invasive procedures in hemophilia A or B patients and in acquired hemophilia (Mittal & Watson, 2006). It has also been used to treat coagulopathy in trauma patients. Two of the studies in trauma demonstrated a reduction in RBC use in the presence of rVIIa while failing to show reduction in mortality (Boffard et al., 2005; Hauser et al., 2010). On that premise, there is no sufficient evidence to support the use of rFVIIa as routine treatment for bleeding due to trauma.

1.6 Risks and adverse events associated with transfusion

1.6.1 Infectious complications

Although transfusion of blood and blood products is lifesaving, it also carries several risks causing morbidity and mortality in recipients. Bacterial contamination is common in platelet transfusion than in RBCs as the storage temperature for platelets is 20-24°C, where many bacteria can survive and multiply at this temperature (Brecher & Hay, 2005; Hillyer et al., 2003). The contamination mainly arises from the skin of the donors, the bacteria being mostly *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae* and Group B Streptococci causing sepsis and hemolytic reactions (Palavecino, Yomtovian, & Jacobs, 2010). The most common viral infections associated with blood products transfusion are Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and West Nile virus (WNV) (O’Brien et al., 2007). Parasitic infections are also found to be transmitted via blood transfusion (Kitchen & Chiodini, 2006). Malaria caused by plasmodium species is the most common infection transmitted via blood transfusions in developing countries.
Chagas disease transmitted by *Trypanosoma cruzi*, is endemic to Central and South America as well as parts of Mexico causes flu like symptoms, hepatosplenomegaly and lymphadenomegaly (Leiby, 2004; Leiby, Herron, Jr., Read, Lenes, & Stumpf, 2002). Other parasites such as *Babesia microti* and *Leishmania donovani* have been shown to be transmitted by blood cause clinical illness in immunocompromised patients (Leiby, 2004; Cardo, 2006). Variant Creutzfeldt-Jacob disease (vCJD), the etiologic agent of transmissible spongiform encephalopathy is transmissible by blood transfusion (Dodd, 2007). However transmission of this disease through blood transfusion is found to be very rare limiting to four cases in the United Kingdom (Turner & Ludlam, 2009).

1.6.2 Immune mediated complications

Transfusion related Immune mediated complications are rare but life threatening. Some complications are minor such as febrile illness with an incidence rate of 1 in 50-300 transfusion units and urticaria with incidence rate of 1 in 50-100 transfusion units (Eder et al., 2007; Klein, Spahn, & Carson, 2007). The severe and life threatening complications, though less common, include acute hemolytic reactions, anaphylaxis, TRALI, transfusion associated circulatory overload (TACO), transfusion related graft versus host disease (GvHD) and transfusion related immune modulation (TRIM).

Acute hemolytic transfusion reactions occur due to ABO incompatible blood transfusions and result in increased degradation of red cells following transfusion resulting in hemoglobinemia, hemoglobinuria, increased lactate dehydrogenase and serum glutamic-oxaloacetic transaminase levels and low hemoglobin concentration (Davenport, 2005). Incidence rate of 1: 38,000 RBC transfusions are ABO incompatible due to incorrect transfusions to the patient (Linden, Wagner, Voytovich, & Sheehan, 2000). Anaphylaxis is rare but severe form of allergic reaction which occur in 1:20,000 to 1:50,000 transfusions causing shock and hypotension (Domen & Hoeltge, 2003; Klein et al., 2007). Transfusion related acute lung injury occurs due to antileukocyte antibodies in plasma obtained from multiparous female donors (Popovsky & Moore, 1985). The clinical presentation is characterized by respiratory distress, hypotension, fever and cyanosis which occur within six hours of transfusion (Bux & Sachs, 2008; Popovsky & Moore, 1985). Variations in incidence of TRALI has been reported, however it is estimated to be approximately...
1:5000 blood component transfusions for antibody mediated TRALI (Popovsky & Moore, 1985). Majority of TRALI are immune mediated which results from blood products containing human anti-neutrophil antibodies (HNA) or anti-leukocyte antibodies (HLA) that cross-react with recipients’ related antigens. As majority of TRALI are immune-mediated, excluding donors having Human Leucocyte Antigen (HLA) or Human Neutrophil Antibodies (HNA) has been implemented. Transfusion associated circulatory overload is a rare occurrence (<1%) and can cause pulmonary edema secondary to congestive heart failure within six hours of transfusion (Bux & Sachs, 2008). Transfusion related graft versus host disease is also a rare occurrence and occurs following transfusion of cellular blood products containing lymphocytes with high fatality rates. The progression of disease is dependent on engraftment and proliferation of donor T-lymphocytes. Transfusion related immune modulation leads to transient immune suppression in recipient, following blood transfusion, known to be mediated by allogeneic leukocytes. The exact incident rates are unknown.

1.7 Summary

Despite advancement in haemostatic resuscitation, exsanguinating hemorrhage continues to be the leading cause of preventable early deaths. Severely injured bleeding patients with established TIC on arrival are likely to require more transfusion, develop more severe in-hospital complications, and have higher mortality rates. While maintenance of hemostasis is important, homeostatic resuscitation on its own carries significant risks. Treatment options should be tailored based on the extent of traumatic injury and circumstances surrounding exsanguination. Early recognition and prompt treatment are paramount for improved outcomes.

Limitations of current laboratory assays and their clinical utility to diagnose and identify patients who may be coagulopathic have led to investigation of more advanced real time viscoelastic methods such as ROTEM® to diagnose and guide resuscitative measures. There is a growing body of evidence on the use of ROTEM® in cardiac surgery and liver transplant procedures. Its use in the area of trauma is rapidly emerging (Levrat et al., 2008; Schochl et al., 2009; Schochl et al., 2010; Schochl et al., 2010; Theusinger et al., 2011). With new diagnostic modalities and management options, rapid identification, control of bleeding and correction of coagulopathy may help save lives from hemorrhage following traumatic injury.
CHAPTER 2 A systematic review on the rotational thrombelastometry (ROTEM®) values for the diagnosis of coagulopathy, prediction and guidance of blood transfusion and prediction of mortality in trauma patients

An abridged version of the following chapter has been published in October 2016 (Veigas et al., 2016).


Permission was received to publish this manuscript in this dissertation.
2.1 Abstract

**Introduction:** Viscoelastic assays have been promoted as an improvement over traditional coagulation tests in the management of trauma patients. Rotational thromboelastometry (ROTEM®) has been used to diagnose coagulopathy and guide hemostatic therapy in trauma. This systematic review of clinical studies in trauma investigates the ROTEM® parameters thresholds used for diagnosing coagulopathy, predicting and guiding transfusion and predicting mortality.

**Methods:** Systematic literature search was performed using MEDLINE, EMBASE and Cochrane databases. We included studies without restricting year of publication, language or geographic location. Original studies reporting the thresholds of ROTEM® parameters in the diagnosis or management of coagulopathy in trauma patients were included. Data on patient demographics, measures of coagulopathy, transfusion and mortality were extracted. We reported our findings according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines. Quality assessment and risk of bias were performed using Newcastle Ottawa Scale (NOS) and the quality assessment of diagnostic accuracy studies (QUADAS-2) tools, respectively.

**Results:** A total of 13 observational studies involving 2835 adult trauma patients met the inclusion criteria. Nine studies were prospective and four were retrospective. There were no randomized controlled trials. The quality of the included studies was moderate (mean NOS 5.92, standard deviation 0.26). Using QUADAS-2, only 1 study (7.6 %) had low risk of bias in all domains, and 9 studies (69.2 %) had low risk of applicability concerns. Outcomes from 13 studies were grouped into three categories: diagnosis of coagulopathy (n = 10), prediction of massive transfusion or transfusion guidance (n = 6) and prediction of mortality (n = 6). Overall, specific ROTEM® parameters measured (clot amplitude and lysis) in the extrinsically activated test (EXTEM) and the fibrin-based extrinsically activated test (FIBTEM) were consistently associated with the diagnosis of coagulopathy, increased risk of bleeding and massive transfusion, and prediction of mortality. Presence of hyperfibrinolysis by ROTEM® was associated with increased mortality.
Conclusions: Most of the evidence indicates that abnormal EXTEM and FIBTEM clot amplitude (CA5, CA10) or maximal clot firmness (MCF) diagnose coagulopathy and predict blood transfusion and mortality. The presence of fibrinolysis (abnormal lysis index [LI30] or maximum lysis [ML]) was also associated with mortality. ROTEM® thus, may be of value in the early management of trauma patients.

Keywords: Acute trauma coagulopathy, Thromboelastometry, Transfusion, Threshold, Bleeding

2.2 Overview

The overall goal of this work was to investigate the use of ROTEM® parameters for early resuscitation of significantly bleeding trauma patients with the future intention to develop transfusion guidelines.

To achieve this goal, we needed to know the existing published thresholds of ROTEM® used in the diagnosis of coagulopathy, guiding transfusion including massive transfusion and predicting mortality in significantly bleeding trauma patients. The study was also geared towards understanding the definitions used to describe coagulopathy of trauma, transfusion of blood products, massive transfusion and mortality.

This systematic review may form a strong base for future research activities which aim to improve outcomes in significantly bleeding trauma patients to tailor hemostatic therapy or to withhold futile transfusion with the aid of viscoelastic testing methods. To have a role in resuscitation these test thresholds require proper evaluation with a strong evidence base. Conducting systematic reviews is an effective means of achieving this as they provide strong research evidence that forms the basis of evidence-based practice.

Policy makers, scientists and physicians are loaded with vast amount of scientific information where there is a need for systematic reviews to collate existing evidence and provide current data for decision making (Cynthia D Mulrow, 1994). A systematically performed literature review collates evidence on the effectiveness of intervention by assessing the consistency and generalizability of research findings from multiple studies in an unbiased manner (Woolf, 1992). Systematic reviews are the foundations for meta-analyses and clinical guidelines. Clinical guidelines should have strong evidence base and this is achieved by a systematic and unbiased
thorough review of literature. The important principles of a systematic review include an explicit and reproducible search strategy, choice of search engines, predefined eligibility criteria and quality assessments.

The objective of this chapter is to systematically review the defined thresholds of ROTEM® used in the diagnosis of coagulopathy, guiding transfusion including massive transfusion and predicting mortality in trauma patients. However due to scarcity of data from prospective studies, a meta-analysis was deemed inappropriate and a descriptive review was considered. The outcomes of this systematic review were used to explore viscoelastic test variables as independent predictors of transfusion requirements and 24h mortality in trauma patients.

2.3 Introduction

The degree of injury and hypoperfusion are implicated as initiators of the Acute Coagulopathy of Trauma/Shock (ACoTS) that occurs immediately after injury (Brohi et al., 2003; Kauvar, Lefering, & Wade, 2006). ACoTS occurs in approximately 25% of all severely injured patients and is associated with a three-fold increase in mortality (Brohi et al., 2003). Patients with ACoTS have complex coagulation defects, higher transfusion requirements, organ dysfunction, longer hospital stays and poorer outcomes including higher mortality rates (Brohi et al., 2003; MacLeod et al., 2003; Maegele et al., 2007; Sauaia et al., 1995).

Current standards for the management of ACoTS are based mainly on results of standard coagulation tests (SCTs) (Armand & Hess, 2003; Dzik, 2004; Frankel et al., 1994; Rossaint et al., 2010) such as prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (APTT), platelet count (PLT), and fibrinogen level (Duchesne et al., 2008).

Currently, many trauma centers use INR >1.5 and PLT <100x10⁹ L⁻¹ to establish the presence of coagulopathy (Spahn & Rossaint, 2005). These values are widely used and incorporated into existing guidelines, despite lacking robust evidence support (Dzik et al., 2011; Rossaint et al., 2010; Spahn & Rossaint, 2005; Spahn et al., 2013). Standard coagulation tests have long turnaround times (TAT), entail transportation of the sample to the laboratory, necessitate separation of plasma from the red cells and detect only the initial phases of clot formation (Dzik, 2004). These tests were originally developed with an intention to diagnose coagulation...
abnormalities in congenital bleeding disorders and to monitor anticoagulation therapy, and their role in guiding transfusion therapy in trauma have not been validated (Dzik, 2004). Patients with ACoTS would benefit from a test that could quickly identify coagulation abnormalities, permit transfusion guidance, reduce exposure to allogeneic blood products and improve clinical outcomes by guiding rapid correction of any hemostatic defect. Thromboelastography (TEG®) and rotational thromboelastometry (ROTEM®) have been used in cardiovascular surgery and liver transplantation (Gorlinger et al., 2011; Gorlinger et al., 2012; Gorlinger, 2006; Tanaka, Bolliger, Vadlamudi, & Nimmo, 2012) and, more recently, in trauma. The use of these devices may reduce hemorrhage as reported in a recent Cochrane systematic review (Afshari, Wikkelso, Brok, Moller, & Wetterslev, 2011) in patients requiring massive transfusion.

ROTEM® has recently been used as point-of-care (POC) test to optimize haemostatic resuscitation in trauma patients (Luddington, 2005). It utilizes a small volume of whole blood to assess hemostatic function from initiation of clot formation, clot propagation and clot lysis (Johansson et al., 2009). Additionally, ROTEM® provides information on platelet number/function and fibrinogen reserve. Luddington, in a review on ROTEM® assays and parameters, offers additional methodologic details about the test (Luddington, 2005). There are four assays which are run simultaneously: extrinsically activated test using tissue factor as activator (EXTEM) which detects defects associated with extrinsic pathway; intrinsically activated test using ellagic acid (INTEM) which detects defects of the intrinsic pathway; fibrinogen test (FIBTEM) using cytochalasin-D as platelet inhibitor which detects the contribution of fibrinogen to the clot; and a test using aprotinin inhibitor (APTEM), a test for hyperfibrinolysis (HF). In other clinical settings such as in liver transplantation, cardiac and vascular surgery, the use of ROTEM® has been linked to a reduction of exposure to allogeneic blood products and improvement of outcomes (Kang et al., 1985; Spalding et al., 2007; Trzebicki et al., 2010; Olde Engberink et al., 2014; Tanaka et al., 2012). In trauma, recent investigations suggest that ROTEM® can be used in the diagnosis of coagulopathy, prediction and guidance of transfusion and reduction of unnecessary exposure to allogeneic blood products (Doran, Woolley, & Midwinter, 2010; Schochl et al., 2009; Schochl et al., 2010; Woolley et al., 2013; Brenni et al., 2010; Schochl et al., 2011b; Schochl et al., 2011a). Though results of ROTEM® parameters are used for treatment decisions, the reported thresholds used to diagnose coagulopathy and to guide transfusion vary substantially among reports.
There is a need for determining ROTEM® parameters and their thresholds that establish the presence of coagulopathy, predict bleeding, guide the hemostatic resuscitation and predict mortality. We conducted a systematic review of literature to summarize the reported ROTEM® parameters and their thresholds for this purpose. The primary goal was to determine evidence-based thresholds that could be incorporated into ROTEM® algorithms in the trauma resuscitation protocols.

2.4 Methods

This descriptive systematic review was reported in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009).

2.4.1 Information sources and search technique

Two reviewers (PVV and LTDL) performed a systematic review of the indexed literature on ROTEM® studies that reported thresholds of ROTEM® parameters in trauma patients. The highly sensitive search strategy was developed by the review team in consultation with the health information specialist (Online supplement-Data sources and search strategy). We searched MEDLINE, EMBASE and EBM Reviews (Cochrane Database of Systematic Reviews) from 1946 to March 2016 without restricting language or geographic location. The reviewers checked titles, abstracts, full texts, and personally contacted the authors (or manufacturer's representative) to retrieve or clarify required information.

1 - Data sources and search strategy

Database: Ovid MEDLINE(R) <1946 to March Week 2 2016>

----------------------------------------------------------------------------------------------------------------------------

1 exp Thrombelastography/ (4040)

2 (thromb?elastomer* or thromb?elastogra* or (thromb* adj2 elastometr*) or (thromb* adj2 elastogr*) or ROTEM or TEG
or ROTEG).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word,
protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (5234)

3 1 or 2 [ROTEM] (5234)

4 Trauma.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (185523)

5 exp Trauma Centers/ (7479)

6 4 or 5 [TRAUMA] (185523)

7 3 and 6 (365)

8 limit 7 to yr="2015 -Current" (40)

9 remove duplicates from 8 (35)

10 limit 9 to english language (35)

Database: Embase Classic+Embase <1947 to 2016 Week 12>

---------------------------------------------------------------------------------------

1 exp Thrombelastography/ (6716)

2 (thromb?elastometr* or thromb?elastogra* or (thromb* adj2 elastometr*) or (thromb* adj2 elastogr*) or ROTEM or TEG
or ROTEG).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug
manufacturer, device trade name, keyword] (9720)

3  1 or 2 [ROTEM] (9720)

4  Trauma.mp. [mp=title, abstract, heading word, drug trade name, original title, device
manufacturer, drug

manufacturer, device trade name, keyword] (270348)

5  exp traumatology/ (9989)

6  exp emergency health service/ (76742)

7  4 or 5 or 6 [TRAUMA] (340246)

8  3 and 7 (852)

9  limit 8 to yr="2015 -Current" (122)

10 remove duplicates from 9 (121)

11 limit 10 to english language (119)

12 exp Thrombelastography/ (6716)

13 (thromb?elastometr* or thromb?elastogra* or (thromb* adj2 elastometr*) or (thromb* adj2 elasto* or ROTEM or

TEG or ROTEGL).mp. [mp=title, abstract, heading word, drug trade name, original title, device
manufacturer, drug

manufacturer, device trade name, keyword] (9720)

14  12 or 13 [ROTEM] (9720)

15  Trauma.mp. [mp=title, abstract, heading word, drug trade name, original title, device
manufacturer, drug
manufacturer, device trade name, keyword] (270348)

16 exp traumatology/ (9989)

17 exp emergency health service/ (76742)

18 15 or 16 or 17 [TRAUMA] (340246)

19 14 and 18 (852)

20 limit 19 to yr="2015 -Current" (122)

21 remove duplicates from 20 (121)

22 limit 21 to english language (119)

23 21 not 22 (2)

**Database: EBM Reviews - Cochrane Central Register of Controlled Trials <February 2016>**

1 exp Thrombelastography/ (181)

2 (thromb?elastometr* or thromb?elastogra* or (thromb* adj2 elastometr*) or (thromb* adj2 elastogr*) or ROTEM or TEG or ROTEG).mp. [mp=title, original title, abstract, mesh headings, heading words, keyword] (417)

3 1 or 2 [ROTEM] (417)

4 Trauma.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword] (6145)

5 exp Trauma Centers/ (134)
4 or 5 [TRAUMA] (6145)

3 and 6 (17)

limit 7 to yr="2015 -Current" (3)

remove duplicates from 8 (3)

limit 9 to english language (3)

Database: EBM Reviews - Cochrane Database of Systematic Reviews <2005 to March 16, 2016>

(thromb?elastometr* or thromb?elastogra* or (thromb* adj2 elastometr*) or (thromb* adj2 elastogr*) or ROTEM or TEG or ROTEG).mp. [mp=title, abstract, full text, keywords, caption text] (14)

Trauma.mp. [mp=title, abstract, full text, keywords, caption text] (1473)

1 and 2 (7)

limit 3 to last year (1)

2.4.2 Eligibility criteria and study selection

We searched for observational studies and randomized controlled trials (RCTs) in trauma where cut off values of ROTEM® parameters were reported in: (1) diagnosing coagulopathy; (2) predicting or guiding transfusion; and, (3) predicting mortality. We excluded animal studies, studies assessing patients with thermal injuries, case reports, case series involving <10 patients, and abstracts from conferences. Outcomes included accuracy in diagnosing coagulopathy, predicting massive transfusion, diminishing exposure to allogeneic blood products and predicting mortality. The reviewers independently screened titles and abstracts of all articles in a hierarchical manner by following the PRISMA guidelines (Liberati et al., 2009; Moher, Liberati, Tetzlaff, & Altman, 2010). Titles were categorized as “include”, “exclude” or “undetermined”
using an excel spreadsheet. The articles classified as “include” and “undetermined” by either
reviewer were included for full text evaluation at the next level. Any discrepancies concerning
agreement at both levels were resolved by discussion, consensus or consultation with a third
reviewer (SR). Inter-rater agreement for inclusion was assessed using Cohen’s Kappa (Cohen,
1968).

2.4.3 Data abstraction and analysis

The reviewers independently collected data using standardized forms developed in collaboration
with the study team. Reviewers were not blinded to the author or publication source of studies.
The following data were collected: author, year, country, design, control group, patient
population, duration of study, sample size, study objective, patient characteristics, and outcomes
(coagulopathy, exposure to allogeneic blood products, and mortality). We also retrieved
information regarding ROTEM® parameters, data on accuracy (sensitivity and specificity) of
SCTs thresholds and ROTEM® thresholds used to diagnose coagulopathy, guide transfusion,
predict exposure to allogeneic blood products, and predict mortality. The methodological quality
of the studies was assessed using the Newcastle-Ottawa Scale (2016) and Quality Assessment of
Diagnostic Accuracy Studies-2 tool (QUADAS-2) (Whiting et al., 2011) by both reviewers
(PVV, LTDL).

2.5 Results

The search strategy identified a total of 1220 citations through MEDLINE, Cochrane and
EMBASE data bases. We excluded 1179 citations as they were either unrelated to our question
or were duplicates. Forty one citations were deemed relevant and were reviewed at full text level.
Thirteen studies enrolling a total of 2835 patients met the eligibility criteria and were included
(Figure 2.1) (Rugeri et al., 2007; Levrat et al., 2008; Schochl et al., 2009; Leemann et al., 2010;
Doran et al., 2010; Schochl et al., 2010; Tauber et al., 2011; Davenport et al., 2011; Rourke et
al., 2012; Woolley et al., 2013; Hagemo et al., 2015; Schochl et al., 2011c; Schochl et al.,
2011a). We searched the references within each included study, and no additional studies were
identified. The Kappa statistics for inter-rater agreement for titles plus abstracts, and full
manuscript screening was 0.84 and 0.76, respectively.
2.5.1 Study characteristics

Ten studies (Table 1) were conducted in Europe (Davenport et al., 2011; Leemann et al., 2010; Levrat et al., 2008; Rourke et al., 2012; Rugeri et al., 2007; Schochl et al., 2009; Schochl et al., 2010; Schochl et al., 2011c; Schochl et al., 2011a; Schochl et al., 2011b; Schochl et al., 2011c; Schochl et al., 2011a; Schochl et al., 2011c) and two in Afghanistan, conducted by United Kingdom (UK) military researchers (Doran et al., 2010; Woolley et al., 2013) and one study included sites in both Europe and the UK (Hagemo et al., 2015). All 13 studies were conducted in adult patients, included blunt and penetrating injuries, in the civilian (Davenport et al., 2011; Leemann et al., 2010; Levrat et al., 2008; Rourke et al., 2012; Rugeri et al., 2007; Schochl et al., 2009; Schochl et al., 2010; Tauber et al., 2011; Hagemo et al., 2015; Schochl et al., 2011a; Schochl et al., 2011c) or in the military settings (Doran et al., 2010; Woolley et al., 2013). A single study included patients above 13 years of age (Davenport et al., 2011) and no study was conducted exclusively in pediatric trauma patients.

![Figure 2.1: Flow diagram of included studies](image-url)
All but two (Rourke et al., 2012; Hagemo et al., 2015) studies were single centered. (Davenport et al., 2011; Leemann et al., 2010; Levrat et al., 2008; Rugeri et al., 2007; Schochl et al., 2009; Schochl et al., 2010; Tauber et al., 2011; Schochl et al., 2011a; Schochl et al., 2011c) The sample sizes varied from 25 (Doran et al., 2010) to 808 (Hagemo et al., 2015) (median sample size 88, interquartile range 53 - 323). The median age of patients included in the studies ranged from 21 (IQR 18-35) (Doran et al., 2010) to 47 (IQR 26-66) years (Schochl et al., 2011c). The percent of male patients ranged from 67% (Levrat et al., 2008) to 100% (Woolley et al., 2013). All studies provided information on injury severity score (ISS), and the median ISS ranged from 12 (IQR 4-25) (Davenport et al., 2011) to 75 (IQR 75-75) (Levrat et al., 2008).
Table 2.1 – Summary of studies included in the review

<table>
<thead>
<tr>
<th>Author, region</th>
<th>Study design, centers, patients, years</th>
<th>N</th>
<th>Objective</th>
<th>ISS (mean ±SD or median/IQR)</th>
<th>Age (mean or median)</th>
<th>Sex Male, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rugeri, 2006 (Rugeri et al., 2007) France</td>
<td>Single center prospective, civilian, Jul 2004-Oct 2004</td>
<td>88</td>
<td>Detect coagulopathy guide transfusion</td>
<td>22 (12-34)</td>
<td>34 (±16)</td>
<td>68 (77%)</td>
</tr>
<tr>
<td>Schochl, 2009 (Schochl et al., 2009) Austria</td>
<td>Single center prospective, civilian, Jan 2003-Dec 2007</td>
<td>33</td>
<td>Diagnosis of HF Predict mortality</td>
<td>47 ± 14</td>
<td>45(20-88)</td>
<td>22 (67%)</td>
</tr>
<tr>
<td>Leemann, 2010 (Leemann et al., 2010) Zurich</td>
<td>Single center retrospective, civilian, Jan 2006-Dec 2006</td>
<td>53</td>
<td>Predict MT</td>
<td>31.1 ± 1.7</td>
<td>39.6 (±2.5)</td>
<td>40 (75.5%)</td>
</tr>
<tr>
<td>Schochl, 2010 (Schochl et al., 2010) Austria</td>
<td>Single center retrospective, civilian, Jan 2005-Apr 2009</td>
<td>13</td>
<td>Guide transfusion</td>
<td>38 ± 15</td>
<td>46 ± 18</td>
<td>96 (73%)</td>
</tr>
<tr>
<td>Tauber, 2011 (Tauber et al., 2011) Austria</td>
<td>Single center, prospective, civilian, Jul 2005-Jul 2008</td>
<td>33</td>
<td>Detect coagulopathy Predict RBC transfusion</td>
<td>34 (24-45)</td>
<td>43(27-56)</td>
<td>260 (77.8%)</td>
</tr>
<tr>
<td>Schochl, 2011 (Schochl et al., 2011a) Austria</td>
<td>Single center retrospective, civilian, Jan 2005-Dec 2010</td>
<td>32</td>
<td>Predict mortality</td>
<td>Non-MT group: 27 (20-34)</td>
<td>44 (26-59)</td>
<td>245 (78%)</td>
</tr>
<tr>
<td>Schochl, 2011 (Schochl et al., 2011a) Austria</td>
<td>Single center retrospective, civilian, Jan 2005-Dec 2010</td>
<td>32</td>
<td>Predict mortality</td>
<td>MT group: 42 (34-50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Study Design</td>
<td>Sample Size</td>
<td>Detect Coagulopathy</td>
<td>Predict MT</td>
<td>MT Data</td>
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</tr>
<tr>
<td>Rourke, 2012 (Rourke et al., 2012) England</td>
<td>Multicenter prospective, civilian, Jan 2008-Dec 2010</td>
<td>51</td>
<td>Detect coagulopathy</td>
<td>14 (8-27)</td>
<td>36 (23-51)</td>
<td>405 (78%)</td>
</tr>
<tr>
<td>Hagemo 2015 (Hagemo et al., 2015)</td>
<td>Multi center prospective civilian, Jan 2007-Nov 2011</td>
<td>80</td>
<td>Detect coagulopathy, Predict MT</td>
<td>16 (20)</td>
<td>38 (28)</td>
<td>625 (77.4%)</td>
</tr>
</tbody>
</table>

**Legend:** FC – fibrinogen concentrate, CA5 – amplitude of the clot at 5 minutes, CA10 – amplitude of the clot at 10 minutes, ISS – injury severity score, HF – hyperfibrinolysis, MCF – maximum clot firmness; MT – massive transfusion, ROTEM® – rotational thromboelastometry, SLTs – standard laboratory tests, TBI – traumatic brain injury
2.5.2 Methodological quality

There were no randomized controlled trials identified. Nine cohort studies were prospective (Davenport et al., 2011; Doran et al., 2010; Levrat et al., 2008; Rourke et al., 2012; Rugeri et al., 2007; Schochl et al., 2009; Tauber et al., 2011; Woolley et al., 2013; Hagemo et al., 2015) and 4 were retrospective (Leemann et al., 2010; Schochl et al., 2010; Schochl et al., 2011c; Schochl et al., 2011a). The studies had moderate methodological quality as determined by the Newcastle Ottawa scale (NOS) (Table 2) with a mean score of 5.92 (SD = 0.26), with a possible range of 1 to 9. Ten studies enrolled consecutive patients (Schochl et al., 2011c; Schochl et al., 2011a). (Davenport et al., 2011; Leemann et al., 2010; Levrat et al., 2008; Rourke et al., 2012; Rugeri et al., 2007; Schochl et al., 2009; Schochl et al., 2010; Tauber et al., 2011) All studies had no comparable control group as defined in the NOS scale. Two studies used healthy volunteers as controls (Rugeri et al., 2007; Woolley et al., 2013) and one study used hospitalized polytrauma patients, and compared to patients with isolated brain injury. (Tauber et al., 2011) All studies were assessed for quality of diagnostic accuracy using QUADAS-2 tool (Whiting et al., 2011) (Table 3, Figures 2A-B). Considering the domains of patient selection, index test, reference standard, flow and timing, only 1 study (7.6%) had low risk of bias in all domains (Tauber et al., 2011); 7 studies (53.8%) had low and unclear risks (Davenport et al., 2011; Leemann et al., 2010; Rourke et al., 2012; Schochl et al., 2009; Woolley et al., 2013; Schochl et al., 2011c; Schochl et al., 2011a); and 5 studies (38.4%) had high risk of bias in at least 1 domain (Doran et al., 2010; Hagemo et al., 2015; Levrat et al., 2008; Rugeri et al., 2007; Schochl et al., 2010). In terms of applicability concerns, 9 studies (69.2%) (Davenport et al., 2011; Hagemo et al., 2015; Leemann et al., 2010; Rourke et al., 2012; Schochl et al., 2009; Schochl et al., 2010; Woolley et al., 2013; Schochl et al., 2011c; Schochl et al., 2011a) had low concerns and 4 studies (30.7%) (Doran et al., 2010; Levrat et al., 2008; Rugeri et al., 2007; Tauber et al., 2011) had at least 1 domain with high concern.
Table 2.2 – The Newcastle Ottawa scale for the cohort studies included in the review

