The Clinical Utility of Viscoelastic Tests of Coagulation (TEG® & ROTEM®) in Liver Disease and Liver Surgery

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I, Susan Veronica Mallett confirm that the work presented in this thesis is my own. Help and contribution of others to this work is specified in the acknowledgement section. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

This thesis is dedicated to my husband Tim, and also to my family for their unending support throughout my career. I also wish to express gratitude to my boss at UPMC, Yoogoo Kang, who introduced me many years ago to the concept of using viscoelastic tests to monitor coagulation in patients undergoing liver transplantation.

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List of Abbreviations used in the text

ADP	Adenosine diphosphate
ALF	Acute liver failure
ALI	Acute liver injury
aPTT	Activated partial thromboplastin time
AT	Antithrombin
CAT	Calibrated automated thrombogram
COX	Cyclo-oxygenase
CCT	Conventional coagulation tests
CLD	Chronic liver disease
DVT	Deep vein thrombosis
EACA	Epsilon aminocaproic acid
EPCR	Endothelial protein C receptor
FF	Functional fibrinogen
FFP	Fresh frozen plasma
FDPs	Fibrin degradation products
GAG	Glycosaminoglycan
Gp-Ib	Glycoprotein
HLE	Heparin like effect
INR	International normalized ratio
LMWH	Low molecular weight heparin
LT	Liver transplantation
MELD	Model of end stage liver disease
NAFLD	Non alcoholic fatty liver disease
PAI-1	Plasminogen activator inhibitor
PAR	Protease activated protein
PBC	Primary biliary cirrhosis
PC	Protein C
PE	Pulmonary embolus
PS	Protein S
PSC	Primary sclerosing cholangitis
PCC	Prothrombin complex concentrate
PT	Prothrombin time

POC	Point of care
POD	Post operative day
rVIIa	Recombinant factor VIIa
ROTEM®	Thromboelastometry
TA	Tranexamic acid
TAFI	Thrombin activatable fibrinolysis inhibitor
TAT	Thrombin antithrombin
TEG®	Thromboelastography
TM	Thrombomodulin
TF	Tissue factor
tPA	Tissue plasminogen activator
UKELD	United Kingdom model of end stage liver disease
VET	Viscoelastic tests
VWF	Von Willebrand factor

TEG/ROTEM parameters:

TEG

r time: reaction time (2mm clot)

K time: coagulation time (20mm clot)

MA: maximum amplitude

CLI: clot lysis index

N: native

NH: native heparinase

K: kaolin

TTG: Total thrombin generation

MRTG: Maximum rate of thrombin generation

TMRTG: Time to maximum rate of thrombin generation

ROTEM

CT: clotting time (2mm clot)

CFT: clot formation time (20mm clot)

MCF: maximum clot firmness

ML: maximal lysis

General Introduction

The liver plays a central role in haemostasis, producing the majority of both pro and anticoagulant proteins. Liver disease has been seen to be the archetypal acquired coagulopathy as it has been assumed that the associated abnormalities in conventional coagulation tests such as prothrombin time (PT) and international normalized ratio (INR) are indicative of hypocoagulability and a bleeding diathesis. However, directly observed study of liver bleeding times (1), and a systematic review of the association of bleeding with abnormal coagulation tests (2) demonstrate that these tests are in fact very poor predictors of bleeding risk. Further evidence that these tests are inadequate for assessing potential bleeding risk has accrued as an increasing number of patients with end stage liver disease undergo liver transplantation without the need for transfusion of blood or blood products (3).

The complexity of the coagulation changes in chronic liver disease was first highlighted in 2005, when Tripodi *et al*. demonstrated using a novel assay of coagulation, the thrombin generation test, that people with cirrhotic liver disease generated similar amounts of thrombin as a normal control population (4). Indeed, as liver disease advances, some of these patients demonstrate enhanced thrombin generation (5), and it is now appreciated that these patients should not be considered “auto-anticoagulated” solely because they have an elevated PT/INR (6). Over the last decade a new paradigm of coagulation in liver disease has emerged, and haemostasis is now described as being “re-balanced” (7). This re-balancing is due to the concomitant decrease in both pro and anticoagulants, and also increases in factor VIII and Von Willebrands factor. The conventional coagulation tests are very responsive to falls in procoagulant factors, but fail to capture the parallel reduction in

anticoagulants, and may therefore over-estimate the bleeding risk in patients with liver disease. Although haemostasis is described as “re-balanced”, it must be appreciated that this is a relatively fragile balance, and as the large haemostatic reserve seen in healthy individuals is significantly reduced, the balance can readily be tipped towards either bleeding or thrombosis, if the system is stressed in any way, as for example by infection.

In liver disease, and also following major liver resection, mild to moderate prolongations of PT/INR are common, and although the evidence that they are predictive of bleeding is poor to non-existent, these tests are routinely used in clinical practice as the basis for decision making, including whether or not to administer fresh frozen plasma prior to invasive procedures, or when to initiate pharmacological thromboprophylaxis. As a consequence, a significant amount of fresh frozen plasma is used in patients with cirrhosis for prophylaxis without evidence of utility or efficacy (8). In addition, there is some reticence to institute thromboprophylaxis due to the perception that bleeding risk is increased as a direct consequence of an elevated PT/INR. To complicate matters even further, there is wide inter laboratory variation in the INR in patients with liver disease(9), and this also has an impact on the calculated MELD score. In terms of defining bleeding and thrombotic risk, it is clear that standard coagulation tests have many limitations when used to direct clinical practice in the setting of liver disease.

Given the complexity of the changes that occur in the haemostatic system in these patients, the question arises whether global viscoelastic tests of coagulation (thromboelastography [TEG] and thromboelastometry [ROTEM]) which are performed in whole blood, and which incorporate all the cellular elements involved in coagulation, may provide more comprehensive and clinically useful information about the coagulation status, and thus of

bleeding and thrombotic risk, than the conventional tests of coagulation. The purpose of this thesis is to explore this hypothesis in more detail.

Outline of this thesis

This thesis focuses on what is currently known about the haemostatic changes that occur in liver disease, and also those that follow major liver resection. Although there has been considerable work on the changes in coagulation in both acute and chronic liver disease in recent years, there has been far less attention to hepatic resection. This is also an important area for study as an elevation of PT/INR is common in the first few days after resection, yet these patients are known to have a high incidence of thromboembolic complications in the early post operative period, and this risk appears to increase with the extent of liver parenchyma resected(10).

It is the central hypothesis of this thesis that global viscoelastic tests (TEG/ROTEM) by facilitating assessment of all the cellular components of the coagulation process in an integrated manner, and their summative effect on ultimate clot formation, strength and stability, provide more clinically relevant information than do conventional coagulation tests which only assess single end points of coagulation in plasma rather than in whole blood.

Chapter one focuses on models of coagulation (traditional cascade model and the newer cell based model of haemostasis) and the limitations of traditional coagulation tests in liver disease.

Chapter two discusses the principles of viscoelastic tests, their limitations and also their correlation with conventional coagulation tests. It also highlights that these tests can detect

"hypercoagulability" which may relate to an increased thrombotic risk, and also fibrinolysis, neither of which is readily detected using conventional tests of coagulation.

Chapter three is a review and critical appraisal of the available literature on the utility of viscoelastic tests of coagulation in patients with both acute and chronic liver disease. The majority of patients with liver disease have "normal" coagulation as assessed by these tests, and this may be seen as supporting the hypothesis of "re-balanced" haemostasis. Aspects such as hypercoagulability and the relation to thrombotic risk, and endogenous heparinoids as markers of infection and endothelial injury are highlighted.

Chapter four is a review and critical appraisal of the available literature on the utility of viscoelastic tests of coagulation in patients undergoing liver transplantation. The literature is reviewed to determine their efficacy in predicting bleeding risk, and also to guide haemostatic therapy in the presence of active bleeding.

Chapter five is a retrospective study to assess the prevalence of fibrinolysis in patients undergoing liver transplantation, and how this relates to subsequent need for blood transfusion. Historically aprotinin (Trasylol) was given to high risk liver transplant patients to minimise the bleeding associated with fibrinolysis. Aprotinin was withdrawn from clinical practice in 2008, and since that time treatment with antifibrinolytic therapy has generally moved towards a treatment only regime, rather than prophylaxis. Comparing retrospective propensity matched cohorts (prophylactic versus treatment only with anti-fibribrinolytics agents) the impact of fibrinolysis on transfusion requirements was investigated, and also whether the timing of the appearance of fibrinolysis at different stages of the operation has different prognostic significance.

Chapter six describes the prevalence of hypercoagulability during liver transplantation, as determined by thromboelastography performed at the start of the procedure, and at various time points during the operation, in a series of 100 consecutive patients undergoing liver transplantation. This information gives an indication of the association of hypercoagulability with underlying disease aetiology, and also whether there are changes in this baseline profile, or de-novo appearance of hypercoagulability during the intraoperative period.

Chapter seven describes the sequential changes in coagulation parameters (conventional coagulation tests, pro and anticoagulant factor levels, thrombin generation, and thromboelastometry (ROTEM) in a prospective series of patients undergoing major hepatic resection. Given that the INR is frequently prolonged in the early post operative period, the question is whether this represents a true bleeding risk, as is currently assumed, or if other tests of coagulation suggest that this assumption should be re-evaluated.

Chapter eight describes the efficacy *in vitro* of two different dose regimes of fresh frozen plasma (FFP) to correct coagulopathy, as determined by a prolonged INR, after major hepatic resection, and also the effect of FFP on viscoelastic tests in the same group of patients. These patients will frequently receive prophylactic FFP prior to procedures, but there is little data on the effect of typical dose regimes on either conventional or viscoelastic coagulation tests. This information could be of value in determining if FFP is useful, or indeed even necessary, in these patients prior to undergoing an invasive procedure.

Chapter nine summarises the findings from these chapters, and discusses whether viscoelastic coagulation tests do indeed give more valuable oversight of the haemostatic profile in patients with liver disease and following major hepatic resection. Directions for future research based on these findings are also considered.

Chapter 1

Models of coagulation and limitations of standard coagulation tests in liver disease

1.1 Introduction

Coagulation and haemostasis is a dynamic process with interplay between primary haemostasis and platelet plug formation, and secondary haemostasis with thrombin generation resulting in the formation of a stable haemostatic clot, with several control mechanisms responsible for the modulation and termination of the activated coagulation cascade. Finally the mechanism of fibrinolysis is responsible for organising and removing the formed clot to restore vessel patency.

Coagulation is a complex, carefully orchestrated and highly sophisticated process, involving numerous checks and balances. Early models of coagulation concentrated on the role of the coagulation proteins, as these are fundamental to describing and understanding the hereditary coagulopathies, such as haemophilia, and for developing coagulation tests that could identify these deficiencies and also be used for monitoring oral anticoagulant therapy with warfarin.

The new model, or cell based model, emphasises the critical importance of cells, mainly platelets, but also white blood cells and vascular endothelial cells, and their vital role in the initiation and subsequent evolution of clot (11). It is apparent from this model that conventional coagulation tests run on citrated plasma samples, with single end points, are wholly inadequate for the purposes of determining, or fully understanding, the haemostatic process *in vivo.*

1.2 Primary Haemostasis

Platelets play an important role in localising clotting reactions because they adhere and aggregate at the sites of injury where tissue factor (TF) is exposed, and they provide the primary surface for generation of the burst of thrombin required to produce effective haemostasis during the propogation phase of coagulation. Platelet localisation and activation are mediated by Von Willebrand factor (VWF), thrombin, platelet receptors, and vessel wall components such as collagen (12). Circulating platelets become in close contact to the injured vessel wall by attachment of the platelet surface glycoprotein Ib (GP-Ib) to VWF in the wound. VWF is a large molecule, synthesised by endothelial cells, that circulates in complex with coagulation factor VIII (FVIII). After binding to exposed collagen in a wound it becomes structurally altered and able to bind GP-Ib on the platelet surface.

During high shear, platelets “roll” along the wound surface, and lose speed and eventually bind irreversibly to the wound surface. This binding is facilitated by the attachment of other platelet surface glycoproteins, the GP-Ia/IIa complex and GP-VI to collagen, resulting in platelet activation. Platelets are also able to bind to fibrinogen and fibrin via the GP-IIb/IIIa receptor. Once activated, platelets release a number of substances from their granules including adenosine diphosphate (ADP), serotonin and thromboxane A2, which stimulate and recruit additional platelets to the area. The thrombin that is generated as a result of activation of the coagulation proteins on the surface of platelets, is also an extremely potent platelet activator. The commonly used anti-platelet drugs interfere with primary haemostasis at various points. Aspirin inhibits cyclo-oxygenase (COX), the enzyme responsible for the formation of thromboxane from arachidonic acid in platelet membrane phospholipids. The thenopyridine derivatives (clopidogrel and prasugrel) inhibit ADP

induced platelet activation by binding irreversibly to the P2Y12 receptor. The most potent anti-platelet drugs, such as abciximab and tirofiban, block GP-IIb/IIIa receptors.

1.3 Secondary Haemostasis

1.3.1 The coagulation cascade

The model of coagulation that was conventionally taught was the "waterfall" or cascade model of a series of proteolytic reactions that act as a biological amplifier, originally proposed in the 1960's. This model conceptualised the process of coagulation as being primarily dependant on adequate levels of the coagulation proteins. The interaction of the coagulation proteins are described in the classic "Y" shaped scheme, with distinct "intrinsic" and "extrinsic " pathways, activated by factor XII and Factor VIIa/ tissue factor (TF) respectively. The pathways converge on a final common pathway at the Factor Xa/Factor Va (prothrombinase) complex, resulting in conversion of prothrombin to thrombin which cleaves soluble fibrinogen to form fibrin strands (Figure 1.1)

The coagulation complexes also generally require the presence of calcium and phospholipids for their activity. This model was eventually, and mistakenly, taken to represent a literal model of haemostasis *in vivo*, and abnormalities of coagulation tests based on the intrinsic system (aPTT) and extrinsic system (PT) taken to be accurate indicators of bleeding risk. However, when examined critically, there is limited evidence to support the supposition that they are useful for predicting bleeding risk in the setting of invasive interventions (2).

The limitations of this model of the haemostatic process become evident when certain clinical observations are taken into account. Patients deficient in the initial components of the intrinsic pathway (factor XIII or pre-kallikrein) have a prolonged aPTT, but no bleeding tendency. However, some components of the intrinsic pathway clearly have an essential

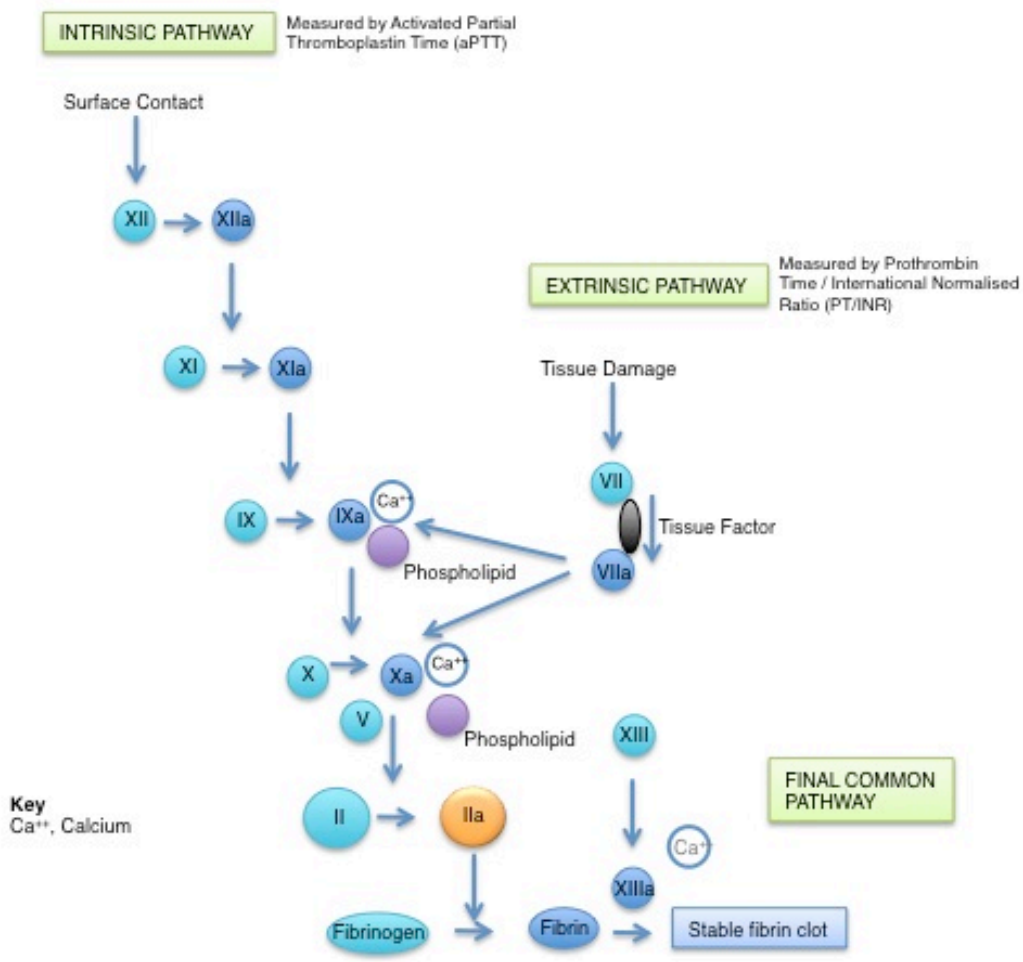


Figure 1.1 Conventional coagulation cascade

role in haemostasis, as patients deficient in factor VIII (Haemophila) or IX (Christmas Disease) have a serious bleeding tendency even though the extrinsic pathway is intact. Similarly, patients with a deficiency of factor VII also have a serious bleeding tendency, even

though the intrinsic pathway is intact. The two pathways therefore cannot be operating as independent, redundant pathways *in vivo* as they appear to do in the cascade model. It was also recognised from the earliest studies on coagulation that cells are important participants in this process, and that normal haemostasis requires cell associated tissue factor (TF) and platelets, in addition to the proteins of the coagulation cascade. Although the coagulation cascade model is a useful way of illustrating the interactions of the coagulation proteins, it is far too simplistic a way to portray the coagulation process. It does not explain the complex part coagulation has in the overall response to injury, nor the fact that it is primarily cells that control the duration, intensity and localization of the haemostatic process.

1.3.2 The cell based model of coagulation

This new model of coagulation was proposed in 2001 by Hoffman and Munroe, and has become the accepted description of how haemostasis takes place *in vivo* (11). It describes how cells, rather than the coagulation proteins, direct and control the coagulation process (Figure 1.2). The cell based model of haemostasis describes the process by which the protease cascade waterfall events of the coagulation pathways occur on, and are controlled by, ligands expressed on the surfaces of various cell types.

Haemostasis requires the formation of an impermeable platelet and fibrin plug at the site of injury, and requires that the powerful procoagulant substances activated in the process remain localised to the site of injury which is achieved by localising the procoagulant reactions to the surface of cells. *In vivo* the coagulation reactions occur on specific cell surfaces, rather than on phospholipid surfaces as they do in the PT and aPTT assays. Different cells have different roles, platelets play a major role in supporting procoagulant

reactions, whereas the vascular endothelial cells play a key role in maintaining the anticoagulant properties of the vasculature. The endothelium paves the wall of the vasculature and controls its surrounding tissues blood flow and also creates a reactively permeable barrier. The plasma facing surface of the endothelium is lined by the glycocalx

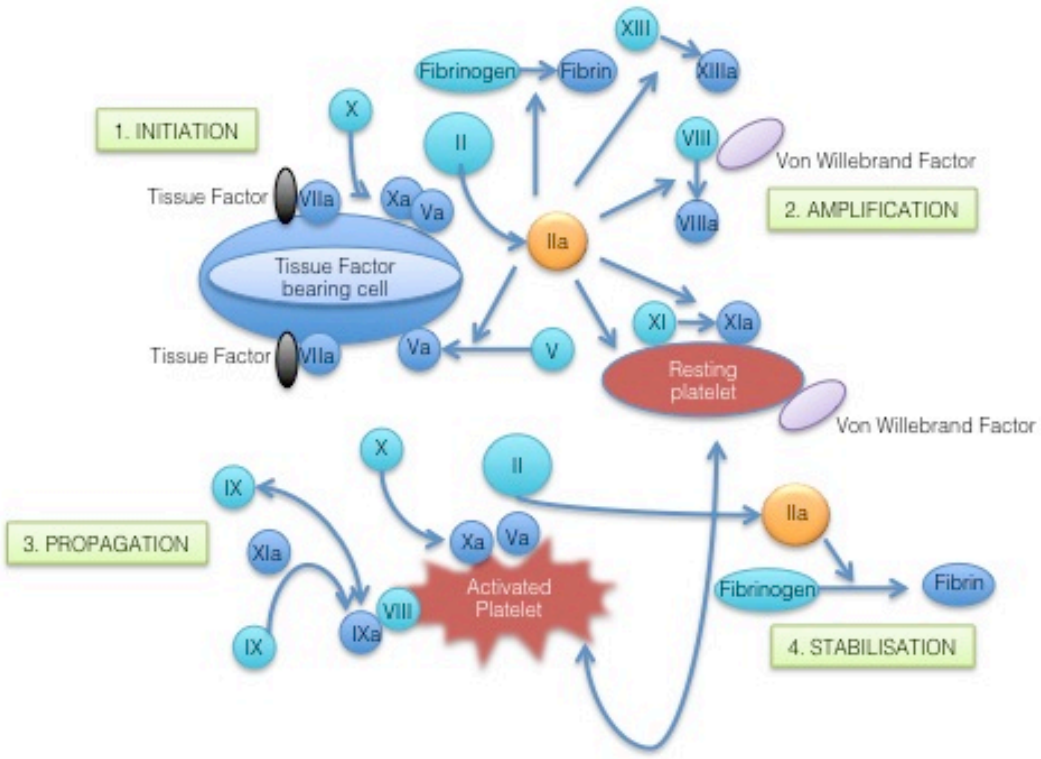


Figure 1.2 Cell based model of coagulation

which is made up of proteoglycans that possess surface charge that repel serine proteases. Embedded and attached to its surface are heparin glycosaminoglycans (GAGs). Heparan binds circulating antithrombin, activates it and further creates an anti-inflammatory/anti-thrombotic surface.

The cell base model proposes that haemostasis occurs in three distinct, but overlapping steps: initiation, amplification and propogation. The process requires two cell types, platelets and tissue factor bearing cells. These cells are kept separated until injury makes activation of coagulation a desirable occurrence.

1.3.3. Initiation, amplification and propogation of coagulation.

Initiation: the generation or exposure of tissue factor (TF) at the wound site, and its interaction with FVII is the primary physiological event in initiating coagulation (13). The initiation step is localised to cells that express TF which are normally kept outside the vasculature.TF is a trans-membrane protein that acts as a receptor and co-factor for factor VII. Once bound to TF factor VII is activated, and this complex of TF/VIIa catalyses activation of factor X and IX. VIIa also interacts with its co-factor, activated Va to generate small amounts of thrombin on the TF bearing cells. In effect, the extrinsic system acts *in vivo* to initiate coagulation (Figure 1.3).

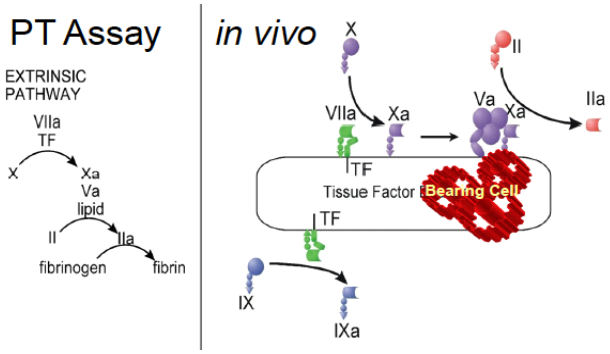


Figure 1.3 Initiation of coagulation

Low levels of IXa, Xa and thrombin are produced on TF bearing cells all the time, and this is known as “coagulation idlying”. These activated factors are normally separated from other key components of the coagulation system by an intact vessel wall, as platelets and factor VIII, bound to von Willebrand factor (vWF) are such large molecules that they only enter the extravascular compartment when an injury disrupts the vascular endothelial wall (glycocalyx). When a vessel wall is disrupted, platelets bind to the exposed collagen and other extracellular matrix components at the site of injury and become partially activated. This process forms the platelet plug that provides primary haemostasis. At this point, small amounts of thrombin generated on TF bearing cells then interact with platelets and the VIII/vWF complex to initiate the haemostatic process that ultimately enmeshes the primary platelet plug in a stable fibrin clot (secondary haemostasis).