<table>
<thead>
<tr>
<th>Reference</th>
<th>Representativeness of the exposed cohort</th>
<th>Selection of Non-exposed Cohort</th>
<th>Ascertainment of Exposure</th>
<th>Outcome not present at start</th>
<th>Comparability of controls</th>
<th>Assessment of outcome</th>
<th>Adequate follow up</th>
<th>Loss to follow up</th>
<th>Total score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rugeri 2007 (Rugeri et al., 2007)</td>
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<td>Levrat 2008 (Levrat et al., 2008)</td>
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<td>Schöchl 2009 (Schochl et al., 2009)</td>
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<td>Leemann 2010 (Leemann et al., 2010)</td>
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<td>Tauber 2011 (Tauber et al., 2011)</td>
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<td>Schochl 2010 (Schochl et al., 2011c)</td>
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<td>Davenport 2011 (Davenport et al., 2011)</td>
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<td>Schöchl 2011 (Schochl et al., 2011a)</td>
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<td>Woolley 2012 (Woolley et al., 2013)</td>
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<td>Hagemo 2015 (Hagemo et al., 2015)</td>
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<td>6/9</td>
</tr>
</tbody>
</table>

Legend: Refer to http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp, for a description of Newcastle-Ottawa Quality Assessment Scale for cohort studies. In general, more stars denote higher quality. ‘Representativeness’ is awarded a star if the cohort is truly or somewhat representative of the population of
interest. For selection of the non-exposed cohort, a star is awarded if it is drawn from the same population as the exposed cohort. The relevant exposure in this review is management using ROTEM®; we considered a non-exposed cohort to be one that was managed without ROTEM®; other studies used healthy or other hospitalized controls to examine associations between ROTEM® abnormalities and outcomes. (Rugeri et al., 2007; Tauber et al., 2011; Woolley et al., 2013) Exposure is satisfactorily ascertained if data are collected from a secure record. A star is awarded if the outcome is not present at the start of the study. A maximum of two stars can be given for ‘Comparability of controls’ for controlling of confounders in either the design (matching) or analysis (statistical adjustment) phase. We also gave one star when selection criteria appeared to create comparable groups via restriction. ‘Assessment of outcome’ is awarded a star if the outcomes were assessed by independent blind assessment or record linkage; we also considered the outcome of mortality to be adequately assessed in all studies reporting it due to low risk of bias. The duration of follow-up was considered adequate if it was long enough for the outcomes to occur. Completeness of follow-up was considered adequate if all patients were accounted for or if the number lost to follow-up was sufficiently low to be unlikely to introduce bias.
Table 2.3 – QUADAS-2 Tool: Summary of assessment of risk of bias and applicability concerns

<table>
<thead>
<tr>
<th>Reference</th>
<th>Patient selection</th>
<th>Index test</th>
<th>Reference standard</th>
<th>Flow and timing</th>
<th>Patient selection</th>
<th>Index test</th>
<th>Reference standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rugeri 2007 (Rugeri et al., 2007)</td>
<td>☺</td>
<td>☺</td>
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<td>Levrat 2008 (Levrat et al., 2008)</td>
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<td>Schöchl 2009 (Schochl et al., 2009)</td>
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<td>Doran 2010 (Doran et al., 2010)</td>
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<td>Leemann 2010 (Leemann et al., 2010)</td>
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<td>Schochl 2010 (Schochl et al., 2010)</td>
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<td>Davenport 2011 (Davenport et al., 2011)</td>
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<td>Schöchl 2011 (Schochl et al., 2011a)</td>
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<td>Tauber 2011 (Tauber et al., 2011)</td>
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<td>Rourke 2012 (Rourke et al., 2012)</td>
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<td>Woolley 2012 (Woolley et al., 2013)</td>
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<td>Hagemo 2015 (Hagemo et al., 2015)</td>
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</tr>
</tbody>
</table>

Legend: ☺ denotes high risk of bias, ☻ denotes low risk of bias, and ? denotes unclear risk of bias.
Figure 2.2 Proportion of studies with low, high or unclear risks of bias

Figure 2.3: Proportion of studies with low, high or unclear applicability concerns
2.5.3 Outcomes

We found ten studies addressing ROTEM® thresholds for diagnosis of coagulopathy (Davenport et al., 2011; Doran et al., 2010; Hagemo et al., 2015; Levrat et al., 2008; Rourke et al., 2012; Rugeri et al., 2007; Schochl et al., 2009; Tauber et al., 2011; Woolley et al., 2013; Schochl et al., 2011c), 6 studies addressing thresholds for prediction or guidance of transfusion (Davenport et al., 2011; Hagemo et al., 2015; Leemann et al., 2010; Schochl et al., 2010; Tauber et al., 2011; Schochl et al., 2011a), and 6 studies addressing prediction of mortality (Levrat et al., 2008; Rourke et al., 2012; Schochl et al., 2009; Tauber et al., 2011; Schochl et al., 2010; Schochl et al., 2011c). Two studies used 4 ROTEM® assays (EXTEM, INTEM, FIBTEM and APTEM) (Doran et al., 2010; Rugeri et al., 2007; Schochl et al., 2011c; Schochl et al., 2011a); 4 studies used 3 assays (EXTEM, INTEM, and FIBTEM) (Doran et al., 2010; Rugeri et al., 2007; Schochl et al., 2011c; Schochl et al., 2011a); 4 studies used 2 assays (EXTEM and FIBTEM) (Rourke et al., 2012; Schochl et al., 2010; Tauber et al., 2011; Hagemo et al., 2015); 1 study used 2 other assays (EXTEM and INTEM) (Leemann et al., 2010) and 2 studies used 1 assay (EXTEM) (Davenport et al., 2011; Woolley et al., 2013).

2.5.3.1 Studies addressing thresholds of ROTEM® parameters to diagnose ACoTS

Definition of coagulopathy by SCTs and ROTEM® parameters varied across all studies. Five studies (Davenport et al., 2011; Doran et al., 2010; Rugeri et al., 2007; Hagemo et al., 2015; Schochl et al., 2011c) used different SCTs as gold standards, with different cut off values to define coagulopathy. For example, one study used INR >1.6 and/or aPTT >60sec and/or a platelet count <100x10^9 L^-1 and/or fibrinogen<1g/L (Rugeri et al., 2007). In contrast, another study used a prothrombin time index (PTI) test <70% (a value of <70% in PTI is equivalent to INR >1.3), aPTT >35sec, and fibrinogen <1.50g/L (Schochl et al., 2011c). Lastly, coagulopathy was defined by Davenport and Hagemo as an INR >1.2. (Davenport et al., 2011; Hagemo et al., 2015)

In ROTEM®, 10 studies reported thresholds of parameters in detecting the various defects in ACoTS (Davenport et al., 2011; Doran et al., 2010; Hagemo et al., 2015; Levrat et al., 2008; Rourke et al., 2012; Rugeri et al., 2007; Schochl et al., 2009; Tauber et al., 2011; Woolley et al., 2013; Schochl et al., 2011c) (Table 2.4). However, there was a wide variation on the parameters chosen, and their cut off values. Better designed studies used SCTs as controls when determining
thresholds and cut-off values. Other studies used the recommendations from previous consensus meetings. Finally, in other studies, authors used previous institutional experience, or cut-off values pre-established by the ROTEM® manufacturer. Studies that used SCTs as reference standards for comparisons are described here. Table 2.4 describes all evidence in details.
<table>
<thead>
<tr>
<th>Study</th>
<th>Comparator</th>
<th>ROTEM® thresholds used</th>
<th>Accuracy of threshold</th>
<th>AUC</th>
<th>Key findings</th>
</tr>
</thead>
</table>
| Rugeri 2006 (Rugeri et al., 2007) | PTR>1.5, FIB<1.0g/L | EXTEM CA15=32mm, FIBTEM A10=5mm | 87/91, 100/85 | 0.98/0.96 | 1 – Significant correlation between EXTEM CA15<32mm and PT >1.5 (r=0.66, p<0.0001) and of FIBTEM CA10<5mm and Fibrinogen <1.0g/L (r=0.85, p<0.0001).  
2 – EXTEM A15=32 and FIBTEM A10=5mm had a higher sensitivity and specificity to detect PTR>1.5 and fibrinogen <1.0g/L |
| Levrat 2008 (Levrat et al., 2008) | ELT<90sec | EXTEM MCF≤18mm, LI30≤71%, APTEM MCF ↑by 7% | 100/75, 100/100 | 1.00/0.87 | 1 – MCF correlated well with ELT when compared with amplitude and CLI.  
2 – HF patients exhibited greater ROTEM® abnormalities, lower INR, lower fibrinogen levels and were more severely injured (↑ ISS) compared to the control group (all p<0.05) |
| Schochl 2009 (Schochl et al., 2009) | | EXTEM and INTEM ML=100% | NA/NA | NA | 1 – Fulminant HF confirmed by complete clot lysis within 30 min by ROTEM® trace |
| Doran 2010 (Doran et al., 2010) | PT>18sec, aPTT>38sec | EXTEM MCF<45mm | NA/NA | NA | 1 – ROTEM® detected coagulation abnormalities in 64% patients vs. 10% detected by SCTs as compared to test reference ranges? (p=0.0005).  
2 – MCF<45mm in 100% of MT patients |
| Davenport 2011 (Davenport et al., 2011) | PTR>1.2 | EXTEM CA5≤35mm | 77/79, NA/NA | NA/NA | 1 – EXTEM CA5≤35mm detected coagulopathy with 77% sensitivity and a false positive rate of 13% |
| Tauber, 2011 (Tauber et al., 2011) | INR>1.5, aPTT>50sec | EXTEM MCF<45mm, FIBTEM MCF<7mm | 72/86, 76/71 | 0.83/0.89 | 1 - Prevalence of low fibrinogen, impaired fibrin polymerization and reduced MCF was 26%, 30%, and 22%, respectively, higher than the prolonged INR (14%)  
2 – There was ↑ F1+2 and TAT and low AT levels, indicating ↑ thrombin formation among all patients |
<table>
<thead>
<tr>
<th>Study, Year (Author, Year)</th>
<th>PTI&lt;70%, aPTT&gt;35sec, PLT&lt;100x10^3, FIB&lt;1.5g/L</th>
<th>EXTEM CT&gt;80sec, CFT&gt;159sec, MCF&lt;50mm</th>
<th>INTEM CT&gt;240sec, CFT&gt;110sec, MCF&lt;50mm</th>
<th>FIBTEM MCF&lt;9mm</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
</table>
| Schochl, 2011 (Schochl et al., 2011c) | | NA | NA | 0.77 | 1 – Coagulopathy was characterized by abnormal values in most or all ROTEM® measurements as compared to reference range vs. SCT.  
2 – Significantly low CA5-CA30 min, MCF in EXTEM, INTEM and FIBTEM assays in non survivors vs. survivors (p<0.01) |
| Rourke 2012 (Rourke et al., 2012) | FIB<1.5g/L | EXTEM CA5<36mm | 53 | 87 | NA | 1 – ROTEM® parameters correlated with fibrinogen level.  
2 – Ex vivo fibrinogen administration reversed coagulopathy by ROTEM®. |
| Woolley 2012 (Woolley et al., 2013) | PT>1.5 | EXTEM CA5<32mm, EXTEM A10<40mm | 96 | 58 | NA | 1 – EXTEM MCF < 40 mm and interim values of EXTEM A5 and A10 predicted coagulopathy (A15: sensitivity/specificity of 96%/58% and for A10: sensitivity/specificity 100%/ 70%) |
| Hagemo 2015 (Hagemo et al., 2015) | INR>1.2 | EXTEM CA5<37mm, FIBTEM CA5<8mm | NA | NA | 0.79 | 1 – Highest ROTEM® AUC values were found for EXTEM CA5 and FIBTEM CA5 for detecting ACoTS  
2 – EXTEM CA5≤37mm had a detection rate of 66.3% and FIBTEM CA≤8mm had a detection rate of 67.5% of ACoTS |

2.5.3.1.1 **ROTEM® thresholds determined with comparison to standard controls (SCTs).**

In a study conducted by Rugeri (Rugeri et al., 2007), thresholds were determined by evaluating the extent of correlation between ROTEM® parameters with corresponding SCTs (CA15-EXTEM with PT: \( r=0.66, p<0.0001 \); clot formation time [CFT]-INTEM with aPTT: \( r=0.91, p<0.0001 \); CA10-FIBTEM with fibrinogen level: \( r=0.85, p<0.0001 \); CA15-INTEM with PLT count: \( r=0.57, p<0.0001 \)). The group found cut-off values of EXTEM CA15<32mm and FIBTEM CA10<5mm to detect laboratory PT>1.5 and fibrinogen level <1g/L, with high sensitivity (87% and 91%) and specificity (100% and 85%), respectively. In another study, Levrat determined the cut off values by assessing correlation between ROTEM® parameters and euglobin lysis time (ELT), used as the gold standard control (Levrat et al., 2008). In this study, a threshold of 18mm (MCF-EXTEM), 71% (CLI30) AND 7% increase of MCF-APTEM, sensitivity was, 100%, 75% and 80%, respectively with a specificity of 100%. Moreover, Davenport(Davenport et al., 2011) and Rourke(Rourke et al., 2012) used ROTEM® CA5<35mm as threshold based on correlation with normal PT values to discriminate normal from the abnormal curves in patients with ACoTS. Hagemo used ROTEM® threshold value of EXTEM CA5≤37mm and FIBTEM A5≤8mm to detect ACoTS (Hagemo et al., 2015). These authors used INR>1.2 and fibrinogen concentration of ≤1.61g/L to define ACoTS, respectively (Hagemo et al., 2015). A feasibility study (Doran et al., 2010) in a deployed military trauma setting demonstrated that an abnormal CA10 was associated with a subsequent development of an abnormal MCF (<45mm). MCF <45mm was present in a 100% of MT patients. When PT>18sec and aPTT >60sec were used as the gold standard for ACoTS, only 10.5% of patients were defined as coagulopathic. By comparing these results with ROTEM® results, it was found that 64% were coagulopathic (FIBTEM-MCF<45mm), (\( p=0.0005 \)). Another study (Davenport et al., 2011) reported that the threshold EXTEM CA5≤35mm predicted INR>1.2 in 77% of cases. In TBI patients, Schochl (Schochl et al., 2011c)reported a cut off value of EXTEM-CT>80sec, compared to PTI<70%, to define coagulopathy (\( p=0.003 \)). Finally, in another study in the military setting (Woolley et al., 2013) the authors compared PT>18 seconds (gold standard) to diagnose coagulopathy and identified that early CA5<32mm and CA10<40mm predicted the hypocoagulation state with a sensitivity/specificity of CA5 96%/58% and CA10 100%/70%, respectively, compared to SCTs.
2.5.3.1.2 Hypofibrinogenemia

Two studies investigated the use of FIBTEM CA10 <5mm and FIBTEM CA5 <10mm for diagnosing different degrees of hypofibrinogenemia. In the first study (Rugeri et al., 2007), FIBTEM CA10<5mm diagnosed fibrinogen levels below 1.0g/L with sensitivity of 87% and specificity of 91%. The second study (Rourke et al., 2012) reported EXTEM CA5<36mm with a sensitivity of 53% and specificity of 87% for discerning patients with fibrinogen levels <1.5g/L. For FIBTEM CA5 <10mm, the reported sensitivity and specificity were 78% and 70% respectively for predicting a fibrinogen level below 1.5g/L.

2.5.3.1.3 Hyperfibrinolysis

Three studies (Levrat et al., 2008; Schochl et al., 2009; Tauber et al., 2011) reported thresholds of different ROTEM® parameters to diagnose degrees of hyperfibrinolysis (HF) such as mild, moderate and fulminant. Two studies compared their findings with SCTs. The first study (Levrat et al., 2008) defined HF as euglobulin lysis time (ELT) <90 minutes (used as gold standard) in a series of 23 patients. The authors used EXTEM MCF≤18mm, clot lysis index at 30 minutes (CLI30)<71% and APTEM MCF 7% increase to define hyperfibrinolysis (HF) (sensitivity 100%, 75%, 80% and specificity 100% for all, respectively) in these patients with an abnormal ELT test. The second study (Schochl et al., 2009) enrolled 33 trauma patients diagnosed with HF by ROTEM®. They used clot lysis in EXTEM and INTEM assays at different time points across the ROTEM® tracing to define the three patterns of HF, confirmed by the APTEM test. A complete clot lysis (ML=100%) within 30 minutes was used to define patients with fulminant HF; complete clot lysis between 30 and 60 minutes defined intermediate HF and complete clot lysis after one hour to define late HF. The median values of laboratory fibrinogen was lower in fulminant HF group and intermediate HF group when compared with late HF group (fulminant HF: 0.5 g/L; intermediate HF: 0.49 g/L compared with late HF 1.04 g/L, p=0.048 for both).

2.5.3.1.4 Platelet count

A single study (Rugeri et al., 2007) evaluated the correlation between platelet count and INTEM CA15 (r=0.57, p<0.0001). However, the threshold value of INTEM CA15=46mm showed poor
positive predictive (PPV) values in the diagnosis of laboratory platelet count below $50 \times 10^9 \text{L}^{-1}$ (sensitivity: 100% [95%CI 71-100], specificity 83% [95% CI 82-83]; PPV 17% [95% CI 12-17], negative predictive value [NPV] 100% [95% CI 98-100]; AUC 0.92).

### 2.5.3.2 Studies addressing thresholds of ROTEM® parameters in predicting or guiding transfusion

#### 2.5.3.2.1 Predicting transfusion

Six studies reported ROTEM® thresholds either in predicting transfusion (Davenport et al., 2011; Leemann et al., 2010; Tauber et al., 2011; Hagemö et al., 2015; Schochl et al., 2011a), including MT (Davenport et al., 2011; Leemann et al., 2010; Hagemö et al., 2015; Schochl et al., 2011a), or guiding transfusion (Schochl et al., 2010) (Table 5). Massive transfusion was defined by the need for transfusion of $\geq 10$U of RBCs within the first 12 hours (Davenport et al., 2011) or 24h (Leemann et al., 2010; Schochl et al., 2011a) of hospital admission in three studies. Values outside the reference range for EXTEM and INTEM CT, CFT, CA at 10, 20 and 30 minutes, as well as reduced MCF were more likely in patients who required a MT vs. patients who did not (p<0.0001, for all) (Leemann et al., 2010; Schochl et al., 2011a). The reference ranges used in this study were the same established by the same group, in a previous study that used SCTs as control (Schochl et al., 2009).

Davenport (Davenport et al., 2011) demonstrated that EXTEM CA5≤35mm predicted the need for MT with higher detection rate compared to INR>1.2 (71% vs. 43%, p<0.001). Schochl (Schochl et al., 2011a), using threshold pre-established in a previous study by the same group (Schochl et al., 2009) reported that both FIBTEM A10≤4mm (ROC AUC=0.83) and FIBTEM MCF≤7mm (ROC AUC=0.84) were predictive of the need for MT. Lastly, Hagemö (Hagemö et al., 2015) demonstrated that threshold values of EXTEM CA5≤40mm predicted MT in 72.7% and FIBTEM CA5≤9mm predicted MT in 77.5%, respectively. However detection rate for MT was found to be highest for INR, as compared to EXTEM CA5 (51.1% and 45.5%, respectively). The optimum threshold value for fibrinogen in predicting MT was $\leq 1.90 \text{g/L}$ with a detection rate of 77.8% and a positive predictive value of 14.
Table 2.5: Studies addressing ROTEM® thresholds used to predict or guide blood transfusion.

<table>
<thead>
<tr>
<th>Study</th>
<th>Comparator</th>
<th>ROTEM® thresholds used</th>
<th>Accuracy of threshold</th>
<th>ROC/AUC</th>
<th>Key findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Massive transfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leemann 2010 (Leemann et al., 2010)</td>
<td>aPTT&gt;36sec, PLT&lt;100x10^3, INR&gt;1.2</td>
<td>EXTEM/INTEM CA10, CA20, CFT, MCF as per manufacturer</td>
<td>NA, NA</td>
<td>0.82</td>
<td>2 – INTEM MCF 37.5±2.9 associated with MT requirements within 24h</td>
</tr>
<tr>
<td>Tauber, 2011 (Tauber et al., 2011)</td>
<td>FIB 1.50g/L, INR&gt;1.5</td>
<td>FIBTEM MCF&lt;7mm</td>
<td>71, NA</td>
<td>0.80</td>
<td>1 – FIBTEM MCF&lt;7mm associated with RBC use (OR 0.92, 95% CI 0.87–0.98)</td>
</tr>
<tr>
<td>Schochl 2011 (Schochl et al., 2011a)</td>
<td>PLT≤161x10^3, aPTT≤35.2 sec, FIB≤1.4g/dL</td>
<td>FIBTEM A10≤4mm, FIBTEM MCF≤7mm</td>
<td>63.3, 83.2</td>
<td>0.83</td>
<td>1 – 85% patients with FIBTEM MCF 0-3mm received MT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 – FIBTEM A10 (0.83) and FIBTEM MCF (0.84) showed high predictive value for MT</td>
</tr>
<tr>
<td>Davenport 2011 (Davenport et al., 2011)</td>
<td>PTR&gt;1.2</td>
<td>EXTEM CA5≤35mm</td>
<td>71.4%, NA</td>
<td>NA</td>
<td>1 – CA5 identified patients who required MT with detection rate of 71% vs. 43% for PTR&gt;1.2, p&lt;0.001</td>
</tr>
<tr>
<td>Hagemo 2015 (Hagemo et al., 2015)</td>
<td>INR&gt;1.2</td>
<td>EXTEM CA5≤40mm, FIBTEM CA5≤9mm</td>
<td>72.7%, 77.5%</td>
<td>0.75</td>
<td>1 – ROTEM® CA5 is a valid predictor for MT.</td>
</tr>
<tr>
<td>Any transfusion</td>
<td></td>
<td></td>
<td></td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Schochl, 2010 (Schochl et al., 2010)</td>
<td>PT(11-13.5sec), aPTT(26-35sec), FIB (2-4.5 g/L), PLT(150-350)</td>
<td>FIBTEM MCF&lt;10mm, EXTEM CT&gt;1.5x normal</td>
<td>NA, NA</td>
<td>NA</td>
<td>1 – ROTEM® guided FC and PCC transfusion, associated with favorable survival (24.4% vs. 33.7%; p=0.032)</td>
</tr>
<tr>
<td>Davenport 2011 (Davenport et al., 2011)</td>
<td>PTR&gt;1.2</td>
<td>EXTEM CA5≤35mm</td>
<td>33.3%, NA</td>
<td>NA</td>
<td>1 – CA5 ≤ 35mm predicted RBC and plasma</td>
</tr>
</tbody>
</table>
CT > 94 sec  
Alpha < 65°

transfusion. Patients with CA5 ≤ 35 mm
received more RBC (46% vs. 17%, p < 0.001)
and plasma (37% vs. 11%, p < 0.001)
transfusions.

2 – CA5 ≤ 35 mm received more RBC (4 U vs.
1 U, p < 0.001) and FFP (2 U vs. 0 U, p < 0.001)

Legend: aPTT – activated partial thromboplastin time, CA – CA – clot amplitude (measured at 5, 10, 15, 20 min, etc), CT – clotting time, CFT – clot formation
time, ED – emergency department, EXTEM – extrinsically activated test with tissue factor; FIB – fibrinogen, FC – fibrinogen concentrate, FFP – fresh frozen
plasma, FIBTEM -fibrin-based extrinsically activated test with tissue factor and the platelet inhibitor cytochalasin D; GCS – Glasgow coma scale, HGB –
hemoglobin, INR – international normalized ratio, ISS – injury severity score, MCF – maximum clot firmness, MT – massive transfusion, NA – not available,
PC – platelet concentrate, PCC – prothrombin complex concentrate, PLT – platelets, RBC – red blood cells, PT – prothrombin time, PTI – prothrombin time
index, PTR – prothrombin time ratio, RBC – red blood cells
2.5.3.2.2 Guiding transfusion

Schochl (Schochl et al., 2010), in a retrospective analysis of trauma patients who received ≥5U RBCs within 24h, and whose coagulation management was guided by ROTEM®, developed a clinical practice guideline using thresholds of ROTEM® parameters to guide transfusion. The group used a threshold of FIBTEM MCF<10mm to guide transfusion of fibrinogen concentrate (FC) and used EXTEM CT>1.5 times normal to guide PCC administration. Reference ranges used for these ROTEM® tests’ parameters were previously determined in a multi-center investigation by Lang (Lang et al., 2005). The authors were able to demonstrate a reduction in the number of RBC units transfused. The use of RBC units was avoided in 29% of patients receiving FC and PCC therapy compared to only 3% avoided in the group receiving fresh frozen plasma (FFP) (p<0.001).

2.5.3.2.3 Studies addressing thresholds of ROTEM® parameters in predicting mortality

Six studies evaluated ROTEM® thresholds in predicting mortality (Table 6) (Levrat et al., 2008; Rourke et al., 2012; Schochl et al., 2009; Schochl et al., 2010; Tauber et al., 2011; Schochl et al., 2011c). These studies evaluated mortality at different time points, including: within 24h of arrival (Tauber et al., 2011); death in hospital (Schochl et al., 2009; Tauber et al., 2011); death within 24hr and 28 days (Rourke et al., 2012), 30 days (Tauber et al., 2011), and two studies did not define the time to death (Schochl et al., 2010; Levrat et al., 2008). Two studies reported that trauma patients with the diagnosis of HF had higher rates of mortality (Schochl et al., 2009; Levrat et al., 2008). The studies defined HF differently: Schochl defined HF as a complete clot lysis (ML=100%) on ROTEM® at different time intervals as fulminant HF, intermediate HF and late HF as described above (Schochl et al., 2009). Finally, Levrat defined HF as an ELT<90 min (Levrat et al., 2008). We describe here under the studies that adjusted their findings for confounders, or compared findings with previously validated trauma scores. The full description of the evidence is on Table 6.

Tauber (Tauber et al., 2011) found a significant increase in mortality with FIBTEM<7 mm (21% vs. 9%, p=0.006) and EXTEM MCF<45 mm (25.4% vs 9.4%, p<0.001). Similarly, EXTEM MCF was independently and negatively associated with early mortality (OR 0.94, 95% CI 0.9–0.99). The author additionally reported 85.7% mortality in patients with fulminant HF (ML100%
within 30 minutes), and 11.1% mortality in patients with moderate HF (ML100% between 30 and 60 minutes). Rourke (Rourke et al., 2012) reported that a low FIBTEM A5<9.5mm was an independent predictor of 24hr and 28 days mortality ($p<0.001$).