Amplification: During the amplification process, the small amounts of thrombin formed on the TF bearing cells promote maximal platelet activation, and also activate additional coagulation co-factors on the surface of the platelet, “priming” the clotting system for the subsequent thrombin burst on the platelet surface by activating V,VIII and XI. The activation of XI by thrombin on platelet surfaces explains why XII is not necessary for normal haemostasis. Factor IXa, activated by both TF bearing cells and by the platelet surface factor XIa binds to factor VIIIa on the platelet surface to assemble IXa/VIIIa (“tenase”) complexes. By the end of the amplification phase, the stage is set for large scale thrombin generation in the propagation stage.

Propogation: The “burst” of thrombin generation needed for effective haemostasis is produced on platelet surfaces during the propogation phase of coagulation [Figure 1.4]. Factor IXa activated during initiation, binds to VIIIa on the platelet surface, and additional

IXa is supplied by the platelet bound XIa. Because Xa cannot effectively move from the TF bearing cell to the platelet, it is provided directly on the platelet surface by the IXa/VIIIa (Tenase) complex, and then Xa rapidly associates with platelet surface Va and produces a burst of thrombin generation of sufficient magnitude to stabilize the initial platelet plug in a durable meshwork of fibrin strands. The intrinsic pathway thus acts on the platelet surface to generate large amounts of thrombin (propagation).

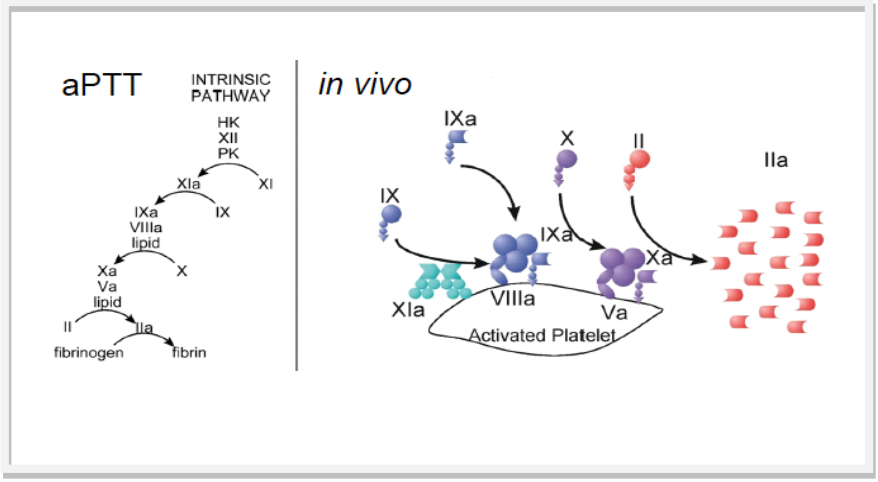


Figure 1.4 Propagation of coagulation

Cell based versus cascade models of haemostasis: The cell based model suggests that there are indeed “intrinsic” and “extrinsic” systems, but that they are closely and mutually inter-related, and they are not independent as suggested by the cascade model. The extrinsic, or TF pathway consists of the VIIa/TF complex and the Xa/Va complex and operates on the TF bearing cell to initiate and amplify coagulation. In contrast, the components of the intrinsic system operate on the activated platelet surface to produce the burst of thrombin that

causes the formation and stabilization of the fibrin clot. Thus two different cell types, TF bearing cells and platelets, are required and necessary for effective haemostasis.

The central role of thrombin: Thrombin plays two very distinctive roles depending on where and when it is generated. The small amount of thrombin produced on TF bearing cells is critical in amplifying the procoagulant response and that initiation of coagulation is successful. Once formed, thrombin can move from the TF bearing cell to nearby platelets, where it binds to its high affinity receptor, GPIb (14). This protein serves as a scaffolding that facilitates the interaction of thrombin with substrates on the platelet surface, setting the stage for subsequent large scale thrombin generation. Platelet surface thrombin cleaves protease activated protein 1 (PAR-1), which plays a key role in platelet activation, and also activates factor VIII and releases it from vWF and activates FXI.

This large amount of thrombin generated on the platelet surface is responsible for producing a stable haemostatic clot. Thrombin on the platelet surface continues to amplify the procoagulant response, but as increased amounts are produced, some leaves the platelet and acts to promote the stabilization of the platelet plug in the fibrin mesh. Platelet produced thrombin has multiple actions in addition to converting fibrinogen to fibrin. It also stabilizes the clot by activating factor XIII, activating thrombin activatable fibrinolysis inhibitor (TAFI), cleaves the platelet PAR 4 receptor, and is incorporated into the structure of the clot.

Studies *in vitro* show that the structure and stability of the fibrin clot are closely related to the amount of thrombin added to a fibrinogen solution to initiate clotting. However, *in vivo*, thrombin generation is an ongoing process, and the amount generated builds up as activated factors and co-factors accumulate on the platelet surface. The amount of

thrombin in the system is constantly changing during the process of clot formation and it is the rate of thrombin generation which is a major determinant of ultimate clot structure (15). Whilst clot formation begins after only a small amount of thrombin has been produced, the structure of the clot evolves and remodels in response to the levels of thrombin achieved after fibrin polymerization has begun. The ultimate clot structure is a complex function of the pattern of thrombin generation and not just the total amount produced.(16) It is has been proposed that different patterns of abnormal thrombin generation produce clots with altered fibrin structure, and that these changes are associated with an increased risk of bleeding or thrombosis (17). Reduced levels of thrombin generation have been associated with bleeding in surgical patients (18). Conversely, high levels of thrombin generation are associated with an increased tendency to thrombosis (19).

1.3.4 Controlling active coagulation: the critical role of plasma protease inhibitors

Failure to properly limit or localise thrombin generation can lead to thrombosis. The vitally important role of protease inhibitors in controlling the haemostatic process, is largely overlooked in the early cascade models of coagulation. Several mechanisms act to localise coagulation reactions to the site of an injury. Firstly, rapid localization and adhesion of platelets to the site of injury, brings the platelet surface into close proximity with the TF bearing cells, which are normally extravascular, thus removing the barrier to the movement of pro-coagulant proteases from the initiating cell surface to the platelet surface.

Secondly, plasma protease inhibitors are much less effective in inactivating coagulation proteases on the surface of cells than when they are in solution. Consequently, activated factors that diffuse away from the appropriate cellular location are susceptible to rapid inhibition. Thirdly, an array of anti-thrombotic mechanisms tends to prevent propagation of

coagulation on healthy intact vascular endothelium. These mechanisms include the endothelial thrombomodulin (TM)/ protein C/ protein S system that inactivates Va and VIIIa, and endothelial surface heparinoids that bind and enhance the activity of plasma antithrombin (AT)(20). Protein C and S, are synthesised by hepatic parenchymal cells. Protein C is localised to endothelial cell surfaces by a specific endothelial protein C receptor (EPCR). Thrombomodulin (TM) is a cell surface receptor for thrombin that is also bound to healthy endothelial cells. When thrombin escapes from the site of injury onto nearby intact endothelial cells, it is bound by TM. This thrombin /TM complex can no longer carry out pro-coagulant reactions, and the complex activates protein C (aPC) which then binds to protein S. The complex cleaves and inactivates any factor V that has been activated on the endothelial surface. As Va is essential for activation of prothrombin by Xa, inactivation of Va disables thrombin production on the endothelial surface and prevents propagation of the procoagulant reactions throughout the vascular tree. While they are often called "anticoagulant" proteins, they primarily act to prevent normal endothelial cells from acting as a site for thrombin generation, ie they act in an antithrombotic capacity. The level of expression of these different antithrombotic mechanisms varies between vascular beds, and can be modulated by inflammatory stimuli and vascular pathology (21). Antithrombin (AT) is one of the major natural inhibitors of thrombin, and as it only binds with thrombin, and not prothrombin, it is only active when coagulation is activated. In addition, AT binds to the activated coagulation factors, Xa, XIa and the VIIa/TF complex to form high molecular weight complexes that are stable and inactive. As mentioned previously, the reaction between thrombin and AT is accelerated by heparin, whether exogenous or endogenous.

Active thrombin remains associated with the fibrin/platelet clot and is thereby protected from inhibition by AT. This provides a reservoir of procoagulant activity should the clot be disrupted by physical trauma. Thrombin not only participates in the haemostatic process, but also has cytokine and growth factor activities that play a role in inflammation and wound healing (22).

1.4 Fibrinolysis

Activation of fibrinolysis is part of the normal haemostatic response to vascular injury. Plasmin is the final effector of fibrinolysis, and is produced by cleavage of its inactive precursor plasminogen by various activators. The most important of these is tissue plasminogen activator (tPA) which is released from endothelial cells. Both tPA and its substrate plasminogen bind to the evolving fibrin polymer, where plasminogen is converted to plasmin by the tPA, which then cleaves fibrin into soluble fibrin degradation products (FDPs) resulting in dissolution of the fibrin clot. One of the major FDPs is D-Dimer, which consists of D domains from adjacent fibrin monomers that were cross linked by activated factor XIIIa. The process of fibrinolysis is closely controlled in health, just as is the process of coagulation. The cross linking and polymerization of fibrin fibres to form a dense meshwork is activated by factor XIIIa, which increases resistance of the clot to fibrinolysis. The major inhibitor of fibrinolysis is plasminogen activator inhibitor (PAI), which is a rapid and irreversible inhibitor of both tPA and uPA(urokinase like plasminogen inhibitor). Any free circulating plasmin is inactivated by the potent inhibitors alpha -2 antiplasmin and alpha -2 macroglobin (23). Thrombin also inhibits fibrinolysis by activating thrombin activatable fibrinolysis inhibitor (TAFI) that removes lysine residues from fibrin, thereby impairing the capacity of fibrin to bind to plasminogen and tPA (Figure 1.5).

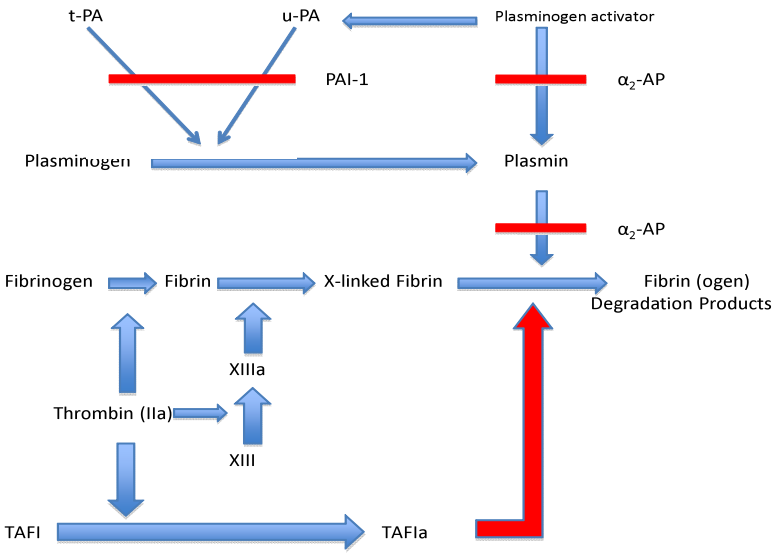


Figure 1.5 Fibrinolysis: Fibrin is broken down by the action of plasmin into fibrin degradation products. Figure illustrates key activators and inhibitors of the fibrinolytic pathway

1.5 Coagulation and Liver Disease

The concept of a causal relationship between abnormal tests of coagulation, such as PT and INR, and increased bleeding risk is widely accepted amongst clinicians, as demonstrated by the common practice of using these tests to screen patients prior to invasive procedures and treating abnormal values with transfusion of fresh frozen plasma (24). Although an increased bleeding diathesis has been considered a traditional hallmark of acute and chronic liver disease (25), it is now recognised that systemic hypercoagulability and thrombosis can also be present, and these patients cannot be considered "auto-anticoagulated"(6). The typical patient with cirrhosis has multiple and opposing factors that influence haemostasis and clot formation, and defects are seen in all components of the haemostatic system.

Stable patients with liver disease exhibit finely tuned "re-balancing" of their haemostatic profile (7) and this is reflected in an increasing number of patients with chronic liver disease (CLD) who undergo major abdominal surgery, such as liver transplantation, without the need for blood or blood product transfusion [Figure 1.6](26). However, the haemostatic balance is precarious and both endogenous and exogenous factors can readily tip the balance towards either a bleeding tendency or a prothrombotic state, as these patients lack the buffering capacity of a large functional reserve with its associated regulatory mechanisms that is seen in health(27).

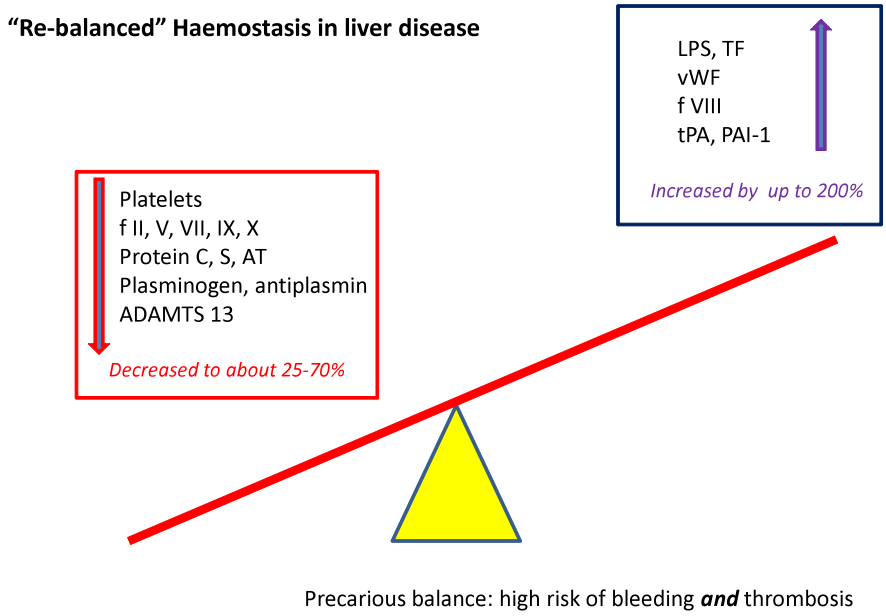


Figure 1.6 Schematic of "re-balanced" haemostasis in liver disease.

Quantifying this imbalance is the key to establishing a clinically useful paradigm for managing patients with liver disease(28). Infection plays a pivotal role in variceal bleeding,

causing abnormalities in coagulation through endogenous heparinoids (29). Renal failure and endothelial dysfunction are other contributory factors to the haemostatic imbalance in cirrhotic patients(30).

1.5.1 Primary Haemostasis in Liver Disease

Platelets exert important haemostatic functions, including primary platelet plug formation (adhesion/aggregation) and provide a membrane surface for the assembly of complexes necessary for thrombin generation. Alterations in the primary haemostatic system include abnormal platelet numbers and function. In chronic liver disease, platelet numbers decrease progressively in patients due to portal hypertension and hypersplenism with associated splenic sequestration, and also impaired hepatic synthesis of thrombopoetin. In addition there can be abnormalities in platelet function. However, increased levels of von Willebrand factor (vWF) and reduced activity of its cleaving enzyme ADAMTS- 13 , produced by hepatic stellate cells, compensate for some of these changes .(31). Platelet hyperactivity has been reported in patients with cholestatic liver disease(32, 33).

VWF is an adhesive glycoprotein secreted by the vascular endothelium in an ultra large, multimeric form (ULVWF), which unfolds under conditions of high sheer stress, exposing sites to which platelets avidly adhere and aggregate to promote haemostasis. Normally this ultra large VWF is rapidly cleaved by ADAMTS13, enabling release of smaller, less active forms of VWF in to the circulation. Deficiency of ADAMTS13 results in a loss of VWF regulatory control, and can, if extreme, lead to vessel occlusion by hyper reactive ULVWF and platelet thrombi. It has been speculated that this mechanism may contribute to, and exacerbate portopulmonary hypertension, and can also result in catastrophic reactions to platelet transfusions (34). In a series of post mortem studies in patients who had sudden

cardiovascular collapse when undergoing liver transplantation, extensive platelet aggregates were found occluding small pulmonary arterioles and alveolar capillaries, with no macroscopic evidence of thrombi. A sudden fall in platelet count, and rise in pulmonary arterial pressure were reported in most of these cases (35). It is of note that platelet transfusion is a major independent risk factor for mortality in liver transplantation, and acute lung injury was the only mechanism identified to account for this observation (36).

A systematic review evaluating qualitative and quantitative aspects of platelet function (31) concluded that primary haemostasis is not normally defective in cirrhosis. Therefore a low platelet count should not necessarily be considered as indicating an increased risk of bleeding, with the caveat that with severe thrombocytopenia, correction is advised if bleeding occurs, or prior to performing invasive procedures. There is consensus that platelet transfusion is indicated in cirrhotic patients with low platelet counts (50,000 or less) during active bleeding (37). The evidence for the commonly set lower cut off values for platelet count is sparse and limited by small sample size. A pre-procedure platelet count of 50,000 is considered adequate(38) and this is reinforced by endogenous thrombin generation studies (39). Giannini studied 121 consecutive patients who were being evaluated for liver transplantation and were undergoing invasive procedures. Bleeding occurred in 31% with severe thrombocytopenia and in none of those with moderate thrombocytopenia (40). Platelet function was traditionally assessed by bleeding time (BT). (41) However in cirrhosis there is a poor association between platelet count and BT and a prolonged BT can be seen in patients with platelet counts > 100,000 and vice versa. (42) As platelet activation is not diminished but may be increased in some patients with cirrhosis, it is possible that BT prolongation is also a result of changes in vasoreactivity and /or arterial dysfunction (42)

1.5.2 Secondary haemostasis and liver disease

In chronic liver disease (CLD) most pro-coagulant factors concentrations are decreased, except factor VIII, which is elevated. Decreased levels of pro-coagulants are accompanied by a concomitant decrease of the naturally occurring anticoagulants (antithrombin, protein C and S. (4) In normal conditions the coagulation system is balanced by the two opposing drivers and thrombin generation is no different or even increased in stable liver disease compared to healthy individuals when the test is modified to incorporate the natural anticoagulant pathways. (4, 5) This apparent paradox is explained by the fact that Protein C (PC) and antithrombin (AT) need to be activated to exert their full anticoagulant activity with thrombomodulin and with glycosaminoglycans (GAGs) (43, 44) which are located on the vascular endothelium. This aspect is not evaluated in the majority of coagulation analyses and this pitfall is particularly important in cirrhosis where both anti-coagulants and procoagulants are reduced.(45)

1.5.3 Fibrinolysis and liver disease

Fibrinolysis is an important component of haemostasis and is a complex physiological process involving the interaction and balance between a number of different activators and inhibitors. In cirrhotic liver disease there is an enhancement of fibrinolysis due to a shift in balance between pro and anti fibrinolytic factors (46). The increased fibrinolytic activity and clot instability are due to increased levels of tissue plasminogen activator (tPA), due to increased synthesis by the vascular endothelium and also decreased hepatic clearance and the low levels of fibrinolytic inhibitors, alpha 2 antiplasmin and thrombin activatable fibrinolysis inhibitor (TAFI), together with low levels of factor XIII, that is required for effective polymerization and stabilisation of the fibrin clot. However, this is balanced by

increased levels of the acute phase reactant plasminogen activator inhibitor (PAI-1), which is the major inhibitor of tPA. Levels of PAI-1 are particularly high in acute liver failure and in cholestatic liver disease, and significant fibrinolyisis is rare in these groups (47, 48). In addition, plasminogen, that is converted to plasmin by tPA, is low due to impaired hepatic synthesis.

1.6 Limitations of standard coagulation tests in patients with liver disease

The in vitro tests of activated partial thromboplastin time (aPTT) and prothrombin time (PT) are the most commonly used tests of coagulation and measure the time elapsed from activation of the coagulation cascade at different points to the generation of fibrin. Citrated plasma, an activator (tissue factor for PT and phospholipids for aPTT) are added together and incubated at 37^{O}C. Calcium is added, and the time required for clot formation is measured. The aPTT is used to assess the contact activation and the integrity of the intrinsic coagulation pathway (factors XII, XI, IX and VIII) and final common pathway (factor II (prothrombin), V,X and fibrinogen. A prolonged aPTT is found with isolated deficiencies of (or inhibitors of) intrinsic and common pathway factors, and after heparin administration. The PT is used to assess the integrity of the extrinsic pathway, which consists of TF and VIIa, and coagulation factors of the common pathway. Causes of isolated prolongation of the PT are inherited or acquired deficiencies of factor VII, vitamin K antagonist administration or vitamin K deficiency (factors II, VII, IX and X),liver disease and inhibitors of factor VII. The PT was standardised (for warfarin control) through the use of the international normalised ratio. When both the aPTT and PT are prolonged, inherited or acquired deficiency of factors X,V, prothrombin or fibrinogen may be the cause.

Prolongation of the PT and PT in liver disease reflects the impaired synthesis of clotting factors by the diseased liver and is widely used in scoring systems (Child-Pugh, MELD and UKELD) in chronic liver disease and as a prognostic tool and for monitoring of liver function in acute liver failure. As both these tests are more effected by procoagulant rather than anticoagulant levels, they will be prolonged even when the reduction in procoagulants is matched by a concomitant reduction in anticoagulant levels, resulting in normal thrombin generation and "balanced" haemostasis (7). They therefore give a false impression of an increased bleeding risk in these patients.

The PT/INR was developed to monitor oral anticoagulant therapy with the vitamin K antagonist Warfarin, and the PTT to investigate the inheritable single factor deficiencies eg. haemophilia, and to monitor heparin therapy. These tests were never intended to model *in vivo* haemostasis or to assess perioperative bleeding risk. Many patients with liver disease have a normal PTT, despite mild baseline deficiencies of multiple procoagulant factors. This may be due to the elevated levels of factor VIII which shorten PTT and compensate for the multiple procoagulant factor deficiencies.(49) The PT/INR is widely used to assess the risk of bleeding in patients with liver disease, however, the evidence from clinical practice and the literature is that it does not correlate with bleeding after liver biopsy or other procedures (1, 2). Despite this, transfusion of fresh frozen plasma (FFP) is often used in an attempt to correct the INR(1, 50). Epidemiological studies suggest that patients with chronic liver disease have the greatest individual risk of transfusion related acute lung injury (TRALI) compared to other populations (51). Observational studies show that even major procedures, such as liver transplantation, can be performed without administration of FFP despite an increased INR (52). Most importantly, the INR value varies between laboratories

in patients with liver disease, so defining a set cut off value is problematic (53) Other limitations of PT/INR are that it is not possible to estimate the overall strength and stability of the clot because these tests are read at the initiation of fibrin polymerisation which happens at very low levels of thrombin generation of about 10 to 20nM, which is less than 5% of the total thrombin that can be generated .(54)

The INR threshold of 1.5 for bleeding risk is derived from studies that originally used a PT threshold and thromboplastin reagents which had an international sensitivity index (ISI) greater than or equal to 2. Whilst the calculated INR of 1.5 mathematically corresponds to a PT ratio of 1.5 for thromboplastin reagents with an ISI of 1.0 as used currently, this does not take into account the fact that many of the earlier studies on PT threshold were done with less sensitive thromboplastins and the corresponding INR would actually be 2.25 to 4.0 (49) This, together with the fact that the INR does not reflect the concurrent reduction in anticoagulant levels in patients with liver disease, may explain why there is no consistent relationship between bleeding and a mild to moderate increase in INR in patients with CLD.

There is no good evidence for administering prophylactic FFP according to baseline INR or indeed to improve outcomes.(55, 56) This leads to unnecessary and wide variability in the use of FFP. Tripodi assessed the effects of *in vitro* addition of pooled normal plasma (PNP) to the plasma of 58 adult patients with advanced cirrhosis and showed that although the PT ratio shortened in many patients, there was no change in thrombin generation. These results cast doubt on the efficacy of FFP to reduce the bleeding risk in patients with liver disease who are undergoing invasive procedures (57)and is an area that needs urgent research.

Standard coagulation tests have been shown to be inadequate for the purposes of stratifying bleeding and thrombotic risk in patients with liver disease, and this mandates a search for alternative means of assessment which better reflect functional changes in coagulation.(58)

1.7 Alternative global methods of monitoring coagulation

1.7.1 Thrombin generation assays

Thrombin generation assays are global coagulation tests that measure the dynamics of thrombin production. Platelet free plasma is incubated with small amounts of tissue factor as a coagulation trigger and phospholipids that act as platelet substitutes (59).Thrombin generation tests have been used to identify patients at increased risk of thrombosis (60-62), and a high endogenous thrombin potential (ETP) is associated with an increased risk of recurrent thrombosis. Conversely, reduced thrombin generation is documented in patients with a bleeding tendency (63, 64). The normal, or even enhanced, thrombin generation in stable patients with CLD explains, at least in part, why many of these patients do not have a significant increased bleeding risk and may be at increased risk of thrombosis. (62) Following Tripodi's landmark paper, in which thrombin generation in cirrhosis was shown to be the same as in healthy people when thrombomodulin was added to activate protein C (39), further papers indicate that thrombin generation may actually be increased.(65) Gatt et al(66) studied 73 adult patients with cirrhosis and also 38 healthy individuals. Thrombin generation was assessed using the calibrated automated thrombography (CAT)(67). Rather than thrombomodulin, Protac® modified TG was used. (Protac is a snake venom extract that activates PC).This study showed a hypercoagulable TG profile in plasma in cirrhosis, with an increased velocity of TG and higher endogenous thrombin potential (ETP) ratios**.** This is the

same profile as patients with protein C / protein S deficiency and factor V Leiden, in whom a greater risk of thrombosis is well documented (68). Overall, the data on TG velocity and Protac resistance demonstrate a prothrombotic tendency in plasma of patients with cirrhosis. These findings are in keeping with reports that patients with liver disease are not protected against thrombosis despite a raised INR (69, 70) and have an increase thrombotic risk compared with age matched controls. Although thrombin generation studies have increased our understanding of the coagulopathy in liver disease, they are for the time being, mainly research tools that are laboratory based.

1.7.2 Viscoelastic tests of coagulation

These global viscoelastic and point of care coagulation tests have the potential to overcome many of the limitations of routine coagulation tests, as they measure the entire coagulation process, from fibrin formation through to final clot strengthening and retraction or fibrinolysis. In addition, as the test is performed with whole blood, the plasmatic coagulation system interacts with platelets and red cells, and therefore they more closely reflect the situation *in vivo*. The principles of these tests, together with a discussion regarding the potential advantages and limitations of these tests will be reviewed in the next chapter.

Chapter 2

Principles of Viscoelastic Tests of Coagulation

2.1 Introduction

Although routine coagulation tests (PT, PTT) provide valuable information regarding the quantitative status of procoagulant proteins, they ignore the interaction of cellular elements and endogenous anticoagulant factors (71). At the time these tests are concluded, only a fraction (< 5%) of the total thrombin that will be generated during the process of coagulation has formed, thus they can give only limited information about the overall haemostatic status (19). In addition, they are insensitive to most thrombin dependent reactions associated with normal haemostasis, that is platelets, protein C and factor V and VIII activation(72). Haemostatic assays that demonstrate the interactivity of the major phases and components of the haemostatic process better represent haemostatic capacity and differentiate the mechanisms related to clotting abnormalities (73).

2.2 Mechanical properties of the clot and relation to haemostasis

Clot formation involves the dynamic interaction between the vascular endothelial wall, platelets, vWF, pro and anticoagulant factors and blood flow. The location and the mechanical properties of the formed clot are essential for appropriate haemostasis. A clot consists of a three dimensional network of cross linked fibrin fibres with platelets and other blood cells trapped within the mesh of fibres. This fibrin network is fundamentally rigid with sufficient elasticity to resist deformation by shear forces. This resistance to deformation is measured by the shear elastic modulus, an index of clot strength. Platelets enhance the elastic properties of the fibrin network by binding to fibrin through specific platelet receptors (Glycoprotein IIb/IIIa). In the cell based model of haemostasis, thrombin generation, fibrin structure and fibrinogen interaction with platelets are mutually dependant processes that contribute to clot strength (74). It is thought that platelet

function is of greater importance to clot strength and stability than platelet number (75). Abnormalities of clot strength are associated with both bleeding and thrombotic events.

The cell based model of haemostasis (11), as opposed to the traditional description of intrinsic and extrinsic pathways, emphasizes the role of platelets in intact thrombin generation and highlights the importance of the dynamics of thrombin generation influencing the quality and stability of thrombus formed. The multifactorial aetiology and sometimes unpredictable nature of many perioperative coagulopathies means that CCT are inadequate for the purposes of monitoring, diagnosing and treating coagulopathy in a goal directed fashion. For haemostasis to occur effectively there must be sufficient thrombin generation (coagulation factors and platelets), adequate substrate (fibrinogen) and clot stability. Viscoelastic haemostatic tests measure changes in clot tensile strength over time and give information on the dynamics of clot formation (coagulation factor and anticoagulant activity), clot strength (platelets and fibrinogen) and clot stability (fibrinolysis and factor XIII).

2.3 Principles of Thromboelastography

There are two commercially available devices, both based on Hartert's invention in 1948 (76), the TEG® (Haemonetics Corporation, Braintree, MA, USA) and the ROTEM® (TEM International GmbH, Munich, Germany). These viscoelastic tests provide rapid information about global clotting in whole blood and have become widely used as a point of care (POC) monitor in the setting of liver transplantation, cardiac surgery, trauma and obstetrics. In this chapter the term thromboelastography will be used to describe general principles of the

common technology, but the difference between the two systems will be specified as TEG or ROTEM respectively.

2.3.1 Basic principles

Thromboelastography (TEG) & thromboelastometry (ROTEM) measure the viscoelastic properties of a developing clot in a sample of whole blood after adding a specific activator, under low sheer conditions. They provide real time information about the quality of the clot and the kinetics of its formation. The viscoelastic (tensile) force between the cup and the immersed pin results from the interaction between activated platelet glycoprotein (GP) IIb/IIIa receptors and polymerising fibrin during endogenous thrombin generation and fibrin degradation by fibrinolysis (77). In both machines, 0.37 micromls of whole blood are pipetted into a heated cup into which a pin is suspended.

In the TEG, the platform on which the cup is placed oscillates through an angle of $4^{o}45'$ and each rotation cycle lasts 10 seconds. A stationary pin, connected to a torsion wire, is suspended into the blood sample. Once blood starts to clot, fibrin strands start to couple the motion of the cup to the pin and the change in torque is converted via a mechanical-electronic transducer into an electronic signal. The output is directly related to the strength of the formed clot (Figure 2.1).

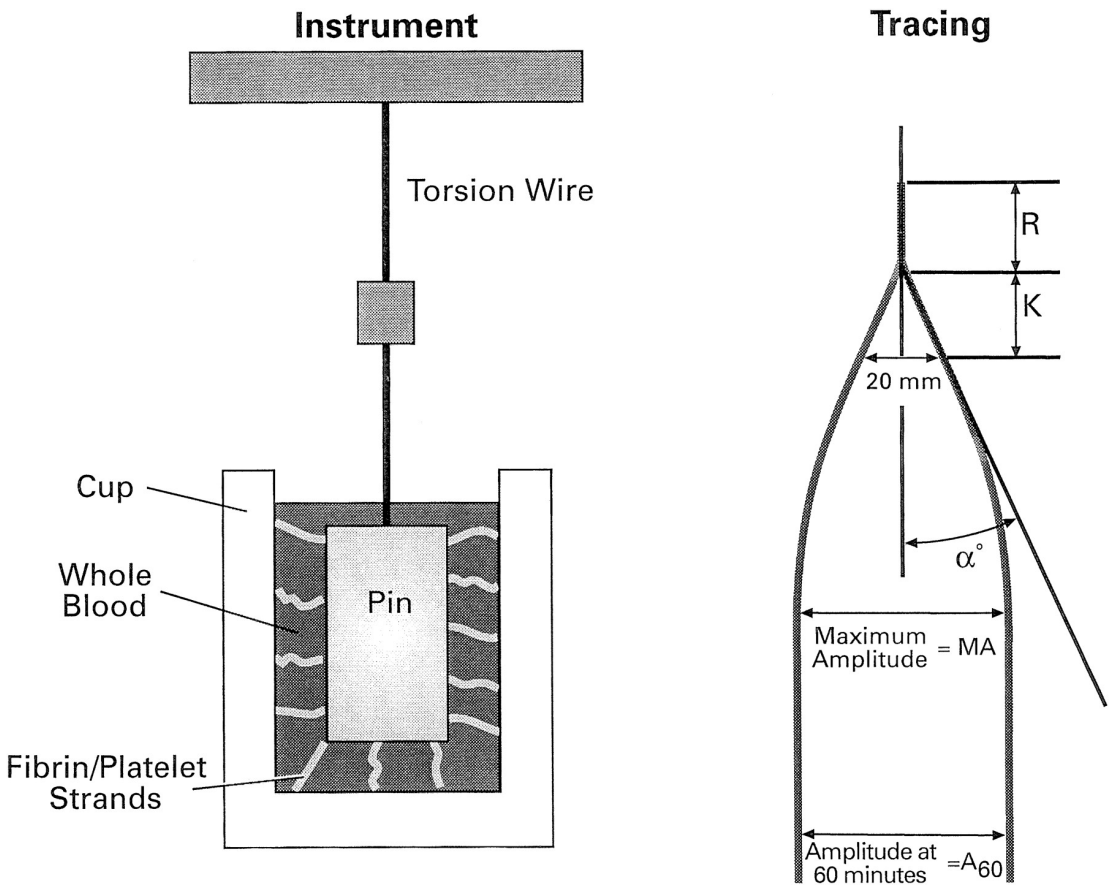


Figure 2.1 Principles of thromboelastography (TEG®)

In the ROTEM, the pin is fixed to a rotating shaft, and the cup is stationary. As clot starts to form and develop, the resistance to rotation of the shaft increases, and this is detected by the angle of reflection of light onto a mirror (Figure 2.2).

The rate of polymerisation and overall clot strength is displayed visually on the TEG/ROTEM trace and also numerically and provides a complete picture of clot initiation, formation and stability (78). Once blood starts to clot, fibrin strands start to couple the motion of the cup to the pin. The change in torque is detected electronically in TEG and optically in ROTEM. Dissociation of fibrin strands from the cup wall due to clot retraction or the degradation of fibrin by fibrinolysis decreases the torque (79). The computer processed signal is presented as a tracing of clot formation and if present, clot dissolution.

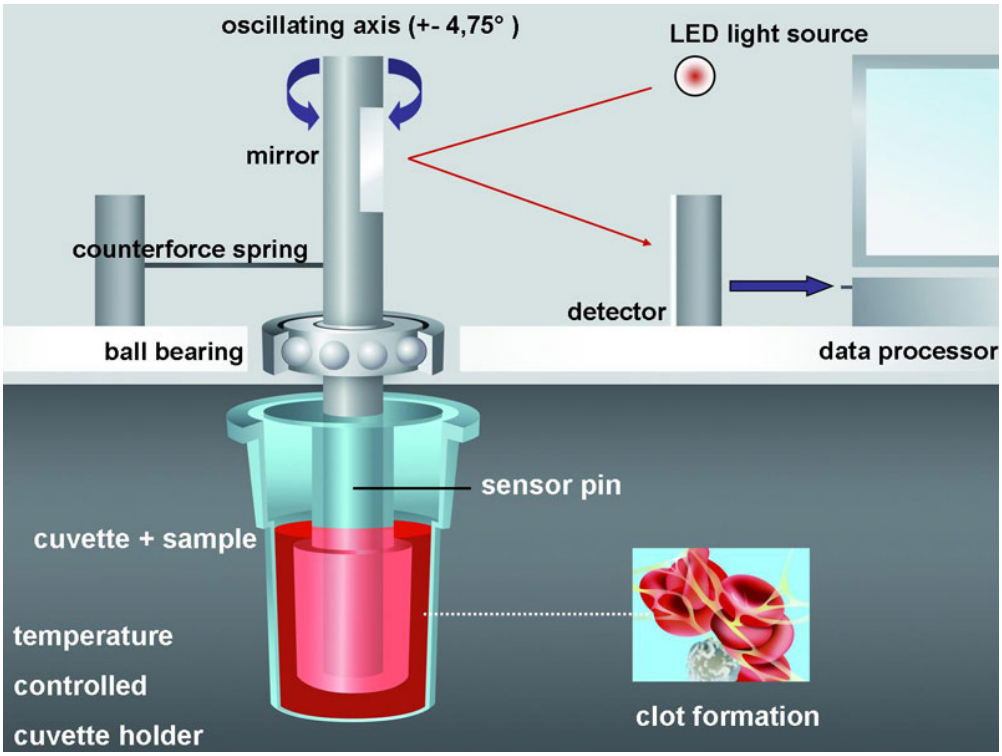


Figure 2.2 Principles of thromboelastometry (ROTEM®)

The initial torque is assumed to be zero (i.e. no clot) for the signal processing and it is therefore essential to start the measurement immediately after a coagulation activator is added to the sample. The parameters produced of the coagulation profile by VETs are identical for TEG and ROTEM, but the terminology used to name the individual parameters is slightly different for each machine (Figure 2.3). Each parameter identifies a specific stage of the coagulation process (Table 2.1).

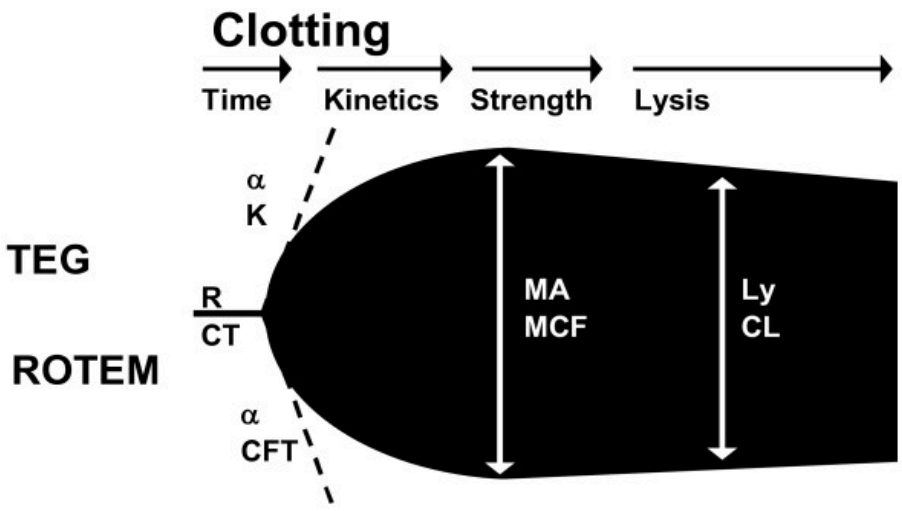


Figure 2.3 Schematic of TEG/ROTEM parameters

	TEG	ROTEM	
Clot initiation or Clotting time	R (reaction time)	CT (clotting time)	Period of initial fibrin formation
Clot Kinetics	K (K value)	CFT (clot formation time)	A measure of the speed to reach a specific level of clot strength
	α (angle in degrees)	α	Measures the rate of clot formation, reflects fibrin rate of fibrin build up and cross linking
Clot strength	MA (maximum amplitude)	MCF (maximum clot firmness)	Represents the ultimate strength of the clot (platelets & fibrin) function of maximum dynamic properties of fibrin & platelet bonding via GPIIb/IIIa receptors
Clot stability	Ly30 (Lysis at 30 minutes as ratio of MA)	CLI (Clot lysis index)	Measures rate of amplitude reduction from MA at 30 minutes, detects fibrinolysis

Table 2.1 Description of TEG/ROTEM parameters. These parameters measure stages of coagulation from clot initiation (R/CT), through clot development and kinetics of its formation (k/CFT and alpha angle), to final clot strength (MA/MCF). Indices of clot retraction or pathological fibrinolysis are described by Ly30/CLI.

Specific haemostatic defects give very characteristic traces on the TEG (Figure 2.4). The addition of different activators or reagents improves the diagnostic capability of these tests [Figure 2.5].

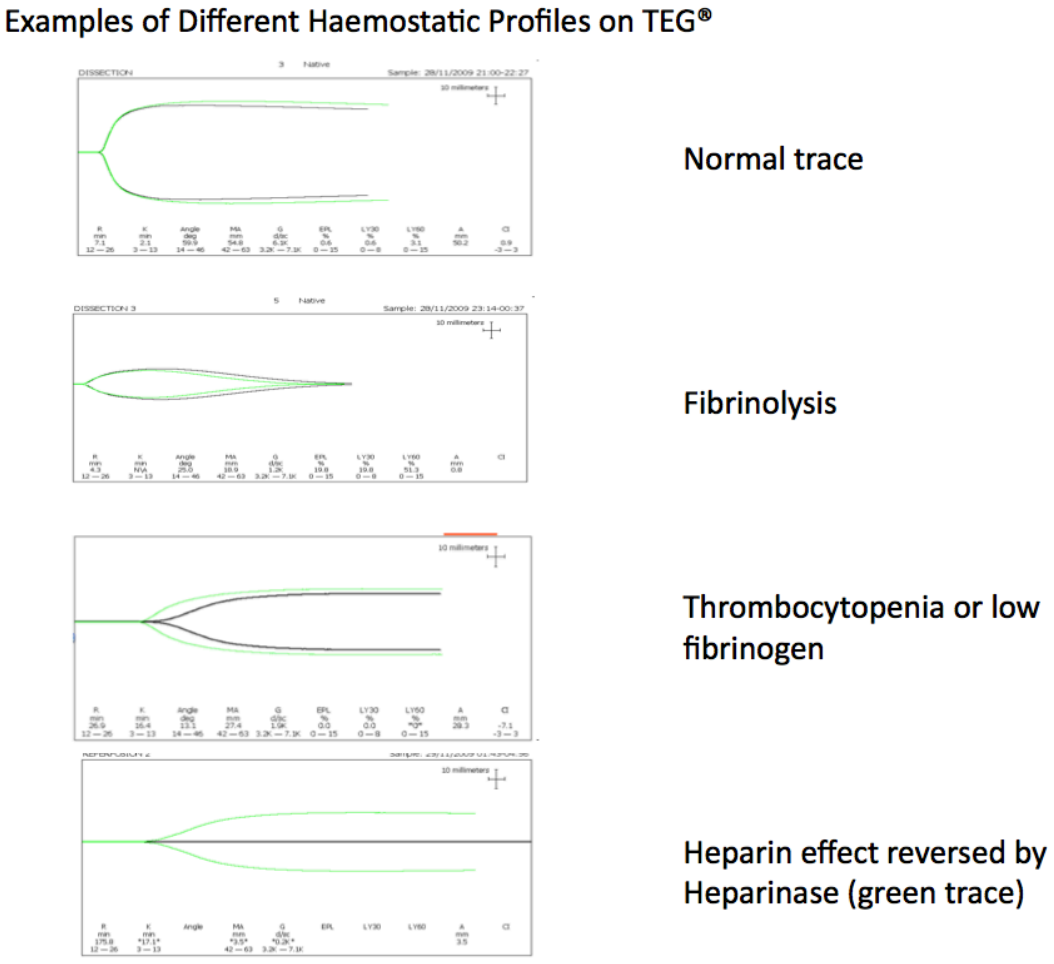


Figure 2.4 Different haemostatic profiles on TEG

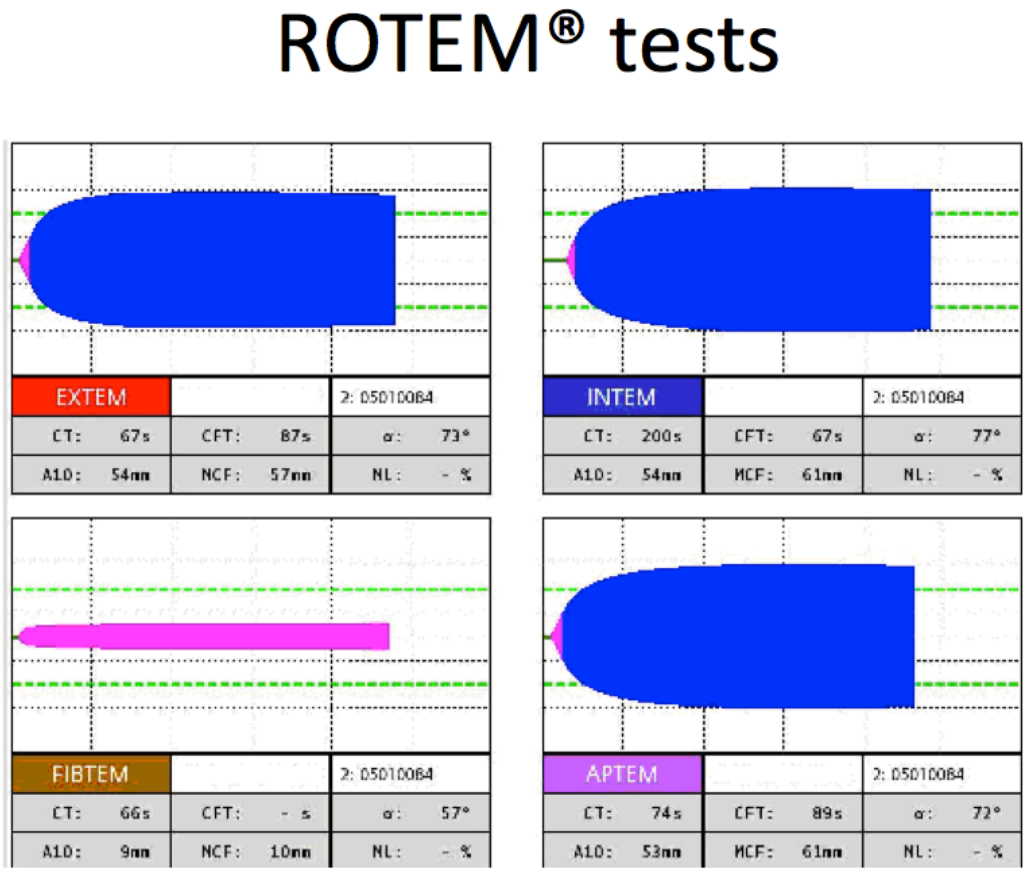


Figure 2.5 ROTEM traces

EXTEM (activated with TF), INTEM (contact activation), FIBTEM (TF and cytochalasin D to remove platelet contribution), APTEM (TF and aprotinin, in vitro fibrinolysis inhibition)

Although the two techniques provide essentially equivalent information, algorithms based on one, are not directly transferrable to the other, as they use different activating reagents, and the cups have slightly different physico-chemical properties (80, 81) . Clinical reference values differ between the two systems and must be interpreted appropriately (82). Native whole blood was used in the earlier studies with TEG(83), and is still used in some institutions, as the lack of activating agents allows more subtle changes in coagulation status to be better appreciated. However, this requires that the blood is placed in the cup for testing 2 minutes after withdrawal of the sample. The use of citrated whole blood is now

standard for most thromboelastographic systems. Before testing, citrated samples are recalcified by adding 20µL of 0.2mmol/l calcium chloride, this does result in minimal dilution (10%) of the sample, in addition the timing of sample analysis is important, and the stability and reproducibility of tests is best between 30 minutes and 2 hours following sampling(84) Sample collection methods need to be standardised and reference ranges for the specific method must be established. The possibility of using different reagents increases the diagnostic capabilities of these tests: eg heparinase to reverse underlying heparin (heparinase TEG/ HEPTEM), tissue factor to accelerate clot initiation (rapid TEG), and aprotinin to reverse fibrinolysis (APTEM).