In a study in brain injury patients, Schochl (Schochl et al., 2011c) demonstrated an independent association between FIBTEM MCF<9mm (ROC: 0.77; 95% CI, 0.665-0.850, $p<0.001$) and aPTT>35sec (ROC 0.79; 95% CI 0.686-0.868, $p<0.001$), and mortality. Moreover, in this study, ROTEM® revealed shorter CT in EXTEM and INTEM ($p<0.001$), shorter CFT in EXTEM and INTEM ($p<0.0001$), and higher MCF in EXTEM and INTEM ($p<0.01$) in survivors compared with non-survivors. Finally, in another study conducted the Schochl (Schochl et al., 2010), where trauma patient resuscitation was guided by ROTEM® with FC and PCC, a reduction in the observed mortality than the predicted mortality by TRISS and RSS was demonstrated (24.4% vs. 33.7%, $p=0.032$).
Table 2.6 - Studies addressing ROTEM® thresholds for the prediction/reduction of mortality

<table>
<thead>
<tr>
<th>Study</th>
<th>Comparator</th>
<th>Optimal ROTEM® Parameter and cut off</th>
<th>Accuracy of threshold</th>
<th>Key findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levrat (Levrat et al., 2008)</td>
<td>ELT&lt;90min</td>
<td>EXTEM MCF≤18mm</td>
<td>Sensitivity Specificity AUC</td>
<td>1 – Patients with HF had higher mortality rate (100%, CI: 48-100% vs. 11% CI: 5-20%, p&lt;0.05)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LI30≤71%</td>
<td>100 100 1.00</td>
<td>2 – ↑CFT and ↓PLT contribution to MCF associated with ↑mortality (p=0.042 and p=0.026 respectively)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>APTEM MCF ↑ by 7%</td>
<td>80 100 0.80</td>
<td>3 – FIBTEM MCF&lt;7mm and EXTEM MCF&lt;45mm associated with higher mortality (21% vs. 9% SCTs, p=0.006 and 25.4% vs. 9.4% SCTs, p&lt;0.001, respectively)</td>
</tr>
<tr>
<td>Schochl 2009 (Schochl et al., 2009)</td>
<td>No comparator</td>
<td>ML = 100%</td>
<td>NA NA NA</td>
<td>4 – EXTEM MCF had strong association with early deaths (OR 0.94, 95% CI 0.9-0.99)</td>
</tr>
<tr>
<td>Schochl 2010 (Schochl et al., 2010)</td>
<td>No comparator</td>
<td>FIBTEM MCF&lt;10mm, EXTEM CT&gt;1.5xnormal</td>
<td>NA NA NA</td>
<td>5 – Observed mortality was lower than the predicted mortality by TRISS (24.4% vs.33.7%, p=0.032) with a favourable survival rate.</td>
</tr>
<tr>
<td>Tauber, 2011 (Tauber et al., 2011)</td>
<td>PT=70% FIB=1.82g/L</td>
<td>FIBTEM MCF&lt;7mm, EXTEM CT 91sec, EXTEM CFT 218sec, EXTEM MCF 46mm</td>
<td>NA NA 0.8</td>
<td>6 – FIBTEM MCF&lt;7mm and EXTEM MCF&lt;45mm associated with higher mortality (21% vs. 9% SCTs, p=0.006 and 25.4% vs. 9.4% SCTs, p&lt;0.001, respectively)</td>
</tr>
<tr>
<td>Schochl, 2011 (Schochl et al., 2011c)</td>
<td>aPTT &gt; 35 sec</td>
<td>FIBTEM MCF &lt; 9 mm</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-------------</td>
<td>------------------</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>1 – Decrease in clotting times in EXTEM and INTEM (p &lt; .001), decreased CFT in EXTEM and INTEM (p &lt; .0001), and increased MCF in EXTEM, INTEM, and FIBTEM (p &lt; .01) were noted in survivors compared with non-survivors, in patients with severe isolated TBI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rourke, 2012 (Rourke et al., 2012)</th>
<th>FIB &lt; 1.5 g/L</th>
<th>EXTEM CA5 &lt; 36 mm</th>
<th>53</th>
<th>87</th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIBTEM CA5 &lt; 10 mm</td>
<td>78</td>
<td>70</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 – Fibrinogen level was independently associated with higher mortality at 24h and 28 days (p&lt;0.001). ROTEM® could detect hypofibrinogenemia early and rapid replacement of fibrinogen may improve outcomes.</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Legend:**  
aPTT – activated partial thromboplastin time, APTEM – EXTEM test inactivated using aprotinin, CA5 – clot amplitude at 5 min, CT – clotting time, CFT – clot formation time, ELT – euglobulin lysis time, EXTEM – EXTEM – extrinsically activated test with tissue factor; FIB – fibrinogen, FIBTEM – FIBTEM -fibrin-based extrinsically activated test with tissue factor and the platelet inhibitor cytochalasin D; HF – hyperfibrinolysis, INTEM - intrinsically
activated test; LI30 – lysis index at 30 min, MCF – maximum clot firmness, ML – maximum lysis, NA – not available, OR – odds ratio, PC – platelet concentrate, ROC – receiver operating curve, s – seconds, SCTs – standard coagulation tests, TRISS – Trauma injury severity score.
2.6 DISCUSSION

2.6.1 Main findings

We performed a systematic review of the literature to ascertain the existing evidence on the reported thresholds of ROTEM® parameters in diagnosing coagulopathy, predicting or guiding transfusion and predicting mortality in trauma patients. Thirteen studies evaluating 2835 patients met our inclusion criteria. Overall, the methodological quality of the included studies was moderate. In general, the patient populations were different across studies. We found studies using different ROTEM® parameters and different thresholds for the same ROTEM® parameter for the diagnosis of coagulopathy and guidance of component transfusions. We did not find any randomized controlled trials, and the majority of the retrospective or prospective cohort studies found in the review did not have a gold standard coagulation test used for comparison. Where SCTs results were used as the gold standard, there were no studies done to validate the chosen cut-off. We were not able to pool the data and conduct meta-analysis due to the marked clinical heterogeneity among the studies. Considering the limited number of studies and the moderate methodological quality, we concluded that there is still no robust evidence supporting the thresholds of ROTEM® parameters reported in the literature in diagnosing coagulopathy, guiding or predicting transfusion, and predicting mortality.

For diagnosis of coagulopathy, the most properly designed studies, using a control (SCTs), identified several different parameters and thresholds. However, definition of coagulopathy by SCTs was not standardized. Most common parameters used to define coagulopathy across the studies were EXTEM-CA5, CA10, CA15, which were correlated with PT and INR. The cut-off values varied from 5mm in CA5 to 35mm in CA15. Of note, several other studies used arbitrary values obtained from previous expert group meetings, previous author’s experience or from reference values from the manufacturer.

Hypofibrinogenemia was diagnosed, in general, with FIBTEM CA5 and CA10 (<10mm and <5mm, respectively). Gold standards SCTs used for comparison were fibrinogen <1.0g/L or 1.5g/L. In the assessment of hyperfibrinolysis, complete clot lysis (ML100%) and LI60<85% were used as definition of hyperfibrinolysis, with the ELT<90min as the gold standard for comparison.
For prediction of transfusion, the best designed study established EXTEM-CA5 ≤35mm using INR as control. The other studies did not use SCTs as gold standards for control. In those studies, the parameters and their cut off values were established from previous author’s experiences or from the manufacturer of the ROTEM® device. Values outside the reference range for EXTEM and INTEM CT, CFT, CA at 10, 20 and 30 minutes, as well as reduced MCF were more likely in patients who required a MT, as compared to patients who did not. Other parameters used were EXTEM CA5≤35mm and FIBTEM-MCF≤7mm that were also associated with the need for MT.

In transfusion guidance, FIBTEM MCF<10mm and EXTEM CT>1.5 times normal were used to guide administration of FC and PCC, respectively, with a reduction of the number of RBC units used in the FC/PCC group, compared to fresh frozen plasma (FFP) group (p<0.001). No other study reported on ROTEM® metrics utilized to guide transfusion.

Mortality was assessed in different studies, and overall, an association between hyperfibrinolysis and mortality was demonstrated (maximum lysis of 100%, defined using ELT as control). Multiple parameters were found to be associated with mortality, including: FIBTEM<7mm/<9mm/<9.5mm, EXTEM-MCF<45mm; shorter EXTEM-CT, INTEM-CT, EXTEM-CFT and INTEM-CFT; higher EXTEM-MCF, INTEM-MCF.

Two systematic reviews of ROTEM® and TEG®, the similar viscoelastic currently mostly used in United States, exist for nontrauma populations. A Cochrane review (Afshari et al., 2011) included nine RCTs, mostly in cardiac surgery, that compared transfusion guided by ROTEM® and TEG® with transfusion guided by clinical judgment, SCTs, or both in severely bleeding patients. This review found that ROTEM® and TEG® reduced blood loss by a mean of 85 ml (95% CI, 29 to 141ml) but had no effect on mortality. Another systematic review(Muller, Meijers, Vroom, & Juffermans, 2014) included 16 observational studies and two RCTs in patients with sepsis and concluded that ROTEM® and TEG® (compared with SCTs) may detect impaired fibrinolysis, which may help to discriminate between sepsis and systemic inflammatory response syndrome (SIRS). Aside from current moderate quality, the evolving trauma literature brings evidence that ROTEM® has the potential to diagnose ACoTS, and predict and guide transfusion faster than the SCTs due to the point of care nature of the tests. Cut-off values of various ROTEM® parameters may diagnose the different nuances of ACoTS such as the different
causes for hypocoagulation (low levels of clotting factors, fibrinogen and platelets, and platelet dysfunction), and hyperfibrinolysis.

2.6.2 Strengths and weaknesses of this study, and future research

Major limitations of this review are related to the quality of the included studies, which were not powered with proper sample sizes for detection of differences, for example. The studies were only observational, and without appropriate control groups. No randomized trials were found in the trauma population. Studies also included different transfusion triggers and transfusion protocols, limiting direct comparisons when evaluating prediction for transfusion. Reproducible technical standards for the performance of ROTEM® were lacking in the included studies. Inconsistent reporting data precluded calculation of summary diagnostic test-performance measures and exploration of threshold effects. Different cut-off values and different parameters were used, what makes standardization and interpretation difficult. A major problem faced by diagnostic studies of ACoTS is the ambiguous nature of the gold standard, given that SCTs may not provide an adequate description of all associated abnormalities or may be inferior to ROTEM® parameters. Important treatment differences between many included studies and contemporary practice include substitution of FFP for clotting factors concentrate such as PCC, FC, and cryoprecipitate, what contributed to the clinical heterogeneity across the studies. Although this review found intense heterogeneity, clinically useful and seemingly valid conclusions were reported, and will be useful in designing future studies and future clinical practice guidelines. Our findings add to the current literature importantly, as we were able to summarize and critically appraise the evidence on the threshold values of ROTEM® parameters use in trauma and demonstrate that the accuracy of the current parameters and their cut-off values need further research to be consolidated.

The information obtained in this review may be useful in designing properly and adequately powered clinical trials to detect differences in laboratory and clinical endpoints, such as bleeding, morbidity, and mortality. Confirmation to whether a resuscitation process guided by ROTEM® parameter thresholds will result in less exposure to allogeneic blood products, as compared to resuscitation guided by SCT or with a blind formula resuscitation is still warranted. Additionally, determination of specific ROTEM® parameter thresholds as independently
predictors of the need for massive transfusion and mortality, assisting the trauma team with prognostication soon after arrival to hospital, are needed.

2.7 CONCLUSION

In summary, this systematic review finds that, consistently across all manuscripts reviewed, abnormal EXTEM and FIBTEM clot amplitude (CA5, CA10, CA20) and MCF are capable of diagnosing ACoTS (compared to SCT tests), predict the need for massive transfusion, and predict mortality. Furthermore, the presence of lysis, diagnosed by abnormal LI30 or ML is also strongly associated with mortality. Thus, based on the current available evidence we reviewed, it could be extrapolated that clinical practice guidelines using ROTEM® parameters thresholds to guide blood component transfusion could be clinically useful. Goal-directed component transfusion approach guided by ROTEM® may reduce the exposure to allogeneic blood products and the complications derived from inappropriate resuscitation. However, due to the use of arbitrary cut-off values, lack of randomized controlled trials, cohort studies with small sample sizes, without comparable controls, and heterogeneous patient populations, no further conclusions can be drawn from the literature to date. Better designed prospective studies comparing ROTEM®-guided transfusion protocols with conventional massive transfusion protocols or transfusion guided by SCTs are warranted to determine optimal parameters and accurate thresholds.

An abridged version of the following chapter has been previously published (Inaba et al., 2015).

The citation is: Kenji Inaba, MD, Sandro Rizoli, MD, Precilla V. Veigas, MSc, Jeannie Callum, MD, Ross Davenport, MD, John Hess, MD, Marc Maegele, MD, and the Viscoelastic Testing in Trauma Consensus Panel,* Los Angeles, California. 2014 Consensus conference on viscoelastic testYbased transfusion guidelines for early trauma resuscitation: Report of the panel. J Trauma Acute Care Surj Volume 78 (6): 1226-1229

Permission was received to publish in this dissertation.
3.1 Abstract

There has been an increased interest in the use of viscoelastic testing to guide blood product replacement during the acute resuscitation of injured patient. Currently, no uniformly accepted guidelines exist for how this technology should be integrated into clinical care. In September 2014, an international multidisciplinary group of leaders in the field of trauma coagulopathy and resuscitation was assembled for a 2-day consensus conference in Philadelphia, Pennsylvania. The conference panel included trauma surgeons, hematologists, blood bank specialists, anesthesiologists, and the lay public. Nine questions regarding the impact of viscoelastic testing in the early resuscitation of trauma patients were developed before the conference by panel consensus. Early use was defined as baseline viscoelastic test result thresholds obtained within the first minutes of hospital arrival when conventional laboratory results are not available. The available data for each question were then reviewed in person using standardized presentations by the expert panel. A consensus summary document was then developed and reviewed by the panel in an open forum. Finally, a two-round Delphi poll was administered to the panel of experts regarding viscoelastic thresholds for triggering the initiation of specific treatments including fibrinogen, platelets, plasma, and prothrombin complex concentrates. This report summarizes the findings and recommendations of this consensus conference.
3.2 Overview

The overall goal of this work was to investigate the use of ROTEM® parameters for early resuscitation of significantly bleeding trauma patients. The previous chapter systematically reviewed the reported thresholds of ROTEM® in the diagnosis of coagulopathy, guiding transfusion including massive transfusion and predicting mortality.

Systematically developed transfusion guidelines recommended by experts are important to direct transfusion decision in critically bleeding trauma patients. A patient can benefit from implementation of transfusion guidelines with the right choice of blood products based on their clinical condition (Woolf, Grol, Hutchinson, Eccles, & Grimshaw, 1999). For healthcare provider, the guidelines will offer explicit guidance about rapidly identifying a patient requiring the right amount and type of blood product (Woolf et al., 1999). This will help in making a quick decision instead of waiting for time consuming laboratory test results and save costs associated with inappropriate transfusion. As transfusion practices vary among trauma centers, an unbiased opinion from experts in the area of research interest is necessary in order to reduce the gap between current knowledge and practice. Thromboelastometry is a new diagnostic test which has the ability to diagnose coagulation abnormalities faster than the standard laboratory tests and aid in guiding transfusion while reducing inappropriate transfusions. Thromboelastometry based transfusion has shown to reduce the use of RBC, the major blood products that is used in many major trauma centers for emergency transfusion such as massive transfusion (Schochl et al., 2010; Schochl et al., 2010; Schochl et al., 2011b).

The next step of this thesis was to summarise the reported thresholds and share institutional experience from experts in the area. To achieve this goal, in September 2014 (September 9-10), an international group of leaders in the field of trauma coagulopathy and resuscitation was gathered for a two-day consensus conference in Philadelphia, Pennsylvania. We followed the NIH guidelines for organizing a consensus conference. The multidisciplinary panel included 25 world experts in the fields of trauma surgery, blood transfusion, blood conservation, hematology, thromboelastometry, surgery, anaesthesia and Critical Care. Two lay public representatives were also involved in the conference. Ten experts presented the current evidence based on their institutional experience; all participants shared experience of their institution and seven members
summarized and wrote the conclusions and consensus guidelines which was subsequently submitted for publication. The consensus panel formulated the questions which encompasses issues surrounding ROTEML® in the use of transfusion management. Formulation of recommendations was done by expert consensus based on the recommendations and extent of their strength, assessment of risks and benefits. Institutional support was obtained for this purpose.

On day two of the conference 25 experts agreed to participate in a two round anonymous Delphi poll. In round one, all 25 participants partook while in round two, 24 participants responded to the poll. The selected Delphi panel is shown in the table 3.1.

<table>
<thead>
<tr>
<th></th>
<th>Title</th>
<th>First name</th>
<th>Last name</th>
<th>Stakeholder Groups</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dr.</td>
<td>Kenji</td>
<td>Inaba</td>
<td>Critical Care Physician</td>
<td>North American Civilian</td>
</tr>
<tr>
<td>2</td>
<td>Dr.</td>
<td>Sandro</td>
<td>Rizoli</td>
<td>Trauma surgeon</td>
<td>North American Civilian</td>
</tr>
<tr>
<td>3</td>
<td>Dr.</td>
<td>Jeannie</td>
<td>Callum</td>
<td>Transfusion medicine physician</td>
<td>North American Civilian</td>
</tr>
<tr>
<td>4</td>
<td>Dr.</td>
<td>Ross</td>
<td>Davenport</td>
<td>Trauma surgeon</td>
<td>International Civilian</td>
</tr>
<tr>
<td>5</td>
<td>Dr.</td>
<td>John</td>
<td>Hess</td>
<td>Trauma surgeon</td>
<td>North American Civilian</td>
</tr>
<tr>
<td>6</td>
<td>Dr.</td>
<td>Hebert</td>
<td>Schochl</td>
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<td>International Civilian</td>
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<tr>
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<td>Dr.</td>
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<td>Brohi</td>
<td>Trauma and vascular surgeon</td>
<td>International Civilian</td>
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<tr>
<td>8</td>
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<td>Goerlinger</td>
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<tr>
<td>9</td>
<td>Dr.</td>
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<tr>
<td>16</td>
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<td>Cohen</td>
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<td>Dr.</td>
<td>Andrew</td>
<td>Cap</td>
<td>Trauma surgeon</td>
<td>North American Military</td>
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<tr>
<td>18</td>
<td>Dr.</td>
<td>Keyvan</td>
<td>Karkouti</td>
<td>Anaesthesiologist</td>
<td>North American Civilian</td>
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3.3 Introduction

Despite all of the major advances in resuscitation science that have been integrated into contemporary trauma care, hemorrhage remains the leading cause of preventable death after injury (Gruen, Jurkovich, McIntyre, Foy, & Maier, 2006; Sauaia et al., 1995; Teixeira et al., 2009a). Because of this, the primary focus of any management strategy for an injured patient is the recognition, control, and mitigation of acute blood loss. One of the key components of this is the correction of any systemic coagulation defects, a problem that is common even at admission (Brohi, 2009; MacLeod et al., 2003; Brohi et al., 2003; Niles et al., 2008), with the potential to become even more pronounced across time. Although, currently, the correction of coagulation defects in most severely bleeding patient is often achieved with empirical use of fixed ratios of complementary blood products, in ideal circumstances, hemostatic interventions targeting specific defects guided by laboratory testing would be preferable.

Current laboratory assays have limitations that restrict their usefulness in guiding early resuscitation of injured patients who are actively bleeding. A promising alternative, viscoelastic testing, uses whole blood and provides both dynamic and timely information through measurement of clot formation and dissolution across time that can be performed as a point-of-care whole-blood test. Originally described by Hartert (Hartert, 1951) in 1948, two commercially available products dominate the current market. The first is the TEG 5000 (Haemonetics Corporation, Braintree, MA), which is used predominantly in the United States, and the other is
ROTEM® (TEM International GmbH, Munich, Germany), which has a large presence in Europe and Canada. Both of these devices use a pin suspended in a cup of recalcified or native whole blood. As the pin and cup rotate relative to each other with repetitive, controlled, low shear movements, the formation and eventual dissolution of clot are captured as changes in torque, which are then transduced into a visual tracing. Although the mechanical principles underlying the two tests are similar, the different hardware and activators result in different output values (Table 3.2) and reference ranges, with results that cannot be used interchangeably. These principles and reference ranges have been extensively reviewed (Haas et al., 2014; Bolliger, Seeberger, & Tanaka, 2012; Theusinger, Nurnberg, Asmis, Seifert, & Spahn, 2010).

Table 3.2: Reference Ranges

<table>
<thead>
<tr>
<th></th>
<th>CT(s)</th>
<th>CFT(s)</th>
<th>α Angle(°)</th>
<th>A10(mm)</th>
<th>A20(mm)</th>
<th>MCF(mm)</th>
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<tr>
<td>INTEM</td>
<td>122-208</td>
<td>45-110</td>
<td>70-81</td>
<td>40-60</td>
<td>51-72</td>
<td>51-72</td>
</tr>
<tr>
<td>EXTEM</td>
<td>43-82</td>
<td>48-127</td>
<td>65-80</td>
<td>40-60</td>
<td>50-70</td>
<td>52-70</td>
</tr>
<tr>
<td>FIBTEM</td>
<td></td>
<td></td>
<td></td>
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<td>7-24</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>R(min)</th>
<th>K(min)</th>
<th>α Angle(°)</th>
<th>MA(mm)</th>
<th>LY30(%)</th>
<th>G(dynes/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEG</td>
<td>5-10</td>
<td>1-3</td>
<td>53-72</td>
<td>50-70</td>
<td>0-8</td>
<td>4.5-11.0k</td>
</tr>
</tbody>
</table>

Despite its long history, the use of this technology in trauma care has only recently generated widespread interest. Despite the growing evidence base supporting its use, many facets of its integration into clinical care remain unclear and no universally accepted guidelines for its use exist. While this is also true for conventional laboratory test based markers of coagulopathy (Haas et al., 2015; Ciavarella et al., 1987; McCully et al., 2013; Murray, Olson, Strauss, & Tinker, 1988) with the rapidly expanding literature base on viscoelastic testing, and the potential that this technology could change resuscitation practices, a consensus conference was organized. In September 2014, an international group of leaders in the field of trauma coagulopathy and resuscitation was assembled for a 2-day consensus conference in Philadelphia, Pennsylvania. This multidisciplinary panel included trauma surgeons, hematologists, blood bank specialists,
anesthesiologists, and the lay public. Nine specific questions regarding the role of viscoelastic testing in trauma patients were developed before the conference by panel consensus and posted for public review. Each of these questions was then reviewed in person using standardized presentations of the relevant evidence by the expert panel. The evidence focused on the early use of viscoelastic testing in civilian adult trauma. Early use was defined as baseline viscoelastic test result thresholds obtained within the first minutes of hospital arrival when conventional laboratory results are not available. At the conclusion of the presentations, a consensus summary document was developed and reviewed by the panel in an open forum. The conference was held just before a large international trauma meeting and was open to all members and the general public.

At the conclusion of the consensus conference, a two round Delphi poll was administered to the panel of experts regarding the use of viscoelastic thresholds for triggering the initiation of specific treatments including fibrinogen, platelets, plasma, and prothrombin complex concentrates (PCCs). The Delphi method is a structured interactive communication technique coordinated by a facilitator. The panel of experts answered a set of questions in a preset number of rounds, with an anonymous summary of the results being presented to the expert panel after each round. The experts were encouraged to revise their answers in light of the responses from the other panel members. Responses were collected and analyzed, and conflicting viewpoints were identified. During this process, the range of answers narrows and the group progresses toward a consensus. The results of the Delphi poll will be presented under each appropriate category.

The purpose of this consensus conference was to develop a set of viscoelastic test based thresholds (Fig. 3.1) that would indicate the need for hemostatic interventions during the acute early phase of resuscitation based on current evidence and expert practice. High-value areas to be targeted for future research were also discussed (Table 3.4). The specific goals of the conference were to do the following:

1. Summarize and consolidate the existing evidence on optimal viscoelastic test threshold values used to diagnose coagulopathy, guide hemostatic interventions including blood product transfusion, and predict mortality in injured patients.
2. Present institutional experience regarding the use of viscoelastic testing.
3. Develop guidelines for the early resuscitation of trauma patients based on the viscoelastic test threshold values.

Figure 3.1: ROTEM® algorithm for early trauma resuscitation

The key recommendations of the Delphi panel members are listed in Table 3.3.
Table 3.3: Key Recommendations

<table>
<thead>
<tr>
<th>Recommendation</th>
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<tbody>
<tr>
<td>Consideration be given to the use of viscoelastic testing during the early phases of trauma resuscitation.</td>
</tr>
<tr>
<td>While clotting time may detect abnormalities in clot initiation, the current evidence is insufficient to recommend an exact threshold for FFP or PCC transfusion.</td>
</tr>
<tr>
<td>While the available data is insufficient to support a precise threshold for fibrinogen replacement, the expert recommendation is to consider cryoprecipitate or fibrinogen concentrate transfusion when abnormalities in clot strength are detected on viscoelastic testing.</td>
</tr>
<tr>
<td>Viscoelastic testing is highly specific for fibrinolysis and should be used during early trauma resuscitation to identify injured patients with systemic hyperfibrinolysis. Any evidence of hyperfibrinolysis during early resuscitation should warrant consideration of antifibrinolytic medications.</td>
</tr>
<tr>
<td>The panel recommends against the use of viscoelastic testing for withholding antifibrinolytic therapy.</td>
</tr>
<tr>
<td>Hypercoagulable or normal results during early resuscitation should lead to the consideration of withholding blood product transfusion.</td>
</tr>
<tr>
<td>Cost effectiveness has been demonstrated in the non-trauma setting and is in large part due to the decrease in the consumption of blood products, which may result from improved hemostatic management.</td>
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<tr>
<td>Normal viscoelastic test results can be seen in patients with mechanical bleeding or significant coagulopathy due to hypothermia, acidosis, warfarin and other oral anticoagulant medications, Von Willebrand Disease, platelet inhibitors and platelet dysfunction.</td>
</tr>
<tr>
<td>The optimal location for equipment setup is institution dependent but should allow for rapid testing and communication to the clinical team if it is to be used for the early resuscitation of bleeding</td>
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trauma patients. Viscoelastic testing can be used as a point of care test.

Testing should also meet the local regulations that govern lab testing in this location.

The person(s) performing the tests and interpreting the results should be trained and educated adequately.

<table>
<thead>
<tr>
<th>Table 3.4: Future Research Opportunities</th>
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### 3.4 Questions to the panel

#### 3.4.1 Question 1. Is there a viscoelastic test parameter that can predict mortality or the need for a massive transfusion in trauma patients?

#### 3.4.1.1 Mortality

The acute coagulopathy of trauma as diagnosed by conventional laboratory testing has been associated with a significant increase in mortality (MacLeod et al., 2003; Niles et al., 2008). Viscoelastic testing in these patients produces a signature trace of traumatic coagulopathy (Davenport et al., 2011) and is also associated with increased mortality. However, like with conventional testing, strict threshold cut-off values remain elusive as the available data has been generated through the testing of arbitrary thresholds rather than rigorous cut-point analyses. In a
prospective analysis of 334 patients evaluated with ROTEM® (Tauber et al., 2011), 24h mortality was increased when MCF FIBTEM ≤7mm (21% vs 9%, p=0.006), CT EXTEM ≥100s (45.5% vs 8.4%, p<0.001), CFT EXTEM ≥200s (27% vs 8.7%, p<0.001) and MCF EXTEM ≤45mm (25.4% vs 9.4%, p=0.001) were examined. After adjustment for confounding variables, these values and LI60 EXTEM were independently associated with death. In patients with isolated traumatic brain injury, MCF FIBTEM had a strong association with mortality (Schochl et al., 2011c). For TEG, retrospective analysis of TEG values on admission demonstrated low clot strength (MA<50mm) (Nystrup, Windelov, Thomsen, & Johansson, 2011) and derived G (Pezold et al., 2012), to be independently associated with mortality. Hyperfibrinolysis (Chin et al., 2014; Brohi et al., 2008; Kashuk et al., 2010) in particular has been demonstrated by both tests to be tightly associated with mortality. In work by Schochl (Schochl et al., 2009) using ROTEM®, hyperfibrinolysis (Maximum Lysis=100%) was separated into three groups based on the time it was diagnosed [<30min (fulminant), 30-60min (intermediate) and >60min (late)], demonstrating a stepwise increase in mortality from 73% for late, 91% for intermediate and 100% for fulminant. Theusinger demonstrated that hyperfibrinolysis associated with trauma had a significantly higher mortality than patients with hyperfibrinolysis from non-trauma causes or matched patients without hyperfibrinolysis (Theusinger et al., 2011). In the Tauber study (Tauber et al., 2011), hyperfibrinolysis was associated with a high degree of mortality, especially for those with complete dissolution of the clot within 60min. In a prospective study from France, ROTEM® was demonstrated to be highly sensitive and specific for the detection of hyperfibrinolysis which was associated with a significantly higher mortality (100% vs 11%, p<0.05) (Levrat et al., 2008; Kashuk et al., 2010). In two studies using admission TEG data from Denver (Chapman et al., 2013), one from Los Angeles (Ives et al., 2012) and another from Houston (Cotton et al., 2012), hyperfibrinolysis was demonstrated to be a major predictor of death. In the Denver study (Chapman et al., 2013), for those requiring a Massive Transfusion (MT), a LY30 of 3% or greater was associated with substantially higher mortality (45.5% vs 4.8%, p=0.001). Reduced clot strength and hyperfibrinolysis are strong predictors of mortality in trauma patients and may be used to prognosticate early in the resuscitation of major hemorrhage. Viscoelastic testing is important because conventional coagulation testing is not practical for diagnosing hyperfibrinolysis during
the acute resuscitation phase of care. The traditional Euglobin Clot Lysis test is time consuming
and is not available at most centers around the clock. This will be further expanded upon in
Question 4, which addresses hyperfibrinolysis and its treatment with antifibrinolytic medications.

### 3.4.1.2 Massive Transfusion

The ability to predict the need for a Massive Transfusion is clinically relevant, facilitating
communication with the Blood Bank and providing lead-time for the preparation of blood
products. For research study design, this also allows for improved screening for injured patients
that will require blood products. The difficulty in answering this question rests with the
uncertainty surrounding the optimal definition of what constitutes a Massive Transfusion. Both
the volume of blood and the time span over which it is administered, are actively being debated.
A more pragmatic question may be; what viscoelastic test values predict the need for blood
product transfusion? The majority of the available data has focused on the MA or MCF as a
reflection of clot strength. In a study by Schochl (Schochl et al., 2011a) of 323 patients, using
>=10 units of packed red blood cells (PRBC) in 24h as the definition of a MT, admission
FIBTEM A10 and MCF were highly predictive of a MT. Davenport has demonstrated that an
EXTEM A5 <=35mm could predict the need for a MT faster and more accurately than the INR
(Davenport et al., 2011). In a study by Tauber et al (Tauber et al., 2011), the MCF FIBTEM was
independently associated with the need for early blood transfusion within the first 6 hours.

For TEG, Cotton (Cotton et al., 2011) demonstrated an ACT >128sec predicted the need for
>=10PRBC in the first 6 hours (OR 5.15, 95% CI 1.36-19.49, p=0.01) and that an ACT<105sec
predicted not needing any transfusion in the first 24h (OR 2.80, 95% CI 1.02-7.07, p=0.04). In
another study from this institution (Holcomb et al., 2012), an alpha angle<56° was highly
predictive of the need for a MT (OR 8.99, 95% CI 2.86-28.29, p<0.001) defined as
>=10PRBC/6h. Pezold (Pezold et al., 2012) using the same definition of a MT showed that TEG
derived G as a marker of clot strength was an independent predictor as well. In a recent
comparison of ROTEM® to TEG A10 (Meyer et al., 2014) values and FIBTEM MCF appeared
to be the best discriminator of the need for a MT.