2.3.2 Limitations of VETs

***Variation in reference ranges due to influence of type of activator and patient factors*:**

Many factors affect the TEG/ROTEM traces, and this is inevitable due to the "global" nature of these tests. Using whole blood means the test can be influenced by all components of whole blood, including white and red cell content, platelet number and function, fibrinogen concentration as well as coagulation protein function and balance(85). The concentrations of all cellular and plasmatic elements in whole blood should be taken into account when interpreting results from VETs.

Normal values for thromboelastographic parameters depend on pre-analytical factors such as re-calcification and time from blood sampling (86), and also on the type and final concentration of the activator (85), which vary substantially between the two systems. In particular, clotting time and clot formation time are strongly dependant on the type of activator used, and the concentration of tissue factor will influence the thromboelastographic parameters (87). The variation of test results is lower after extrinsic

activation with tissue factor (coefficient of variance [CV] 3-5%), but substantially higher with intrinsic activation with kaolin or ellagic acid (CV 12-15%). The variation is least for MA/MCF (CV 5%) independent of the activator used (88). For all assays, final clot strength is dependent on many factors, including the activator used, other factor concentrations such as fibrinogen, haematocrit, and the amount and speed of initial thrombin generation.

Reference ranges need to be constructed for the specific population under study, as it is known that gender, pregnancy, age and underlying co-morbid conditions all influence VET parameters. Paradoxically, increasing haematocrit results in a slight reduction in overall clot strength as measured by the maximum amplitude (89), this is possibly due to a looser clot structure when increasing amounts of RBC are incorporated into the fibrinogen mesh network (90). There are significant gender related differences in TEG variables, with a trend of increasing coagulability through men, nonpregnant women to pregnant women(91). Threshold values for clinical outcomes or haemostatic interventions should be locally evaluated for each system and relevant patient population.

Methodology not standardized and issues with quality assurance: These viscoelastic tests have never undergone all the validation procedures that are mandatory for conventional haemostatic tests, such as variability and repeatability, calibration and quality controls.(92) A major criticism of these devices was the fact that they were not well standardised, especially in relation to pre-analytical and analytical factors. In an attempt to demonstrate reproducibility and consistency using these devices, the international TEG/ROTEM working group was formulated, and laboratories from a number of countries blind tested panels from normal pooled and factor VIII deficient plasma (93). The CV varied for different VET parameters, with K or CFT, being associated with the highest CV. There was also significant

inter laboratory variance with CV in excess of 10%. (Figures 5 and 6). In the UK, steps have been taken to evaluate the provision of external quality assessment (EQA) material for these devices, using lyophilised plasma samples to improve quality assurance and quality control. It was of note that some centres returned results that were sufficiently different from other participants to predict alterations in patient management decisions. It was concluded that a mechanism of providing EQA and also regular proficiency testing of individuals performing these tests was highly desirable (94). The issue of EQA and participation in formal NEQAS monitoring is problematic as the material usually provided for EQA purposes is lyophylized plasma, whereas whole blood samples are routinely analysed. Internal QC is available for both TEG and ROTEM. These are used to check daily variation and permit early detection of test problems that could affect patient results.

To bring these analysers to the next level, several improvements are desirable, including full automation to improve ease of use, simultaneous testing with multiple activators to more accurately define the nature of any underlying coagulopathy, integrated analysing software, and increased robustness of these devices.

2.4 Correlation of conventional coagulation tests and viscoelastic tests

2.4.1 PT/INR

The plasma based tests, PT and aPTT, reflect the lag time for non polymerised fibrin gel formation after extrinsic (tissue factor) and intrinsic (ellagic acid, kaolin) activation respectively. Correlation between reaction time and clotting time (R/CT) and PT/INR is weak [r= 0.24-0.37](95-98). This can be partly explained by the use of different activators, but also by the fact that R/CT unlike PT/INR reflects the balance of both pro and anticoagulants. This

may partially explain why the R/CT is not sensitive to mild to moderate increases in INR (=<1.6)(99, 100) and why there is no useful correlation between these CCT and the viscoelastic parameters R and CT. In models of dilutional coagulopathy, an increase in CT occurs only when clotting factor concentrations are reduced to levels below 30% ((101). The exponential relationship of coagulation factors on PT/INR is not always appreciated and is one reason why fresh frozen plasma (FFP) does not contribute sufficient coagulation factors to correct PT/INR by 50% when there is a minimally prolonged PT/INR (102). The R/CT may therefore be a better reflection of true bleeding potential than INR, as a prolongation (in the absence of excess anticoagulants) usually is seen only if procoagulant levels are less than the haemostatic threshold of 30% (103). In addition, in contrast to plasma based CCT, the inclusion of platelets (ie whole blood) in VET will affect the onset (R/CT) and rate (K,CFT) of fibrin polymerization, due to the platelet mediated procoagulant reactions and platelet-fibrinogen interactions. In liver disease, wide derangements in INR are not often mirrored by similar changes in VET parameters and this reflects the fact that the INR is a poor predictor of clinically important bleeding (2).

2.4.2 Platelet count

Clot strength as assessed by the maximum amplitude (MA) or maximum clot firmness (MCF) is highly influenced by both fibrinogen levels and platelet count. VET are useful for evaluating the overall interaction between platelet GPIIb/IIIa receptors and fibrinogen(104) as activated platelets provide binding sites for fibrinogen. The minimal platelet count for normal clot formation on VET is not certain and is markedly affected by the fibrinogen level. In a study in patients with idiopathic thrombocytopenic purpura (ITP), it was found that the critical cut off for platelet count to affect MCF was 31 x 10^9 and the critical fibrinogen level

was 375mg dl^{-1} (105) The MCF was found to be the most important parameter in predicting bleeding in patients with ITP. Others have found that the MCF is greatly decreased when the platelet count falls below 50,000 $x10^9$ (106) . In liver disease, where fibrinogen levels are usually within the normal range, platelet count may have a more significant impact on changes in MA/MCF. Tripodi et al found that in stable patients with cirrhosis the correlation of platelet count with MCF was 0.691 compared to 0.590 for fibrinogen(98). As clot strength (MA/MCF) is a composite reflection of platelet –fibrinogen interaction, even if there is a low platelet count, adequate clot strength may still be achieved if the fibrinogen levels are at the high end of normal or raised. The combination of both a low platelet count and a low fibrinogen always results in a reduced MA/MCF and is strongly associated with an increased bleeding tendency. (107)

2.4.3 Clauss fibrinogen

Preoperative fibrinogen levels vary greatly among patients and low levels may be predictive of bleeding during surgery. The Clauss method is currently the gold standard for determination of fibrinogen. It is turbidometric and depends on thrombin induced fibrin formation. It is affected by multiple factors including the presence of colloidal solutions (starch and gelatins) and also direct thrombin inhibitors (108, 109). In the ROTEM FIBTEM test, the addition of cytochalasin D inhibits GPIIb/IIIa interaction thereby removing the platelet contribution to MCF and has good correlation with plasma fibrinogen levels (110). A functional fibrinogen (FF) assay is also available for the TEG.(111). However, until recently, it has not routinely been used in TEG analyses, and the lack of a standardised protocol on the TEG to distinguish hypofibrinogenaemia from thrombocytopenia is a major limit in determining the need for fibrinogen replacement therapy (112) . A preliminary

observational study in liver transplant patients found that FF correlated strongly with Klauss fibrinogen (r=0.9) at baseline, but overestimated levels after graft reperfusion, when plasma fibrinogen levels are frequently less than 1g/l (113).

A fibrinogen level of 1.5g/l or less increases the risk of bleeding, and is considered borderline for major surgery (114). In trauma induced coagulopathy a FIBTEM amplitude after 10 minutes (A10) of less than 5mm was reported to be a good predictor of low plasma fibrinogen (<1g/l) with a sensitivity of 91% and a specificity of 85% (95). The ability to monitor and determine fibrinogen levels at the bedside has led to increased use of fibrinogen concentrates and cryoprecipitate in cardiac surgery and also in severe trauma with a reduction in both total and massive transfusions (107, 115, 116). In dilution and massive bleeding fibrinogen is the first factor to reach critical levels (117). Bollinger investigated the minimum fibrinogen concentration above which clot formation normalises and found that fibrinogen concentrations above 2g/l are required (118). All VET parameters are progressively affected during haemodilution or blood loss because viscoelastic strength is highly dependent on fibrin polmerization, illustrating that fibrinogen is an indispensible substrate of thrombin and critical for haemostasis. Fibrinogen supplementation may also compensate for defects in fibrin polymerisation and also for low platelet counts(119) and thereby reduce blood loss. In a prospective observational study in 20 liver transplant patients administered fibrinogen concentrate to maintain the MCF, the transfusion of blood, FFP and platelets was reduced by more than half and the percentage of patients who received no transfusion increased from 3.5% to 20% compared to a historical cohort from the previous year. (120)

2.4.4 Fibrinolysis

The euglobulin clot lysis time was the original method used to assess fibrinolysis, and reflects overall fibrinolytic activity in the plasma, but has largely been superseded by specific functional and immunological assays. Validation of VET to assess fibrinolytic activity and to determine the underlying individual susceptibility to fibrinolysis is an area of increasing interest (121, 122). Clinically significant fibrinolysis is detected by the clot lysis index (CLI), when there is a rapid decline in MA/MCF over time. A CLI of >15% (the decrease in MA/MCF over 1 hour is more than 15%) is considered hyperfibrinolysis (123). The ROTEM test which uses an assay containing aprotinin (APTEM) confirms the diagnosis and in addition, by reversing any fibrinolysis, allows pre-assessment of the coagulation profile after antifibrinolytic therapy has been administered to the patient, thus enabling earlier administration of other prohaemostatic therapy if necessary. (88) Because plasma normally contains high concentrations of plasminogen activator inhibitor (PAI-1) and alpha 2 anti-plasmin, the fibrinolytic response is normally limited to the surface of the thrombus and the absence of significant fibrinolysis on VET does not exclude fibrinolysis in a localised vascular bed, however it does suggest that the systemic concentration of tPA is not high enough to induce *ex vivo* hyperfibrinolysis.(124) The fibrin clot is more susceptible to fibrinolysis after massive haemodilution because of the progressive loss of endogenous fibrinolysis inhibitors (125).

2.5 Thrombin generation

The rate and amount of thrombin generation is considered predictive for both thrombosis and haemorrhage. A thrombus velocity curve or V curve can be obtained from the TEG waveform using a software programme. The V curve is plotted from the first derivative of changes in clot resistance expressed as a change in clot strength per of unit time (dynes/ $cm^{2/}$ s) representing the maximum velocity of clot formation. Parameters obtained are total thrombus generation (TTG), maximum rate of thrombus generation (MRTG) and time to maximum rate of thrombus generation (TMRTG). This gives similar, but not identical information, as the automated calibrated thrombogram.

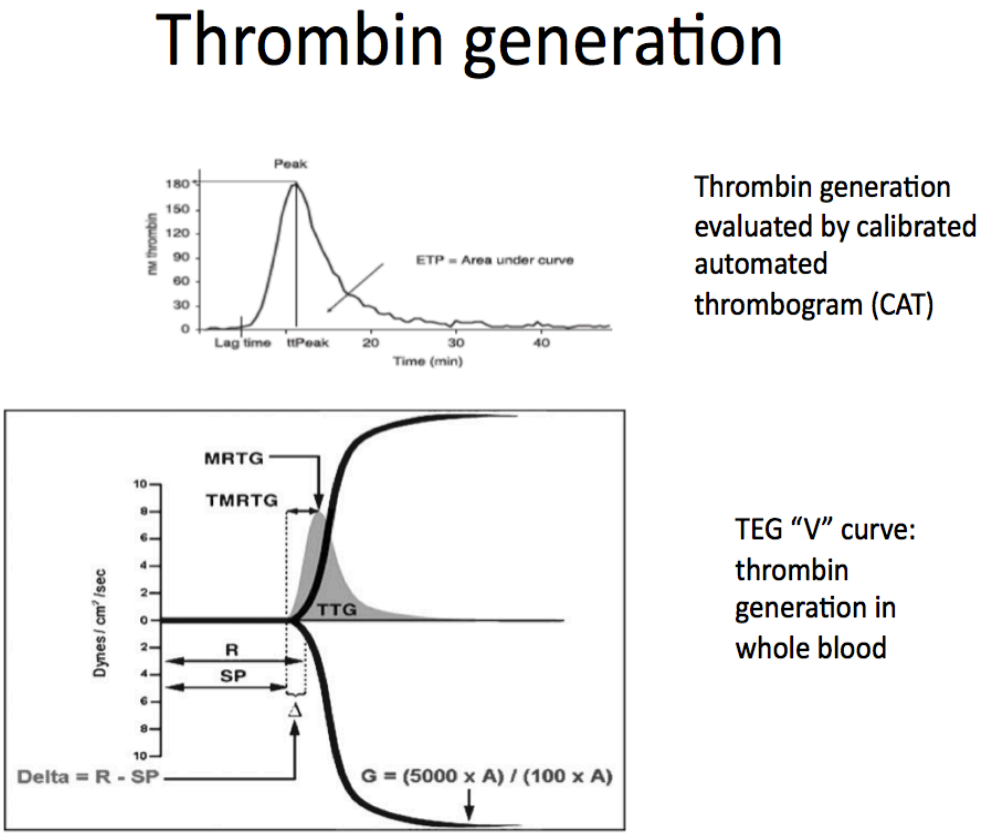


Figure 2.6 Thrombin generation and TEG "V" curve

A small study in healthy volunteers demonstrated that thrombin-antithrombin (TAT) complexes, a surrogate marker for thrombin generation, correlated well with TMRTG and TTG (77). However, the rate of clot formation on the TEG can only be assumed to be directly proportional to the rate of thrombin generation if the platelet count, fibrinogen and factor XIII levels are normal. This means that thrombin generation data derived from the V curve, in the setting of liver disease, should be interpreted with care as the platelet count affects not only final clot firmness (MA) but also the rate of clot propagation. The TEG has also been used to assess thrombin generation in haemophiliac patients (126, 127). In patients undergoing surgery, the fibrinogen level and platelet count can change rapidly and changes in the first derivative of the TEG could be due to either hypofibrinogenaemia or thrombocytopenia as well as a decrease in thrombin generation. Thrombin generation and fibrin clot formation are closely interlinked and reductions in one or both will predispose patients to bleeding complications(64) and explains why a reduced MA/MCF has such a significant relationship with increased bleeding tendency.

2.6 Conclusions

Understanding the principles of how VETs provide information on clot formation, strength and stability is key to interpreting the results generated by these tests, and to using them in an informed and consistent manner. It is important to appreciate the factors that can influence the test results, and to also have some understanding of the limitations of these tests. It is essential that the equipment is maintained to adequate standards for point of care tests with quality assurance and standard operating procedures, and that all personnel that use these machines are adequately trained, and regularly tested for proficiency.

Chapter 3

Review of the clinical utility of viscoelastic tests of coagulation in patients with liver disease.

The basis of this review was published in Liver International 2013

Mallett SV, Chowdary P, Burroughs AK. Clinical utility of viscoelastic tests of coagulation in patients with liver disease. Liver Int 2013;33:961-974

3.1 Introduction

The prothrombin time (PT) and internationalised normalised ratio (INR) are used in scoring systems (Child-Pugh, MELD, UKELD) in chronic liver disease and as a prognostic tool and for dynamic monitoring of hepatic function in acute liver disease. These tests are known to be poor predictors of bleeding risk in liver disease, however they continue to influence clinical management decisions. Recent work on coagulation in liver disease, in particular thrombin generation studies, has led to a paradigm shift in our understanding and it is now recognised that haemostasis is relatively well preserved. Whole blood global viscoelastic tests (TEG®/ROTEM®) produce a composite dynamic picture of the entire coagulation process and have the potential to provide more clinically relevant information in patients with liver disease.

We searched MEDLINE and the Cochrane Library for papers published in English on coagulation and liver disease from 1 January 1980 to 31 January 2015 using the following keywords “liver disease”, “liver surgery”, “coagulation”, “coagulopathy”, “thromboelastometry” and “thromboelastography”. Although the vast majority of studies are observational , small in size, and limited to single centres, it is clear that VET provide additional information that is in keeping with the new concepts of how coagulation is altered in these patients. This review provides the basis for large scale, prospective outcome studies to establish the clinical value of these tests.

3.1 Introduction

Conventional coagulation tests (CCT) are abnormal in acute and chronic liver disease and are interpreted as demonstrating an underlying bleeding diathesis, this is because abnormal results, and therefore a presumed "coagulopathy" as demonstrated by conventional coagulation tests, are used interchangeably. However, standard coagulation tests do not predict bleeding, nor do they provide sufficient information to optimise the management of bleeding events (128, 129). The shortcomings of this classical interpretation of the coagulopathy of liver disease have been increasingly recognised in recent years (130). There has been a paradigm shift in our understanding of haemostasis in these patients, and it is now described conceptually as being "re-balanced" (131). Thrombin generation in patients with liver disease is much better conserved than previously thought when the test conditions were adapted to reflect the contribution of the anticoagulant pathways (4) and it is now recognised that there is an increased risk of thromboembolism in chronic liver disease(70).

Thrombin generation tests (TGT) have revealed important new information on haemostasis in liver disease, but these tests are not readily available, and therefore have poor clinical applicability and furthermore, there are no studies comparing the TGT to a clinical endpoint. In addition, TG assays are generally performed in platelet poor plasma and therefore lack information on protein cell interactions. Whole blood global viscoelastic tests (VET) of coagulation are increasingly used for point of care (POC) analysis of the complex coagulopathies that can occur during cardiac surgery and following major trauma (132, 133). They differ from CCT as they evaluate the kinetics of coagulation from initial clot formation to final clot strength. These dynamic tests provide a composite picture reflecting the

interaction of plasma, blood cells and platelets, and more closely reflect the situation *in vivo* than do CCT, as these are performed solely in plasma and measure only isolated end points. In addition VET provide valuable information on the presence and severity of fibrinolysis and also hypercoagulability (134). Since the early 1980's, VET have been used for POC coagulation monitoring during orthotopic liver transplantation (OLT)(83). The possibility that there may be more clinical benefit in using VET rather than CCT to assess and stratify bleeding or thrombotic risk in patients with liver disease is an idea that is gaining increasing traction, but requires prospective clinical outcome studies to determine the validity of such an approach (98).

The purpose of this chapter is to review relevant published studies on VET and liver disease, in the context of the current understanding of the coagulopathy of liver disease, to establish evidence if VET could be used as routine coagulation tests in this setting. To this end we performed a systematic review of all relevant studies that have used viscoelastic tests (VET) of coagulation in patients with liver disease. Although many studies are observational and small in size, it is clear that VET provide additional information that is in keeping with the new concepts of how coagulation is altered in these patients.

3.2 Viscoelastic tests and Chronic Liver Disease (CLD)

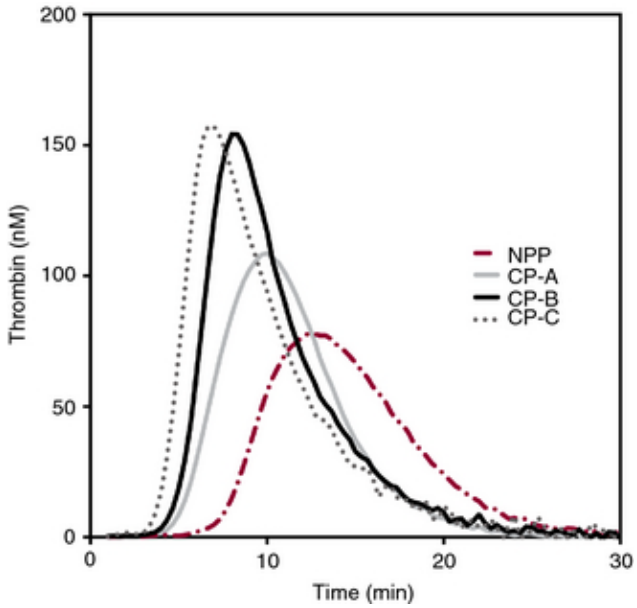
3.2.1. VET parameters and CLD

Because TEG/ROTEM are global tests providing a composite analysis that reflect function of plasma, blood cells and platelets, they are increasingly viewed as an appropriate tool to investigate the coagulopathy of chronic liver disease. In agreement with the concept of rebalanced haemostasis, patients with cirrhosis often maintain global haemostasis as

assessed by TEG. In a cohort of 273 patients with stable cirrhosis, it was found that mean and median TEG parameters were all within normal limits, although the maximum amplitude decreased in proportion to the severity of thromobocytopenia and severity of liver disease (135). In a subset of 48 patients with more decompensated, but stable cirrhosis (INR>1.5) the mean maximum amplitude was below normal limits, presumably due to lower platelet counts in this sicker population. Tripodi et al. compared ROTEM parameters between 58 healthy volunteers and 51 adult patients with cirrhosis (98). Abnormal ranges were defined as above the 95th percentile for CT and CFT or below the 5th percentile for MCF. ROC curves were constructed to identify patients with cirrhosis (true positives) from healthy individuals (true negatives). The CT did not distinguish between healthy and cirrhotic individuals and there was no correlation between PT and CT (r = -0.264) and only 27% of patients with cirrhosis had any prolongation of CT despite the fact that PT was prolonged. MCF was a good discriminator and 76% of patients with cirrhosis had an abnormal (low) value. The MCF also correlated well with MELD score. There was good correlation between platelet count and MCF (r=0.691) and also CFT (r= 0.741). Clauss fibrinogen correlated reasonably well with MCF (r= 0.590). It was concluded that VET may be useful to assess the severity of chronic liver disease and can be used to distinguish between healthy and cirrhotic individuals.

Others have also found that many patients with compensated cirrhosis have normal TEGs (136), and this supports the observation that overall haemostasis is relatively well preserved in these patients and that the compensatory mechanisms that occur in liver disease act to maintain a state of balanced haemostasis. Another study evaluated plasma thrombin generation (CAT), and whole blood clot formation (ROTEM activated with TF, with and

without tPA) in 73 patients with all cause cirrhosis (Child Pugh A = 52, B = 15, C = 6)and compared the results to 20 healthy controls. Activity of the coagulation pathway was measured by assay of factor VIIa and Xa –antithrombin complexes. Thrombin generation was increased with increasing severity of cirrhosis, whilst there was a progressive delay in clot formation rate and reduced clot strength as the severity of cirrhosis increased [Figure 3.1]. There was increased generation of VIIa, without apparent increased factor X activation. The results indicated cirrhotic patients have an overall procoagulant plasma milieu, but a reduced whole blood clot formation capacity with an apparently unaltered resistance to clot lysis (137).



Thrombin generation curves:
Kleinegris et al, 2014)

Normal plasma

Plasma from patients with Child Pugh grade A,B,C.