**Recommendation of the Consensus Panel**
We recommend that consideration be given to the use of viscoelastic testing during the early phases of trauma resuscitation for the following reasons:

1. There is a strong association between early abnormal results (in particular clot strength and systemic hyperfibrinolysis), and mortality as well as the need for massive transfusion.

2. This is the only practical test capable of diagnosing hyperfibrinolysis with a high degree of specificity within the time constraints of early trauma resuscitation.

3. The time to onset of hyperfibrinolysis, with earlier being worse, and the magnitude of hyperfibrinolysis, correlates to increased mortality.

Further considerations such as cost, time, accuracy and variables affecting results, are addressed in Questions 7 and 8.

3.4.2 Question 2. *Is there a viscoelastic test parameter threshold that can be used to guide the administration of plasma or Prothrombin Complex Concentrate?*

As a point of care whole blood test, viscoelastic testing would theoretically allow for the rapid evaluation of the coagulation profile of an injured patient, allowing a targeted infusion of specific blood products to correct specific deficits. Plasma and the importance of its early role in the resuscitation of the injured patient has been extensively studied however, studies using ROTEM® or TEG to guide its transfusion are lacking. The available data attempts to retrospectively correlate specific viscoelastic test findings to the “need” for plasma as evaluated by the amount of plasma given to a patient over a fixed time after the test was run. This study design does not allow a direct evaluation of the impact of plasma infusion for a specific viscoelastic test threshold value.

Using TEG, Nystrup (Nystrup et al., 2011) demonstrated that low clot strength correlated with an increased transfusion of plasma within the first 24h. In a study by Holcomb (Holcomb et al., 2012) all output values except for LY30 predicted plasma transfusion in the first 6 hours. In a study from Denver (Kashuk et al., 2012), Maximum Rate of Thrombin Generation \( \geq 9.2 \text{mm/min at three hours} \) was associated with a decrease in plasma usage. Not all of the available studies support this association. In a study evaluating 161 injured patients, none of the
output values differed between those who did and did not get transfused (Carroll et al., 2009). ROTEM\textsuperscript{®} has also been studied in this manner. Davenport has demonstrated that patients with an EXTEM A5 <=35mm were more likely to receive a plasma transfusion (37\% vs 11\%, p<0.001. (Davenport et al., 2011).

For PCC, two retrospective studies were available for review. Schochl (Schochl et al., 2010) examined 131 patients who received fibrinogen concentrate as the initial treatment. Of these, 98 additional patients received PCC when EXTEM CT was prolonged by 1.5 times normal. A blunt comparison of the observed mortality against TRISS predicted mortality demonstrated favorable outcomes. In a further study, ROTEM\textsuperscript{®} guided PCC transfusion was compared to patients receiving plasma empirically demonstrating a decrease in exposure to blood products(Schochl et al., 2011b).

**Recommendation of the Consensus Panel**

While the clotting time may detect abnormalities in clot initiation, the current evidence is insufficient to recommend an exact threshold for FFP or PCC transfusion.

The two-round Delphi poll revealed little agreement amongst the participants when asked about the viscoelastic triggers for the transfusion of plasma or PCC. Of the participant group, 15/23 (65\%) would consider transfusion when EXTEM CT ≥ 80 seconds (plus EXTEM A10 ≥ 40 mm or EXTEM MCF ≥ 50 mm). The major concern amongst the participants was the lack of rigorous data to support this recommendation and the differences between plasma and PCC. Low platelet count and low fibrinogen levels may also prolong EXTEM CT and should be considered in the decision to use this value as a transfusion trigger for plasma or PCC

3.4.3 **Question 3: Is there a viscoelastic test parameter threshold that can be used to guide the administration of fibrinogen concentrate or cryoprecipitate?**

Fibrinogen is essential for hemostasis and is one of the first clotting factors to fall to critical values following major traumatic hemorrhage (Hagemo et al., 2014; Rourke et al., 2012; Harr et al., 2013). Depressed levels have been associated with poor outcomes including mortality. (Hagemo et al., 2014; Inaba et al., 2013; Rourke et al., 2012; 1994; Brohi et al., 2008; Harr et al., 2013). There is a lack of consensus on the optimal serum level that would require replacement...
with trigger values ranging from 0.8 to 2.0 g/dL (Levy, Welsby, & Goodnough, 2014). The current standard for conventional fibrinogen testing is the Clauss method of fibrinogen measurement. Although it can take upwards of 30-60 minutes to obtain the result, FIBTEM correlates well with this value (Rourke et al., 2012; Rugeri et al., 2007). In the presence of artificial colloids in particular, the Clauss method may over-estimate fibrinogen levels thus favoring the use of viscoelastic testing (Fenger-Eriksen et al., 2010). Pre-clinical animal models demonstrate ROTEM® guided fibrinogen supplementation in trauma can be effective (Bolliger et al., 2009; Haas et al., 2008; Velik-Salchner et al., 2007). Clinically, because ROTEM® can identify low levels of functional fibrinogen, it can help direct replacement therapy in fibrinogen depleted states (Lang et al., 2009; Velik-Salchner et al., 2007). Rugeri has shown that there is good correlation between fibrinogen and FIBTEM A10, with a trigger value of 1g/dL equal to a cut point of 6mm (Rugeri et al., 2007). Other groups have used a FIBTEM A10 of <7mm or MCF<7mm (18) or a FIBTEM MCF of 10-12mm (Schochl et al., 2010; Schochl et al., 2010). The direct impact on survival of using these thresholds for fibrinogen replacement is not known.

**Recommendation of the Consensus Panel**

While the available data is insufficient to support a precise threshold for fibrinogen replacement, the expert recommendation is to consider cryoprecipitate or fibrinogen concentrate transfusion when abnormalities in clot strength are detected on viscoelastic testing.

The two-round Delphi poll revealed agreement among 20/23 (87%) of the participants who would consider the administration of a fibrinogen concentrate or cryoprecipitate in patients with the following viscoelastic testing parameters: FIBTEM A10 < 10 mm corresponding to a FIBTEM MCF < 12 mm (plus abnormal low EXTEM A10 < 40 mm corresponding to an EXTEM MCF < 50 mm). There was concern expressed by 3/23 (13%) participants regarding the threshold. These participants favored a lower treatment threshold of FIBTEM A10 or FIBTEM MCF < 7 mm to avoid excessive fibrinogen administration (Fig 3.1).

The considerations supporting this recommendation include:
1. Early abnormalities in clot strength on viscoelastic testing correlate with conventional laboratory fibrinogen measurements.
2. Viscoelastic testing results can be more rapidly obtained than conventional Clauss method measurements.
3. Viscoelastic testing can detect functional deficits in fibrin polymerization.

3.4.4 Question 4. Is there a viscoelastic test parameter threshold that can be used to guide the administration of antifibrinolytic medications?

Fibrinolysis is an integral part of the coagulation process. After injury, excess fibrinolysis has been implicated as an important component of early trauma induced coagulopathy, and, as discussed in Question 1, is tightly associated with mortality. This becomes important because there are pharmacologic antifibrinolytic agents available to mitigate this hyperfibrinolysis, which tested in a large multinational randomized controlled trial was shown to decrease mortality (Shakur et al., 2010). Due to the study design and lack of mechanistic coagulation data however, universally accepted indications for antifibrinolytic administration have not been established and viscoelastic testing in theory may provide guidance. Conventional coagulation testing is not practical for diagnosing hyperfibrinolysis. The Euglobin Clot Lysis test is not available in most centers at all times of the day, and takes 90 minutes of observation before the test is complete. Using viscoelastic testing however, an estimate of systemic fibrinolysis can be obtained with good correlation to the Euglobin Clot Lysis values. In the study by Levrat (Levrat et al., 2008), 87 trauma patients were prospectively studied using both admission ROTEM® and Euglobulin Clot Lysis time with hyperfibrinolysis defined at <90 minutes. Using threshold values of EXTEM MCF 18mm, CLI30 71% and increase of APTEM MCF 7%, a sensitivity of 100%, 75% and 80% respectively with a specificity of 100% was found. In work done by Raza, the magnitude of fibrinolysis may in fact be greater than that detected by viscoelastic testing when plasmin-antiplasmin complex and D-dimer level analysis is utilized as the gold standard for measurement (Raza et al., 2013). The optimal cut-off for treatment or additional doses of antifibrinolytic medications if required however has not been studied. The original LY30 threshold of 7.5% (Cotton et al., 2012) may be too high. Work done by the group in Denver using TEG (Chapman et al., 2013; Moore et al., 2014) suggests that at an LY30 of 3% or
greater, may be a more sensitive cut-off value for the detection of hyperfibrinolysis. To date however, a validation of the outcomes associated with viscoelastic test guided anti-fibrinolytic therapy has not been performed. Likewise, the sensitivity of viscoelastic testing is insufficient to support its use for withholding antifibrinolytic therapy if hyperfibrinolysis is absent.

**Recommendation of the Consensus Panel**

Viscoelastic testing is highly specific for fibrinolysis and should be used during early trauma resuscitation to identify injured patients with systemic hyperfibrinolysis.

The panel recommends against the use of viscoelastic testing for withholding antifibrinolytic therapy.

While waiting for viscoelastic test results may delay timely antifibrinolytic therapy, the panel recommendation is that any viscoelastic evidence of hyperfibrinolysis during the early resuscitation of a bleeding trauma patient should warrant consideration of antifibrinolytic medication administration if not yet already administered.

The two-round Delphi poll revealed agreement amongst 21/23 (91%) of the participants who would consider the use of an antifibrinolytic medication in patients where there is viscoelastic evidence of lysis (Fig 3.1). Viscoelastic parameters consistent with fibrinolysis include: ML ≥ 5% within one hour (corresponding to an LI60 ≤ 95%), EXTEM A5 < 35 mm (corresponding to an EXTEM A10 < 45 mm or an EXTEM MCF < 55 mm) or a flat-line in FIBTEM (FIBTEM CT > 600 seconds).

### 3.4.5 Question 5. Is there a viscoelastic test parameter threshold that can be used to guide the administration of platelets?

For the administration of platelets, viscoelastic testing has been correlated to platelet counts that are commonly utilized as a transfusion trigger (50 x 10⁹/L and/or 100 x 10⁹/L). In a retrospective study of 44 patients where the platelet count was compared to the MA (Kashuk et al., 2009a), correlation with a k-value of 0.48 was found. When transfusion thresholds were compared, platelet count <100x10⁹/L and MA <52mm demonstrated near identical impact on transfusion decision making. In a study of 90 patients(Levy et al., 2014) , platelet count was correlated to
INTEM A15 (r=0.57) and EXTEM A15 (r=0.56). An INTEM A15 <46mm correlated well to a platelet count of 50 x 10^9/L with a sensitivity of 100% and specificity of 83%. In another study (Meyer et al., 2014), platelet count correlated best with INTEM A10 (Spearman’s p=0.54) and EXTEM A10 (Spearman’s p=0.51). Several reports have published cut-off values for platelet transfusion however these are based on expert opinion. ROTEM® values of EXTEM A10 <40mm (Schochl, Schlimp, & Voelckel, 2014), EXTEM A10 <35-42mm (Stensballe, Ostrowski, & Johansson, 2014), INTEM A10 <40mm (Theusinger, Spahn, & Ganter, 2009) and TEG values of MA <45-49mm (Stensballe et al., 2014) and MA <55mm (Gonzalez et al., 2014) have been published. The differing cut-off values tested in these studies and the lack of uniformity in platelet transfusion practices, make the determination of a threshold viscoelastic test value unattainable.

**Recommendation of the Consensus Panel**

The available data does not support an exact threshold for treatment however, the two-round Delphi poll revealed agreement amongst 21/23 (92%) of the participants who would consider platelet transfusion in patients where viscoelastic testing demonstrated an abnormally low EXTEM A10 or EXTEM MCF (plus normal FIBTEM A10). It was noted by the participants that this was an indirect measure of platelet function. An abnormality in these measures may be specified as any EXTEM A10 or EXTEM MCF below the reference range (EXTEM A10 < 40 mm corresponding to an EXTEM MCF < 50 mm).

The considerations supporting this recommendation are the following:

1. There is a correlation between clot strength as measured by viscoelastic testing and platelet count.
2. A high fibrinogen level can mask abnormal platelet count or function on clot firmness.

**3.4.6 Question 6. Is there a viscoelastic test parameter threshold that can be used to withhold the transfusion of blood products?**

In the acute resuscitation phase of a trauma patient, as long as there is no clinically apparent bleeding, normal viscoelastic test values should allow for the withholding of blood products.
There are no studies available to support this however the administration of plasma or platelets to a hemodynamically normal patient with no evidence of bleeding and normal ROTEM® or TEG values would be difficult to justify. In the study from Houston, an ACT <105sec was associated with no need for transfusion in the first 24h (Cotton et al., 2011). For the hypercoagulable patient, the administration of blood products would be contraindicated. The hypercoagulable state can be diagnosed by viscoelastic testing but not by conventional coagulation tests (Pommerening et al., 2014; Schreiber, Differding, Thorborg, Mayberry, & Mullins, 2005; Cotton et al., 2012; Branco et al., 2014; Kashuk et al., 2009b). This state can be seen early on admission blood sampling, and would preclude the need for any blood product transfusion acutely unless otherwise dictated by the patient’s clinical presentation.

**Recommendation of the Consensus Panel**

While there is no data to support the use of viscoelastic testing results to withhold transfusion, the panel recommendation is that hypercoagulable results during early resuscitation should lead to the consideration of withholding blood product transfusion. There is also evidence that hypercoagulable viscoelastic testing results are associated with a reduced need for hemostatic resuscitation.

3.4.7 **Question 7. Is there a practical advantage in terms of cost, time or logistics when viscoelastic testing is compared to conventional laboratory testing?**

3.4.7.1 **Cost**

Cost data in general is lacking. There are complex factors that need to be considered which include equipment costs, maintenance, training, personnel, sample handling, and data entry, all of which can vary according to the setup of the system within a specific institution. The migration from a standard conventional test based protocol for the transfusion of components to one utilizing a viscoelastic test based algorithm in the cardiac surgery literature (Despotis et al., 1994) reduced costs at one institution by $267,658 per year. In a study out of Houston, as emphasized by the authors, cost and charge values vary widely between institutions, however at this hospital, the charge for a set of conventional lab tests was comparable to that for TEG ($286 vs $317) (Holcomb et al., 2012). The National Institute for Health and Care Excellence Diagnostics
Assessment Program in the UK performed an evidence review for viscoelastic testing (Detecting, managing and monitoring haemostasis: viscoelastometric point-of-care testing. Nice Diagnostics Guidance 13. Available at: http://www.nice.org.uk/dg13August2014. Accessed December 14, 2014). They found in their cost-effectiveness analysis that this test would be cost effective if more than 80 tests were performed each year.

3.4.7.2 Time

The time to data output can be critical if viscoelastic testing or any other coagulation parameter is to be effectively integrated into the early phase of resuscitation. This has long been espoused as one of the advantages of viscoelastic testing over conventional coagulation tests, especially if performed as a point of care test. When platelets were examined, the median turnaround time for the standard platelet count was 13 minutes (IQR 9-22 minutes) as compared to 12 minutes for EXTEM A5 (Olde Engberink et al., 2014). In a different study, the conventional test took 16 minutes (SD 10.3), compared to 63.7 minutes (SD 19.1) for the full tracing, with preliminary data becoming available at approximately 30 minutes (Welsh et al., 2014). In this study, a conventional coagulation lab panel took 20 minutes (SD 9.9) as compared to the 60 minutes for the full tracing.

For fibrinogen and fibrinolysis, viscoelastic testing however may have a distinct advantage. The turnaround time for Clauss fibrinogen in one study was 37 minutes (IQR 31-54) as compared to 12 minutes for FIBTEM A5 (Olde Engberink et al., 2014). In a pediatric study, fibrinogen testing took 53 minutes (IQR 45-63) whereas the A10 measurement took only 23 minutes (IQR 21-24) (Haas et al., 2012). Of note, the standard Clauss fibrinogen assay can be modified to provide a more rapid result (Chandler, Ferrell, Trimble, & Moody, 2010). For fibrinolysis, TEG and ROTEM® remain the only practical tests available in the acute resuscitation phase as the standard Euglobin Clot Lysis test is not available in most centers at all times, and takes 90 minutes of observation before the test is complete.

3.4.7.3 Accuracy
The quality control process is a major issue for viscoelastic testing, especially if it is used in a satellite setting away from the core laboratory as a point of care test by a clinical care provider. An external proficiency testing report (Kitchen, Kitchen, Jennings, Woods, & Walker, 2010) from the United Kingdom’s National External Quality Assessment Scheme (NEQAS) raised major concerns regarding the lack of precision of both TEG and ROTEM®. Overall, 18 TEG and 10 ROTEM® users were sent a total of 8 blood samples including both normal and abnormal samples. They found a lack of uniformity in the output values with coefficients of variance ranging from 7.1-39.9% for TEG and 7.0-83.6% for ROTEM® (Kitchen et al., 2010). The results between centers were so different that if transfusion decisions were made based on these results, the treatments would have differed between centers. There may be differences between the two available technologies. In one study performed in cardiac patients, ROTEM® analyses were found to be more reproducible than those found on TEG (Anderson et al., 2014).

**Recommendation of the Consensus Panel**

**Question 3.4.7.1 Cost**

The costs of starting a program may range from 100-125K USD. Ongoing costs are comparable to other conventional coagulation tests.

Cost effectiveness has been demonstrated in the non-trauma setting and is in large part due to the decrease in the consumption of blood products, which may result from improved hemostatic management.

**Question 3.4.7.2 Time**

There is a time advantage with the use of viscoelastic testing for the measurement of fibrinogen and fibrinolysis and overall screening of hemostatic competence.

**Question 3.4.7.3 Accuracy**

Accuracy and reproducibility of viscoelastic testing in the trauma setting requires further study
3.4.8 Question 8. Are there patient variables that can interfere with the interpretation of viscoelastic test results?

Patient variables can affect the results obtained from viscoelastic testing. Because viscoelastic tests work by activating plasma coagulation which in turn recruits platelets with the strong platelet activator thrombin, problems in the normal weak platelet activation pathways may not be detected. Platelet adhesion defects in a high shear situation such as von Willebrand’s disease can be missed. Likewise, drug related coagulopathy due to aspirin, clopidogrel or hydroxyethyl starch will also go undetected (Schramko et al., 2015). However GPIIb-IIIa inhibitors will be detected. Therefore, in clinical practice, where the coagulopathy is being driven by defects in platelet adhesion or adhesion coupled activation, the platelet count may be a better marker for thrombocytopenic bleeding. MCF, as measured on ROTEM® has also been demonstrated to correlate with the changes in clot strength due to anemia (Ogawa et al., 2012; Solomon, Rahe-Meyer, Schochl, Ranucci, & Gorlinger, 2013). Alcohol has also been demonstrated to result in a hypocoagulable state, with decreased fibrin formation, clot strength and rate of fibrin cross-linking (Spoerke et al., 2010). This may be gender specific with a positive correlation to alcohol seen in men. The presence of gender differences in normal viscoelastic testing is unclear (Lang et al., 2005). For pediatric reference ranges, above one year of age, minimal differences have been noted with increasing age(Oswald et al., 2010).

Pre-analytic variables include the technique of drawing and the use of anticoagulation (Flatland, Koenigshoff, Rozanski, Goggs, & Wiinberg, 2014; Woolley et al., 2013; Keene, Nordmann, & Woolley, 2013). Unlike the elective blood draw for conventional testing which is performed by a trained clinician gently using large bore silicone lined needle, sample acquisition and handling in the chaotic trauma bay may result in platelet activation by shear with subsequent loss of activity and alteration of the test result (Keene et al., 2013). This has been demonstrated by testing a sample repeatedly over the first hour after the draw (Woolley et al., 2013). If a non-anticoagulated sample is used, differences in the time from draw to test will result in variability in the test results (Martin, Schuster, Moessmer, Kochs, & Wagner, 2012). If anticoagulated samples are used, the choice of 1.8 or 3.6 mL citrated tubes has been shown to not make a difference however, care should be taken to fill the tubes completely (Andreasen, Pistor-Riebold, Knudsen, Ravn, & Hvas, 2014). Analytic variability also exists. Basic test preparation such as
ensuring the ROTEM® machine in the mobile mode is up to temperature can be easily controlled. User variability in pipetting, reagent stability, and the maintenance and calibration of equipment however are much more difficult to control and can all impact the output values. Individual tests run on different units, between different channels of the same unit, between morning and afternoon measurements show a high degree of variability (Nagler et al., 2014). In the NEQAS surveys, as discussed earlier, high variability and poor reproducibility of the test results was seen even when the same sample was run on the same machine by the same technologist (Kitchen et al., 2010).

**Recommendation of the Consensus Panel**

There are multiple factors that impact viscoelastic testing results.

Normal viscoelastic test results can be seen in patients with mechanical bleeding or significant coagulopathy due to hypothermia, acidosis, warfarin and other oral anticoagulant medications, Von Willebrand Disease, platelet inhibitors and platelet dysfunction.

**3.4.9 Question 9. What is the best location for the viscoelastic testing unit and who should operate this equipment?**

These viscoelastic tests can be run either as point of care tests at the bedside or as core laboratory based tests. Having the unit located in the ER or OR allows for faster time to delivery of results however this requires a trained technologist or clinical team member in this location available to run the test. In the United States, the Clinical Laboratory Improvements Act of 1988 (CLIA), allows the device to be used as a point of care test or as a laboratory instrument. In Europe, where hematology and blood bank are a combined specialty for laboratory technologists, having the device in the Blood Bank allows the transfusion staff to monitor the need for components and work with a transfusion medicine specialist to manage the blood therapy. Even if performed at a centralized core laboratory, these results can be immediately processed and visualized in real time by the clinical team on a variety of platforms including clinician hand held devices. To date, there is insufficient data comparing bedside and core lab run samples. Ultimately how viscoelastic testing is integrated into a specific system will depend on local factors such as the physical infrastructure and human resources.
Recommendation of the Consensus Panel

The optimal location for equipment setup is institution dependent but should allow for rapid testing and communication to the clinical team if it is to be used for the early resuscitation of bleeding trauma patients. Viscoelastic testing can be used as a point of care test.

Testing should also meet the local regulations that govern lab testing in this location.

The person(s) performing the tests and interpreting the results should be trained and educated adequately.

Further refinement of the technology for performing the test, improving throughput and for the transfer of results to the medical record are required.
Chapter 4: Risk factors of blood products transfusion, Massive transfusion and 24h mortality: Methodology, Development and external validation of Prediction models
4.1 Abstract

Introduction: Early identification of patients with trauma induced coagulopathy (TIC) and resourceful blood transfusion management is an ongoing problem for many trauma centers. Rotational thromboelastometry is an emerging and promising solution to identify patients with TIC. We aimed to assess if ROTEM® variables along with readily available clinical parameters could predict the need for 24h blood products transfusion among bleeding trauma patients.

Methods: Following ethics approval, we performed a retrospective cohort analysis of adult trauma patients presenting with blunt or penetrating injuries and admitted to two level-I trauma centers. Patients who did not receive admission ROTEM® and who died within one hour of admission without ROTEM® or blood products transfusion were excluded. The derivation cohort consisted of trauma patients admitted to Sunnybrook Hospital between August 2011 and March 2013. The validation cohort consisted of trauma patients admitted to St. Michael’s hospital between November 2014 and October 2015. We developed and validated prediction models for 24h transfusion of blood products using readily available ROTEM® and clinical variable using multivariable logistic regression analysis.

Results: During the study 1146 patients were included in the derivation cohort and 779 in validation cohorts. Groups were similar in patient and clinical characteristics. ROTEM® FIBTEM A10 was the predictor of 24h plasma (aOR: 0.90(95% CI: 0.83, 0.98, P=0.0171) and cryoprecipitate transfusions (aOR: 0.80, 95% CI: 0.71, 0.91, p=0.0005) while adjusting for other variables. The sensitivity, specificity, and negative predictive value of the model for 24h plasma transfusion at a probability threshold of 0.06 were 89%, 88% and 99% respectively. The sensitivity, specificity, negative predictive value of the model for 24h cryoprecipitate transfusion at a probability threshold of 0.02 was 96%, 85% and 99.9% respectively. ROTEM® was not the predictor of RBC and platelet transfusions.

Conclusions: ROTEM® predicted the transfusion of 24h plasma and cryoprecipitate; however it was not a predictor of RBC and platelet transfusion. More robust studies are required to confirm these findings.
4.2 Overview

The overall aim of this work was to investigate the use of thromboelastometry for the initial resuscitation of significantly bleeding trauma patients. The previous chapter described and summarized the existing evidence on optimal thresholds of viscoelastic tests used to diagnose coagulopathy guided transfusion and predict mortality. The third specific aim involved analysis of Sunnybrook Health Sciences Center’s trauma registry to explore the relationship between viscoelastic test parameters and early available clinical variables with 24h transfusion of blood products (RBC, plasma, platelets, and cryoprecipitate), massive transfusion (MT) and 24h mortality to develop prediction models for each of these outcomes. Next, these prediction models were validated in an independent patient population admitted to St. Michael’s hospital, Toronto.

This chapter first presents our institutional experience with thromboelastometry in a cohort of trauma patients admitted from August 2011 to March 2013. The evidence from systematic review and the consensus conference will then be incorporated to evaluate the association between specific risk factors and the need for blood products transfusions, MT and 24h mortality. This chapter also describes the methodology involved in developing and validating the prediction models for our six outcomes. The prediction models for MT and 24h mortality are presented separately in chapter five.

4.3 Introduction

Traumatic injury is the leading cause of mortality, morbidity as well as resource use (Sauaia et al., 1995). Exsanguinating hemorrhage following a traumatic injury is the second leading cause of death, accounting for nearly 40%-50% of all trauma related in-hospital deaths, most of which occur within the first 24h of injury (Kauvar et al., 2006; Sauaia et al., 1995; Acosta et al., 1998). Traumatically injured patients are at higher risk for developing trauma induced coagulopathy TIC, which is seen in a quarter of patients (Brohi et al., 2003; Maegele et al., 2007; Frith et al., 2010). Trauma induced coagulopathy is independently associated with requirement for increased transfusion requirements including massive transfusion (MT), in hospital complications, longer hospital stay and higher mortality (Brohi et al., 2003; MacLeod et al., 2003; Rugeri et al., 2007; Maegele et al., 2007).
Trauma patients, especially coagulopathic patients, are the top consumers of blood transfusion products and experience higher in-hospital morbidity and mortality (Hess & Zimrin, 2005; McLaughlin et al., 2008; Como, Dutton, Scalea, Edelman, & Hess, 2004). Apart from higher morbidity and mortality, allogenic red blood cells and plasma transfusion on its own carries several risks such as transfusion-related lung injury (TRALI), graft versus host reaction, allergic reaction as well as infections and post injury organ failure (Dunne, Malone, Tracy, & Napolitano, 2004; Claridge, Sawyer, Schulman, McLemore, & Young, 2002; Malone et al., 2003; Moore, Moore, & Sauaia, 1997). Current use of routine coagulation tests such as PT, aPTT, fibrinogen, platelet count etc., usually take 30-40 minutes for the results to be available to the physician (MacLeod et al., 2003). In a constantly changing physiologic condition they may not reflect the actual coagulation status of the patient.

Currently there is no reliable tool for early identification of patients who may or may not require individual blood product transfusion, MT or to recognize patients who may die early during the hospital stay. Lack of early determinants of these outcomes may result in underestimation or failure to identify coagulopathic bleeding and thus causing delay in treatment strategy and result in poor outcome. Rapid identification of significantly bleeding patients and a consequent utilization of transfusion including MT may be directed toward better therapeutic management strategies and faster stabilization of their impaired hemostatic status while saving valuable blood products and improving patient outcome. Similar strategies may be used to manage patients who die early upon hospital admission. Previous investigations which attempted to evaluate risk factors for early transfusion of blood products, MT and early mortality in trauma patients have several limitations such as use of time consuming laboratory tests, radiological examinations such as computed tomography, Focussed Assessment with Sonography in Trauma (FAST), complex mathematical computations etc. No studies in trauma have developed prediction models using ROTEM® variables for blood products transfusion, MT and early mortality.

Given that coagulopathic patients are at higher risk for receiving transfusion and early deaths, identifying viscoelastic assay parameters of early transfusions and early mortality may allow the clinician to tailor transfusion needs or withhold transfusion.
The objective of this study was to estimate the incidence and risk factors associated with transfusion of blood products within the first 24h of hospital admission, MT and 24h mortality.

4.4 Methods
4.4.1 Patient setting, study design and description of derivation cohort

Sunnybrook Health Sciences Center (SHSC) is one of the leading level I adult trauma center and a teaching hospital affiliated to the University of Toronto, Ontario, Canada. The trauma center provides trauma management and care to nearly 1200 trauma patients a year. Following research ethics board approval we performed a retrospective cohort analysis of prospectively collected data of consecutive trauma patients admitted to SHSC between August 2011 and March 2013 which constitutes the derivation cohort.

4.4.2 Description of validation cohort

In order to have good generalizability to population’s different from those in which it was developed, external validation of the model is necessary. The validation cohort consisted of trauma patients admitted to St. Michael’s hospital (SMH) between November 2014 and October 2015. The trauma and ROTEM® data were obtained from the trauma registry following ethics approval from St.Michael’s Hospital, a leading level I trauma center in Toronto. The models were externally validated to estimate the performance measures in this validation data set meeting the same eligibility criteria.