Figure 3.1 Thrombin generation curves in cirrhosis

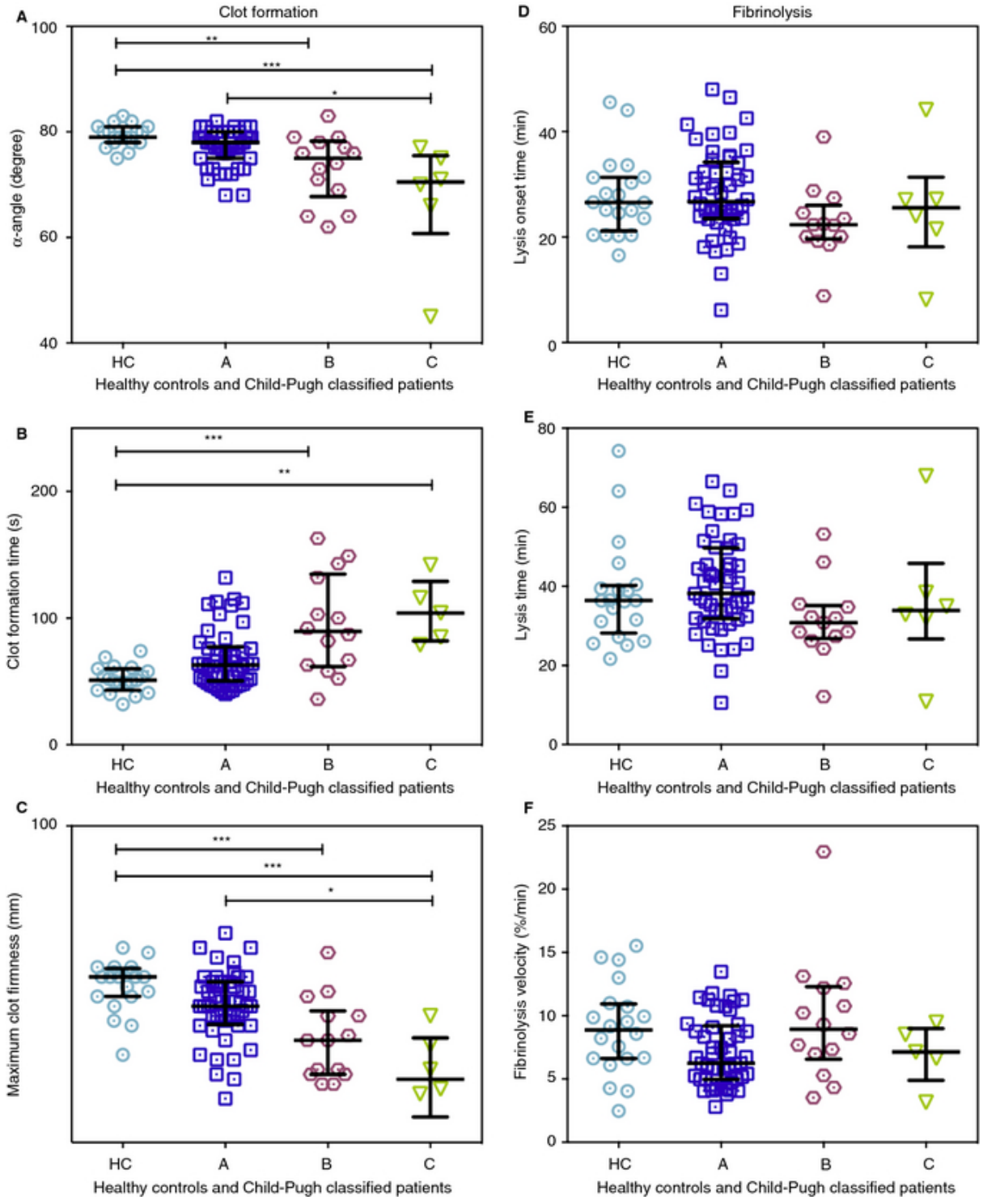


Figure 3.2 Changes in ROTEM parameter in cirrhosis *after Kleinegris et al. 2014*) There is a progressive increase in clot formation time and a decrease in maximun clot firmness with increasing severity of liver disease. There is no change in susceptibility to fibrinolysis.

The trigger for prophylactic platelet transfusion prior to invasive procedures, such as liver biopsy, is commonly set at a platelet count of 50 x 10^9 (38). However, this does not take into account the multiple changes in the haemostatic profile of patients with liver disease, such as the elevated levels of VWF, or higher levels of fibrinogen that can occur in some patients with liver disease, and result in relatively normal clot firmness, despite thrombocytopenia.(138, 139). The increased levels of VWF seen in patients with cirrhosis means that simple platelet counts can be misleading as a diagnostic tool for predicting bleeding in patients with liver failure. A randomised controlled study in patients with cirrhosis of Eltrombopag, a thrombopoetin receptor agonist, was terminated prematurely because of thrombotic complications in the treatment group (140). It was thought that elevated levels of VWF together with normalized platelet counts were the major contributing factor.

The value of prophylactic platelet transfusion in preventing bleeding as a result of invasive procedures in cirrhotic patients, has been largely taken for granted until recently, but without any confirmatory evidence. In a small observational study of 26 thrombocytopenic patients with cirrhosis, undergoing variceal ligation who were given one standard adult dose platelet transfusion, the effects on thrombin generation and ROTEM were evaluated. Although there was a small increment in platelet count, from 39 (16-64) to 52 (19-91) there was no significant effect on TG, and only very modest improvements in ROTEM parameters with none reaching normal values following platelet transfusion (141). The success of conservative transfusion policies employed in patients undergoing liver transplantation, where very much lower platelet counts are tolerated, unless there is active bleeding, calls the practice of prophylactic platelet transfusions for less invasive procedures into serious

question (142). A recent randomised controlled study in 60 patients with cirrhosis published in 2015, demonstrated that prophylaxis with FFP and/or platelets prior to invasive procedures is significantly reduced when using TEG as compared to standard coagulation tests [platelet count ≤ 50,000 and INR ≥1.8], without any increase in bleeding complications (De Pietri L et al. E-pub Hepatology 2015). Only 16.7% of the TEG group received any transfusion, whereas all patients in the standard of care group received a transfusion. There was only one post procedural bleeding episode (high volume paracentesis) and this was in the group using conventional coagulation parameters.

3.2.2. Heparin like effect (HLE) and CLD:

The native TEG is extremely sensitive to the presence of heparin and heparin like substances. Coppell et al investigated the effects of unfractionated heparin (UFH), low molecular weight heparin(LMWH) and danaparoid on native and heparinase TEGS. The difference between parameters in these two tests was able to differentiate between a range of low concentrations (0.005-0.05U/ml) of these heparin like substances and demonstrated a clear dose response, and in the case of UFH there was greater sensitivity than with anti-Xa activity. (143). Although the standard assay for monitoring LMWH is by inhibition of factor Xa (anti-Xa activity), this test is not routinely available at all institutions, and there are some concerns relating to inter-assay variability. Whilst native TEG is undoubtedly the most sensitive method to detect low concentrations of heparin, kaolin activated TEGS have also been found to be a useful method to monitor and guide LMWH therapy in sick hospitalized patients, where co-morbid conditions can impact on both the pharmacodynamics and pharmacokinetics of LMWH (144)

In recent years there has been increasing interest in the detection of, and the significance of endogenous heparins. Under conditions of endothelial stress, such as surgery or sepsis, endogenous release of very small quantities of glycosoaminoglycans (GAGS) may be detected systemically (145). Minor disturbances of the endothelial glycocalyx can lead to the selective cleavage of heparan and chondrotin sulphate sidegroups from the luminal layer of the glycocalyx. Where there is more significant damage to the vascular endothelium from ischaemia or sepsis, systemic activation of coagulation is promoted, and it is thought that this shedding of GAGS into the circulation is an adaptive response to keep a progressively more pro-coagulant microvasculature open by means of endogenous heparinization (146). Five percent of patients with severe traumatic injury have evidence of acute endogenous heparinization on TEG, and given that their levels of syndecan 1 are also significantly increased, this can be mechanistically linked to endothelial glycocalyx degradation.(147). When shed, the glycocalyx GAGs retain their anticoagulant activity and this is detectable by a prolonged R value on TEG analysis. These endogenous GAGS may represent an increase bleeding risk for some patients (29, 148) and demonstration of their presence may provide clinically useful information. In a prospective observational study in 30 patients with cirrhosis, Mancuso et al demonstrated that citrated samples, (allowing a delay in running the analysis) give comparable results to samples that are run immediately and facilitates the logistics of using TEG when it is not close to the patient.(149). Bacterial infection in cirrhosis induces a HLE detected by TEG (29) and this reverses with antibiotics and resolution of the infection [Figure 3.3]. Of 30 patients with infection, 28 had significantly improved TEG parameters in the heparinase modified TEG, indicating a significant heparin effect, this HLE disappeared after the infection resolved.

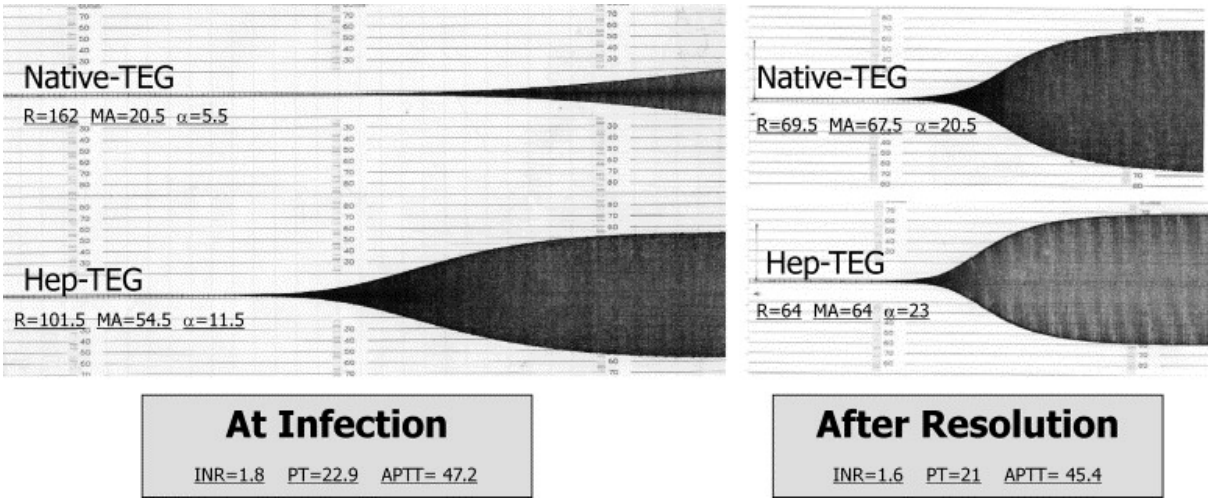


Figure 3.3 Heparin like effect (HLE) demonstrated in cirrhotic patients with infection after *Montalto P et al. J Hepatology 2002*)

HLE is associated with detectable anti-Xa activity (150, 151) and appears to differentiate patients at increased risk of variceal re-bleeding(152).In contrast, none of the standard laboratory tests of haemostasis (INR, PTT and platelet count) differed between those that re-bled and those that did not.

A transient HLE in systemic venous blood after transjugular intrahepatic portosystemic shunt (TIPS) has been reported, suggesting a high concentration of heparinoids in the portal venous system prior to TIPS placement. (153)

3.2.3 Hypercoagulability and CLD

Hypercoagulability may have an important role in many aspects of liver disease and intrahepatic microthrombi have been implicated in the progression of fibrosis (154). Portal vein thrombosis (PVT) is a common complication of liver cirrhosis, with an incidence of 10-25%, with a greater tendency to thrombosis with more severe liver disease (155). Reduced portal blood flow and blood vessel damage may play an important role in the increased risk

of PVT (156), but the haemostatic status may also be an important contributory factor. In cirrhosis, the ratio of the two most powerful pro and anticoagulants in the plasma, factor VIII and protein C respectively, show a balance strongly in favour of factor VIII indicating hypercoagulability (157) and it is now appreciated that the risk of venous thrombosis is often greater in patients with cirrhosis than in those without liver disease (158)

Ben-Ari et al evaluated hypercoagulability in patients with primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) using TEG. 28% of patients with PBC and 43% of patients with PSC were found to be hypercoagulable compared to only 5% of non cholestatic cirrhosis and none in healthy controls. (32) In contrast, INR did not differ between patients with cholestatic versus non cholestatic cirrhosis. These observations may explain why patients with PSC and PBC have fewer bleeding complications and lower intraoperative transfusion requirements during liver transplantation. The relative hypercoagulability is ascribed to increased platelet reactivity and higher fibrinogen concentrations in patients with cholestatic liver disease (33)

In a prospective, observational study in non alcoholic fatty liver disease (NAFLD) using TEG, a significantly stronger clot development was found in patients compared to healthy controls (MA 58.3 +-6.3*vs*52+-10 mm p=0.01,) and the platelet contribution to overall clot strength was higher in NAFLD patients with a trend to reduced inducible clot lysis (p=0.03) (159). It has been well described that patients with NAFLD are relatively prothrombotic and have an increased incidence of thrombosis (160). In a prospective study in 23 patients with obstructive jaundice, 80% were found to be hypercoagulable on TEG analysis (increased MA) and this was independent of prolonged PT times. A repeat TEG three weeks after a biliary drainage procedure, showed all TEG parameters had returned to normal range.(161)

The clinical implications of these findings have yet to be evaluated. However, emerging evidence suggests that hypercoagulability detected by VET puts patients in an “at risk” group for both venous and arterial thrombotic events. (134, 162, 163) . A recent systematic review of 10 studies in surgical patients showed an increased MA to be the most important parameter to predict postoperative TE events. However, there was considerable variability as to which parameters were used to define hypercoagulability and no study was adequately powered. Nevertheless, the vast majority of patients who had a TE event were hypercoagulable on one or more TEG parameters (164) and future prospective studies are recommended.

3.3 Viscoelastic Tests and Acute Liver Disease (ALD)

3.3.1 VET parameters and ALD

In over 1000 patients reviewed by The Acute Liver Failure Study Group (ALFSG) the mean INR was 3.8 (165) Patients with ALF are assumed to have a bleeding diathesis based on an elevated INR. However, clinically significant bleeding is rare. Although blood clot formation by TEG is generally preserved in stable patients with cirrhosis (29) patients with acute liver injury (ALI) and ALF have not been extensively studied.

As an ancillary project of the ALFSG, Stravitz prospectively studied 51 patients with ALI/ALF with kaolin initiated TEG (166). Despite a mean INR of 3.4 (range 1.5 -9.6) mean TEG parameters were within normal limits for the entire study population, and all 5 individual TEG parameters were completely normal in 63% of patients suggesting that the dynamics of clot formation are generally well preserved. Moreover, 8% of patients were hypercoagulable. The TEG was significantly more sensitive than INR for predicting bleeding,

with the R time being significantly more prolonged in those that bled (6.4 vs 4.5 secs) [Table 3.1], whereas the INR was not significantly different in those who bled and those who did not.

Complication		N	R Time (min)	K Time (min)	α-Angle (degrees)	Maximum Amplitude (mm)
Encephalopathy	ALI	14	4.2 ± 1.1	3.3 [0.9-20.0]	56.6 ± 14.5	50.4 ± 12.8
	ALF	37	4.9 ± 2.2	1.6 [0.8-10.5]**	66.3 ± 10.2**	56.8 ± 9.7*
Infection	Absent	38	4.3 ± 1.2	1.8 [0.9-20.0]	64.1 ± 11.5	54.1 ± 10.6
	Present	13	6.0 ± 3.0**	1.6 [0.8-10.5]	62.4 ± 14.6	57.7 ± 11.8
Renal Failure	No CVVH	30	3.9 ± 1.1	2.0 [0.9-7.6]	64.8 ± 7.9	53.6 ± 8.6
	CVVH	21	5.8 ± 2.3***	1.5 [0.8-20.0]	62.0 ± 16.7	57.0 ± 13.4
Thrombosis	Absent	40	4.5 ± 1.5	1.8 [0.8-20.0]	64.2 ± 11.7	54.7 ± 10.5
	Present	11	5.7 ± 3.0	1.5 [1.1-10.5]	61.6 ± 14.4	56.3 ± 12.5
Bleeding	Absent	45	4.5 ± 1.6	1.8 [0.8-20.0]	63.2 ± 12.7	54.8 ± 11.1
	Present	6	6.4 ± 3.5*	1.6 [1.9-3.3]	67.1 ± 7.7	56.8 ± 9.7
Overall Survival	Alive	37	4.3 ± 1.4	1.8 [0.8-20.0]	64.7 ± 11.8	55.2 ± 10.8
	Dead	14	5.9 ± 2.6**	1.7 [1.0-10.5]	61.0 ± 13.2	54.7 ± 11.5

Table 3.1 TEG parameters and complications in patients with ALI/ALF *(Todd Stravitz et al. J Hepatology 2011*)

The MA was higher in ALF than ALI and correlated with increasing severity of liver injury. The preservation or even increase in MA in patients with ALI/ALF may be due to increased factor VIII levels, decreased ADAMTS13 activity, increased vWF and increased levels of fibrinogen and or platelets as acute phase reactants. As the severity of the SIRS response increased there was a corresponding increase in MA [Figure 3.4].

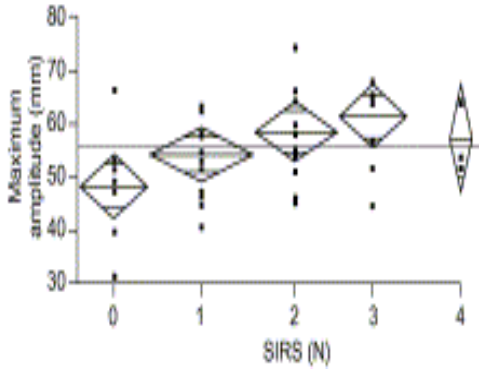


Figure 3.4 Change in MA with increasing severity of SIRS response

This important study demonstrates TEG parameters in ALF/ALI are generally well preserved and potentially provides an explanation for why clinical bleeding is rare despite the elevated INR. The authors conclude that INR, although a valid indicator of prognosis, is not a good guide for administration of procoagulant therapy.

In a prospective study, in our own institution of 20 patients with ALF admitted to the intensive care unit, coagulation analysis was performed on admission and at 48 hours. CCT suggested a markedly hypocoagulable state with a significantly raised INR (mean 4.3), however TEG values were hypocoagulable in only 20% of patients, whilst 45% had normal and 35% had hypercoagulable profiles. All patients with hypocoagulable TEGs had platelet counts < 100,000 (167). The fact that 80% of these patients with ALF had normal, or even hypercoagulable TEG profiles is evidence of the rebalancing of haemostasis, with the fall in procoagulant levels counterbalanced by low levels of anticoagulant proteins, together with significant increases in VWF and factor VIII [Figure 4].

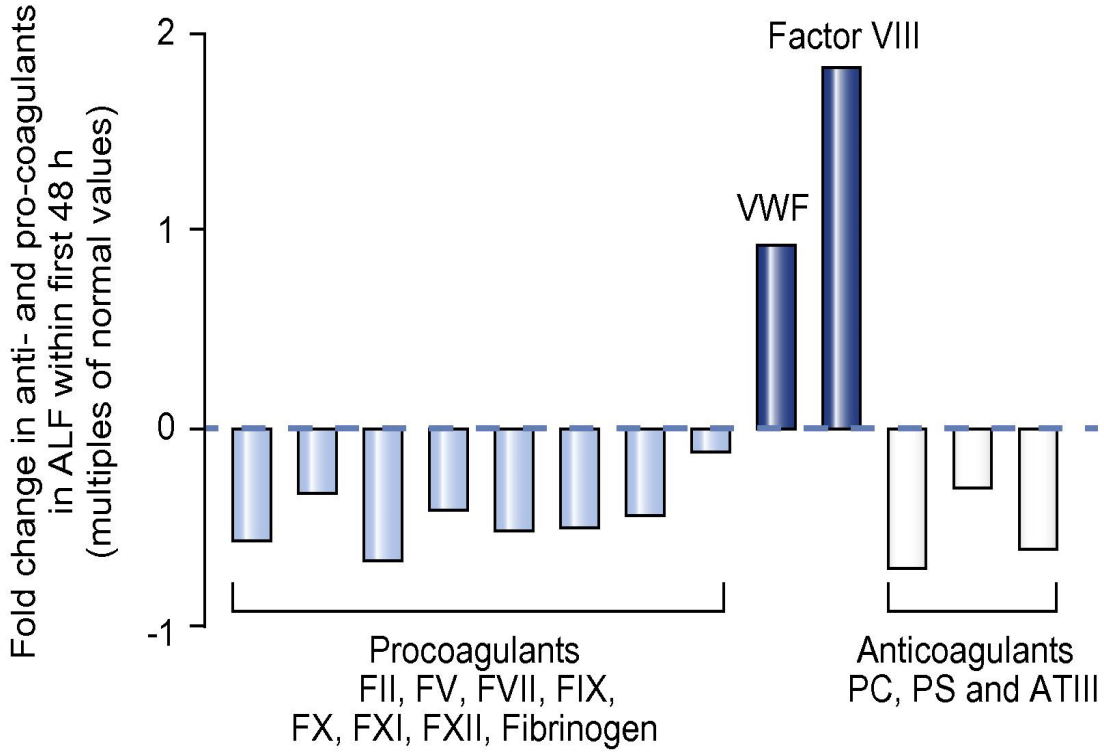


Figure 3.5 Pro & anticoagulant levels in acute liver failure *(Banwari et al. J Hepatology 2012*)

3.3.2 Heparin like effect & ALF

A HLE is commonly seen in ALF (168, 169). This HLE is thought to be due to the release of endogenous heparinoids and reflects the vascular endothelial injury inherent with acute liver injury . In ALF, the R time is significantly increased in the presence of infection, renal failure and in those with bleeding complications (166). In an observational study comparing TEG parameters in ALF to those in cirrhosis, Senzolo et al found that R and K time and alpha angle on native TEGs were significantly more hypocoagulable in ALF patients undergoing OLT compared to control stable patients with cirrhosis. These TEG changes were ascribed to endogenous heparinoids as heparinase reversed these differences (170). Using the TEG "V" curve as a surrogate for thrombin generation, TTG was generally found to be similar to normal controls. Therefore, although endogenous heparinoids slow the velocity of initial

clot formation, they did not ultimately affect final clot strength. Heparinase modified TEG should be considered as a useful adjunct in the assessment of coagulopathy in ALF.

3.4 Anticoagulation and Liver Disease

Patients with liver failure have traditionally been managed with no, or minimal anticoagulation, because the abnormal clotting tests are perceived to reflect an increased bleeding risk. However, patients with cirrhosis can develop DVT despite a prolonged INR and can do so even when receiving antithrombotic prophylaxis (171). In addition, as many as 5-20% of patients with advanced liver disease will develop portal vein thrombosis (PVT) each year (172). Anticoagulant drugs should be administered with caution in patients with liver disease. The bioavailability of heparin and LMWH cannot be assumed to be stable in patients with liver disease as this will be affected by fluctuations in liver synthetic function and also changes in hepatic clearance and renal function(173). Anticoagulation with heparin to reduce the incidence of vascular thrombosis after liver transplantation, is well known to be difficult to control, and despite monitoring ACT levels , neither thrombotic, and particularly bleeding complications, are avoided (173). Alternative methods of monitoring the anticoagulant status in these patients is a real necessity, that is yet unmet. It is known that patients with cirrhosis show an increased response to LMWH and this correlates with the severity of liver disease. (174)

LMWH are routinely prescribed for VTE prophylaxis in general medical and surgical patients in a standardised dose and monitoring is generally thought to be unnecessary. However, in patients with liver disease, effective and safe dosing is more problematic. Anti-factor Xa levels are the gold standard for monitoring LMWH activity but these tests are not routinely available, they are expensive and standardisation between different laboratories can be a

problem (175). In addition monitoring anti-Xa levels in cirrhosis is unreliable due to the low levels of antithrombin(176). An increasing number of published papers suggest that VET may be a useful way to assess the efficacy of LMWH therapy in general surgical patients with various co-mobidities that will affect the pharmacokinetics of these drugs . Van et al, measured anti-factor Xa levels and performed simultaneous kaolin and heparinase TEGS in 61 surgical ICU patients (261 time points) all receiving prophylactic therapy with Enoxaparin: 17 patients developed a DVT. Overall there was a mean increase in TEG R value in the kaolin trace compared to the heparinase trace, demonstrating that TEG is able to quantify functional anticoagulation. In the group that developed a DVT, there was no significant difference between R values of kaolin TEG and heparinase TEG, suggesting that these patients were not receiving adequate thromboprophlyaxis(177). Performing simultaneous kaolin and heparinase TEGs appears to be a sensitive methodology for detecting evidence of anticoagulation with LMWH, and at the low doses used for prophylaxis is a better differentiator than anti-factor Xa.