4.4.3 Inclusion and exclusion criteria

We included patients presenting with blunt and/or penetrating mechanism of injury and who received an admission ROTEM® test early upon hospital arrival. Patients who did not have admission ROTEM® test and those died within one hour without receiving any blood products transfusions and ROTEM® test were excluded to avoid survivor bias.

4.4.4 Study data, quality and variable definition (risk factors/predictor variables)

Both data sets contained prospectively collected data and captured into respective trauma registries. The trauma registries in both centers capture in-depth data on individual trauma
patients from the time a patient is admitted to the hospital’s Emergency Department (ED) through hospital discharge. ROTEM® analyser captures the test data in designated software which was merged with trauma data. Quality of data was assessed for accuracy and completeness. Any outlier values were verified by revisiting the patient records and any typographical errors were corrected. Missing values were obtained from patient charts when possible and patients with actual missing values were retained in the analysis. Patient demographics, physiologic data, standard coagulation tests, ROTEM® measurements and outcomes data were collected for descriptive purposes.

Potential readily available demographic, clinical variables and ROTEM® variables were included in the analysis based on prior research evidence, clinical expertise and clinical rationale that have been associated with the outcomes (Davenport et al., 2011; Larson et al., 2010; Maegele et al., 2012; McLaughlin et al., 2008; Nunez et al., 2009; Leemann et al., 2010; Dutton, Lefering, & Lynn, 2006; Dutton & Carson, 2006; Schreiber et al., 2007; MacLeod et al., 2004; Mica L, 2012; Mica, Rufibach, Keel, & Trentz, 2013; Barbosa et al., 2011; Dutton et al., 2006; Rourke et al., 2012; Tauber et al., 2011). The variables used in building prediction models are defined in table 4.1. A set of risk factors from the table were used in model building process for each dependent variable, depending on clinical relevance and consensus. The risk factors are described for respective outcomes.

Table 4.1: Risk factors included in the model building process

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition and unit of measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics and baseline physiological characteristics</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>Continuous variable; years</td>
</tr>
<tr>
<td>Sex</td>
<td>Categorical variable; Male/Female</td>
</tr>
<tr>
<td>Systolic blood pressure (SBP)</td>
<td>Continuous variable; mmHg</td>
</tr>
<tr>
<td>Injury Severity Score (ISS)</td>
<td>Continuous variable; ISS is a numerical scale to assess trauma severity, and is assessed by the level of trauma from Anatomical Injury Scores to the three most severely injured regions of the body; ISS ranges from 1 to 75 (a score of &gt;15 is classified as major trauma)</td>
</tr>
<tr>
<td>Injury type</td>
<td>Categorical variable; Blunt/penetrating</td>
</tr>
</tbody>
</table>
Glasgow Coma Scale Score (GCS) | Continuous variable; GCS is a neurological scale used to assess the conscious state of a patient and results in a score between 3 (indicating deep unconsciousness) and 15 (indicating that the patient has good eye, verbal and motor responses)

<table>
<thead>
<tr>
<th><strong>Standard Coagulation tests</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>International Normalized Ratio (INR)</td>
<td>Continuous variable; number</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>Continuous variable; g/L</td>
</tr>
<tr>
<td>Platelet count</td>
<td>Continuous variable; X $10^9$/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>ROTEM® Variables</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>EXTEM A10</td>
<td>Continuous variable; clot amplitude measured at 10 min in EXTEM assay expressed in millimeters</td>
</tr>
<tr>
<td>EXTEM CT</td>
<td>Continuous variable; clotting time in EXTEM assay measured in seconds</td>
</tr>
<tr>
<td>EXTEM CFT</td>
<td>Continuous variable; clot formation time in EXTEM assay measured in seconds</td>
</tr>
<tr>
<td>FIBTEM A10</td>
<td>Continuous variable; clot amplitude measured at 10 minutes in FIBTEM assay expressed in millimeters</td>
</tr>
</tbody>
</table>

EXTEM A10 – clot amplitude in EXTEM measured at ten min; EXTEM CFT – clot formation time; EXTEM CT – clotting time in EXTEM assay; FIBTEM A10 - clot amplitude in FIBTEM measured at ten min

### 4.4.5 Outcome measures and definitions

The following six outcome measures were collected for developing the risk prediction models and a set of risk factors for each outcome were chosen based on prior research and clinical relevance which are described for each outcome:

1. 24h RBC transfusion (response: yes or no) is defined as delivery of any units of RBCs within 24h of hospital arrival. The patient population was subdivided into two groups: patients who received any RBC transfusion within the first 24h and those who did not. The following risk factors were chosen a priori for bivariate analysis followed by multivariable logistic regression analyses: age, systolic blood pressure, ISS, injury type, GCS, INR, hemoglobin, platelet count, EXTEM A10 and FIBTEM A10.
2. 24h Fresh Frozen Plasma (FFP) transfusion (response: yes or no) is defined as delivery of any FFP within 24h of hospital arrival. The patient population was subdivided into two groups: patients who received any FFP transfusion within the first 24h and those who did not. The following risk factors were chosen a priori for bivariate analysis followed by multivariable logistic regression analyses: age, systolic blood pressure, ISS, injury type, GCS, INR, hemoglobin, platelet count, EXTEM CT, EXTEM A10 and FIBTEM A10.

3. 24h platelets transfusion (response: yes or no) is defined as delivery of any platelets within 24h of hospital arrival. The patient population was subdivided into two groups: patients who received any platelets transfusion within the first 24h and those who did not. The following risk factors were chosen a priori for bivariate analysis followed by multivariable logistic regression analyses: Age, systolic blood pressure, ISS, injury type, GCS, hemoglobin, platelet count, EXTEM CT and EXTEM A10.

4. 24h cryoprecipitate transfusion (response: yes or no) is defined as delivery of any cryoprecipitate within 24h of hospital arrival. The patient population was subdivided into two groups: Patients who received any cryoprecipitate transfusion within the first 24h and those who did not. The following risk factors were chosen a priori for bivariate analysis followed by multivariable logistic regression analyses: age, systolic blood pressure, ISS, injury type, GCS, INR, hemoglobin, platelet count, EXTEM A10 and FIBTEM A10.

5. Massive transfusion status (response: yes or no) is defined as delivery of ten or more units of red blood cells (RBCs) within 24h of hospital arrival, the most popularly used definition used in published studies (Stainsby et al., 2000; Malone et al., 2006; Huber-Wagner et al., 2007). The patient population was subdivided into two groups: patients who received MT and those who did not. The following risk factors were chosen a priori for bivariate analysis followed by multivariable logistic regression analyses: gender, age, systolic blood pressure, ISS, injury type, GCS, INR, hemoglobin, platelets count, EXTEM A10 and FIBTEM A10.
6. 24h mortality status (response: yes or no) is defined as death occurring within 24h of hospital arrival and calculated based on time of arrival to the hospital’s emergency department. The patient population was subdivided into two groups: patients who died within 24h and those who survived greater than 24h. The following risk factors were chosen *a priori* for bivariate analysis followed by multivariable logistic regression analysis: gender, age, systolic blood pressure, ISS, injury type, GCS, INR, hemoglobin, platelet count, EXTEM CFT, EXTEM A10 and FIBTEM A10.

### 4.5 Statistical analysis

Data were analysed using Statistical Software SAS (version 9.3 SAS Institute, Cary, NC). The patients admitted to SHSC between August 2011 and March 2013 were used as derivation cohort while the patients admitted to SMH between November 2014 and October 2015 were used as validation cohort. All continuous variables were evaluated for normality using Kolmogorov-Smirnov and Shapiro-Wilk test. Descriptive statistics for continuous variables were reported as medians and interquartile range (25th and 75th interquartile range (IQR)) as the data was not normally distributed. Categorical variables were reported as absolute numbers and percentages. Characteristics of groups were compared by using the Wilcoxon test for continuous variables and the Chi-square test or Fisher’s exact test for categorical variables. The unadjusted association of risk factors with each outcome were performed using student t-test or Wilcoxon Rank-Sum test for continuous variables and Chi-square or Fisher’s exact test was used for categorical variables. Statistical significance was considered at a two sided p-value of <0.05.

Multivariable model building techniques were used to select the significant risk factors for constructing the prediction model and to assess the risk of transfusion, MT and 24h mortality (Wasson, Sox, Neff, & Goldman, 1985; Laupacis, Sekar, & Stiell, 1997). Initially all continuous variables were assessed for multicollinearity by examining the variance inflation factor (VIF) statistics with a cut-off value of 0.5. Spearman correlations were also assessed. When a pair of variables with VIF >0.5 and spearman correlation of greater than 0.5 were found, clinical rationale was used to select a single appropriate variable in the multivariable model. Maximum likelihood ratio method was used to ascertain the significant contribution of variables to the model. The variables which did not contribute significantly to the model were removed. The
significant variables were then subjected to multivariable modeling technique using manual backward selection methods to select most parsimonious model to assess independent risk factors with each outcome.

The predictive ability of the prognostic model was assessed in terms of calibration and discrimination. Calibration assesses the agreement between observed risks versus predicted risks. The predictive ability (calibration) of the model was evaluated using the Hosmer-Lemeshow goodness-of-fit statistic (Olde Engberink et al., 2014; Steyerberg et al., 2010). Discrimination show whether patients at low risk can be differentiated from those at high risk. The discriminative performance was quantified using C-statistic which is equivalent to the area under the receiver-operating characteristic (AUC) curve (Olde Engberink et al., 2014; Steyerberg et al., 2010; Harrell, Jr., Lee, & Mark, 1996; Steyerberg & Vergouwe, 2014). A value of 1 indicates that the model can perfectly predict the outcome where as a value 0.5 indicates no discrimination (Steyerberg & Vergouwe, 2014). The key assumptions of logistic regression were assessed. The best fit model was presented as an adjusted odds ratios (aORs) and 95% confidence intervals (CIs) to estimate the association between risk factors and the outcomes (24h transfusion of blood products, MT and 24h mortality) to determine the robustness of the model.

The final model was used to calculate the predicted probability of 24h transfusion of blood products, massive transfusion and 24h mortality for each patient in the derivation data set. The sensitivity and specificity of the model at various probability cut offs were examined and the optimal probability cut off was selected for prediction of each outcome in both, derivation and validation, cohorts. Those values below the cut off would be predicted as not to require any blood transfusion within the first 24hr of hospital admission, do not require MT, and survive 24h.

4.5.1 Missing data

Whenever possible, missing values were obtained by re-visiting the patient’s medical records. Less than 10% of our data were missing in majority of variables chosen. We used the entire patient data set for analysis. Due to large number of missing temperature and heart rate data, these variables were used only to report descriptive statistics and were excluded from our model building process.
### 4.6 Results

Between August 2011 and March 2013, 1890 patients were admitted to SHSC. After excluding patients without an admission ROTEM® (n=718) and who died within one hour without receiving ROTEM® test (n=26), 1146 patients were included for analysis in the derivation cohort. The validation cohort consisted of 1148 patients admitted to SMH between November 2014 and October 2015. After excluding patients without an admission ROTEM® (n=350), died within one hour without ROTEM® (n=18) and drowning (n=1), 779 patients were included in the validation cohort (figure 4.1).

![ CONSORT (Consolidated Standards for Reporting Trials) diagram describing patient enrollment, eligibility criteria in derivation and validation cohorts.](image)

**Figure 4.1:** The CONSORT (Consolidated Standards for Reporting Trials) diagram describing patient enrollment, eligibility criteria in derivation and validation cohorts.

**Patient and clinical characteristics of derivation cohort: Full cohort, patients receiving ROTEM® vs. not receiving ROTEM® test on admission**

The clinical characteristics of the patients transferred to SHSC are described in Table 4.2. Patient population was predominantly males (72%) and the median age was 42 (IQR, 27-58) years. The median ISS was 17 (IQR, 9-26) and 84% (n=1587) patients sustained blunt trauma. The median RBC, FFP, platelet and cryoprecipitate transfusion was 0 (IQR 0-0). The overall all-cause in-
hospital mortality was 9.4% (n=178). Of the 1890 trauma admissions, 1146 received admission ROTEM® while 744 did not (Table 4.2). The groups were similar in demographic, clinical and laboratory variables.

Table 4.2: Patient characteristics in derivation data: Full cohort, patients receiving ROTEM® Vs. NO-ROTEM®

<table>
<thead>
<tr>
<th>Variable</th>
<th>Full cohort (n=1890)</th>
<th>ROTEM® (n=1146)</th>
<th>No-ROTEM® (n=744)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic and vital signs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yrs</td>
<td>42 (27, 58)</td>
<td>41 (26, 58)</td>
<td>42.5 (26, 58)</td>
<td>0.529</td>
</tr>
<tr>
<td>Gender, male n (%)</td>
<td>1369 (72)</td>
<td>836 (73)</td>
<td>533 (72)</td>
<td>0.56</td>
</tr>
<tr>
<td>Injury type-blunt, n (%)</td>
<td>1587 (84)</td>
<td>951 (83)</td>
<td>636 (85)</td>
<td>1.48</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>140 (124, 159)</td>
<td>142 (126, 160)</td>
<td>140 (123, 156)</td>
<td>0.082</td>
</tr>
<tr>
<td>ISS</td>
<td>17 (9, 26)</td>
<td>17 (9, 26)</td>
<td>17 (10, 26)</td>
<td>0.914</td>
</tr>
<tr>
<td>GCS</td>
<td>15 (13, 15)</td>
<td>15 (13,15)</td>
<td>15 (13, 15)</td>
<td>0.5517</td>
</tr>
<tr>
<td>Temperature, ºC</td>
<td>36 (35, 36)</td>
<td>36 (35.4, 36.5)</td>
<td>36.1 (35.6, 36.6)</td>
<td>0.007</td>
</tr>
<tr>
<td>Heart rate</td>
<td>90 (76, 104)</td>
<td>90 (76, 104)</td>
<td>89 (76, 102)</td>
<td>0.036</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>18 (17, 20)</td>
<td>18 (18, 20)</td>
<td>18 (16, 20)</td>
<td>0.197</td>
</tr>
<tr>
<td><strong>Standard Coagulation Tests</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INR</td>
<td>1.07 (1.0, 1.2)</td>
<td>1.07 (1.0, 1.2)</td>
<td>1.07 (1.0, 1.2)</td>
<td>0.961</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>138 (123,149)</td>
<td>138 (123, 149)</td>
<td>137 (122, 149)</td>
<td>0.059</td>
</tr>
<tr>
<td>Platelet count, x10⁹/L</td>
<td>228 (185, 275)</td>
<td>231 (192, 275)</td>
<td>222 (179, 273)</td>
<td>0.257</td>
</tr>
<tr>
<td><strong>ROTEM® Measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>EXTEM A10, mm</td>
<td>52 (47,57)</td>
<td>52 (47, 57)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>EXTEM CT, sec</td>
<td>50 (44,59)</td>
<td>50 (44, 59)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>EXTEM CFT, sec</td>
<td>100 (82,122)</td>
<td>100 (82, 122)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>FIBTEM A10, mm</td>
<td>13 (10,16)</td>
<td>13 (10, 16)</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>24h Transfusion data</strong></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PRBC, U</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>0.372</td>
</tr>
<tr>
<td>FFP, U</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>0.351</td>
</tr>
<tr>
<td>Platelet, U</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>0.396</td>
</tr>
<tr>
<td>Cryoprecipitate, pooled units</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>0.314</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Mortality</strong></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause in-hospital mortality,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n(%)</td>
<td>178 (9.4)</td>
<td>100 (9)</td>
<td>78 (10)</td>
<td>0.2011</td>
</tr>
</tbody>
</table>

Data are presented as medians with interquartile ranges (25th-75th percentile) for continuous variables. Categorical variables are presented as absolute numbers and percentages.

EXTEM A10- clot amplitude measured at ten minutes after in EXTEM assay; EXTEM CT- clotting time in EXTEM assay; EXTEM CFT- clot formation time in EXTEM assay; FIBTEM A10- clot amplitude measured at ten minutes in FIBTEM assay; GCS-Glasgow Coma Scale Score; INR-international normalized ratio; ISS-injury severity score; SBP-systolic blood pressure

### 4.6.1 Patient and clinical characteristics of derivation cohort and validation cohort

Table 4.3 reports the patients’ characteristics for the derivation cohort and the validation cohort.
Patients in derivation and validation cohorts differed in injury severity score (17(9-26) vs. 9(2-19). In general patients in both cohorts were of similar age and sex, experienced blunt trauma, had similar coagulation profiles in SCTs and ROTEM® tests, and had similar blood product consumption results. Two percent of patients in derivation cohort received MT and died within 24h while 1% in validation cohort received an MT and died within 24h.

4.3 Patient characteristics: Derivation and Validation cohorts

<table>
<thead>
<tr>
<th>Variable</th>
<th>Derivation cohort</th>
<th>Validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic and vital signs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient (n)</td>
<td>1146</td>
<td>779</td>
</tr>
<tr>
<td>Gender, male n (%)</td>
<td>836 (73)</td>
<td>566 (73)</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>41 (26-58)</td>
<td>42 (27-58)</td>
</tr>
<tr>
<td>Injury type-blunt, n (%)</td>
<td>951 (83)</td>
<td>618 (79)</td>
</tr>
<tr>
<td>Injury type-penetrating, n(%)</td>
<td>195 (17)</td>
<td>161 (21)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>142 (126-160)</td>
<td>135 (121-149)</td>
</tr>
<tr>
<td>ISS</td>
<td>17 (9-26)</td>
<td>9 (2-19)</td>
</tr>
<tr>
<td>GCS</td>
<td>15 (13-15)</td>
<td>15 (14-15)</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>36.00 (35.4-36.5)</td>
<td>36 (35.2-36.6)</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>90.00 (76.00-104.0)</td>
<td>89 (78-100)</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>18 (18-20)</td>
<td>18 (16-20)</td>
</tr>
<tr>
<td><strong>Standard Coagulation Tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Value 1</td>
<td>Value 2</td>
</tr>
<tr>
<td>------------------------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>INR</td>
<td>1.1 (1-1.2)</td>
<td>1 (1-1.1)</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>138 (123-149)</td>
<td>138 (126-149)</td>
</tr>
<tr>
<td>Platelet count, $\times 10^9$/L</td>
<td>231 (192-275)</td>
<td>237 (199-277)</td>
</tr>
<tr>
<td><strong>ROTEM® Measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EXTEM A10, mm</td>
<td>52 (47-57)</td>
<td>55 (51-59)</td>
</tr>
<tr>
<td>EXTEM CT, sec</td>
<td>50 (44-59)</td>
<td>69 (61-76)</td>
</tr>
<tr>
<td>EXTEM CFT, sec</td>
<td>100 (82-122)</td>
<td>85 (72-100)</td>
</tr>
<tr>
<td>EXTEM MCF, mm</td>
<td>60 (56-64)</td>
<td>63 (59-66)</td>
</tr>
<tr>
<td>FIBTEM A10, mm</td>
<td>13 (10-16)</td>
<td>15 (12-18)</td>
</tr>
<tr>
<td>FIBTEM MCF, mm</td>
<td>14 (10-17)</td>
<td>16 (13-20)</td>
</tr>
<tr>
<td><strong>24h Transfusion data</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC, U</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Plasma, U</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Platelet, U</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Cryoprecipitate, pooled units</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Red Blood Cells, n(%)</td>
<td>172 (15)</td>
<td>81 (10)</td>
</tr>
<tr>
<td>Fresh Frozen Plasma, n(%)</td>
<td>64 (6)</td>
<td>30 (4)</td>
</tr>
<tr>
<td>Platelets, n(%)</td>
<td>60 (5)</td>
<td>24 (3)</td>
</tr>
<tr>
<td>Cryoprecipitate, n(%)</td>
<td>30 (3)</td>
<td>35 (4)</td>
</tr>
</tbody>
</table>
Data are presented as medians with interquartile ranges (25th-75th percentile) for continuous variables. Categorical variables are presented as absolute numbers and percentages.

EXTEM A10- clot amplitude measured at ten minutes after in EXTEM assay; EXTEM CT- clotting time in EXTEM assay; EXTEM CFT-clot formation time in EXTEM assay; EXTEM MCF – maximum clot firmness in EXTEM assay; FIBTEM A10- clot amplitude measured at ten minutes in FIBTEM assay; FIBTEM MCF- maximum clot firmness in FIBTEM assay; GCS-Glasgow Coma Scale Score; INR-international normalized ratio; ISS-injury severity score; SBP-systolic blood pressure; RBC-red blood cells.

Blood products consumption within the first 24h in derivation cohort and validation cohort is as follows: Fifteen percent patients (172/1146) in derivation cohort and 10% (81/779) in validation cohort received any RBC in the first 24h of hospitalization. 6% patients (64/1146) in derivation cohort and 4% (30/779) in validation cohort received plasma transfusion within 24h of hospitalization. 5% patients (60/1146) in derivation cohort and 3% (24/779) in validation cohort received any platelet within 24h of hospitalization. 3% (30/1146) in derivation cohort and 4%(35/779) in validation cohort received any cryoprecipitate within 24h of hospitalization.

4.6.2 24h transfusion of Red Blood Cells, bivariate analysis

Of the1146 patients assessed, 172 (15%) patients received any RBC transfusion while 974 (85%) did not receive any RBC transfusions within the first 24h. Table 4.4 shows bivariate association of risk factors with 24h RBC transfusion. Patients who received any RBC within 24h were older, had lower systolic blood pressure, higher ISS and lower GCS (Table 4.4). Likewise, patients who received any RBC in 24h had abnormal laboratory coagulation test results and ROTEM®
results compared to patients who did not receive any RBCs in 24h. There were no meaningful differences in terms of age and penetrating mechanism of injury.

Table 4.4: Bivariate association of risk factors associated with 24h Red Blood Cells (RBC) transfusion

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Any RBC in 24h (n=172)</th>
<th>No RBC in 24h (n=974)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>44 (24, 64)</td>
<td>41 (26, 56)</td>
<td>0.4810</td>
</tr>
<tr>
<td>Systolic Blood Pressure (SBP), mmHg</td>
<td>120 (94, 142)</td>
<td>146 (128, 160)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Injury Severity Score (ISS)</td>
<td>29 (22, 38)</td>
<td>14 (6, 24)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Injury type, penetrating</td>
<td>30 (17%)</td>
<td>165 (17%)</td>
<td>0.8718</td>
</tr>
<tr>
<td>GCS</td>
<td>14 (4, 15)</td>
<td>15 (14, 15)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Laboratory results**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Any RBC in 24h (n=172)</th>
<th>No RBC in 24h (n=974)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>International Normalized Ratio</td>
<td>1.2 (1.1, 1.4)</td>
<td>1.1 (1, 1.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>117.0 (102, 132.5)</td>
<td>141 (129, 151)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelet count X10^9/L</td>
<td>233.0 (164.0, 280.0)</td>
<td>231.0 (195, 275)</td>
<td>0.1000</td>
</tr>
</tbody>
</table>

**ROTEM® measurements**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Any RBC in 24h (n=172)</th>
<th>No RBC in 24h (n=974)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXTEM A10, mm</td>
<td>50.00 (43, 55)</td>
<td>52.00 (47,57)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FIBTEM A10, mm</td>
<td>11 (8, 14)</td>
<td>13 (10,17)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are presented as medians with interquartile ranges (25th - 75th percentile) for continuous variables. Categorical variables are presented as absolute numbers and percentages.

EXTEM A10 – clot amplitude in EXTEM measured at ten min; FIBTEM A10 - clot amplitude in FIBTEM measured at ten min; GCS - Glasgow Coma Scale Score
After adjustment in a multivariable logistic regression model, the variables that were found to be associated with 24h RBC transfusion were: low systolic blood pressure (aOR=0.98, 95% CI: 0.98-0.99, p<0.0001), low hemoglobin (aOR=0.96, 95% CI: 0.96 - 0.97, p<0.0001) and higher injury severity score (aOR=1.09, 95% CI:1.07 - 1.11, p<0.0001) and penetrating mechanism of injury (aOR=2.17, 95% CI: 1.22, 3.92, p=0.0100). FIBTEM A10 was not a significant predictor of 24h RBC transfusion (aOR 0.98 (95% CI: 0.94, 1.01, p=0.2295). The C statistic for the model was 0.88 and Hosmer and Lemeshow-goodness-of-fit was 0.43 (Table 4.5).

Table 4.5: Multivariable logistic regression analysis of risk factors associated with 24h RBC transfusion:

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Adjusted OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>0.98 (0.98, 0.99)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.96 (0.96, 0.97)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ISS</td>
<td>1.09 (1.07, 1.11)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FIBTEM A10</td>
<td>0.98 (0.94, 1.01)</td>
<td>0.2295</td>
</tr>
<tr>
<td>Injury type (penetrating vs. blunt)</td>
<td>2.17 (1.22, 3.92)</td>
<td>0.0100</td>
</tr>
</tbody>
</table>

Chi square = 305.87df(5), p <0.0001; C-statistic: 0.88; Hosmer Lemeshow goodness-of-fit statistic: 0.43

OR-odds ratio; SBP-systolic blood pressure; ISS-Injury severity score; FIBTEM A10-clot amplitude measured at ten minutes in FIBTEM assay

The sensitivity, specificity, positive and negative predictive value were calculated across a range of predicted probability thresholds for the derivation cohort are presented in table 4.6 (Table 4.6). The model utilized in the analysis had a sensitivity of 83%; specificity of 80% and negative predictive value of 96.49% with the optimal threshold on the probability of risk for 24h RBC transfusion was considered at 0.1413.
### Table 4.6: Accuracy of the 24h RBC transfusion model at various probability cut off levels in the derivation data

<table>
<thead>
<tr>
<th>Probability level</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1413</td>
<td>83.23</td>
<td>80.15</td>
<td>42.14</td>
<td>96.49</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>89.44</td>
<td>71.74</td>
<td>35.47</td>
<td>97.51</td>
</tr>
<tr>
<td>0.2</td>
<td>73.91</td>
<td>87.27</td>
<td>50.21</td>
<td>95.06</td>
</tr>
<tr>
<td>0.3</td>
<td>57.76</td>
<td>92.66</td>
<td>57.76</td>
<td>92.66</td>
</tr>
<tr>
<td>0.4</td>
<td>51.55</td>
<td>95.25</td>
<td>65.35</td>
<td>91.88</td>
</tr>
<tr>
<td>0.5</td>
<td>39.75</td>
<td>97.09</td>
<td>70.33</td>
<td>90.27</td>
</tr>
<tr>
<td>0.6</td>
<td>31.06</td>
<td>98.17</td>
<td>74.63</td>
<td>89.13</td>
</tr>
<tr>
<td>0.7</td>
<td>19.88</td>
<td>98.49</td>
<td>69.57</td>
<td>87.62</td>
</tr>
<tr>
<td>0.8</td>
<td>13.04</td>
<td>99.14</td>
<td>72.41</td>
<td>86.78</td>
</tr>
<tr>
<td>0.9</td>
<td>6.83</td>
<td>99.57</td>
<td>73.33</td>
<td>86.02</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>100</td>
<td>85.2</td>
<td></td>
</tr>
</tbody>
</table>

NPV - negative predictive value; PPV - positive predictive value

For the validation data at this probability level, sensitivity was 69%, specificity 86% and negative predictive value (NPV), the probability that a patient who does not truly require 24h RBC transfusion was 95.99% (Table 4.7). The model’s discriminative ability assessed by area under the ROC curve for both cohorts was 0.88 which showed good discrimination between patients who received 24h RBC and those who did not (Fig 4.2). The calibration statistics based on the regression of the predictive versus the true response based on the optimal threshold had calibration slope of 1.18, significantly different from zero (p-value <0.0001).
Table 4.7: Accuracy of the 24h RBC transfusion model at various probability cut off levels in the validation data

<table>
<thead>
<tr>
<th>Probability level</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.14</td>
<td>69.14</td>
<td>85.67</td>
<td>35.90</td>
<td>95.99</td>
</tr>
<tr>
<td>0.00</td>
<td>95.06</td>
<td>1.58</td>
<td>10.08</td>
<td>73.33</td>
</tr>
<tr>
<td>0.10</td>
<td>76.54</td>
<td>80.66</td>
<td>31.47</td>
<td>96.74</td>
</tr>
<tr>
<td>0.20</td>
<td>54.32</td>
<td>91.69</td>
<td>43.14</td>
<td>94.53</td>
</tr>
<tr>
<td>0.30</td>
<td>38.27</td>
<td>96.56</td>
<td>56.36</td>
<td>93.09</td>
</tr>
<tr>
<td>0.40</td>
<td>28.40</td>
<td>98.71</td>
<td>71.88</td>
<td>92.24</td>
</tr>
<tr>
<td>0.50</td>
<td>17.28</td>
<td>99.14</td>
<td>70.00</td>
<td>91.17</td>
</tr>
<tr>
<td>0.60</td>
<td>13.58</td>
<td>99.57</td>
<td>78.57</td>
<td>90.85</td>
</tr>
<tr>
<td>0.70</td>
<td>8.64</td>
<td>99.86</td>
<td>87.50</td>
<td>90.40</td>
</tr>
<tr>
<td>0.80</td>
<td>4.94</td>
<td>99.86</td>
<td>80.00</td>
<td>90.05</td>
</tr>
<tr>
<td>0.90</td>
<td>3.70</td>
<td>100.00</td>
<td>100.00</td>
<td>89.95</td>
</tr>
<tr>
<td>1.00</td>
<td>100.00</td>
<td>89.60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NPV-negative predictive value; PPV-positive predictive value
4.6.3 24h transfusion of plasma, bivariate analysis

Of the 1146 patients assessed, 64 (6%) patients received any plasma transfusion within the first 24h of hospital arrival while 1082 (94%) did not receive any plasma transfusions within the first 24h. Unadjusted analyses (bivariate association) revealed that all variables were significantly associated with 24h plasma transfusion (Table 4.8). Patients who received any plasma within 24h were younger, had lower systolic blood pressure, higher ISS and lower GCS. Likewise, patients who received any plasma in 24h had abnormal laboratory coagulation test results and ROTEM® results compared to patients who did not receive any plasma in 24h. There were no meaningful differences in terms of penetrating mechanism of injury.

Table 4.8 Bivariate association of risk factors associated with receiving 24h plasma transfusion

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Any Plasma in 24h (n=64)</th>
<th>No Plasma in 24h (n=1082)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>30 (23, 58)</td>
<td>42 (26,58)</td>
<td>0.0317</td>
</tr>
<tr>
<td></td>
<td>Median (25th - 75th percentile)</td>
<td>Median (25th - 75th percentile)</td>
<td>p value</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>Systolic Blood Pressure (SBP), mmHg</strong></td>
<td>104 (80, 130)</td>
<td>144 (126, 160)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Injury Severity Score (ISS)</strong></td>
<td>37 (29, 50)</td>
<td>17 (9, 26)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Injury type, penetrating</strong></td>
<td>13 (20%)</td>
<td>182 (17%)</td>
<td>0.4701</td>
</tr>
<tr>
<td><strong>GCS</strong></td>
<td>7 (3, 15)</td>
<td>15 (13, 15)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Laboratory results**

<table>
<thead>
<tr>
<th></th>
<th>Median (25th - 75th percentile)</th>
<th>Median (25th - 75th percentile)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>International Normalized Ratio (INR)</strong></td>
<td>1.45 (1.2, 1.71)</td>
<td>1.07 (1, 1.15)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Hemoglobin, g/dL</strong></td>
<td>109.5 (94, 129.5)</td>
<td>139 (126, 150)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Platelet count X10⁹/L</strong></td>
<td>215 (145, 245)</td>
<td>232 (194.5, 277)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**ROTEM® measurements**

<table>
<thead>
<tr>
<th></th>
<th>Median (25th - 75th percentile)</th>
<th>Median (25th - 75th percentile)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EXTEM CT, sec</strong></td>
<td>68.5 (53.5, 87)</td>
<td>49 (43, 58)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>EXTEM A10, mm</strong></td>
<td>43 (31, 51)</td>
<td>52 (47, 57)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>FIBTEM A10, mm</strong></td>
<td>8 (6, 11)</td>
<td>13 (10, 17)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are presented as medians with interquartile ranges (25th - 75th percentile) for continuous variables. Categorical variables are presented as absolute numbers and percentages.

EXTEM A10 – clot amplitude in EXTEM measured at ten min; EXTEM CT – clotting time in EXTEM; FIBTEM A10-clot amplitude in FIBTEM measured at ten min; GCS- Glasgow Coma Scale Score.

After adjustment in a multivariable logistic regression model, variables that were found to be associated with 24h plasma transfusion were: younger age (aOR=0.98, 95% CI: 0.97 – 1.00, p<0.0359), lower systolic blood pressure (aOR=0.98, 95% CI:0.97 – 0.99, p<0.0002), low hemoglobin levels (aOR=0.97, 95% CI: 0.95 - 0.98, p<0.0001), high INR (aOR=1.68, 95% CI: 1.09-2.60, p=0.0201), higher injury severity (aOR=1.09, 95% CI: 1.06-1.12, p<0.0001) and lower clot amplitude at ten minutes in FIBTEM (aOR=0.90, 95% CI: 0.83 - 0.98, p=0.0171). The C
statistic for the model was 0.95 and Hosmer and Lemeshow-goodness-of-fit was 0.72 (Table 4.9).

Table 4.9 Multivariable logistic regression analysis of risk factors associated with 24h plasma transfusion

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Adjusted OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.98 (0.97, 1.00)</td>
<td>0.0359</td>
</tr>
<tr>
<td>SBP</td>
<td>0.98 (0.97, 0.99)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.97 (0.95, 0.98)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>INR</td>
<td>1.68 (1.09, 2.60)</td>
<td>0.0201</td>
</tr>
<tr>
<td>ISS</td>
<td>1.09 (1.06, 1.12)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FIBTEM A10</td>
<td>0.90 (0.83, 0.98)</td>
<td>0.0171</td>
</tr>
</tbody>
</table>

Chi square = 193.39 df (6), p < 0.0001; C-statistic: 0.95; Hosmer-Lemeshow goodness-of-fit statistic: 0.72

INR- international normalized ratio; ISS-injury severity score; SBP-systolic blood pressure

Table 4.10 reports the sensitivity, specificity, positive and negative predictive values across a range of predicted probability thresholds for the derivation cohort for 24h plasma transfusion.

Using a probability level of 0.06, the sensitivity of the derivation model to identify patients who would require 24h plasma transfusion was 89% and specificity 88% and the NPV was 99% (Table 4.10).
Table 4.10: Accuracy of the 24h plasma model at various probability cut off levels in the derivation data

<table>
<thead>
<tr>
<th>Probability level</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06</td>
<td>89.09</td>
<td>87.91</td>
<td>28.65</td>
<td>99.33</td>
</tr>
<tr>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
<td>5.17</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>72.73</td>
<td>92.17</td>
<td>33.61</td>
<td>98.41</td>
</tr>
<tr>
<td>0.20</td>
<td>63.64</td>
<td>96.13</td>
<td>47.30</td>
<td>97.98</td>
</tr>
<tr>
<td>0.30</td>
<td>49.09</td>
<td>97.92</td>
<td>56.25</td>
<td>97.24</td>
</tr>
<tr>
<td>0.40</td>
<td>41.82</td>
<td>98.51</td>
<td>60.53</td>
<td>96.88</td>
</tr>
<tr>
<td>0.50</td>
<td>34.55</td>
<td>98.81</td>
<td>61.29</td>
<td>96.52</td>
</tr>
<tr>
<td>0.60</td>
<td>27.27</td>
<td>99.11</td>
<td>62.50</td>
<td>96.15</td>
</tr>
<tr>
<td>0.70</td>
<td>21.82</td>
<td>99.41</td>
<td>66.67</td>
<td>95.89</td>
</tr>
<tr>
<td>0.80</td>
<td>7.27</td>
<td>99.60</td>
<td>50.00</td>
<td>95.17</td>
</tr>
<tr>
<td>0.90</td>
<td>5.45</td>
<td>99.60</td>
<td>42.86</td>
<td>95.08</td>
</tr>
<tr>
<td>1.00</td>
<td>0.00</td>
<td>100.00</td>
<td>94.83</td>
<td></td>
</tr>
</tbody>
</table>

NPV-negative predictive value; PPV-positive predictive value

For the validation cohort, the sensitivity at probability level of 0.06 was 50% ; specificity 95% and the NPV 97.94% (Table 4.11). The AUC of the predictive model for the derivation cohort was 0.95 whereas for validation cohort was 0.91 indicating good discrimination (Fig 4.3). The calibration had a slope of 1.0344 for the regression of predicted probability to the actual response, the risk for receiving plasma transfusion with a p value of <0.0001.
Table 4.11: Accuracy of the 24h plasma model at various probability cut off levels in the validation data

<table>
<thead>
<tr>
<th>Probability level</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06</td>
<td>50.00</td>
<td>95.06</td>
<td>28.85</td>
<td>97.94</td>
</tr>
<tr>
<td>0.00</td>
<td>93.33</td>
<td>3.20</td>
<td>3.72</td>
<td>92.31</td>
</tr>
<tr>
<td>0.10</td>
<td>36.67</td>
<td>97.86</td>
<td>40.74</td>
<td>97.47</td>
</tr>
<tr>
<td>0.20</td>
<td>30.00</td>
<td>99.47</td>
<td>69.23</td>
<td>97.26</td>
</tr>
<tr>
<td>0.30</td>
<td>23.33</td>
<td>99.87</td>
<td>87.50</td>
<td>97.02</td>
</tr>
<tr>
<td>0.40</td>
<td>13.33</td>
<td>99.87</td>
<td>80.00</td>
<td>96.64</td>
</tr>
<tr>
<td>0.50</td>
<td>13.33</td>
<td>99.87</td>
<td>80.00</td>
<td>96.64</td>
</tr>
<tr>
<td>0.60</td>
<td>13.33</td>
<td>100.00</td>
<td>100.00</td>
<td>96.65</td>
</tr>
<tr>
<td>0.70</td>
<td>10.00</td>
<td>100.00</td>
<td>100.00</td>
<td>96.52</td>
</tr>
<tr>
<td>0.80</td>
<td>6.67</td>
<td>100.00</td>
<td>100.00</td>
<td>96.40</td>
</tr>
<tr>
<td>0.90</td>
<td>3.33</td>
<td>100.00</td>
<td>100.00</td>
<td>96.27</td>
</tr>
<tr>
<td>1.00</td>
<td>100.00</td>
<td></td>
<td></td>
<td>96.15</td>
</tr>
</tbody>
</table>

NPV-negative predictive value; PPV-positive predictive value
4.6.4 24h transfusion of platelets, bivariate analysis

Of the 1146 patients assessed, 60 (5%) patients received any platelet transfusion within the first 24h of hospital arrival while 1086 (95%) did not receive any FFP transfusions within the first 24h. Unadjusted analyses (bivariate association) revealed that all variables except EXTEM clotting time were significantly associated with 24h platelet transfusion (Table 4.12).

Table 4.12 Bivariate association of risk factors associated with receiving 24h platelet transfusion

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Any Platelets in 24h (n=60)</th>
<th>No Platelets in 24h (n=1086)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>34 (24, 60)</td>
<td>41 (26, 58)</td>
<td>0.4997</td>
</tr>
<tr>
<td>SBP</td>
<td>106 (84, 130)</td>
<td>144 (126, 160)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Injury Severity Score (ISS)</td>
<td>34 (26, 48)</td>
<td>17 (9, 26)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Injury type (penetrating)</td>
<td>12 (20%)</td>
<td>183 (17%)</td>
<td>0.5274</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------</td>
<td>-----------</td>
<td>--------</td>
</tr>
<tr>
<td>GCS</td>
<td>12 (3, 15)</td>
<td>15 (13, 15)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Laboratory results**

<table>
<thead>
<tr>
<th>Hemoglobin, g/dL</th>
<th>111 (96, 130)</th>
<th>139 (126, 150)</th>
<th>&lt;0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count X10⁹</td>
<td>195 (122, 241)</td>
<td>232.00 (195, 278)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**ROTEM® measurements**

<table>
<thead>
<tr>
<th>EXTEM A10, mm</th>
<th>43.50 (34, 50)</th>
<th>52 (47, 57)</th>
<th>&lt;0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXTEM CT, sec</td>
<td>61 (47, 84.5)</td>
<td>50 (43, 58)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are presented as medians with interquartile ranges (25th - 75th percentile) for continuous variables Categorical variables are presented as absolute numbers and percentages.

EXTEM A10 – clot amplitude in EXTEM measured at ten min; EXTEM CT – clotting time in EXTEM; SBP-systolic blood pressure, GCS- Glasgow Coma Scale Score

Table 4.13 shows the results of multivariable logistic regression in predicting 24h transfusion of platelets. The variables found to be independently associated with 24h platelet transfusion in multivariable logistic regression were: low systolic blood pressure (aOR=0.98, 95% CI: 0.97 - 0.99, p<0.0001), low platelet count (aOR=0.99, 95% CI: 0.99 - 1.00, p<0.0140), low hemoglobin (aOR=0.97, 95% CI: 0.96 - 0.99, p=0.0003), high ISS (aOR=1.10, 95% CI: 1.07 – 1.14, p<0.0001), higher GCS (aOR=1.13, 95% CI: 1.04 – 1.22, p=0.0042) had an increasing odds of receiving platelet transfusion in the first 24h of hospital admission. ROTEM® variables were not predictors of 24h platelet transfusion. The C statistic for the model was 0.92 and Hosmer and Lemeshow-goodness-of-fit was 0.15.
Table 4.13: Multivariable logistic regression analysis of risk factors associated with 24h platelet transfusion

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Adjusted OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>0.98 (0.97, 0.99)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelet count</td>
<td>0.99 (0.99, 1.00)</td>
<td>0.0140</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.97 (0.96, 0.99)</td>
<td>0.0003</td>
</tr>
<tr>
<td>ISS</td>
<td>1.10 (1.07, 1.14)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GCS</td>
<td>1.13 (1.04, 1.22)</td>
<td>0.0042</td>
</tr>
</tbody>
</table>

Omnibus likelihood ratio 155.68, df(5), p<0.0001. C statistic=0.92; Hosmer Lemeshow goodness of fit: 0.15

GCS- Glasgow coma scale score; ISS- injury severity score; SBP-systolic blood pressure

Table 4.14 reports the sensitivity, specificity, positive and negative predictive values across a range of predicted probability thresholds for the derivation cohort for 24h platelet transfusion.

Using a probability level of 0.05, the sensitivity of the model in derivation cohort to identify patients who would require 24h platelets transfusion was 91% and specificity 85% and the NPV was 99.44% (Table 4.14).

Table 4.14: Accuracy of the 24h platelet transfusion model at various probability cut off levels in the derivation data

<table>
<thead>
<tr>
<th>Probability level</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>90.74</td>
<td>84.47</td>
<td>23.00</td>
<td>99.44</td>
</tr>
<tr>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
<td>4.86</td>
<td></td>
</tr>
</tbody>
</table>
For the validation cohort, the sensitivity at probability level of 0.05 was 79%, specificity 91% and the NPV 99.28% (Table 4.15). The AUC of the predictive model for the derivation cohort was 0.92 whereas for validation cohort it was 0.91 indicating good discrimination (Fig 4.4). The calibration had a slope of 1.0677 for the regression of predicted probability to the actual response, the risk for receiving platelet transfusion with a p value of <0.0001.

Table 4.15: Accuracy of the 24h platelet transfusion model at various probability cut off levels in the validation data

<table>
<thead>
<tr>
<th>Probability level</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>79.17</td>
<td>90.86</td>
<td>21.59</td>
<td>99.28</td>
</tr>
<tr>
<td>0.00</td>
<td>95.83</td>
<td>1.99</td>
<td>3.01</td>
<td>93.75</td>
</tr>
</tbody>
</table>
NPV-negative predictive value; PPV-positive predictive value

The AUC for derivation and validation cohort were 0.92 and 0.91 respectively (Fig 4.4)

Fig 4.4: Model for 24h platelet transfusion –ROC curves for derivation and validation cohorts
4.6.5 24h cryoprecipitate transfusion, bivariate analysis

Of the 1,146 patients assessed, 32 (3%) patients received any cryoprecipitate transfusion within the first 24h of hospital arrival while 1,114 (97%) did not receive any cryoprecipitate transfusions within the first 24h. Unadjusted analyses (bivariate association) revealed that all variables except age (p=0.1968) were significantly associated with 24h cryoprecipitate transfusion (Table 4.16).

Table 4.16 Bivariate association of risk factors associated with receiving cryoprecipitate transfusion within 24h of hospital admission

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Any CRYO in 24h (n=32)</th>
<th>No CRYO in 24h (n=1114)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>28 (21, 55)</td>
<td>41 (26, 58)</td>
<td>0.0981</td>
</tr>
<tr>
<td>Injury Severity Score (ISS)</td>
<td>41 (29, 50)</td>
<td>17 (9, 26)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GCS</td>
<td>7 (3, 15)</td>
<td>15 (13, 15)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Injury type, penetrating</td>
<td>6 (19%)</td>
<td>189 (17%)</td>
<td>0.7912</td>
</tr>
</tbody>
</table>

**Laboratory results**

<table>
<thead>
<tr>
<th></th>
<th>Any CRYO in 24h (n=32)</th>
<th>No CRYO in 24h (n=1114)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>International Normalized Ratio</td>
<td>1.5 (1.3, 1.7)</td>
<td>1.1 (1, 1.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>109.5 (92, 130.5)</td>
<td>139 (124, 150)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelet count X10⁹</td>
<td>195 (92, 241)</td>
<td>231 (193, 276)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**ROTEM® measurements**

<table>
<thead>
<tr>
<th></th>
<th>Any CRYO in 24h (n=32)</th>
<th>No CRYO in 24h (n=1114)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXTEM A10, mm</td>
<td>38 (25.5, 44)</td>
<td>52 (47, 57)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FIBTEM A10, mm</td>
<td>7 (4, 10)</td>
<td>13 (10, 16)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are presented as medians with interquartile ranges (25th - 75th percentile) for continuous variables Categorical variables are presented as absolute numbers and percentages.
EXTEM A10 – clot amplitude in EXTEM measured at ten minutes; FIBTEM A10 - clot amplitude in FIBTEM measured at ten minutes; GCS-Glasgow coma score scale

Table 4.17 shows the results of multivariable logistic regression in predicting 24h transfusion of cryoprecipitate. The variables found to be independently associated with 24h cryoprecipitate transfusion in multivariable logistic regression were: low systolic blood pressure (aOR=0.99, 95% CI: 0.97 – 1.00, p=0.0374), high injury severity score (aOR=1.08, 95% CI: 1.05 - 1.11, p<0.0001) and low FIBTEM A10 (aOR=0.80, 95% CI: 0.71 – 0.91, p<0.0005). The C statistic for the model was 0.95 and Hosmer and Lemeshow goodness-of-fit was 0.91.

**Table 4.17: Multivariable logistic regression analysis of risk factors associated with 24h cryoprecipitate transfusion**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Adjusted OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td>0.99 (0.97, 1.00)</td>
<td>0.0374</td>
</tr>
<tr>
<td>Injury Severity Score</td>
<td>1.08 (1.05, 1.11)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FIBTEM A10</td>
<td>0.80 (0.71, 0.91)</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Omnibus likelihood ratio 85.45, df(3), p<0.0001; C-statistic: 0.95; Hosmer and Lemeshow goodness-of-fit statistic: 0.91

Table 4.18 reports the sensitivity, specificity, positive and negative predictive values across a range of predicted probability thresholds for the derivation cohort for 24h cryoprecipitate transfusion. Using a probability level of 0.02, the sensitivity of the model in derivation cohort to identify patients who would require 24h cryoprecipitate transfusion was 96% and specificity 85% and the NPV was 99.89% (Table 4.18).
Table 4.1: Accuracy of the 24h cryoprecipitate transfusion model at various probability cut off levels in the derivation data

<table>
<thead>
<tr>
<th>Probability level</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>96.15</td>
<td>85.43</td>
<td>13.89</td>
<td>99.89</td>
</tr>
<tr>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
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<td>100.00</td>
<td></td>
<td>97.61</td>
</tr>
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</table>

NPV-negative predictive value; PPV-positive predictive value

For the validation cohort, the sensitivity at probability level of 0.02 was 66%, specificity 96% and the NPV 98.34% (Table 4.19). The AUC of the predictive model for the derivation cohort was 0.95 whereas for validation cohort it was 0.93 indicating good discrimination (Fig 4.5). The calibration had a slope of 1.220 for the regression of predicted probability to the actual response, the risk for receiving platelet transfusion with a p value of <0.0001.
### Table 4.19: Accuracy of the 24h cryoprecipitate transfusion model at various probability cut off levels in the validation data

<table>
<thead>
<tr>
<th>Probability level</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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<td>95.51</td>
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</table>

NPV - negative predictive value; PPV - positive predictive value
4.7 Discussion:

Hemorrhage and subsequent exsanguination is the leading cause of trauma related deaths with 50% of deaths occurring within the first 24h of hospital arrival (Sauaia et al., 1995; Acosta et al., 1998). A quarter of civilian trauma patients arrive to the hospital with TIC which is associated with increased transfusion requirements, in-hospital complications and poor outcomes (Brohi et al., 2007; Niles et al., 2008). Patients with TIC often warrant emergent blood transfusion to compensate the losses suffered by injury.

Early recognition of trauma patients who require urgent and effectual therapeutic intervention may improve outcome. Due to processing delays in obtaining advanced and specific SCTs for individual defects in coagulopathies, use of SCTs have been very challenging in predicting transfusion of allogeneic blood products, MT and early mortality. In an emergency situation such as traumatic injury, trauma care team often rely on time consuming laboratory tests or their clinical judgement in managing significantly bleeding trauma patients. ROTEM® has been increasingly used to monitor coagulation abnormalities and guide therapy with massive transfusion and individual blood products such as PRBC, plasma, platelets and cryoprecipitate in different clinical areas such as surgery, liver transplant procedure as well as traumatic injury (Gorlinger et al., 2011; Grassetto et al., 2012; Johansson et al., 2009; Rugeri et al., 2007; Schochl
et al., 2010; Tauber et al., 2011; Theusinger et al., 2011; Schochl, Voelckel, Grassetto, & Schlimp, 2013; Olde Engberink et al., 2014; Tanaka et al., 2012; Schochl et al., 2011a). ROTEM® has also been shown to be the predictor of mortality (Theusinger et al., 2011). Many studies emphasize the role of low clot amplitude in ROTEM® and its association with increased bleeding, transfusion and mortality.

Using readily available ROTEM® and important clinical variables, we attempted to identify risk factors for 24h transfusion of blood products and developed prediction models which could promptly identify significantly bleeding trauma patients at risk of receiving allogeneic blood products within the first 24h of hospital arrival. To our knowledge this is the first study in Canada that evaluated viscoelastic test variables in the prediction of 24h blood products transfusion including MT and early mortality. All prediction models were externally validated in dataset from a separate level I trauma center.

Patients who arrive with hemorrhagic shock require immediate surgical intervention and rapid delivery of hemostatic intervention during the initial resuscitation phase. Early identification of patients presenting with TIC, and timely substitution of blood products is an important step in the management of significantly bleeding patients. Hemodynamically unstable patients with significant blood loss may require early transfusion of blood transfusion products during early hours of resuscitation. A rapid intervention may save lives as continued hemorrhage and concurrent coagulopathy is a key factor in the hospital course following major injury (MacLeod et al., 2003). Furthermore, a delay in transfusion may be associated with grave outcomes. In order to recognize patients with TIC, who are in need of early transfusion of blood products we developed risk prediction models using ROTEM® and available clinical variables which can be made available within few minutes of hospital admission.

4.7.1 24h RBC transfusion

Our study demonstrates that 15% of trauma patients admitted to our hospital required any RBC transfusion within the first 24h of hospital admission. In our analyses, SBP, hemoglobin, injury severity and penetrating mechanism of injury were found to be the significant predictors of 24h RBC transfusion. Our models demonstrated penetrating mechanism as the strong predictor of likelihood of 24h RBC transfusion. In our study, surprisingly FIBTEM A10 was not the
significant predictor of 24h RBC transfusion. A possible reason for this observation is that a lot of RBC was not necessary. A part of it was also attributed to clinical judgement, which is an error factor. Furthermore it can be speculated that a lot of patients were bleeding due to penetrating mechanism of injury which shows the dominance of penetrating trauma, low hemoglobin levels, and low SBP as risk factors for RBC transfusion. With a sensitivity of 83% and specificity of 80%, the model could predict the patients who are at higher odds of receiving RBC transfusion. The study showed high discrimination in both derivation and validation data (ROC=0.88 for both) indicating that our model for 24h RBC transfusion has good predictive ability. At a probability threshold of 0.14, the model had a high negative predictive value of 96% in both derivation and validation cohort correctly predicting those patients who were not in need of RBCs. It should be noted that a large number of patients with TIC receive high volume of RBC transfusion irrespective of their coagulopathic status which adds to high resource utilization and wastage of blood products.

This study is in alignment with previous studies in identifying the risk factors for 24h RBC transfusion. A series of investigations have pointed out early available clinical variables such as hypotension, lower GCS, hemoglobin as the predictors of RBC transfusion (West, Jurkovich, Donnell, & Luterman, 1989; Grupp-Phelan & Tanz, 1996; Baker, Korn, Robinson, Chan, & Henderson, 2001; Hooker, Miller, Hollander, & Bukowski, 1994; Roubinian et al., 2014).

**4.7.2 24h Plasma transfusion**

Our study demonstrates that 6% of trauma patients admitted to our hospital required any plasma transfusion within the first 24h of hospital admission. SBP, hemoglobin, INR, ISS and FIBTEM A10 were found to be the significant predictors of plasma transfusion. Age was marginally significant. At the probability level of 0.06, a sensitivity of 89% and a specificity of 88% the model was able to predict patients who are at higher risk for receiving plasma transfusion. The discrimination in both derivation (AUC 0.95) and validation data (AUC 0.91) indicated that the model for 24h plasma transfusion had a good predictive ability. Though the prediction model was good, our threshold on the probability of risk of plasma transfusion is non-optimal. In such scenarios one can choose multiple thresholds near the optimal points and take general consensus for the decision of the risks.
There were no studies that developed ROTEM® based prediction model for plasma transfusion. However some studies explored the association and correlation of viscoelastic test variables with plasma transfusion. A prospective study by Davenport et al., at the Royal London Hospital, England has reported that CA5 in EXTEM assay correlated with the PT ratio (Davenport et al., 2011). Patients with CA5≤35mm in EXTEM assay were more likely to receive FFP transfusion (37% vs. 11% p<0.001). This study also found that patients with CA5<25mm received more units of plasma transfusion (2U vs. 0 U, p<0.001). Cotton et al., in their pilot study assessed TEG parameters for predicting plasma and platelet transfusion in the first hour and two hours of hospital arrival (Cotton et al., 2011). However none of the TEG values predicted plasma transfusion in the first hour of hospital arrival. Using simple linear regression and multivariable linear regression, they assessed the TEG parameter’s ability to predict transfusion in the first two hours of hospital arrival. While controlling for age, gender, race, mechanism of injury, FAST examination, ED blood pressure and heart rate, ACT(activated clotting time) predicted plasma transfusion in 2 hours of hospital arrival (coef: 0.089; 95% CI: 0.074–0.104; p <0.001) (Cotton et al., 2011).

4.7.3 24h Platelet transfusion

This study demonstrated that 5 % patients received any platelets within the first 24h of hospital arrival. The variables predictive of platelet transfusion were SBP, platelet count, hemoglobin, ISS and GCS. ROTEM® was not the predictor of platelet transfusion. The model demonstrated good sensitivity (91%) and specificity (84%) at a probability threshold of 0.05 for derivation cohort. The sensitivity and specificity for the validation cohort were 79% and 91% respectively. The AURO for derivation and validation were 0.92 and 0.91 showing good discriminative ability of the model to predict platelet transfusion.

A single study reported correlation (r=0.57, p<0.001) between INTEM – CA15 with platelet counts (Rugeri et al., 2007). However, the thresholds of INTEM CA15 reported poor predictive value in the diagnosis of laboratory platelet count <50 × 10−9L−1 (sensitivity: 100 % [95 % CI 71–100], specificity 83 % [95 % CI 82–83]; PPV 17 % [95 % CI 12–17], negative predictive value [NPV] 100 % [95 % CI 98–100]; AUC 0.92). Cotton et al., used the ability of TEG to predict platelet transfusions in the first 1-2 hours of hospital arrival (Cotton et al., 2011). In their
study all TEG parameters except G-value was predictive of platelet transfusion (P<0.001 for all variables). The evidence of VE tests in predicting platelet transfusion in trauma is very limited with these two studies.

4.7.4 24h Cryoprecipitate transfusion

According to our study only 3% of patients received cryoprecipitate transfusion. The variables predictive of 24h cryoprecipitate transfusion were SBP, ISS and FIBTEM A10. A low FIBTEM A10 is a measure of low fibrinogen level which warrants fibrinogen or cryoprecipitate administration. At a probability threshold of 0.02, the sensitivity of the model in derivation cohort in predicting cryoprecipitate transfusion was 96%, specificity 85% and the NPV was 99.89%. The high NPV indicates that this model is good at predicting patients who do not require cryoprecipitate in 24h. The sensitivity, specificity and NPV in validation cohort were 66%, 96% and 98% respectively. The AUC for the predictive models in both derivation and validation cohort were 0.95 and 0.93 which showed a good discriminative ability.

4.8 Limitations:

Our study has several limitations. The first and foremost being retrospective nature of analysis of data from a single centre, and therefore has all the limitations inherent to this type of study. Furthermore many eligible patients admitted to the trauma center did not undergo ROTEM® tests attributed to inconsistent utilization of ROTEM® or due to logistic issues leading to selection bias.

Second, the trauma registry in developmental cohort did not capture fibrinogen levels which is an important parameter and is associated with patient outcomes. A significant amount of data on initial temperature and respiratory rate was missing in many cases and hence could not be included in our model thus excluding two important predictors of patient outcomes. Although we have built models with good performance statistics these important missing variables affecting the patient outcomes were not included in our model. Furthermore pre-existing comorbidities may also affect the outcomes of trauma patients which were not assessed. However in our defence, given the fact that our models were able to discriminate between the outcomes, the chances that any unadjusted risk factors would change the results is very low.
4.9 Summary and conclusion

Coagulopathy following trauma requires early resuscitative measures and is associated with poor outcomes. Currently majority of trauma centers transfuse hemostatic blood products based on time consuming standard coagulation tests or based on their clinical instinct. ROTEM® is a near patient testing device which is increasingly used to recognize TIC and guide transfusion. Its ease of use and remote monitoring by multidisciplinary team may allow for rapid assessment of coagulopathy and help in guiding transfusion.