A recent prospective randomised control study of fixed dose prophylactic LMWH versus no therapy administered for one year in 70 patients with advanced cirrhosis, demonstrated that no patients in the enoxaparin group developed PVT compared with 17% in the control group. In addition, the incidence of documented bacterial infections was significantly lower in the enoxaparin group (8.8% vs 33.3%). Surprisingly, there were no reports of haemorrhagic complications in the treated group.(178) This study raises interesting hypothesis as to whether LMWH act by improving intestinal microcirculation and thereby reduce the frequency of portal endotoxaemia. In addition, it is possible that systemic anticoagulation reduces the formation of intrahepatic microthrombi which are implicated in

the progression of portal hypertension and parenchymal extinction over time (179). The ability to monitor the efficacy and safety of anticoagulant therapy in patients with liver disease is becoming a real clinical dilemma, a challenge that could be met, in part, by using point of care viscoelastic tests of coagulation.

It is known that following liver transplantation, there can be a temporary hypercoagulable state, due to the imbalance between pro and anticoagulant systems and the post operative fibrinolytic shutdown(180). It has been suggested that these haemostatic changes, as well as technical and surgical factors, may have a role in the early development of hepatic artery thrombosis (HAT)(181). In an observational study of 298 liver transplant patients, high fibrinogen levels and low protein C levels were significantly associated with post-transplant thrombotic events(182). The optimal anticoagulant regime in these patients is still an open question, and in the first week post transplantation using heparin with monitoring based on CCT still leads to significant bleeding complications in certain patients (173, 183). Large scale prospective outcome studies are necessary to evaluate the impact of VET in managing thromboprophylaxis in these groups of patients. Anticoagulant therapy in patients with Budd Chiari syndrome (BCS) is also challenging and major bleeding, especially during invasive procedures, is common (184). A recent case report of a complex patient with BCS and a TIPS occluded with thrombus, describes the use of TEG to guide the successful management of anticoagulant therapy and resultant re-canalisation of the stent(185).

3.5 Conclusions

The complex haemostatic changes that occur in liver disease are difficult to assess using conventional coagulation tests. These tests are known to be poor predictors of bleeding risk and also, importantly, thrombosis. Consequently, the routine use of CCT to assess

coagulation in patients with liver disease needs to be re-assessed. VET have been used for coagulation monitoring and to guide haemostatic therapy in liver transplantation for many years, but to date they have not been used to any great extent in hospitalised patients with liver disease. The summative information provided by these tests has the potential to be used in future clinical studies to determine a means of stratifying bleeding and thrombotic risk in these patients. It is clear that haemostasis is critically dependent on platelet number and function and fibrin clot formation, which are not evaluated by CCT. The current lack of randomised controlled trials of coagulopathy in liver disease is largely due to the inability to develop satisfactory surrogate end points in measuring coagulation. Global coagulation tests such as TEG/ROTEM could provide the basis on which to develop such criteria. It is to be hoped that the new understanding of the haemostatic changes in liver disease, together with the knowledge that VET give more clinically relevant and comprehensive information than conventional coagulation tests will stimulate interest in producing the large prospective outcome studies that are needed to establish the clinical utility of VET in liver disease and to determine threshold values of VET that predict bleeding or thrombosis and thus optimise haemostatic and antithrombotic interventions.

Chapter 4

Liver Transplantation and Viscoelastic Tests of Coagulation

This chapter formed part of a review article published in Seminars of Thrombosis and Haemostasis in 2015

Mallett SV. Clinical utility of viscoelastic tests of coagulation (TEG/ROTEM) in patients with liver disease and during liver transplantation. Semin Thromb Hemost 2015,41:527-37

4.1 Bleeding and coagulopathy during Liver Transplantation.

Historically liver transplantation (LT) was accompanied by substantial blood loss, however, improvements to all aspects of the process, from graft preservation through to surgical techniques and anaesthetic management, have led to an increasing number of patients being able to undergo LT without the need for transfusion of red blood cells or blood products (3, 186), although there continues to be a small, but significant proportion of patients that will require massive transfusion.

Marked variations in inter-institutional transfusion requirements for LT still persist (187). Varying transfusion thresholds, particularly in relation to the use of fresh frozen plasma (FFP), differences in the way coagulation is (or is not) monitored, the use of cell salvage, fluid management strategies, and use of anti-fibrinolytic therapy, all lead to wide variations in blood product use (97, 188). Preoperative hemoglobin is the most significant predictor of the need for red cell transfusion (189). Fluid management is very important, and restrictive fluid administration in the dissection phase can minimise haemodilution, with associated fall in haemoglobin and clotting factor levels, as well as limiting rises in splanchnic and portal pressure that will exacerbate bleeding. The aetiology of liver failure is an independent parameter for the prediction of massive blood loss, for example patients with cholestatic liver disease have reduced bleeding risk compared to patients with viral or alcoholic cirrhosis (190). It is well known that preoperative PT/INR is not predictive of the need for transfusion, although there is a suggestion that higher values of INR (>2.0) may be associated with an increased bleeding risk (191). It is of note that transplant units that report the lowest rate of blood and blood product use, have adopted aggressive fluid

restriction, tolerance of low haemoglobin thresholds, and treat only when there is active bleeding, avoiding prophylactic therapy (3).

There is an obvious need to develop consensus guidelines for transfusion practice in liver transplantation and to determine which method of coagulation monitoring and which transfusion thresholds are optimal (192). The inverse relationship between the number of units of RBC transfused intraoperatively, and patient survival is well known, consequently any reduction in transfusion requirements will impact positively on patient outcome (193). The concepts of Patient Blood Management programs, with a multimodal approach to limiting inappropriate and unnecessary transfusions, should be advanced for all surgical procedures, including liver transplantation , as this leads to reduced exposure to allogeneic products, with their immunomodulatory and other adverse consequences, and ultimately reduces hospital costs (194).

4.2 Viscoelastic tests and coagulation management in Liver Transplantation

Coagulation monitoring with TEG/ROTEM can reduce overall transfusion requirements as empirical therapy is eliminated and specific management of coagulation defects is instituted at an early stage. (83, 195). TEG-guided transfusion algorithms to treat coagulopathy in OLT were first introduced in the early 1980's by Kang at the University of Pittsburgh, who showed that using an algorithm based on TEG, transfusion requirements were reduced by 30% compared to an historical cohort (83). In the context of bleeding during liver transplantation, VETs are particularly useful for detecting the presence of systemic fibrinolysis, and to also to detect poor clot strength, which is often the result of low fibrinogen levels (196). Although there is moderate to good correlation of MCF with Clauss fibrinogen (r= 0.59) and platelet count (r= 0.79), there is no correlation between CT and

PT/INR (r=0.22)(138) This consistent finding questions the routine use of, and need for FFP in liver transplantation. Unless there is massive haemorrhage, the use of FFP may be counterproductive as the volume loading will increase splanchnic pooling and portal pressures and may increase blood loss (197).

Serial coagulation monitoring with VET can detect early deterioration of coagulation and facilitates goal directed treatment therapy. (198) Roullet et al in a prospective observational study of 23 patients undergoing liver transplantation compared standard coagulation tests, coagulation factor levels (II,V,X and VIII and anti-thrombin) and Euglobin clot lysis time (ECLT) and PAI with ROTEM analysis (EXTEM, INTEM, APTEM and FIBTEM) at 6 time points during the procedure. Clot amplitude on EXTEM at 10 minutes (A10) correlated well with platelet count (R^2=0.46) and fibrinogen (R^2=0.52) and FIBTEM A10 showed moderate correlation with Clauss fibrinogen (R^2 =0.55) ROC analysis showed that EXTEM A10 with a threshold of 26mm predicted hypofibrinogenaemia with a sensitivity of 83% and specificity of 75% (199). The authors concluded that ROTEM is helpful for the detection of hypofibringinaemia and thrombocytopenia. Blasi et al, in a prospective observational study of 236 patients undergoing liver transplantation, found that whilst the MCF was reliably able to detect low platelet and fibrinogen levels, correlation of CT and PT was poor and therefore more studies are required to determine when FFP should be administered based on VET (200). A recent small randomised, prospective study in 28 OLT patients showed a significant reduction in transfusion in the TEG monitored group, most notably for the use of FFP, as the trigger threshold for transfusion was reached much more frequently using conventional INR values compared to R values on TEG (201). However transfusion trigger thresholds described for viscoelastic tests have not been validated, and large controlled clinical trials

comparing strategies of coagulation management and cut off values for transfusion of blood product components are needed (202). Transfusion algorithms using specific trigger thresholds developed for use with the ROTEM are not directly transferable to TEG and vice versa (97)

A prospective observational study of 20 OLT patients found that fibrinogen replacement therapy based on VET reduced the requirement for platelet and red cell transfusion compared to historical controls (120) Plasma fibrinogen levels control the mass amount of fibrin formed by thrombin, and hence clot strength, and assessment of fibrinogen by VET is critical for managing bleeding .

Prophylactic recombinant activated factor VII has not been found to reduce transfusion requirements in OLT (203), but it is still occasionally used as “rescue therapy” in situations of uncontrolled blood loss. It is of note that when POC coagulation testing with VET is routine, there is rarely any necessity to use rVIIa as rescue therapy (107). Although rVIIa does effect the physical properties of the clot as measured by VET, this does not necessarily reduce transfusion and may lead to more thromboembolic events, especially arterial TE (204). In a small pilot study in OLT patients, Hendriks et al. found that 80mcg/Kg of recombinant activated factor VIII shortened the INR and also reduced the R value and increased the α angle and MA on the TEG(205), but did not affect transfusion requirements.

The use of goal-orientated algorithms based on VET facilitate targeted transfusion therapies with specific haemostatic agents and avoids the empirical administration of multiple components with potentially hazardous effects , and are recommended (Grade 1C) in recent guidelines for the management of severe bleeding (206). The short turn-around times of VET (10-20 minutes) are important for guiding therapy and preventing inappropriate

transfusion during surgery and on the ITU (207). The use of algorithms, although reducing transfusion requirements compared to historical cohorts, often leads to changes in the type and amount of hemostatic support given, with many European units using increasing amounts of factor concentrates, including prothrombin complex concentrates (PCCs) and fibrinogen concentrates (196, 208). Monitoring coagulation with basic VETs, without assessment of fibrinogen (FIBTEM or TEG functional fibrinogen) can lead to increased transfusion of platelets to increase the MA/MCF (209). Using VETs to specifically assess fibrinogen levels can avoid platelet transfusion, where goal-directed substitution of fibrinogen is more appropriate. This is especially important in liver transplantation as platelet transfusion is associated with significant reductions in one year survival (36). Although there does not appear to be increased risk of thromboembolic events as a result of using fibrinogen concentrates, there remains some concern about the safety profile of PCCs, as in the setting of trauma, it has been demonstrated that in patients receiving PCC, compared to those that did not, endogenous thrombin potential is increased for several days post operatively, and also that Antithrombin (AT) levels remain low, implying a potential prothrombotic state not reflected by standard coagulation tests (210). The results of the PROTON study, a randomised controlled study of PCC in LT are awaited with interest (211).

There may well be a place for PCC in treating severe clotting factor deficiency, such as in dilutional coagulopathy, where there is marked prolongation of the R/CT and loss of the normal thrombin generation profile on VET (increased K/CFT and decreased α angle), but this needs to be validated in future trials. It took a number of years before the increased risk of arterial TE events associated with the use of recombinant factor VIIa (rFVIIa) was

recognized , another drug that acts by enhancing thrombin generation (212). It is now recommended that rFVIIa should only be used as rescue therapy (206), and as noted above, the need to use rFVIIa in the circumstances of uncontrolled haemorrhage, is virtually never necessary when coagulation is routinely monitored with VET, suggesting that this type of monitoring results in earlier, and more effective, haemostatic inteventions (213).

Although there are many reports of the success of VET monitoring in reducing transfusion requirements in LT, this must be seen in the context that the majority of these studies generally compare these results with historical cohorts, where in many cases, there was a relatively high baseline transfusion rate (120, 195, 201). A more recent prospective study, of 60 LT patients, with and without ROTEM monitoring, did not demonstrate any significant differences, but overall transfusion was low, with a significant number of patients receiving no transfusion at all (214). It is difficult to extrapolate the results of earlier studies to the current situation, as bleeding and transfusion management continues to evolve with many interrelated factors leading to lower transfusion rates. It is clear, however, that the implementation of dedicated liver transplant anaesthesia teams and the use of transfusion protocols leads to improved transfusion practice (215).

4.3 Conclusions

The transfusion trigger thresholds described for VET have not been validated, and values may need to be substantially outside normal ranges before intervention is indicated (216). A small proportion of patients undergoing LT will inevitably have massive blood loss, and there is no doubt that VET can be useful in these circumstances to facilitate goal-directed therapy, and assess the efficacy of any treatment intervention. Finally, the fact that up to 60% of VET traces in patients with chronic liver disease are within normal ranges, despite

hypocoagulable CCTs, is commensurate with the concept of “re-balanced” hemostasis, and the reality that an increasing number of patients are able to undergo this major surgery without the need for blood or blood products.

Conventional coagulation tests are unable to give any useful information on either fibrinolysis or the presence of hypercoagulability. We were interested in exploring in more detail the area of diagnosing and managing fibrinolysis during liver transplantation, and the implications for reducing bleeding, and also in determining the relative prevalence of hypercoagulability and the possible implications for bleeding and thrombotic complications in patients undergoing liver transplantation. These two areas are examined in further detail in the next two chapters.

Chapter 5

Fibrinolysis during Liver Transplantation

A retrospective cohort study in liver transplant patients comparing prophylactic versus treatment only with anti-fibrinolytic drugs forms the basis of this chapter. This was published in Liver Transplantation in 2014.

Schofield N, Sugavanam A, Thompson K, Mallett S.V. No increase in blood transfusions during liver transplantation since the withdrawal of aprotinin. Liver Transplantation 2014;20:584-590

5.1 Introduction

Increased fibrinolytic potential is well described in patients with chronic liver disease and it is known that enhanced fibrinolytic activity can occur at any point during liver transplantation, but particularly during the anhepatic period, due to high levels of tissue plasminogen activator (tPA) as hepatic clearance is compromised (217). This is often followed by a dramatic increase in tPA immediately after reperfusion, which can be associated with explosive primary hyperfibrinolysis (218), with some patients developing diffuse uncontrolled bleeding. In the presence of good graft function, hyperfibrinolysis after reperfusion is usually self-limiting and does not require treatment, but in the presence of a poorly functional or marginal graft it may persist, and will require treatment with antifibrinolytic drugs such as tranexamic acid (1-2 g) (219). Fibrinolysis is rarely seen in acute liver failure due to the high levels of PAI-1. (220)

The use of antifibrinolytic therapy with the lysine analogue epsilon aminocaproic acid (EACA) to reduce blood loss was first described in liver transplantation (LT) in the 1980's (83). The success of aprotinin, a serine protease inhibitor of plasmin, and at higher doses kallikrein, in reducing bleeding in cardiac surgery, resulted in its being used in liver transplantation in European centres from the early 1990s onwards, with reports of reduction in the incidence of bleeding caused by fibrinolysis, compared to historical cohorts (221). The first multicenter randomized controlled study, by Porte et al (222), showed that the intra-operative use of aprotinin in patients undergoing OLT, significantly reduced blood and blood product transfusion (FFP) requirements. A subsequent systematic review in 2008 of the use of aprotinin in LT confirmed statistically lower transfusion requirements

compared to control groups, with no difference in the incidence of thrombotic events, however, there were concerns about the power of these studies to satisfactorily exclude this risk (223).

The potential for thrombotic adverse effects from the use of aprotinin in cardiac surgery had been known for some time, and in 2008, the publication of 'The Blood Conservation Using Antifibrinolytics in a Randomized Trial' (BART), showed a strong and consistent negative mortality trend associated with aprotinin, compared to lysine analogues, resulting in the study being terminated early (224, 225). Case studies reported concerns about the potential increased risk of thrombotic events in patients undergoing liver transplantation (226, 227), but a systematic review and meta-analysis did not confirm this association (228), nor did a subsequent observational study in over 1400 LT patients (229). Nevertheless, the data from the cardiac studies was sufficiently compelling that the product license for aprotinin was withdrawn in many countries, with the result that there was a virtually complete cessation of use.

Although the use of antifibrinolytic therapy was standard practice in many centres that undertook liver transplantation, there was, and remains, significant variability as to which drug was used, the dose required, and the timing of administration. At the time aprotinin was withdrawn from the market, most European centres were using it in a prophylactic regime in all high risk cases. The abrupt withdrawal of aprotinin from clinical use led to concerns that this would lead to an increased risk of bleeding and transfusion requirements during LT, and this fear appeared to be confirmed in an observational study comparing transfusion requirements with aprotinin and then after its withdrawal from routine clinical

practice (230). However, as blood transfusion requirements for LT were already steadily decreasing to a mean of 2-4 units per case, many transplant units, including our own, went from a regime of prophylactic treatment with aprotinin or tranexamic acid (TA) to a policy of treatment with TA only in the case of diffuse bleeding and/or evidence of fibrinolysis on viscoelastic tests.

5.2 Aims of Study

The presence of fibrinolysis can be detected using thromboelastography or thromboelastography, by assessing alterations in the clot lysis index. In our institution TEG studies are run at multiple time points, according to a standardized protocol during the peri-operative period, and blood and blood product transfusion is recorded by stage of the procedure. We sought to identify firstly whether the aprotinin ban had had a detrimental effect on blood loss during liver transplantation in our patient population, and secondly to determine the prevalence of fibrinolysis in the absence of prophylactic anti-fibrinolytic therapy, and the impact of fibrinolysis diagnosed on VET monitoring on transfusion requirements.

5.3. Methods

The routine use of aprotinin in our institution was stopped in 2007. This study was based on a retrospective analysis of patients undergoing liver transplantation, between 2004 and 2010. All data routinely collected in our liver transplant database is anonymized, and as all patients consent a priori to data collection for research purposes, institutional approval for this analysis was waived. Consecutive patients treated with aprotinin prior to 2007 (APRO

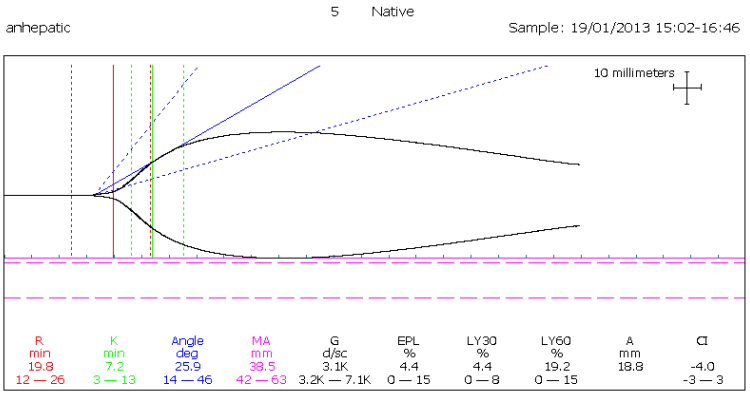
group, n=100) were compared with a group in which aprotinin was not used after 2007 (NO-APRO group, n=100). Patients were excluded from this analysis if aprotinin was not used in the first time period or if there was incomplete data for analysis. A cross over period of two years was used, in which patients were not included, as there was sporadic use of aprotinin during this time. During the first time period (01/05/2004 -01/06/2007) a total of 188 LT were performed. During the second period (01/10/2009 – 01/12/2010) a total of 121 LT were performed. Propensity score matching was performed on each group to identify two matched cohorts, and patients were matched for primary diagnosis and model for end stage liver disease (MELD). This resulted in two matched cohorts of 55 patients in each group.

Information was gathered using the hospital liver transplant database and case note review. Blood product usage by stage of procedure was recorded. Cell saver blood conservation was used during all LT. The anaesthetic and surgical teams were largely unchanged during the study period, and intra-operative management remained similar, although in more recent years there was a trend to more active fluid restriction during the dissection period. Transfusion protocols were the same for all groups of patients using an algorithm based on TEG and point of care testing of haemoglobin and platelet count, taken at set periods during the procedure according to our institutional practice. A transfusion trigger threshold of 80g/l was used for red blood cells (RBC) transfusion, and blood products were administered only in the presence of diffuse bleeding and abnormal TEG findings, or during massive uncontrolled haemorrhage. A prolonged R value was treated with FFP, and a reduced MA treated with either cryoprecipitate (or fibrinogen concentrate) or platelet transfusion, depending on the cause of the reduced clot strength. The presence of fibrinolysis (clot lysis index (CLI) at 30 minutes) was reviewed in all heparinase samples. Fibrinolysis was graded as

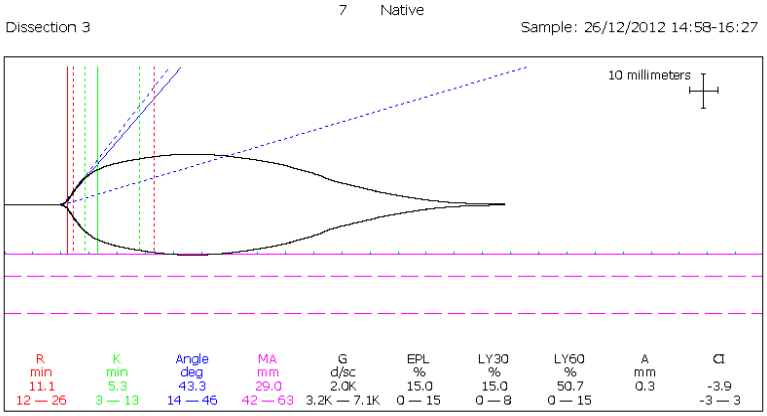
none (CLI <15%), mild (CLI 15-30%), moderate (CLI 30-60%) and severe (CLI >60%) (Figure 5.1).

The timing, usage and dose of alternative antifibrinolytic therapy (tranexamic acid) was correlated with TEG findings and stage of surgery. Data for patients receiving a massive transfusion (six or more units of packed red cells) were also reviewed. Wilcoxon matched-pairs were used for non-parametric transfusion data analysis between the two groups. Fishers exact 2x2 two-tailed test were used for comparison of massive transfusion data and presence of lysis between groups. For comparison of transfusion to lysis a four group Kruskal-Wallis test was used. Parametric data was analysed using unpaired t test. Values of $p<0.05$ were considered statistically significant. Statistical analysis was performed using Graphpad software (GraphPad Software, Inc., La Jolla, CA, USA).

1 Mild Fibrinolysis: CLI $_{60}$ >15 and < 30



2 Moderate Fibrinolysis: CLI$_{60}$ >30 and <60



3 Severe Fibrinolysis: CLI$_{60}$ > 60

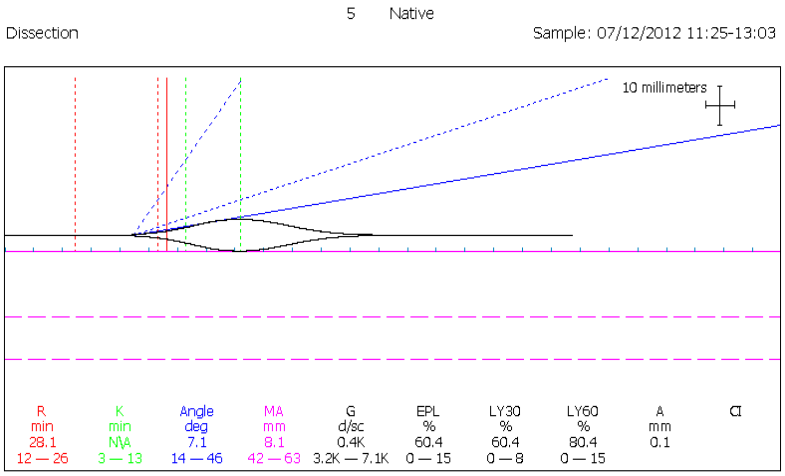


Figure 5.1 Grading of Fibrinolysis on basis of TEG traces

5.4 Results

Demographics

Within the two groups, prior to propensity matching there was evidence of both increased age in the second group, and increased use of DCD grafts. After propensity matching, a group of 55 patients treated with aprotinin were compared with a similar group in which aprotinin was not used. Patient demographics, reason for transplantation, graft type and MELD score are shown (Table 4.1 and 4.2).