We have attempted to demonstrate the role of ROTEM® variables in predicting the need for 24h transfusion of allogeneic blood products using large data sets. However it is still a small study when considering for development of transfusion guidelines and early resuscitation. In this study only 15% received 24h RBC transfusion, 7% received 24h plasma transfusion, 5% received 24h platelet transfusion and only 3% received cryoprecipitate transfusion. Our models were more useful in identifying patients who do not require plasma and cryoprecipitate which may help in reducing the plasma and cryoprecipitate transfusion requirements and save resources. ROTEM® was not a predictor of RBC and platelet transfusion. We need to understand if using these prediction models can change the transfusion practices and reduce the blood product usage in a busy trauma center. From our findings we conclude that large prospective studies may be required to support the use of ROTEM® in guiding transfusion requirements in trauma patients.
Chapter 5: ROTEM®-based prediction model of massive transfusion and early mortality in trauma
5.1 Abstract

Introduction: Coagulopathy occurs in a quarter of all severely injured patients, and significantly reduces survival. Hemorrhage is the most preventable cause of traumatic death, which occurs early (often within 2-3h). Early blind ratio resuscitation (i.e. 1:1) increases survival but also inappropriate blood transfusions. The use of viscoelastic assays (ROTEM®) is increasing to diagnose coagulation defects and guide their treatment. We hypothesize that the ROTEM® clot amplitude measured at 10 minutes of initiating the test is a predictor of both the need for massive transfusion (MT) and 24h mortality.

Methods: Retrospective analysis of adult trauma patients presenting with blunt or penetrating trauma admitted to a Level I trauma center between Aug 2011 and Mar 2013 was conducted. The following variables were analyzed in multivariable logistic regression: age, gender, injury type, injury severity score (ISS), Glasgow Coma Score scale (GCS), systolic blood pressure (SBP), international normalized ratio (INR), hemoglobin, platelet count, ROTEM® A10 EXTEM and A10 FIBTEM. The models were validated in a similar cohort of patients admitted to another level I trauma center between Nov 2014 and Oct 2015.

Results: Of the 1146 patients in derivation cohort, 22 (2%) received MT and 29 (3%) died within 24h of admission. Median age was 41 (IQR 26-58) and 73% were men. MT and 24h mortality patients had more severe injuries, abnormal laboratory and ROTEM® assay results (p<0.05 for all variables) compared to non-MT patients and patients surviving over 24h. Variables independently associated with MT in multivariable logistic regression were: ISS (OR 1.08 95% CI: 1.04-1.10 p=0.0002); SBP (OR 0.98 95% CI: 0.97-0.99 p=0.0038); Hemoglobin (OR 0.97 95% CI: 0.95-1.00 p=0.0270); A10 EXTEM (OR 0.94 95% CI: 0.90-0.97 p=0.0011) and penetrating trauma (a OR 8.31 95% CI: 2.20-31.35, p=0.0018). Variables independently associated with 24h mortality were: GCS (OR 0.68, 95% CI 0.53-0.85, p=0.0011); ISS (OR 1.05, 95% CI: 1.01-1.09, p=0.0212); and FIBTEM A10 (OR 0.74, 95% CI 0.63–0.87, p=0.0002).

Conclusion: In 10 minutes from starting the test (ROTEM®), the measurement of the clot amplitude is capable of predicting both the need for massive transfusion and mortality. While these findings require prospective validation, they indicate that an abnormal clot amplitude result
may be clinically relevant and useful to the clinical decision-making process during early resuscitation.
5.2 Introduction

Trauma is the leading cause of death among young individuals (Sauaia et al., 1995). Exsanguination following a traumatic injury is the second leading cause of death, next to central nervous system injuries, accounting for nearly 40%-50% of all trauma related in-hospital deaths, most in the first 24h (Kauvar et al., 2006; Sauaia et al., 1995; Acosta et al., 1998). Early coagulopathy is common and exacerbates mechanical bleeding, which may result in exsanguination (Brohi et al., 2003; Maegele et al., 2007; Frith et al., 2010). Approximately one fourth of all severely injured patients are coagulopathic on hospital admission (Brohi et al., 2003; Brohi et al., 2007; Brohi et al., 2008; Hess et al., 2008; MacLeod et al., 2003; Maegele et al., 2007). This early trauma-induced coagulopathy is independently responsible for increased transfusion requirements, complications and higher mortality (Brohi et al., 2003; MacLeod et al., 2003; Maegele et al., 2007; Brohi et al., 2007).

Most trauma centers in North America have created and adopted massive transfusion protocols (MTP) that are promptly initiated by the suspicion of significant bleeding. While reducing time to initiating blood transfusion, MTP activation has significant costs and risks. MTP activation involves immediate preparation and transportation of blood products by the Blood Bank, mobilization of anesthetists, porters and other personnel, preparation of the operating room for an eventual surgery and other resource-intense steps such as mobilization of interventional radiology and laboratories. In our experience, only half of the MTP activations actually result in massive transfusions, which are performed in 2%-4% of all civilian trauma population (Holcomb et al., 2007; Frith & Brohi, 2010; Como et al., 2004).

On the other hand, delaying MTP activation carries risk of depriving patients from timely transfusion and hemostatic management. Current standard of care for the initial managements of patients suspected of having significant bleeding is hemostatic resuscitation which comprises of empiric transfusion of blood and blood products at a 1:1:1 ratio of platelets, plasma and red blood cells, until hemostasis is achieved (Duchesne et al., 2008). The definition for achieving hemostasis remains elusive and mostly undefined. Most clinicians use clinical judgement to trigger MTP and determine when an injured patient has stopped bleeding and hemostasis has been achieved.
After initial empiric resuscitation, most clinicians gradually move to a hybrid resuscitation, increasingly utilizing laboratory tests to guide transfusions. The use of standard coagulation tests (SCTs) such as INR, PTT, platelet count and fibrinogen levels however, has been criticized in trauma (Dzik, 2004; Rossaint et al., 2010). These tests are performed in platelet-poor plasma that contrasts with broadly accepted theory of a cell-based hemostasis (Davenport et al., 2011; Toulon et al., 2009). Turnaround time for routine laboratory results is often too long to provide an accurate assessment of hemostasis in rapidly bleeding patients and thus be used to guide transfusions (Davenport et al., 2011). These tests were not engineered for trauma, lacking the ability of measuring lysis and other parts of hemostasis and lack association with bleeding (Bolliger, Gorlinger, & Tanaka, 2010; Schreiber, 2005). These limitations may account for the preference to empiric early transfusions despite the significant rates of inappropriate blood usage.

There is a growing interest in viscoelastic assays such as Thromboelastography (TEG, Haemonetics Corporation, USA) and Rotational thromboelastometry (ROTEM®, TEM International GmbH) in trauma (Luddington, 2005; Brohi et al., 2007; Johansson et al., 2009; Johansson, Stensballe, Vindelov, Perner, & Espersen, 2010; Kaufmann, Dwyer, Crews, Dols, & Trask, 1997; Leemann et al., 2010; Levrat et al., 2008). They may be performed as point-of-care tests providing immediate results that have been associated with ongoing bleeding, need for blood transfusion and mortality (Luddington, 2005; Davenport et al., 2011; Doran et al., 2010; Schochl et al., 2010; Schochl et al., 2010; Schochl et al., 2011b; Schochl et al., 2011a). In cardiac surgery, transfusion-algorithms based on viscoelastic tests have proven to reduce the need for transfusions and arguably even improve patients’ outcome (Karkouti et al., 2016). A few studies have demonstrated the cost-effectiveness of viscoelastic tests (Whiting et al., 2015) and their clinical utility in diagnosing coagulopathy, guiding blood transfusion and predicting transfusion as well as early mortality (Davenport et al., 2011; Doran et al., 2010; Levrat et al., 2008; Schochl et al., 2010; Tauber et al., 2011; Theusinger et al., 2011; Schochl et al., 2011a) in the setting of trauma. Most studies in trauma have significant methodological limitations and a recent systematic review fell short of recommending the use of viscoelastic tests in trauma (Wikkelso, Wetterslev, Moller, & Afshari, 2016). A few studies have attempted to demonstrate the utility of viscoelastic tests to predict outcome (Nystrup et al., 2011; Hampton, Lee, Diggs, McCully, &
Most studies demonstrated a strong association of abnormal results with outcome of interest (transfusion, mortality, etc.), but none have ever validated their findings. A prediction model based on viscoelastic test results could assist clinicians in making early decisions such as activating, or withholding MTP.

We proposed to develop and validate prediction models for the massive transfusion and early mortality using large cohorts of trauma patients admitted to two level 1 Trauma Centers. We hypothesized that the use of rapidly available viscoelastic test parameters and clinical information could discriminate patients at risk of requiring massive transfusion and dying early thus assisting the clinician on the decision to start, end or withhold massive transfusion activation.

5.3 Methods
5.3.1 Study design, setting and description of study cohorts

We performed a retrospective cohort analysis of prospectively collected data on trauma patients admitted to two level I trauma centers in Toronto.

The derivation cohort consisted of consecutive trauma patients admitted to Sunnybrook Health Sciences Center (SHSC), a leading level I trauma center affiliated with the University of Toronto, Ontario, Canada, between August 2011 and March 2013. This trauma center provides trauma management and care to nearly 700 trauma patients a year. We included patients presenting with blunt and/or penetrating mechanism of injury and received an admission ROTEM® test early upon hospital arrival. Patients who did not have admission ROTEM® test and those died within one hour without ROTEM® test were excluded.

The validation cohort consisted of an independent cohort of trauma patients admitted to St. Michael’s Hospital (SMH), a leading level I trauma center affiliated to the University of Toronto between November 2014 and October 2015 meeting similar eligibility criteria.

The trauma registries at both centers capture in-depth data on individual trauma patients admitted to the hospital’s Emergency Department (ED) through hospital discharge. Both centers follow similar MT protocols and guidelines set up by the Advanced Trauma Life Support (ATLS).
Standard coagulation tests and ROTEM® assays were performed in the main laboratory immediately on arrival following the manufacturer’s guidelines by trained laboratory personnel in both centers. For research purpose the following two assays were used routinely in both centers: extrinsically activated assay (EXTEM), which uses tissue factor (TF) as an activator added to citrated sample of blood, and the fibrinogen assay (FIBTEM) where cytochalasin D, a platelet inhibitor was added to detect fibrin component of the clot to prevent contribution of platelets to the clot. These two key assays are routinely used in trauma which provides information on deficiency of coagulation factors, fibrinogen levels and functional contribution of platelets to the clot. Clot amplitude in EXTEM and FIBTEM estimate platelet and fibrinogen levels and are found to be associated with transfusion requirements and mortality.

Patient demographics (age, gender), physiological data (systolic blood pressure, heart rate, temperature, injury severity score (ISS), Glasgow coma scale score (GCS)), SCTs (INR, platelets count) and blood product transfusion (RBC, plasma, platelets and cryoprecipitate) within the first 24h as well as outcome data were collected from trauma registry. The following ROTEM® measurements were recorded: Clotting time (CT), the time from the onset of the test to the appearance of first detectable clot; clot formation time (CFT), the time from the first detectable clot to reach 20mm amplitude; clot amplitude (CA), the amplitude achieved in ten minutes and maximum clot firmness (MCF), the maximum amplitude achieved during the entire duration of the test in EXTEM assay. For FIBTEM assay, A10 and MCF were recorded.

Quality of data was assessed for accuracy and completeness. Any outliers were verified by revisiting the patient records and any typographical errors were corrected. Missing values were obtained from the patient charts when possible and patients with actual missing values were retained in the analysis. The models were externally validated in a similar patient population to estimate the performance measures in this data set using the same eligibility criteria. The ethics boards of both trauma centers approved the study.

Our primary outcome was massive transfusion defined as delivery of ≥10 units of packed red blood cells (PRBCs) within 24h of hospitalisation. Our secondary outcome was early mortality defined as death occurring within 24h of hospitalisation.
5.4 Statistical analysis

All statistical analyses were carried out using SAS (v. 9.3 SAS Institute, Cary, North Carolina). Patient demographics, physiologic data, SCTs, ROTEM® measurements, blood product transfusion within 24h and outcome data were analysed for descriptive purposes. With the exception of temperature and respiratory rate data, <10% missing data were present in all other variables. All patients were included in the analysis.

Variables were analyzed for normal distribution using the Shapiro-Wilk test. Continuous variables are reported as median and interquartile range (IQR) as the data was not normally distributed, and comparison between groups was analysed using Wilkoxon Rank-Sum test. Categorical variables are reported as absolute numbers and percentages. Chi-square or Fisher’s exact test was used to assess for differences in categorical variables between the groups. Statistical significance was defined a priori as a two-tailed p-value of <0.05.

The following predictor variables were included in the multivariable logistic regression based on prior research evidence and clinical rationale for determining the association with MT (Davenport et al., 2011; Larson et al., 2010; Maegele et al., 2012; McLaughlin et al., 2008; Nunez et al., 2009; Leemann et al., 2010; Dutton et al., 2006; Dutton & Carson, 2006; Schreiber et al., 2007): age, gender, injury mechanism (blunt/penetrating), SBP, ISS, GCS, INR, hemoglobin, platelets count, EXTEM A10 and FIBTEM A10. The following predictor variables were included in the multivariable logistic regression for determining the association with 24h mortality (MacLeod et al., 2004; Mica L, 2012; Mica et al., 2013; Barbosa et al., 2011; Dutton et al., 2006; Rourke et al., 2012; Tauber et al., 2011): age, gender, injury mechanism (blunt/penetrating), SBP, ISS, GCS, INR, hemoglobin, platelet count, EXTEM CFT, EXTEM A10 and FIBTEM FIBTEM A10.

All continuous variables were assessed for multicollinearity by examining the variance inflation factor (VIF) statistics with a cut-off value of 5. When a pair of variables with VIF >5 were found, clinical rationale was used to select a single appropriate variable in the multivariable model. Likelihood ratio estimation was used to add the variable to the model, which was further assessed using backward stepwise method to assess the best model for MT and 24h mortality. The results were reported as an adjusted odds ratio (aOR) with 95% confidence interval (CI).
The predictive ability (calibration) of the model was evaluated using the Hosmer-Lemeshow goodness-of-fit statistic. The discriminative performance was quantified using C-statistic which is equivalent to the area under the receiver-operating characteristic (AUC) curve (Harrell, Jr. et al., 1996; Steyerberg & Vergouwe, 2014; Olde Engberink et al., 2014; Steyerberg et al., 2010). A value of 1 indicates that the model can perfectly predict the outcome, a value greater than 0.90 indicates high accuracy where as a value 0.5 indicates no discrimination. (Steyerberg & Vergouwe, 2014) The final models were externally validated in the SMH validation cohort. We compared the discrimination using the area under the ROC curve for both models. Finally we assessed the predicted probability over a series of thresholds and calculated the sensitivity, specificity, positive and negative predictive values for the models in both cohorts.

5.5 Results

Between August 2011 and March 2013, 1890 patients were admitted to the SHSC. After excluding patients without an admission ROTEM® (n=718) and who died within one hour without receiving ROTEM® test (n=26), 1146 patients were included in the derivation cohort. The validation cohort consisted of 1148 patients admitted to SMH between November 2014 and October 2015. After excluding patients without an admission ROTEM® (n=350), died within one hour without ROTEM® (n=18) and drowning (n=1), 779 patients were included in the validation cohort (figure 5.1).
Figure 5.1: The CONSORT (Consolidated Standards for Reporting Trials) diagram describing patient enrollment, eligibility criteria in derivation and validation cohorts.

The demographic and clinical characteristics of derivation cohort and validation cohort are presented in Table 5.1. In general patient characteristics in both cohorts were similar in age and sex, sustained blunt trauma, exhibited similar physiologic conditions and had similar coagulation profiles on admission. Patients in derivation cohort experienced slightly higher ISS, lower EXTEM CT, and higher EXTEM CFT. There were no clinically meaningful differences in SBP, INR, A10 and MCF in EXTEM assay, A10 and MCF in FIBTEM assay and blood product transfusions. The rates of MT (n=21) and early death (n=26) was 2% in derivation cohort while the rates of MT (n=11) and early death (n=7) was 1% in validation cohort. The overall all-cause in-hospital mortality was 8% (n=97) in derivation cohort and 5% (n=40) in validation cohort (table 5.1).
Table 5.1: Patient demographics, clinical characteristics and outcomes in the derivation and validation cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>Derivation cohort</th>
<th>Validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic and vital signs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient (n)</td>
<td>1146</td>
<td>779</td>
</tr>
<tr>
<td>Gender, male n (%)</td>
<td>836 (73)</td>
<td>566 (73)</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>41 (26-58)</td>
<td>42 (27-58)</td>
</tr>
<tr>
<td>Injury type-blunt, n (%)</td>
<td>951 (83)</td>
<td>618 (79)</td>
</tr>
<tr>
<td>Injury type-penetrating, n(%)</td>
<td>195 (17)</td>
<td>161 (21)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>142 (126-160)</td>
<td>135 (121-149)</td>
</tr>
<tr>
<td>GCS</td>
<td>15 (13-15)</td>
<td>15 (14-15)</td>
</tr>
<tr>
<td>ISS</td>
<td>17 (9-26)</td>
<td>9 (2-19)</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>36.00 (35.4-36.5)</td>
<td>36 (35.2-36.6)</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>90.00 (76.00-104.0)</td>
<td>89 (78-100)</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>18 (18-20)</td>
<td>18 (16-20)</td>
</tr>
<tr>
<td><strong>Standard Coagulation Tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INR</td>
<td>1.1 (1-1.2)</td>
<td>1 (1-1.1)</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>138 (123-149)</td>
<td>138 (126-149)</td>
</tr>
<tr>
<td>Platelet count, x10^9/L</td>
<td>231 (192-275)</td>
<td>237 (199-277)</td>
</tr>
<tr>
<td><strong>ROTEM® Measurements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>---------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>EXTEM A10, mm</td>
<td>52 (47-57)</td>
<td>55 (51-59)</td>
</tr>
<tr>
<td>EXTEM CT, sec</td>
<td>50 (44-59)</td>
<td>69 (61-76)</td>
</tr>
<tr>
<td>EXTEM CFT, sec</td>
<td>100 (82-122)</td>
<td>85 (72-100)</td>
</tr>
<tr>
<td>EXTEM MCF, mm</td>
<td>60 (56-64)</td>
<td>63 (59-66)</td>
</tr>
<tr>
<td>FIBTEM A10, mm</td>
<td>13 (10-16)</td>
<td>15 (12-18)</td>
</tr>
<tr>
<td>FIBTEM MCF, mm</td>
<td>14 (10-17)</td>
<td>16 (13-20)</td>
</tr>
</tbody>
</table>

### 24h Transfusion data

<table>
<thead>
<tr>
<th></th>
<th>Median (IQR)</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRBC, U</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>FFP, U</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Platelet, U</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Cryoprecipitate, pooled units</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Massive transfusion, n (%)</td>
<td>21(2)</td>
<td>11 (1)</td>
</tr>
</tbody>
</table>

### Mortality

<table>
<thead>
<tr>
<th></th>
<th>Median (IQR)</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause in-hospital mortality, n (%)</td>
<td>97(8)</td>
<td>40(5)</td>
</tr>
<tr>
<td>Died within 24h, n (%)</td>
<td>26(2)</td>
<td>7(1)</td>
</tr>
</tbody>
</table>

Data are presented as medians with interquartile ranges (25th - 75th percentile) for continuous variables. Categorical variables are presented as absolute numbers and percentages.

A10 - amplitude measured at ten minutes after CT; bpm-beats per minute; CT-clotting time; CFT-clot formation time; EXTEM – extrinsically activated thromboelastometry test; FIBTEM-extrinsically activated thromboelastometry test with cytochalasin-D as an activator; FFP – fresh frozen plasma; GCS - Glasgow Coma Scale score; INR-international normalized ratio; ISS-Injury severity score; MCF- maximum clot firmness; PRBC-packed red blood cells; SBP-systolic blood pressure.
4.2.1.1 Massive transfusion – Bivariate analysis

The unadjusted associations of selected risk factors with MT status in the derivation cohort are described in Table 5.2. Patients in MT group sustained more penetrating trauma compared to non-MT group (29% vs 17). Patients who received MT were slightly younger (39 (IQR 24-42) vs 42 (IQR,26-58)); sustained severe injuries with higher ISS (41(IQR, 29-50) vs 17 (9-26)), had lower systolic blood pressure (80 (IQR, 64-120) vs 142 (IQR, 126-160)) and lower GCS (3 (3-12) vs 15 (13-15)) compared to Non-MT patients. Likewise, MT patients showed significantly abnormal findings in all standard coagulation tests as well as ROTEM® measurements compared to Non-MT patients indicating compromised coagulation status.

Table 5.2: Bivariate association of risk factors associated with massive transfusion and 24h mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>MT</th>
<th>Non-MT</th>
<th>Died &lt;24h</th>
<th>Survived &gt;24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient (n)</td>
<td>21</td>
<td>1125</td>
<td>26</td>
<td>1120</td>
</tr>
<tr>
<td>Gender, male n (%)</td>
<td>15 (71)</td>
<td>821 (73)</td>
<td>18 (69)</td>
<td>818 (73)</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>39 (24-42)</td>
<td>42 (26-58)</td>
<td>51 (27-72)</td>
<td>41 (26-57)</td>
</tr>
<tr>
<td>Injury type-blunt, n (%)</td>
<td>15 (71)</td>
<td>936 (83)</td>
<td>25 (96)</td>
<td>926 (83)</td>
</tr>
<tr>
<td>Injury type-penetrating, n (%)</td>
<td>6 (29)</td>
<td>189 (17)</td>
<td>1 (4)</td>
<td>194 (17)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>80 (64-120)</td>
<td>142(126-160)</td>
<td>106 (56-137)</td>
<td>142 (126-160)</td>
</tr>
<tr>
<td>GCS</td>
<td>3 (3-12)</td>
<td>15 (13-15)</td>
<td>3 (3-3)</td>
<td>15 (13-15)</td>
</tr>
<tr>
<td>ISS</td>
<td>41 (29-50)</td>
<td>17 (9-26)</td>
<td>38 (30-50)</td>
<td>17 (9-26)</td>
</tr>
<tr>
<td>Standard Coagulation Tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INR</td>
<td>1.6 (1.3-1.8)</td>
<td>1.1 (1-1.2)</td>
<td>1.7 (1.2-2.1)</td>
<td>1.1 (1-1.2)</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>102 (85-117)</td>
<td>139 (124-149)</td>
<td>115 (96-132)</td>
<td>139 (124-149)</td>
</tr>
</tbody>
</table>
Platelet count, x10⁹/L

|   | 174 (90-236) | 231 (193-276) | 172 (100-230) | 232 (193-276) |

**ROTEM® Measurements**

<table>
<thead>
<tr>
<th></th>
<th>EXTEM A10, mm</th>
<th>EXTEM CFT, sec</th>
<th>FIBTEM A10, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33 (18-43)</td>
<td>52 (47-57)</td>
<td>7 (2-11)</td>
</tr>
<tr>
<td></td>
<td>52 (47-57)</td>
<td>31 (18-41)</td>
<td>13 (10-16)</td>
</tr>
<tr>
<td></td>
<td>31 (18-41)</td>
<td>52 (47-57)</td>
<td>6 (2-10)</td>
</tr>
<tr>
<td></td>
<td>52 (47-57)</td>
<td>236 (129-328)</td>
<td>13 (10-16)</td>
</tr>
<tr>
<td></td>
<td>236 (129-328)</td>
<td>99 (81-121)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as medians with interquartile ranges (25th - 75th percentile) for continuous variables and were analysed using Wilcoxon Rank-Sum test. Categorical variables are presented as absolute numbers and percentages and were analysed using Chi-square test/Fisher’s exact test.

A10 - amplitude measured at ten minutes; CFT - clot formation time; FFP - fresh frozen plasma; GCS – Glasgow Coma Scale score; INR - international normalized ratio; ISS - injury severity score; MT - massive transfusion; PRBC - packed red blood cells; SBP - systolic blood pressure.

The final logistic regression model for risk of receiving an MT is shown in table 5.3. The variables found to be independently associated with MT in multivariable logistic regression were: higher ISS (aOR 1.08 95% CI: 1.04-1.12 p=0.0002); lower SBP (aOR 0.98 95% CI: 0.97-0.99, p=0.0038); lower hemoglobin concentration (aOR 0.97 95% CI: 0.95-1.00 p=0.0270); reduced clot amplitude in EXTEM A10 (OR 0.94 95% CI: 0.90-0.97, p=0.0011) and penetrating mechanism of injury (aOR 8.31 95% CI: 2.20-31.35, p=0.0018). The model’s ability to predict the need for MT, as assessed by AUC was 0.97 (Fig. 5.2). The Hosmer-Lemeshow goodness-of-fit was 0.99 indicating sufficient fit.

**Table 5.3: Multivariable logistic regression analysis of risk factors associated with Massive Transfusion**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Adjusted OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISS</td>
<td>1.08 (1.04-1.12)</td>
<td>0.0002</td>
</tr>
<tr>
<td>SBP</td>
<td>0.98 (0.97-0.99)</td>
<td>0.0038</td>
</tr>
<tr>
<td>HGB</td>
<td>0.97 (0.95-1.00)</td>
<td>0.0270</td>
</tr>
<tr>
<td>EXTEM A10</td>
<td>0.94 (0.90-0.97)</td>
<td>0.0011</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Injury Type (Penetrating vs. Blunt)</td>
<td>8.31 (2.20-31.35)</td>
<td>0.0018</td>
</tr>
<tr>
<td>Omnibus Likelihood Ratio (Chi sq (df), p value 90.51 (5), &lt;0.0001; Hosmer Lemeshow goodness of fit: 0.99; C-statistics: 0.97</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A10-clot amplitude measured in EXTEM assay at ten min; HGB-hemoglobin; ISS-injury severity score, OR-odds ratio; SBP-systolic blood pressure.

Table 5.4 demonstrates the sensitivity, specificity, positive and negative predictive values across a range of predicted probability thresholds for derivation cohort. Using a probability level of 0.023, the sensitivity of the model to identify patients who would require MT was 89.5% and the specificity, or the ability of the model to identify patients who would not require MT was 92%. The negative predictive value (NPV), the probability that a patient who does not truly require MT was 99.8% (Table 5.4).

**Table 5.4: Accuracy of the Massive transfusion model at various probability cut off levels in the derivation data**

<table>
<thead>
<tr>
<th>Probability level</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.023</td>
<td>89.5</td>
<td>92</td>
<td>16.3</td>
<td>99.8</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>57.9</td>
<td>97.2</td>
<td>26.2</td>
<td>99.2</td>
</tr>
<tr>
<td>0.2</td>
<td>57.9</td>
<td>98.9</td>
<td>47.8</td>
<td>99.3</td>
</tr>
</tbody>
</table>
When the final model was applied to an independent validation cohort using the same eligibility criteria, model’s discrimination assessed by AUC was 0.99 which showed that the ability to discriminate between patients receiving MT and those who did not was fairly strong based on the model utilized (Fig. 5.2). The sensitivity was 82%, specificity, 98% and the negative predictive value was 99.7% (Table 5.5). The calibration measure, which is slope of the regression of the predicted response of MT on to the true response in validation data, was 1.71 (p <0.0001) indicating that the calibration is away from 1 but not very far between the validation and derivation cohorts.

**Table 5.5: Accuracy of the Massive transfusion model at various probability cut off levels in the validation data**

<table>
<thead>
<tr>
<th>Probability level</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>81.82</td>
<td>97.79</td>
<td>34.62</td>
<td>99.73</td>
</tr>
<tr>
<td>0.00</td>
<td>90.91</td>
<td>1.82</td>
<td>1.31</td>
<td>93.33</td>
</tr>
</tbody>
</table>
NPV-negative predictive value; PPV-positive predictive value

Figure 5.2. Comparison of ROC curves for prediction of MT based on derivation cohort and validation cohort
4.2.1.1.2 24h Mortality-Bivariate association

The bivariate association between potential risk factors by 24h mortality are presented in Table 5.2. Patients who died in <24h were older compared to those who survived 24h (51 IQR (27 - 72) vs 41 IQR (26-57)). Sixty nine percent of patients who died in <24h were male vs 73% who survived 24h. Patients who died in <24h had significantly abnormal ROTEM® and SCT results vs those who survived >24h.

Table 5.6 shows the results of multivariable logistic regression in predicting 24h mortality in derivation cohort. The variables found to be independently associated with 24h mortality in multivariable logistic regression were: low GCS (aOR 0.68, 95% CI: 0.53-0.85, p=0.0011); higher ISS (aOR 1.05, 95% CI: 1.01-1.09, p=0.0212); and reduced amplitude in FIBTEM A10 (aOR 0.74, 95% CI 0.63-0.87, p=0.0002). The C-statistics for the model was 0.97 and Hosmer Lemeshow goodness-of-fit statistic was 1.00.