Demographics	Aprotinin	No Aprotinin	P-Value
Age	51.04 (9.65)	52.96 (10.95)	0.37
MELD	15.51 (7.16)	16.22 (7.92)	0.62
Child Pugh	8.47 (2.12)	8.45 (2.35)	0.97
Weight (Kg)	74.22 (16.03)	79.24 (16.06)	0.18
BMI	25.98 (4.93)	27.26 (4.95)	0.17
Cold Ischaemia time (min)	532.4 (149)	475.2 (176)	0.07
DCD, DBD	2,53	2,53	NS

Table 5.1 Baseline characteristics of both groups [mean (standard deviation)]

Primary Diagnosis	**Aprotinin**	**No Aprotinin**	**Significance**
Alchoholic Liver Disease	20	19	NS
Hepatitis C	14	15	NS
Primary Biliary Cirrhosis	6	6	NS
Primary Sclerosing Cholangitis	3	3	NS
Hepatitis B	4	4	NS
Cryptogenic cirrhosis	3	3	NS
Chronic rejection	1	1	NS
Primary graft non function	1	1	NS
Acute liver failure	3	3	NS
Secondary Diagnosis			
Hepatocellular Carcinoma	9	9	NS

Table 5.2 Primary and secondary diagnosis for both groups

5.4.1 Prevalence of Fibrinolysis

No patient in the aprotinin group developed fibrinolysis (CLI <15) during transplantation. In the No-APRO group, 23.6% (n=13) of patients developed lysis at some stage during the operative period, of which 5 patients had mild lysis, 6 had moderate and 2 patients had severe lysis. This difference in fibrinolysis between groups was statistically significant ($p<0.0001$). When the presence of lysis was analysed by stage of operation: 33% occurred in the dissection phase; 27% in the anhepatic phase; and 40% occurred during the reperfusion stage. In the No-APRO group, of the patients that developed lysis, 85% (n=11) had only one episode of lysis and 15% (n=2) had lysis on two samples, which were both consecutive samples. Tranexamic acid (TA)) was used as an anti-fibrinolytic in a dose of 1-2g in 62% (n=8) of the No-APRO group in whom lysis was present, which resolved the fibrinolysis in all but one of these patients. In all but one patient with TEG evidence of fibrinolysis where the decision was taken not to administer TA, the fibrinolysis had resolved spontaneously by the next measurement. There was no significant difference in blood product requirements in the no aprotinin group when analysed by the severity of lysis. However, there was a trend to increased requirements in the two patients with severe lysis, but these numbers were too small to reach any statistical significance.

	RBC	FFP	Platelets	Cryoprecipitate
No lysis (n=42)	4 (1.25,7.5)	4 (2,6)	2 (0,2)	0 (0,0)
All lysis (n=13)	3 (2,6)	4 (2,4)	1 (0,1)	0 (0,0)
Mild lysis (n=5)	3 (2,3)	2 (2,4)	1 (1,1)	0 (0,1.5)
Moderate lysis	3 (1.25,5.5)	3.5 (2.25,5.5)	0 (0,0.75)	0 (0,1.5)
Severe lysis	12.5 (8.25,16.75)	7 (5.5,8.5)	3 (1.5,4.5)	0 (0,0)

Table 5.3 Comparison of blood component transfusion in the No Aprotinin group by degree of lysis (median (inter-quartile range).

5.4.2 Blood Product Transfusion and Blood Results

There was no significant difference in red cell or blood product transfusion between the aprotinin and non aprotinin groups [Table 5.4]. A similar proportion in both groups received no transfusion (19.61 vs 18.18%) (p=0.39) and the percentage of patients requiring a massive transfusion (>6 units) was similar in both groups (23% vs 24%). At the start of the case there was no significant difference in haemoglobin, platelets or INR, but there was a significantly lower Hb and and higher INR in the aprotinin group at the end of the case. There was no significant difference in TEG parameters in the two groups [Table 5.5]

	Aprotinin	No Aprotinin	P value
Total RBC	3 (1.5,5)	4 (2,6)	0.27
Total FFP	4 (3,7)	4 (2,6)	0.72
Total Platelet	1 (0,2)	1 (0,2)	0.07
Total Cryo	0 (0,0)	0 (0,0)	0.25

Table 5.4 Comparison of blood component transfusion between groups (median (interquartile range)

	Aprotinin	No Aprotinin	P Value
Start of case			
Hb	102.8 (16.8)	104 (9.8)	0.95
Platelets (x 10^9)	70.58 (29.77)	87.44 (47.05)	0.15
INR	1.76 (1.06)	1.87 (1.01)	0.57
TEG R time (s)	21.7 (12)	17.3 (9)	0.53
TEG MA (mm)	46.6 (14.1)	40.23 (12.35)	0.06
End of case			
Hb	85.0 (12.3)	107 (21.0)	0.001*
Platelets (x 10^9)	67.75 (34.81)	91.07 (54.66)	0.12
INR	3.03 (1.96)	1.83 (1.41)	0.01 *
TEG R time (s)	20.5 (9.10)	21.3 (10.57)	0.92
TEG MA (mm)	39.5 (6.09)	45.43 (6.550	0.32

Table 5.5 comparison of conventional and viscoelastic coagulation tests in the two groups at the start and end of case. (Mean (standard deviation)).

5.5 Discussion

Routine use of prophylactic antifibrinolytic agents was common in the early history of LT, as the massive blood loss was relatively common, and any potential risk associated with the use of antifibrinolytics was small in comparison. Concerns have always existed about the potential for thromboembolic complications when prophylactic antifibrinolytic therapy is routinely used. However, a review of over 1400 LT patients found no significant difference in arterial or venous thromboembolism in patients receiving aprotinin compared to no treatment (229). In addition a systematic review and meta-analysis of antifibrinolytic

therapy in LT found no increase in thrombotic complications (228). However, the lack of difference in thromboembolic events does not necessarily mean that there is no increased risk associated with the use of antifibrinoytics in a specific subset of patients, as relevant subgroups may be missed in meta-analysis. In addition, different drug doses were used in different studies. The risk-benefit balance has altered now that massive bleeding is less frequent, and there is a move away from prophylactic therapy towards selective (high risk patients) or treatment only. Prediction is difficult as hyperfibrinolysis-induced bleeding may become most pronounced in the post reperfusion stage of the operation and depends to a great extent on the quality of the donor liver, which is not reflected by the preoperative condition of the recipient (231). Treatment with antifibrinolytic therapy is increasingly recommended only when there is evidence of microvascular ooze and/or documented fibrinolysis (CLI > 15) on TEG/ROTEM (196).

We have shown that since the withdrawal of prophylactic aprotinin in 2007, that there is a significant increase in the prevalence of fibrinolysis on TEG analysis in our patients undergoing liver transplantation. However, the clinical significance of this is not entirely clear. The incidence of fibrinolysis in our patients who did not receive prophylactic antifibrinolytic therapy was only 26%, much less than we had expected, and brings into question the clinical case for prophylactic treatment in these patients. Indeed, in all but one case in this study where no antifibrinolytic was administered, the fibrinolysis had resolved spontaneously by the next measurement point. We have also shown that tranexamic acid is an effective alternative to aprotinin, leading to resolution of fibrinolysis in 85% of cases, confirming the findings of other institutions (232).

In the presence of good graft function, fibrinolysis is usually self limiting after reperfusion and does not always require treatment (233). The decision to treat should be based on the presence of diffuse bleeding, severity of fibrinolysis and the stage of the operation. Fibrinolysis occurring during dissection and the early anhepatic phase of surgery is more likely to require treatment, as it is unlikely to resolve spontaneously, and tends to increase in severity. After reperfusion of a marginal graft, fibrinolysis is more common, and can be severe, and we now routinely give tranexamic acid prior to reperfusion when a DCD graft is used (234).

In contrast to expectations, and the findings of some other groups, we did not show an increase in red cell, or other blood component transfusion requirements since the withdrawal of aprotinin from our routine practice. This may be partly explained by the ongoing reduction in transfusion requirements that are seen year by year, such that mean transfusions are now only 2-4 units (235), and the impact of antifibrinolytic therapy correspondingly less pronounced. The number of patients receiving no transfusions was also similar in the two groups, suggesting that the change in antifibrinolytic strategy has not had a detrimental effect on bleeding and transfusion requirements. It is of note that there was a trend to increased amounts of red cell transfusions as the degree of lysis increased, but this was not significant, possibly due to the small number of patients that developed severe lysis, and a type II error cannot be excluded. This is likely to be a useful area for further study as it appears that more severe degrees of lysis warrant treatment with antifibrinolytic drugs, and indeed is a factor in our decision to treat fibrinolysis.

Limitations of this study are the fact that data was compared from two non overlapping cohorts in different but adjacent time periods. Therefore, the risk of unrecognised changes

in practice cannot be excluded completely, but the study design, and the fact that the known variables were comparable, minimise this risk. This observational study enrolled a relatively large number of consecutive patients, however, the study did not have enough power to detect all important differences between the treatment groups.

5.6 Conclusions

In this study we have shown that the withdrawal of aprotinin from use in liver transplantation surgery has not had the predicted deleterious effects with regard to red blood cell and other blood component transfusion requirements. We have demonstrated that a viscoelastic test guided strategy tailored to the individual patient's risk of bleeding is as effective as routine administration of aprotinin to all high risk patients undergoing liver transplantation. Factors which may contribute to an increased risk of bleeding include severity of fibrinolysis, stage of operation, on-going bleeding and disease process. Further study is needed to validate individual risk factors for bleeding.

Chapter 6

Intraoperative hypercoagulabilty in patients undergoing liver transplantation

A retrospective database review of liver transplant patients to determine the prevalence of hypercoagulability as defined by viscoelastic tests is the basis of this chapter and published as a manuscript in Liver Transplantation 2013

Krzanicki D, Sugavanam A, Mallett SV. Intraoperative hypercoagulability during liver transplantation as demonstrated by thromboelastography. Liver Transplantation 2013;19: 852-861.

6.1 Introduction

It is increasingly recognised that patients with liver disease, despite the " coagulopathy" described by conventional coagulation tests, and the implied bleeding diathesis, are also at increased risk of developing thromboembolic complications (6). Although stable liver disease is characterised by a new "re-balanced" haemostatic profile, with a reduction in both pro and anticoagulants proteins, there is a limited quantitative reserve on either side of this coagulation equation. These patients therefore have a reduced ability to compensate for stresses to the system that would be effectively buffered in healthy patients with a larger functional reserve (236).

Hypercoagulability, or a prothrombotic environment, may be associated with macrovascular thrombosis, such as portal vein thrombosis, deep vein thrombosis and pulmonary emobolism. In addition, accumulating data indicates that microvascular thrombosis as a result of dysbalanced coagulation ,and also increased TF expression, may be implicated in non hepatic end organ damage and also in the progression from hepatic inflammation to fibrosis and atrophy (237).

Patients with chronic liver disease often have deranged conventional clotting tests (CCT) including prolonged prothrombin time (PT)/INR and activated partial thromboplastin time (APTT) as well as thrombocytopenia. It is well recognised that the coagulopathy of liver disease does not necessarily translate into excessive bleeding during OLT (26, 238), and an increasing number of patients undergo the procedure without the need for red cell or blood product transfusion (239) The INR reflects only the alterations in procoagulant factors, but

not the concurrent reduction in naturally occurring anticoagulants. It is now known that thrombin generation is normal or even increased in patients with chronic liver disease (4, 240). The changes that occur in both primary haemostasis (thrombocytopenia) and secondary haemostasis (low pro-coagulant factor levels) that might promote bleeding are "counter- balanced" by changes that might promote thrombosis i.e. high levels of the platelet adhesive protein vWF with low levels of its regulator ADAMTS13, reduced levels of protein C and AT, together with high levels of factor VIII (241). However, this balance is relatively precarious, and due to the decreased haemostatic reserve, these patients are more readily tipped into either a hypo- or hypercoagulable state. This rebalancing has also been shown to be the case in patients undergoing liver transplantation (242).

There is emerging evidence that thrombotic complications are common in patients with both cirrhotic and non-cirrhotic liver disease (6, 243). Portal vein thrombosis is a common complication with an incidence of 10-20%, and although altered local flow dynamics probably play a large role, a relative hypercoagulable state and a genetic prothrombotic predisposition may also be relevant (244). The aetiology of thrombosis in liver disease is multifactorial, including flow obstruction, chronic inflammation, shear stress and also insulin resistance in metabolic syndrome (245). Studies have reported the incidence of venous thromboembolism (VTE) in patients with liver disease between 0.5% and 1.9 % (246) representing a relative risk of 1.7-1.9 (247). Northup et al reported a VTE event in 0.5% of cirrhotic inpatients despite an elevated INR.(243). These findings highlight the complexity of coagulation changes in liver disease.

In the setting of liver disease, conventional coagulation tests give no information as to where the balance of coagulation lies, as discussed in detail in chapter 1, and it is has been

shown that these tests can be “hypocoagulable” when in contrast global viscoelastic tests demonstrate hypercoagulability(248). Coagulation monitoring using viscoelastic coagulation tests, (TEG®, thromboelastography and ROTEM®, thromboelastometry) has been used during liver transplantation for many years (249, 250), however, less than 30% of centres use viscoelastic tests routinely (251). Some patients with liver disease are at risk of hypercoagulability and this may potentially be exacerbated during the transplant procedure (242). Different aspects of the coagulation system have been implicated as responsible for this phenomenon; for instance platelet hyper-reactivity rather than plasmatic coagulation in patients with cholestatic liver disease(252). Thromboembolic events during OLT are associated with a high morbidity and mortality(253), and have a multimodal aetiology(254). Case series have identified intraoperative cardiac emboli during transplantation with an incidence between 1.2% and 4.25% and on-table fatal cardiac arrest has been associated with massive pulmonary embolism(255).

VET offer a rapid overview of the cumulative effect of all the individual components of haemostasis and can demonstrate hypercoagulable profiles in some individuals, and it is known that these may be associated with an increased risk of thromboembolic complications (162). Normal to increased thrombin generation has been demonstrated in liver transplant patients (65), and it is well known that thromboembolic compications, including cardiopulmonary emboli and portal vein thrombosis can develop intra-operatively(256).

Demonstration of hypercoagulability on viscoelastic tests has shown to be associated with an increased risk of thromboembolic events, both arterial and venous, in a general surgical population(257), critical care and trauma patients(258) and in cardiac surgical patients(259).

However, the definition of hypercoagulability using viscoelastic parameters is not standard. Some authors use only a shortened r time or clotting time (plasmatic hypercoagulability), whilst others define hypercoagulability based on a high MA (platelet/ fibrinogen interaction) or combinations of various parameters(258, 260).

6.2 Aims

The prevalence of pre-existing hypercoagulability in patients presenting for liver transplantation is unclear, and in addition, little is known about the de novo development of hypercoagulability during the procedure. We sought to examine a sample of our patients to quantify and describe this issue, to examine the relationship between conventional coagulation tests and hypercoagulation, and to investigate adverse thrombotic outcomes. We undertook a retrospective database review of intra-operative TEG traces in 124 patients undergoing liver transplantation in order to determine the prevalence of hypercoagulable VET profiles, the relevance of disease aetiology, the effect of the various stages of the intraoperative procedure, and any association with intraoperative thrombotic events.

6.3 Methods

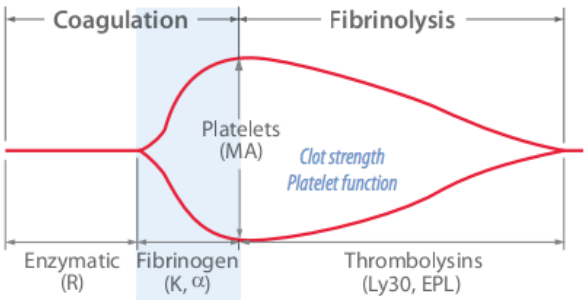
In our institution, intra-operative thromboelastography (TEG®), (Hemonetics Corporation, USA), is performed by dedicated trained operatives throughout liver transplantation according to a standard protocol.

A native and native heparinase TEG is performed at baseline, and during the dissection, anhepatic and reperfusion stages. This is in conjunction with point of care (POC) international normalised ratio – INR (Hemochron Signature Elite, ITC. US), full blood count

(PocH-100i, Sysmex Europe) and arterial blood gas analysis (RapidLab 1265, Siemens AG, Germany). This intra-operative POC data is entered onto a database post-operatively by the anaesthetic liver transplant fellows.

We performed a retrospective analysis of the database for an 18-month period over 2009-2010. All point of care and TEG data was analysed and compared. In advance of analysing this data, advice was sought from the Local Ethics Committee who advised that formal institutional approval was not required, as this was anonymised data that is routinely collected on our liver transplant database.

Thromboelastography generates a number of variables from a sample of blood as it clots. (Figure 6. 1). These describe differing parts of clot formation and dissolution. The r-time demonstrates time to initiation of clotting, the K time clot formation kinetics and MA is the maximal amplitude of clot reflecting the overall strength of clot. The r value represents the initiation of clot formation and plasmatic contribution to coagulation.



Native heparinase TEG parameter	Normal range (units)
r	12-26 (min)
K	3-13 (min)
MA	42-62 (mm)
G	3200-7100 (dyn/sec)

Figure 6.1 A graphical representation of thromboelastography showing the 'r' time and MA. Reference values shown on right.

All thromboelastographic maximum amplitude (MA) data was converted to its respective G value prior to analysis (a mathematical transformation: G=5000 x MA/(100-MA)). G

represents overall clot strength and is a unit of force. The G value allows direct linear comparison of net clot strength between two different values, whereas MA does not. Therefore a G value of 10000 dyn cm^{-2} would reflect a clot twice as strong as one with a G of 5000 dyn cm^{-2}. A twofold increase in MA does not represent a twofold increase in clot strength. We also analysed plasmatic (enzymatic) coagulation by investigating TEG R values across the database.

Definitions of hypercoagulability used in this study were as follows

r value below lower limit of normal value for native heparinase TEG (< 12 mins) = Plasmatic hyper-reactivity.

G value above upper limit of normal range (> 7100 dynes/cm^2) = Increased clot strength (high G): Hypercoagulation.

Given the significant effect of endogenous heparin-like substances on the native TEG, particularly at reperfusion, all calculations were performed on the native-heparinase TEG (261). We identified all patients who were 'hypercoagulable' by TEG G value criteria (G> 7100 dynes cm^{-2}) and further characterised their underlying pathology.

TEG data was also compared to conventional clotting tests and platelet counts.

We reviewed patients' radiological case notes for evidence of investigation for thromboembolism (deep venous, pulmonary and hepatic arterial/venous).

All results were handled and analysed on Microsoft Excel 2008 for Mac with statistical analysis performed at www.wessa.net. (Wessa, P. (2011), Free Statistics Software, Office for Research Development and Education, version 1.1.23-r7, URL http://www.wessa.net/)

6.4 Results

124 consecutive liver transplant operations were included in the study. This reflected 117 patients with 7 (6%) having a re-transplant within the study period. The median Model for end stage liver disease (MELD) was 15 (range 16-39) at the time of listing (not weighted for hepatocellular carcinoma)

Within the re-graft group, 1 was performed within two days of original transplant for primary graft non-function, 4 were performed between 3 and 34 days for hepatic artery thrombosis (HAT), with the others taking place at various time points for chronic rejection. A total of 784 separate TEG analyses were identified in the database, reflecting a mean of 6.5 TEGs per transplant. 108 of the TEG panels were performed at baseline, 258 during the dissection period, 130 during the anhepatic period, and 288 were performed after reperfusion.

6.4.1 Aetiology of liver disease in study population

The indication for the liver transplants is outlined in Table 1. The most common aetiologies were alcoholic liver disease (ALD) and the viral hepatitides. Thereafter, the cholestatic pathologies account for the largest majority of the remainder of transplants.

A substantial proportion of OLTs (21%) were performed for patients with concurrent hepatocellular carcinoma.

Aetiology	**Frequency [n (%)]**	**Concurrent hepatocellular carcinoma [n (%)]**
Alcoholic liver disease	20 (16.1)	1(5)
Alcoholic liver disease plus hepatitis B or C	16 (12.9)	4 (25)
Amyloidosis	3 (2.4)	_
Autoimmune hepatitis	1 (0.8)	_
Epithelioid tumour	1 (0.8)	_
Fulminant hepatic failure	6 (4.8)	_
Hepatitis B	5 (4.0)	3 (60)
Hepatitis C	26 (21.0)	12 (46)
Hepatitis B plus Hepatitis C	2 (1.6)	2 (100)
Nonalcoholic steatohepatitis	8 (6.5)	2 (25)
Primary biliary cirrhosis	7 (5.6)	_
Polycystic liver disease	2 (1.6)	_
Cryptogenic	2 (1.6)	_
Drug induced	1 (0.8)	_
Primary hepatocellular carcinoma	1 (0.8)	_
Nodular regenerative hyperplasia	1 (0.8)	_
Regraft for HAT	4 (3.2)	_
Regraft - other	3 (2.4)	_
Oxalosis	1 (0.8)	_
Primary sclerosing cholangitis	14 (11.3)	_
Total	**124**	**26**

Table 6.1 Aetiology of liver disease in the study population

6.4.2 Prevalence of hypercoagulability

High G values

The median G value was 3716 dyn cm^{-2} (mean 3673) with a range of 107 - 31496 dyn cm^{-2} (reference range 3200-7100 dyn cm^{-2}). Overall, 11.2% of the native TEG G values and 13.1% of the native heparinase TEG G values were above the reference range of 7100 dyne.cm-2 at some stage during the procedure. 27.4% (34/124) of the patients had a high G value on at least 1 native TEG trace during OLT, and 30.6% (38/124) had a high G value on at least 1 NH TEG.

Shortened R Values

Overall, 19.1% and 20.3% of the N and NH TEG R times, respectively, were below the lower reference range (12 minutes) at some stage of the procedure: 59.7% of the patients had a shortened R time on at least 1 native TEG, and 61.3% had a shortened R time on at least 1 NH TEG.

As described previously, when more than 1 TEG panel was performed during a particular stage for a given patient, only the first panel was used for analysis in this article.

6.4.3 Hypercoagulabilty by stage of the procedure

Baseline: The mean values were compared for native and native-heparinase TEG traces. The mean R times were 20.22 and 20.61 minutes respectively (p=0.64 [Student t test]). The mean G values were significantly higher for NH TEG tracings (5322 dyne.cm^{-2}) versus the native tracings (4613 dyne. cm^{-2}, p = 0.001 [Student t test]). This was reflected in the higher

incidence of high G values on NH TEG (20.39%) versus native TEG (15.3%: Table 6.2). There was a 6.80 to 10.68% prevalence of shortened R times in patients presenting for OLT.

TEG Type	Parameter	%	Median (Minutes)	Range (Minutes)
Native	**Short R time**	**10.68**	**10.2**	**8.6-11.9**
	Normal R time	71.84	17.1	12.25.8
	Long R time	17.48	33.55	27.3-58.8
Native-Heparinase	**Short R time**	**6.80**	**10.3**	**7.9-11.3**
	Normal R time	77.67	18	12.1-26
	Long R time	15.53	29.45	26.4-55.4
TEG Type	Parameter	%	Median (dyne-cm^{-2})	Range (dyne-cm^{-2})
Native	**High G Value**	**15.53**	**8986.7**	**7165-16,097**
	Normal G value	45.63	4505.7	3291-7048
	Low G Value	38.83	2407.99	122-3196
Native-Heparinase	**High G value**	**20.39**	**9970.06**	**7106-19,752**
	Normal G value	54.37	4363.33	3116-6904
	Low G value	25.24	2283.51	117-3077

Table 6.2 Native and Native-Heparinase TEG parameters at Baseline

Bolded values are related to those reflecting an enhanced haemostatic potential.