Table 5.6: Multivariable logistic regression analysis of risk factors associated with 24h mortality

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Adjusted OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCS</td>
<td>0.68 (0.53 - 0.85)</td>
<td>0.0011</td>
</tr>
<tr>
<td>Injury Severity Score</td>
<td>1.05 (1.01 - 1.09)</td>
<td>0.0212</td>
</tr>
<tr>
<td>FIBTEM A10</td>
<td>0.74 (0.63 - 0.87)</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

Chi square df(3)= 102.23, p <0.0001; C-statistic: 0.97; Hosmer Lemeshow goodness-of-fit statistic: 1.00

GCS- Glasgow coma scale score

Table 5.7 reports the sensitivity, specificity, positive and negative predictive values across a range of predicted probability thresholds for derivation cohort for early death. Using a probability level of 0.015, the sensitivity of the derivation model to identify patients who would die within 24h was 95%, specificity 89% and the NPV was 99.9% (Table 5.7).
Table 5.7: Accuracy of the 24h mortality model at various probability cut off levels in the derivation data

<table>
<thead>
<tr>
<th>Probability level</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0145</td>
<td>95</td>
<td>88.84</td>
<td>13.67</td>
<td>99.9</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
<td>1.83</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>70</td>
<td>95.35</td>
<td>21.88</td>
<td>99.42</td>
</tr>
<tr>
<td>0.2</td>
<td>45</td>
<td>98.51</td>
<td>36</td>
<td>98.97</td>
</tr>
<tr>
<td>0.3</td>
<td>40</td>
<td>99.35</td>
<td>53.33</td>
<td>98.89</td>
</tr>
<tr>
<td>0.4</td>
<td>35</td>
<td>99.63</td>
<td>63.64</td>
<td>98.8</td>
</tr>
<tr>
<td>0.5</td>
<td>30</td>
<td>99.81</td>
<td>75</td>
<td>98.71</td>
</tr>
<tr>
<td>0.6</td>
<td>20</td>
<td>99.91</td>
<td>80</td>
<td>98.53</td>
</tr>
<tr>
<td>0.7</td>
<td>15</td>
<td>99.91</td>
<td>75</td>
<td>98.44</td>
</tr>
<tr>
<td>0.8</td>
<td>0</td>
<td>100</td>
<td></td>
<td>98.17</td>
</tr>
<tr>
<td>0.9</td>
<td>0</td>
<td>100</td>
<td></td>
<td>98.17</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>100</td>
<td></td>
<td>98.17</td>
</tr>
</tbody>
</table>

NPV - negative predictive value; PPV - positive predictive value

For the validation cohort, the sensitivity was 57%, specificity, 94% and the NPV was 99.59% (Table 5.8). The calibration measures using the calibration-slope based on the regression of the predicted response of 24h mortality on to the true response in validation data showed a slope of
0.5 (p-value <0.0001) indicating that the calibration is good between the validation and derivation cohorts.

**Table 5.8: Accuracy of the 24h mortality model at various probability cut off levels in the validation data**

<table>
<thead>
<tr>
<th>Probability level</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>57.14</td>
<td>94.04</td>
<td>8.00</td>
<td>99.59</td>
</tr>
<tr>
<td>0.00</td>
<td>100.00</td>
<td>98.19</td>
<td>12.50</td>
<td>99.34</td>
</tr>
<tr>
<td>0.10</td>
<td>28.57</td>
<td>99.22</td>
<td>14.29</td>
<td>99.22</td>
</tr>
<tr>
<td>0.20</td>
<td>14.29</td>
<td>99.74</td>
<td>33.33</td>
<td>99.23</td>
</tr>
<tr>
<td>0.30</td>
<td>14.29</td>
<td>99.87</td>
<td>99.10</td>
<td></td>
</tr>
<tr>
<td>0.40</td>
<td>100.00</td>
<td>99.10</td>
<td>99.10</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>100.00</td>
<td>99.10</td>
<td>99.10</td>
<td></td>
</tr>
<tr>
<td>0.60</td>
<td>100.00</td>
<td>99.10</td>
<td>99.10</td>
<td></td>
</tr>
<tr>
<td>0.70</td>
<td>100.00</td>
<td>99.10</td>
<td>99.10</td>
<td></td>
</tr>
<tr>
<td>0.80</td>
<td>100.00</td>
<td>99.10</td>
<td>99.10</td>
<td></td>
</tr>
<tr>
<td>0.90</td>
<td>100.00</td>
<td>99.10</td>
<td>99.10</td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>100.00</td>
<td>99.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NPV-negative predictive value; PPV-positive predictive value

The validation of the predictive risk model for 24h mortality provided using discrimination under C-statistics had AUC of 0.90 whereas the derivation model had an AUC of 0.97(Figure 5.3)
Figure 5.3. Comparison of ROC curves for prediction of 24h mortality based on derivation cohort and validation cohort

5.6 Discussion

Several studies have derived risk factors and scoring systems for recognizing patients requiring MT and early mortality with diverse ranges of accuracy and sensitivity (McLaughlin et al., 2008; Schreiber et al., 2007; Yucel et al., 2006; Cancio, Wade, West, & Holcomb, 2008; Larson et al., 2010; Nunez et al., 2009; Rainer et al., 2011; Vandromme et al., 2011; Mica et al., 2013; Hampton et al., 2014). These studies used variables that combined physiologic, laboratory and procedural data. Some of these studies either required time consuming laboratory tests, computed tomography or complex mathematical computations limiting their practical use as none of the scores/models were able to correctly identify every patient requiring an MT or early death. Predictive models using viscoelastic tests are less studied, arguably due to their recent emerging use in trauma. Two studies explored the use of TEG variables in predicting 30 day and early mortality (Hampton et al., 2014; Nystrup et al., 2011). To our knowledge, no studies to date have developed and validated their models for prediction of MT or early mortality using ROTEM®. We attempted to derive and validate prediction models using readily available clinical and
ROTEM® variables using a large cohort of patients. The primary observations of this study are that a low clot strength (A10) assessed at 10 minutes in EXTEM and FIBTEM was independently associated with MT and 24h mortality in adult trauma patients after adjusting for other relevant variables. In our study approximately 2% of patients received a MT, which is in alignment with published studies from other centers for civilian trauma patients. Our findings add value to the existing evidence regarding the utility of ROTEM® in identifying coagulation abnormalities in patients who ultimately received massive transfusion and who died within 24h.

A model that utilizes rapidly available information could help with the clinical decision making of either activating MT or withholding it. The factors that were independently associated with the need of MT included: ISS, systolic blood pressure, hemoglobin, EXTEM A10 and penetrating mechanism of trauma. All these variables are easy to use and readily available, including EXTEM A10 (available within 10 minutes of initiating the assay), while ROTEM® itself is commonly employed as point of care test. While the exact ISS score cannot be precisely calculated in the trauma bay, most experienced clinicians can crudely estimate the approximate ISS score.

The AUC values (0.97) and Hosmer-Lemeshow goodness of fit statistics (0.99) reported in the model indicate that our model has good ability to predict the outcome. External validation of the model showed a slight decrease in C statistic from 0.99 to 0.97 indicating minimal over-optimism in developing the model. Our model was more useful in predicting patients who are at less risk for MT as indicated by the high NPV. This may be useful in identifying patients who do not require MT and to withhold MT thus avoiding unnecessary exposure to large volume of blood products as well as reducing hospital resources. The high specificity and high NPV is probably attributed to small percentage of patients who received MT.

There is intense resource utilization associated with the activation of the MTP. In most institutions, approximately one hour time is required for plasma to be thawed and delivered to patient’s bed side after it has been ordered. An early (within minutes) and proactive recognition of patient’s need for MT could reduce the time required to have large numbers of plasma being immediately prepared. The same would be valid for platelets, which have short shelf life and once thawed cannot be used again. If an MTP is inappropriately activated, it is associated with
costs such as mobilization of the blood bank personnel, trauma team, and time to prepare blood products for delivery, as well as complications associated with large volume transfusion of blood products. Early identification of patients who require or do not require an MT would enable restricting undue blood product usage and may improve patient outcomes.

We used A10, a measure of clot strength, because it is routinely reported by ROTEM® devices in North America. The equipment can be calibrated to provide the same information earlier, i.e. amplitude at 5 minutes (A5), but this is not currently provided by the equipment we have available. ROTEM® may have some logistic advantages over TEG such as better software that allows for results to be viewed remotely and simultaneously improving communication and timely action. Overall, our experience with both viscoelastic tests, TEG and ROTEM®, has been that no one is superior.

Our findings were similar to that of published studies that reported association of low clot amplitude with MT (Davenport et al., 2011; Hagemo et al., 2015). Davenport et al., defined coagulopathy based on EXTEM A5≤35mm which identified coagulopathic patients and predicted the need for MT with a detection rate of 71.4% compared to 43% detection rate for prothrombin ratio of >1.2 (p<0.001) (Davenport et al., 2011). The findings of this study were further re-evaluated by prospective validation by Hagemo et al. The study confirmed the previous findings, i.e. A5 ROTEM® threshold ≤35mm is a valid trauma-induced coagulopathy marker and predicts the need of MT with a detection rate of 45.5% (Hagemo et al., 2015). Our study is in alignment with these findings where reduced clot amplitude in EXTEM is useful in predicting the need for MT.

The factors that were independently associated with early mortality were: GCS, ISS and FIBTEM A10. The sensitivity of the model to identify patients who would die in 24hr was 95% and the specificity of the model to identify patients would survive >24h was 89%. The negative predictive value was 99.9%. However AUC of the validation model was 0.9047 whereas the original model had AUC of 0.9713 which satisfactory but not as good as in derivation cohort, indicating a poor fit. Furthermore, the probability threshold of risk of death being 0.01453, the sensitivity 0.95, and specificity was 0.88 in the prediction data, where as the respective values for the validation set were 0.94 and 0.57 which were lower than the derivation cohort.
The variables associated with early mortality were low GCS and increasing age. Our results are in alignment with other published studies. Two studies have reported the association between abnormal amplitude in FIBTEM with 24h and 28-day mortality (Rourke et al., 2012; Tauber et al., 2011). A significant increase in 24h mortality was reported by Tauber et al., for FIBTEM A10 <7mm (21% vs 9%, p=0.006) and EXTEM MCF <45mm (25.4% vs 9.4%, p<0.001) (Tauber et al., 2011). They further showed independent association between EXTEM MCF and early mortality (OR 0.94, 95% CI:0.90-0.99) (Tauber et al., 2011). Rourke et al., reported an independent association between FIBTEM A5<9.5mm with 24h and 28-day mortality (p<0.00) (Rourke et al., 2012). A low clot amplitude is associated with increased transfusion requirement and mortality which indicates that a weak clot leads to extensive bleeding due to low fibrinogen reserve causing severe hypoperfusion and death. Immediate intervention to maintain hemostasis in these patients may improve outcome.

ROTEM® provides a rapid and reasonably accurate overall assessment of coagulation abnormalities from clot initiation to breakdown. It provides ongoing assessment of clot strength during the entire coagulation process. Low clot amplitude suggests defects in platelet function and/or fibrinogen content, which is associated with increased bleeding, transfusion requirements and mortality. Our study found that a low clot amplitude in EXTEM and FIBTEM are independent predictor of MT and 24h mortality. Other studies have reported similar findings, with A5<36mm in EXTEM and A5<9mm in FIBTEM diagnosing laboratory fibrinogen<1.5gL⁻¹ which independently predicted mortality at 24h and 28-day respectively (Rourke et al., 2012; Schochl et al., 2011c). Considering the short turnaround time of ROTEM® to deliver results, it can be used for initial assessment of coagulation disorders in unstable bleeding trauma patients. Davenport et al., defined coagulopathy based on ROTEM® value of EXTEM A5≤35 mm, which also predicted MT with a detection rate of 71% compared to 43% detection rate for prothrombin ratio >1.2 (p <0.001) (Davenport et al., 2011). They further emphasized that a reduced A5 is characteristic of acute traumatic coagulopathy and may alert the clinician of MT requirement. Their study also demonstrated that fibrinogen levels decline following severe trauma which is clinically relevant in MT patients. However our study did not have data on fibrinogen levels and hence we could not correlate clot amplitude values with fibrinogen.
Our prognostic model may assist clinicians with decision making on critically bleeding trauma patients in selecting emergency trauma treatment, advanced laboratory tests or rationalize patients who may require operative procedure or massive transfusions. The convenience of remote access to results will enhance collaboration and communication between the trauma team and transfusion medicine department who prepares and delivers blood products on time. It may also enable clinicians to inform the patient/family about the likelihood of outcome or complications and, to decide on pre-emptive therapeutic strategies. It may help in resource utilization such that the patients with higher risk of mortality can be rationalized for immediate alternate care. The model also has a role in saving hospital costs such as avoiding unnecessary thawing of valuable blood products if the likelihood for transfusion is low.

4.2.1.3 Limitations

The present study has the inherent limitations to being retrospective, observational and used registry data that was not primarily developed for the purpose of this study. The derivation registry did not capture potentially important measurements such as fibrinogen levels, lactate, co-morbidities, oral anticoagulant medications etc., which could have an effect on the outcomes. A significant amount of data on variables, including admission temperature and heart rate were missing in some cases and hence were not included in our models. Concerning blood transfusions, it cannot be assumed that every patient transfused in fact needed it and vice versa. This models are relevant to centers that use thromboelastometry only.

4.2.1.4 Key findings

- Coagulopathy assessed by rapidly available viscoelastic parameters such as clot amplitude at 10 minutes (A10) could potentially identify patients who may require massive transfusion or die early.
- Massive transfusion is reliably predicted using data on abnormal hemoglobin levels, low systolic blood pressure, high ISS low EXTEM A10 and penetrating trauma.
- Early death is reliably predicted by low GCS, high ISS and low FIBTEM A10.
- These readily available information, including the ROTEM® A10 result, can assist with the early clinical decision of activating (or withholding) massive transfusion and other interventions.
• Future studies could focus in developing risk scores/index that accurately identify patients as high or low risk of death or requiring MT.

4.2.1.5 Summary and conclusions

Timely diagnosis of massive hemorrhage, underlying coagulopathy and initiation or interruption of hemostatic resuscitation interventions, is an ongoing concern when resuscitating injured bleeding patients. Rapid identification of patients who may require MT and timely activation of hemostatic interventions may improve survival. Our findings suggest that clot amplitude at ten minutes (A10) along with other clinical variables is capable of predicting MT and early mortality. Further studies are warranted.
Chapter 6: Summary and future directions
6.1 Introduction

The overall aim of this dissertation was to investigate the role of rotational thromboelastometry (ROTEM®) in the early resuscitation of trauma patients and to develop transfusion guidelines for early trauma resuscitation. Specifically, the overarching goal of this thesis was to develop transfusion guidelines based on ROTEM® and focus on early resuscitation. In order to achieve this goal, we first had to understand the mechanisms involved in Trauma Induced Coagulopathy (TIC), explore how TIC is diagnosed and managed during early resuscitation, immediately after hospital arrival. Focusing on the use of viscoelastic assays such as ROTEM® and Thromboelastography (TEG), we systematically review the literature in Review/Introduction. We explored which ROTEM® measurements (variables) were used for the diagnosis and management of trauma patients. The systematic review was complemented by an international consensus conference on ROTEM® threshold values relevant for both the diagnosis and management, where the institutional experience of world clinicians and research experts came together to establish the optimal ROTEM® values. Finally the role of ROTEM® in early resuscitation was studied by analyzing the association of ROTEM ± clinical variables with transfusion of allogeneic blood products, massive transfusion (MT) and 24h mortality in a large population of trauma patients from 2 major Canadian Trauma Centres.

The specific research objectives of this thesis were to:

(i) Systematically evaluate the published evidence from the medical literature on the thresholds of ROTEM® parameters for the early diagnosis of TIC, transfusion guidance and mortality prediction.

(ii) Organize and conduct an international consensus conference with a panel of world experts in ROTEM® and TIC to identify the ROTEM® thresholds used by clinicians and investigators in the diagnosis and management of early TIC.

(iii) An analysis of a large cohort of injured patients from two large Canadian Trauma Centres using ROTEM® and readily available clinical variables to assess the risk factors for transfusion of blood products, MT and 24h mortality, followed by external validation of the models.
This chapter will present a summary of the findings for each of the above objectives. The strength and limitations of this dissertation will be presented and the future directions of the work will also be discussed.

6.2 Summary of major findings

6.2.1 Summary by thesis objectives

As per the thesis objectives, a summary of the major findings of this dissertation are presented below. The scientific studies described in this thesis contribute significantly to the body of knowledge regarding the use of ROTEM® in the diagnosis and management of early resuscitation of bleeding trauma patients.

Chapter 1 provided detailed information about TIC, its mechanisms at cellular level, diagnosis and management. This chapter explored the basic and cellular mechanisms involved in the clotting process with special emphasis on TIC. It further described the role of protein C pathway and systemic anticoagulation which causes TIC according to most recent scientific investigations.

Chapter 2 investigated the reported ROTEM® variables and threshold values used for the early diagnosis of coagulopathy, prediction and guidance of blood transfusion, and prediction of mortality in trauma patients. The systematic review identified 13 observational studies involving 2835 adult trauma patients meeting our eligibility criteria with moderate quality as assessed by the Newcastle Ottawa Scale (NOS). There were no randomized controlled studies. None of the identified studies used a comparator as Gold standard laboratory test and were without appropriate control group. The patient’s population consisted of both civilian and military trauma patients. The studies were categorized into three groups according to outcomes: diagnosis of coagulopathy (n=10), prediction of massive transfusion or guidance of blood products transfusion (n=6) and prediction of mortality (n=6). We found that the studies used different ROTEM® thresholds for the same ROTEM® parameters for the diagnosis of TIC and guidance of blood transfusions. The thresholds were used arbitrarily or based on single institution experience with small patient sample size. Majority of these studies reported that ROTEM® clot amplitude and lysis measured in extrinsically activated tests (EXTEM) and the fibrin-based extrinsically
activated tests (FIBTEM) were associated with the diagnosis of coagulopathy, increased risk of bleeding, MT and prediction of mortality. Presence of hyperfibrinolysis assessed in FIBTEM assay was associated with increased mortality. The reported thresholds of ROTEM variables varied between studies as they were either based on institutional experience or based on manufacturer’s information.

Overall the results of this review found that abnormal clot amplitude in EXTEM and FIBTEM measured at 5-10 minutes or maximum clot firmness (MCF) were used to diagnose coagulopathy, predict or guide blood transfusion and predict mortality. The presence of fibrinolysis was found to be associated with mortality. ROTEM® thus, appear to be of use in the early resuscitation of bleeding trauma patients. The results of the systematic review were published in the Scandinavian Journal of Trauma and Emergency medicine.

Chapter 3 described the second aim of the thesis of conducting an international consensus conference on which ROTEM® values are used clinically and for research to diagnose and guide blood transfusions during early trauma resuscitation. Considering the increasing interest in using ROTEM® in early trauma resuscitation, there are no broadly accepted guidelines for its use in trauma. This conference was aimed at exploring viscoelastic test technology and its use in the diagnosis and clinical decision of transfusing trauma patients. The consensus conference extended for two days and took place in Philadelphia, Pennsylvania. The consensus panel included world renowned trauma surgeons, hematologists, blood bank specialists, anesthesiologists, scientists and even patient representative (lay public). Nine questions regarding the use of viscoelastic testing in the early resuscitation of trauma patients were developed before the conference. Nine experts presented their institutional experience for each question in the format of lectures, followed by standardized two round Delphi poll administered to the panel of experts regarding the thresholds of viscoelastic test parameter for triggering specific treatments such as fibrinogen, platelets, plasma, prothrombin complex concentrates and massive transfusion. Finally, a ROTEM®-based algorithm for early transfusion of blood and blood products was developed. Despite the lack of strong evidence supporting a threshold value triggering plasma, prothrombin complex, fibrinogen or cryoprecipitate , the panel supported the use of abnormal clot amplitude for transfusion of cryoprecipitate or fibrinogen transfusion. The diagnosis of systemic hyperfibrinolysis recommended the use of antifibrinolytic medication. The
Panel also recommended withholding blood transfusion when hypercoagulable or normal results were found in viscoelastic testing during early resuscitation. The results of the consensus conference were published in Journal of Trauma.

Chapter 4 and 5 described the development and validation of six prediction models utilizing early available ROTEM and clinical variables for the prediction of blood products transfusion, massive transfusion and 24h mortality. This segment of the thesis work was done in a large population of injured patients admitted in two large Canadian Trauma Centres. ROTEM was found to predict the utilization of plasma, cryoprecipitate transfusion, massive transfusion and 24h mortality. The findings were subsequently externally validated. The risk prediction models reported low clot amplitude in ROTEM as the predictors of plasma transfusion, cryoprecipitate transfusion, MT and 24h mortality while adjusting for other clinical variables. ROTEM was not the predictor of RBC and platelet transfusion.

6.3 Strengths and limitations

6.3.1 Strengths

This dissertation has several strengths, starting with a systematic review of the literature, followed by an international consensus conference and then an in-depth analysis that developed and validated prediction models for six different variables in a large cohort of trauma patients prospectively collected comprehensive data. To our knowledge this is the first study in Canadian trauma center that has evaluated the use of early (immediately after hospital arrival) available ROTEM variables to predict transfusion requirements and early mortality in trauma patients. The systematic review attempted to collate the studies that reported thresholds values of ROTEM to detect coagulopathy, guide transfusion and predict mortality. The international consensus conference brought together and in person, world experts in the areas of trauma, resuscitation, anaesthesia, blood bank, surgery, research and hematology to discuss the thresholds and came up with transfusion algorithms for early resuscitation of trauma patients, which was first of its kind to discuss the use of viscoelastic tests in the early resuscitation of trauma patients. Our own institutional experience gave us an overview of use of ROTEM in resuscitation of trauma patients.
6.3.2 Limitations

While this study has several strengths, it also has limitations pertinent to any broad scope of work. First, we focussed our studies on adult trauma patients presenting with traumatic injuries to any parts of the body and only patients transferred to level I trauma center. Our results may not be generalizable to smaller hospitals that receive less injured trauma patients, or who may transfer them later to a level I trauma center. Second there is no universally accepted definition for coagulopathy based on visco-elastic assays such as ROTEM. Other limitations include the retrospective nature of the studies and use of registry data. Some important variables such as fibrinogen were not captured in the development of the data base during our analysis which has an important association with the outcomes we investigated. Furthermore, our data bases did not capture pre- morbid conditions and medications such as use of anticoagulants the patient might have been taking.

Missing data was another concern in our studies. Even though missing data was below 10 percent, we used the entire data set in our analysis. We were not able to perform cost analysis as ROTEM was used for research purpose only in the developmental dataset while it was used inconsistently for patient care (decision making on whether to transfuse or not) in the validation data. Finally due to lack of robust randomized clinical studies on ROTEM use in trauma, we were not able to develop an algorithm on ROTEM-based transfusion guidelines as the evidence supporting the use of ROTEM and its threshold were lacking in the field of trauma.

6.4 Implications and future directions

6.4.1 Implications

This dissertation will add value to existing literature regarding the use of visco-elastic assays such as ROTEM in bleeding trauma patients. The intent of this section is to focus on the clinical and research opportunities created by the findings of the thesis work.

6.4.1.1 Trauma induced coagulopathy occurs immediately following injury, thus before hospital arrival
In our study we found from both standard coagulation laboratorial tests and ROTEM assays indicated the presence of coagulopathy in a significant number of injured patients, by the time of arrival to the hospital. This fact suggests that the acquired trauma-induced coagulopathy occurred prior to hospital arrival, thus at the time of injury or soon after considering the short pre-hospital time in many of the coagulopathic patients. Patients that were transfused in the first 24h of hospital admission, massively transfused or died within 24h had abnormal findings in both SCTs and ROTEM.

6.4.1.2 ROTEM clot amplitude at 10 minutes (CA10) can detect various clinically significant hemostatic defects or early trauma induced coagulopathy (TIC)

Our studies found that ROTEM may be useful in detecting early trauma-induced coagulopathy in trauma patients. Patients that received 24h transfusion of blood products, massive transfusion and/or died within 24h of hospital admission, had abnormal ROTEM findings. A low clot amplitude at 10 minutes (CA10), is an indicator of abnormal maximum clot firmness (MCF), and is associated with low levels of fibrinogen or platelets, thus coagulopathy, and the need for MT and mortality. Similar to our findings, Rugeri et al in a 300 trauma patient report, observed a significant correlation between clot amplitude at 15 minutes (EXTEM CA15) and other standard coagulation tests, need for transfusion and mortality (Rugeri et al., 2007) These findings suggest that early EXTEM clot amplitude indicates the presence of coagulopathy that affects clinical outcome. The possibility of diagnosing coagulopathy early, within 10 minutes of arrival to hospital, instead of waiting for later measurements such as MCF or other coagulation tests, make ROTEM an attractive option for the clinician having to make quick decisions concerning the management of injured bleeding patients. Rugeri also described a correlation between EXTEM CA15<32mm and prothrombin time (PT)>1.5 (r=0.85, p<0.0001) while Davenport et al reported that a CA15≤35mm diagnosed coagulopathy with 77% sensitivity and false positive rate of 13%.

6.4.1.3 Significantly bleeding patients who are at risk of requiring blood and blood product transfusion, massive transfusion and early deaths can be identified by ROTEM.
Understanding the risk factors that are associated with the risk for requiring transfusion and massive transfusion will allow the clinicians the option of goal-directed management (resuscitation) for bleeding trauma team. Arguably, goal-directed ROTEM-based resuscitation will reduce inappropriate blood and blood product transfusion, improving patients’ outcome and optimizing the use of resources such as blood and hemostatic drugs – possibly even costs. Clot amplitude can promptly be measured within 10 minutes of arriving in the hospital, and thus likely to be used in clinical practice where standard coagulation tests are only available much later. Clot amplitude is associated with significant bleeding and transfusion requirement as well as mortality, all clinically relevant.

6.5 Future directions

This section explains the potential future directions. New questions arose from this dissertation and this section describes the possible means to address these questions and potential for future research are considered.

6.5.1 Improve capability of prompt and early identification of injured patients at risk of requiring blood and blood product transfusions, massive transfusion and 24h mortality

We found that the ROTEM low clot amplitude along with other important promptly available clinical variables such as blood pressure, can help the clinician to identify injured patients at risk of requiring transfusions and early death. The systematic review and the consensus conference have shown that low clot amplitude is associated with increased need for blood products and early mortality. This may influence clinical decision making, including the decision of whether to transfuse, administer drugs or make use of other hemostatic interventions. The current standard of care for rapidly bleeding unstable injured patients is blind blood transfusion according to formulas along with the unrestricted use of hemostatic drugs such as tranexamic acid. Some bleeding injured patients are resuscitated in accordance with the results from standard coagulation tests, which are often only available after prolonged delays, which in rapidly bleeding patients may lead to inappropriately late interventions. Both resuscitation strategies have significant flaws and may account for the poor outcome of many bleeding patients – hemorrhage remains the most common cause of preventable deaths in trauma. At least 1/3 of the blood transfusions in early trauma resuscitation are considered inappropriate or sub optimal
transfusions. We propose that a follow up to our studies is to develop algorithms using visco-elastic tests, promptly available to guide resuscitation.

6.5.2 Develop prospective randomized studies to verify whether ROTEM-based resuscitation can improve injured patients’ outcomes.

We propose that prospective randomized clinical trials should be planned to verify whether ROTEM-based resuscitation can improve the outcome of bleeding injured patients and reduce inappropriate blood usage during early resuscitation. Arguably ROTEM-based resuscitation could increase blood usage overall – considering that some patients not transfused may be found to need transfusions if ROTEM is used. We hypothesize that ROTEM could be used to reduce inappropriate transfusion, which could result in an increase in appropriate use of blood products (individualized or goal-directed resuscitation).

6.5.3 Research potential for exploring the importance of clot amplitude

This study may lead to further investigation in the physiologic meaning and clinical utility of clot amplitude measurement. The physiologic implication of this measurement and the reasons for it occurring in some but not all patients remain unanswered. Further research is needed to study pathophysiology of TIC and implication of ROTEM variables with TIC. We also propose that ROTEM, along with reduced turn-around time SCTs, should be considered by other trauma centers.

6.5.4 Development of risk score/risk index and decision tree analysis

The next step for this work is to apply the models in actual clinical practice in similar level I trauma centers in order to translate our findings in new trauma population. Further research may take the form of a risk score or risk index of early transfusion or early mortality which may be used in trauma population or may follow a decision tree. The main goal will be to develop easy to use guidelines or algorithm that could assist clinical decisions during early resuscitation. Promptly available results trauma physicians could identify patients at risk of requiring transfusion or at risk of death and thus guide resuscitation. Once this task is achieved it should be followed by evaluation of its effectiveness, clinical relevance and usefulness to the trauma team. We need to assess if our models and risk scores in fact are of help to the clinicians in rapidly
identifying patients with TIC and guide their management. Ultimately this work could be used as user friendly application to be used in the trauma bay.

6.6 Summary:

In summary, coagulopathy following traumatic injury is associated with higher transfusion requirements, longer hospital stay, higher in hospital complications and increased mortality. Identification and rapid correction of coagulopathy is important in reducing complications, blood and blood product transfusions and improving survival. TIC is an imbalance of many hemostatic mechanisms and occurs even before hospital arrival, possibly by activation of the protein-C pathway resulting in systemic anticoagulation and hyperfibrinolysis. TIC occurs independently of dilution, acidosis and hypothermia as previously believed. Current coagulation tests such as PT/INR, aPTT, fibrinogen levels, platelet count are used to diagnose coagulopathy and guide transfusion, but with limitations. Viscoelastic tests such as ROTEM are emerging important technologies in identifying patients with TIC by providing real time assessment of ongoing hemostatic abnormalities. Its use in guiding transfusion, MT and predicting mortality has been studied in trauma setting. Injured patients often present with TIC and have abnormal ROTEM and SCT assays. We generated statistical models using ROTEM early measurements, which along with promptly available clinical variables may predict the need for blood transfusion, MT and mortality.

Our studies have shown that low clot amplitude available early during hospital admission may be useful in guiding early resuscitation of trauma patients. Further research should focus on prospective studies to validate our findings, and cost effectiveness analysis of using ROTEM to guide early resuscitation. The results of these studies and future works may help in optimizing transfusion decisions, saving hospital resources and improving patient outcomes in bleeding trauma patients.
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