Dissection: The prevalence of high G values peaked during dissection: 18.49% of native traces and 20.87% of NH traces demonstrated this characteristic. There was a larger increase in the frequency of shortened R times during dissection: 22.69% on native TEG, and 17.39% on NH TEGs.

Anhepatic: During the anhepatic stage, short R times peaked: 29.45% on native TEG and 28.575 on NH TEG. During this stage the prevalence of high G values was low at 8.04% on both native and NH TEGs.

Reperfusion: At reperfusion, the endogenous heparinoid effect was clearly visible, with only 3.31% of native traces showing a short R time and with 74.38% of patients showing a significant HLE with prolonged R time reversed on the NH trace. The NH tracings possibly suggest an ongoing enhanced haemostatic potential with 17.5% of patients displaying a short R time. The prevalence of high G values was lowest immediately after reperfusion.

TEG Type	Parameter	Dissection (%)	Anhepatic (%)	Reperfusion (%)
Native	**Short R time**	**22.69**	**29.46**	**3.31**
	Normal R time	68.91	66.96	22.31
	Long R time	8.40	3.57	74.38
N-Heparinase	**Short R time**	**17.39**	**28.57**	**17.50**
	Normal R time	74.78	67.86	70.83
	Long R time	7.83	3.57	11.67
Native	**High G value**	**18.49**	**8.04**	**4.96**
	Normal G value	57.98	51.79	23.14
	Low G value	23.53	40.18	71.90
N-Heparinase	**High G value**	**20.87**	**8.04**	**9.17**
	Normal G value	61.74	56.25	44.17
	Low G value	17.39	35.71	46.67

Table 6.3 Distributions of normal and abnormal TEG parameters by stage of liver transplant. Bolded values are related to those reflecting an enhanced haemostatic potential. Percentages refer to patients with the characteristic**.**

6.4.4 Laboratory and Transfusion Data

The baseline (preoperative) haematological and clotting data for the cohort are described in Table 6.4. The group as a whole had mild thrombocytopenia with a prolonged INR and

anaemia. The mean fibrinogen level was within the normal range. The median haemoglobin level was lower in the high G group (8.9 versus 9.9 g/dl, p=0.02), whereas the platelet count and fibrinogen levels were significantly higher. There was no significant difference in the baseline INR.

Packed red cell transfusion volumes were equivalent for patients with or without high G values (on NH TEGs). Fresh frozen plasma (FFP) transfusion showed a tendency toward lower volumes in the group with higher G values (p=0.05 [Mann–Whitney U test]). When we compared the likelihood of no transfusion versus any transfusion, there was no difference between the groups for red blood cell transfusions (75.5% for normal G values versus 65% for high G values, p=0.33 [X^2] , but there was a lower chance for transfusions of FFP (75.5% for normal G values versus 50% for high g values, p=0.02 [X^2] and platelets (59.6% for normal G values versus 35% for high G values, p=0.04 [X^2]).

Laboratory tests	**All patients**	**Normal or low G value**	**High G value**	**P Value ***
Haemoglobin g/dl)	9.8 (8.4-11.3)	9.9 (8.8 -11.6)	8.9 (7.5 -10.8)	0.02
Platelets (x 109/l)	86 (54-128.8)	71.6 (51.5-103)	139.5 (99.5-181.5)	<0.001
INR	1.55 (1.3-1.8)	1.60 (1.3-1.825)	1.45 (1.3-1.625)	0.52
Fibrinogen (g/l)	2 (1.5-3.0)	1.9 (1.5-2.7)	3.2 (2.05-4.13)	0.007
Transfused Products	**All patients**	**Normal or low G values**	**High G value**	**P Value***
Packed red cells (U)	3 (0-6)	3 (1-6)	3 (0-8)	0.9
FFP (U)	3 (0-6)	4 (2-6)	1 (0-4)	0.05
Platelets (U)	1 (0-2)	1 (0-2)	0 (0-2)	0.27

Table 6.4 Haematological parameters & transfusion requirements according to G Values at baseline

6.4.5 Influence of the aetiology of liver disease

The phenomenon of high G values was not evenly distributed by aetiology. Table 6.5 shows patients who had prothrombotic TEG results on any tracing during the procedure according to aetiology. Patients with cholestatic pathologies (primary sclerosing cholangitis and primary biliary cirrhosis) had high rates of G values above the reference range (85% and 43%) respectively and also shortened R times. The incidence was also high in patients with fulminant hepatic failure (50%) and patients undergoing regrafting for HAT (50%), although the numbers of these patients was small. Patients with viral or alcoholic liver disease had a different pattern: between 65 and 100% had short or hypercoagulable R times, but only 10-12% had an increased G Value. Only 3 of the 26 patients who had concurrent hepatocellular carcinoma had high G values on TEG (median native G value =4108 dyne.cm^{-2} , native R time= 16 minutes). In contrast, all other aetiologies had similar incidences of both shortened R times and increased G values. Therefore, there appears to be a different distribution of the nature of hypercoagulability that is dependent on the aetiology of liver disease.

Diagnosis	Cases (n) *	Cases with a short R time n (%)	Median Baseline R time (IQR) §	Cases with a high G value n (%)	Median baseline G value (IQR) §
Primary sclerosing cholangitis	14	10 (71.4)	17.8 (14-19.2)	12 (85.7)	8889 (5417-11234)
Primary biliary cirrhosis	7	3 (42.9)	16.8 (14.6-21.7)	3 (42.9)	6236 (3026-7658)
Fulminant hepatic failure	6	3 (50)	16.8 (14.6-21.7)	3 (42.9)	2631 (1174-5450)
Alcoholic liver disease	20	13 (60)	16.8 (14.1-19.5)	2 (10)	_
Hepatitis C	26	18 (69)	17.7 (15.2-27.4)	4 (15.4)	3526 (2331-4704)
Alcoholic liver disease plus hepatitis B or C	16	11 (68.8)	18.7 (13.8-23.3)	2 (12.5)	_
Nonalcoholic steatohepatitis	8	3 (37.5)	17.3 (15.5-18.5)	3 (37.5)	4074 (3375-6069)
Regraft for HAT	4	2 (50)	_	2 (50)	_
Amyloidosis	3	1 (33.3)	_	1 (33.3)	_
Hepatitis B	5	5 (100)	21 (18.4-26.8)	1 (20)	_
Other ◆	12	4 (33)	_	4 (33)	_
Total Transplants	124	75 (60.5)	-	39 (31.5)	_

Table 6.5 Prevalence of hypercoagulability according to disease aetiology

★Cases with an abnormal parameter on any intraoperative TEG examination are listed
§The data are presented as medians and interquartile ranges. Values are provided only when the number of cases was greater than 3
◊ The other category comprises of aetilogies where only two or fewer cases were found in the series.

6.4.6 Hypercoagulability and Conventional Coagulation Tests

A comparison of paired G values with point of care INR tests showed that there was no significant correlation between the 2 parameters (r=-0.33, p=0.001 [Spearman's rank correlation]). Figure 3 shows a scatter plot of the 2 measurements and reveals that TEG traces that were hypercoagulable (ie above the normal reference range) could be associated with an INR between 0.9 and 3.8. G values were compared with platelet counts, and a

moderate correlation was found (r=0.62, p=0.001 [Spearman's rank correlation]). None of the patients with high G traces had platelet counts above the normal reference range. There was no correlation between the R time and INR (r=0.04,p=0.27 [Spearman's rank correlation]).

One hundred sixty two of the 784 TEG R time measurements were found to be shorter than the normal range (12-26 minutes) with a median time of 8.95 minutes. In this group, the median INR was 1.8 with a range of 1.1 to 10. On the basis of INR values, 128 TEG analyses (79%) showing a shortened R time would be described as coagulopathic (INR > 1.5), and 61 (37.7%) would be described as markedly so (INR > 2.0).

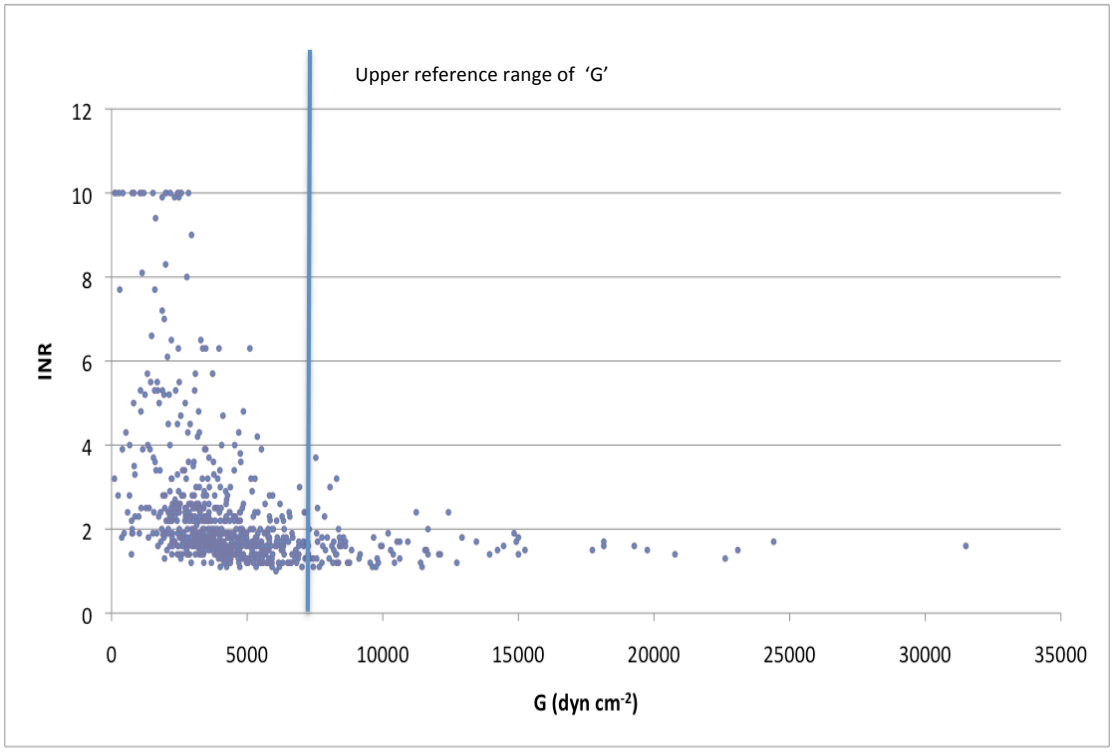


Figure 6.2 X-Y scatter plot of all native heparinase TEG G values against corresponding INR values

A similar plot of paired G values with platelet counts is shown in Figure 6.3. There is a greater correlation between the two as may be expected given the direct contribution of platelets to clot strength (r=0.62, r^2=0.38). The TEG trace takes into account both platelet function and fibrinogen levels and their relative contributions to clot strength, which may account for the less than perfect correlation. None of the patients with hypercoagulable traces had platelet counts above the normal reference range.

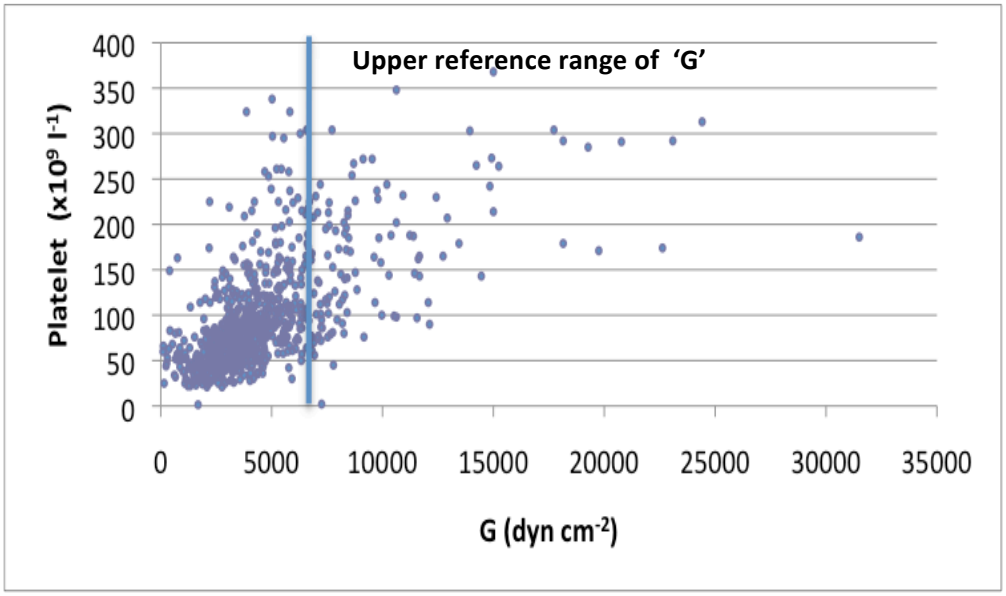


Figure 6.3 X-Y scatter plot of paired G and platelet count

Correlation of R and INR

Figure 6.4 compares the two parameters throughout the study group. There is no correlation between INR and R value.

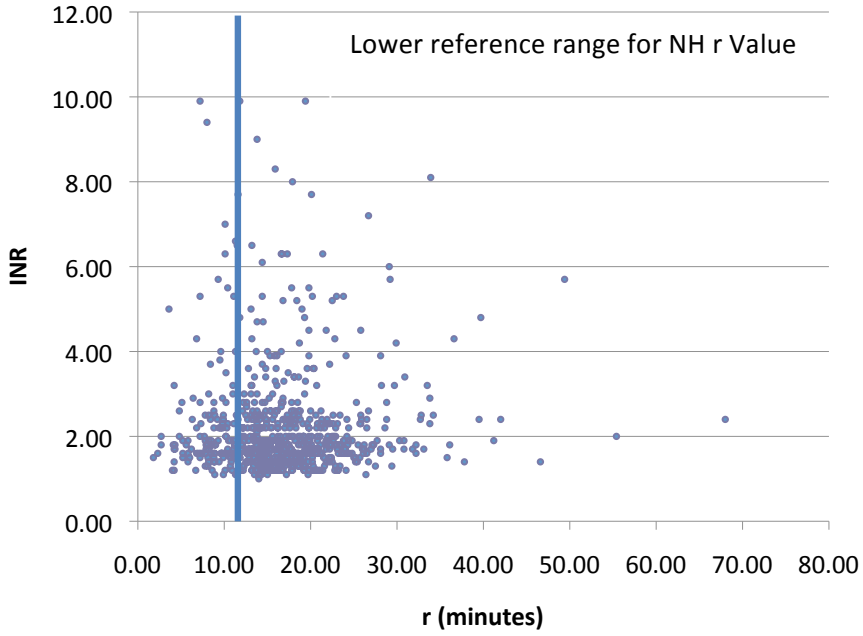


Figure 6.4 X-Y scatter plot of INR and r time

6.4.7 Perioperative Thrombotic events

One patient had an intraoperative portal vein thrombosis that required on table thrombectomy. This was associated with a grossly shortened R time (2.6 minutes) on both native and NH TEGs during early reperfusion. The concurrent G value was within the normal range at 3347 dyne-cm-2. There were no intraoperative pulmonary emboli identified by attending clinicians.

The database review identified thrombotic complications within 30 days of transplant. Among the 117 primary transplants (ie with the exclusion of the 7 regraft procedures), there were 6 cases (5%) of HAT. Four of the 6 required a regraft procedure as a result of HAT. Three of the 6 cases had high G traces during their initial transplant ($p=0.25$ [X^2]) and 4 of the 6 demonstrated shortened R times ($p=0.79$ [X^2]). The underlying aetiology for those patients undergoing regraft for HAT were PSC (2), alcoholic liver disease, and hepatitis C

virus. The aetiologies of the 2 patients who did not undergo regrafting were amyloidosis and hepatitis B cirrhosis. It is notable that the occurrence of hypercoagulable traces in patients who developed HAT was more frequent than that in the general population of patients undergoing liver transplantation.

There were only 2 postoperative pulmonary emboli in the primary transplant cohort, one of whom had a hypercoagulable trace intraoperatively (PSC).

6.5 Discussion

The various thrombotic complications that can occur in the perioperative period, such as HAT, have been traditionally been assumed to be caused mainly by surgical factors or related to graft function. The role of the haemostatic system in the development of perioperative thrombotic complications has, until recently, been largely overlooked, because of the long held belief that a hypocoagulable state is present prior to and during liver transplantation (262).

This study, which we believe is the first to examine such a large number of intra-operative data sets, demonstrates that a significant number of patients with end stage liver disease undergoing liver transplantation, present with, or develop hypercoagulable thromboelastograms during the procedure. In this series, the incidence of patients with baseline G values greater than 7100 dynes-cm-2 was 20.39% on heparinase TEG and 15.53% on native TEG.

Patients with cholestatic disease (PSC or PBC) have a high incidence of hypercoagulability on the basis of increased G values (85.8% and 42.9 % respectively), and this is in keeping with

previous work published on this group of patients (32). Although there were only 6 patients with acute liver failure, 50% of these were hypercoagulable. This may be surprising, given that all these patients had an INR of 2 or more, but is compatible with similar findings reported recently (166, 167). Nearly 40% of patients with non-alcoholic steato-hepatitis (NASH had evidence of both plasmatic hyper reactivity and excessive clot strength). NASH is both increasing in prevalence and as aetiology for chronic liver disease. (263)

The pathophysiology of hypercoagulation is multifactorial. In the cholestatic pathologies, work has demonstrated hyperfibrinogenaemia and also platelet hyperreactivity, (252) whereas in NASH, there is stronger clot development (increased MA) and reduced clot lysis. (159). Many intraoperative factors may contribute to persistence or *de novo* development of hypercoagulation. These include vascular stasis, coagulation activation with endotoxins (within the portal vein) and endothelial cell injury and local inflammation of the graft organ caused by ischaemia reperfusion injury. Models of endotoxaemia have shown that this results in significant shortening of the r time or clotting time, with accelerated initiation of coagulation (264) but that conventional coagulation tests (PT/INR) remain prolonged. (265) Ischaemia reperfusion injury leads to activation of coagulation and also platelets (266) and it is of note that reports of intra-cardiac thrombi are most common around the time of reperfusion. In a review of 27 case reports of thromboembolic events occurring during liver transplantation, TEG profiles (267) were hypercoagulable in over 70% of cases, whereas conventional coagulation tests were all hypocoagulable. (268) In addition, most patients undergoing liver transplantation do not routinely have thrombophilia screening, but a genetic component involving prothrombotic gene polymorphisms may be present in some.

(269) No patient in our series had an intraoperative event suggestive of intra cardiac thrombosis or pulmonary embolism.

VWF levels are known to be elevated in liver disease, and rise further during the LT procedure, as a result of release of VWF from endothelial cells activated by inflammatory processes and surgical stress. In addition, plasma levels of its cleaving protease, ADAMT13, decrease during transplantation, resulting in a profound dysbalance in the VWF/ADAMT13 ratio (270). Such a dysbalance has been linked to thrombotic risk in different disease states, such as myocardial infarction and sepsis (271). Although the risk of post-operative HAT is most probably initiated by local activation of endothelial cells as a result of ischaemia-reperfusion injury linked to surgical risk factors involving the arterial anastomosis (267), a hyper-reactive primary haemostatic system may enhance this risk. If there is a significant deficiency of ADAMTS13, VWF secreted from the vascular endothelium survives in the circulation and is able to promote the formation of platelet rich thombi in the microcirculation. It is of note that extensive platelet aggregates occluding small pulmonary arterioles and capillaries have been found in the lungs of patients who have died suddenly during liver transplantation, and that these cases were usually associated with a sudden fall in platelet count and a rise in pulmonary artery pressure (35).

In this series, the incidence of HAT was 5%. Although the aetiology is known to be multifactorial and significantly related to difficulties associated with the arterial anastomosis and arterial reconstruction, it is of note that of the 6 patients that developed HAT, 4 of them were hypercoagulable at some point during the procedure. We cannot conclude from our

data whether the presence of a procoagulable state predisposes to thrombotic events as the incidence of such events in our dataset is too low to demonstrate any significant association. However, the relatively low incidence of HAT and corresponding small number of cases means that a type 2 error cannot be excluded. It would seem physiologically plausible that such a relationship may exist, but further research is required to demonstrate a conclusive link.

The clinical implications of hypercoagulability occurring during liver transplantation have yet to be evaluated. However, emerging evidence suggests that hypercoagulability detected by viscoelastic tests puts patients in an “at risk” group for both venous and arterial thrombotic events. (134, 162) A recent systematic review of 10 studies in surgical patients showed an increased MA to be the most important parameter to predict postoperative TE events. However, there was considerable variability as to which parameters were used to define hypercoagulability and no study was adequately powered. Nevertheless, the vast majority of patients who had a TE event were hypercoagulable on one or more TEG parameters (164) and future prospective studies are recommended. It has also been shown that TEG can display hypercoagulation in thrombosis prone patients. (272)

The definition of hypercoagulation based on viscoelastic tests is not standard. A routine TEG will generate a number of variables including r time, alpha angle, and maximum amplitude. These values represent different components of the clot formation process. Different researchers have chosen to use differing variables or indeed a combination of more than

one. The G value is a function of the maximal amplitude and represents clot strength rather than initial clot formation kinetics and as such, is used as the definition of hypercoagulation in our paper. The ability to directly compare G values to one another linearly enables a better appreciation of the magnitude in change between different results. Plasmatic (or enzymatic) coagulation is also likely to contribute to a pro-thrombotic state, whereby initial clot formation is accelerated.

Conventional coagulation tests do not provide information about the quality of the clot or the dynamics of its formation. Unless the platelet count or fibrinogen levels are elevated above normal values (which was not the case in any of our patients) CCT are unable to identify a hypercoagulable state. It is clear from the comparisons between the conventional clotting tests (INR) and the TEG parameters that there is no useful correlation between the two. It has consistently been shown that there is only a weak correlation between the r time/clotting time and the PT/INR. (98, 273) Wide derangements in INR may not represent a defect in coagulation by TEG criteria and indeed this reflects the fact that INR is a poor predictive ability of clinically important bleeding. (2) In liver disease, the endogenous anticoagulants, as well as procoagulant factors, are all reduced and the balance of pro to anticoagulants may be altered in favour of a prothrombotic state.(7) We have shown that a significant number of patients with INR values of 1.5 or greater, are hypercoagulable on viscoelastic tests. Solely relying on conventional coagulation tests can only provide the attending physician with a small part of the global picture. Viscoelastic monitoring adds valuable qualitative information to the management of these cases (227)

What remains unclear is what action should be taken when hypercoagulability is demonstrated on TEG. Where there is evidence of significant plasmatic hypercoagulabilty (shortened R value) and a normal or hypercoagulable MA or G, then it is reasonable to give a small dose intravenous dose (3000-5000 units) of heparin. (Andre de Wolf, personal communication). It would also seem prudent to avoid prohaemostatic agents, including FFP and platelets, if there is thromboelastographic evidence of hypercoagulabilty.

The limitations of this study are its retrospective design and therefore potentially we missed minor thrombotic events, such as DVT. In addition we defined hypercoagulability as TEG parameters outside the normal range, whereas others have used values of 2 or more standard deviations (134). Our study did not extend into the post-operative period, and as such it is difficult to comment on persistence of the phenomenon. There are of course many potential issues with both bleeding and thrombosis in this group post-operatively and the phenomenon requires further investigation.

6.6 Conclusions

These results suggest that there may be significant benefits in future research aimed at investigating the phenomenon of hypercoagulability within liver transplantation, and liver disease in general. Crucially, work needs to be targeted towards ascertaining the presence or absence of a causal relationship between this state and thrombotic events and indeed ultimate post-transplant outcome.

Because hypercoagulability is not detected by conventional coagulation tests, and can be present even though conventional coagulation tests indicate *hypocoagulability*, VET should

be used routinely for coagulation monitoring during liver transplantation. As well as identifying coagulopathy early, and allowing specific haemostatic therapy to be instituted if there is clinical evidence of bleeding, the role of TEG may crucially be just as valuable in avoiding unnecessary and potentially harmful transfusion of blood products in the hypercoagulable cohort of patients.

Chapter 7

Alterations in coagulation profile following major liver resection

This study was funded by a grant awarded by the Association of Anaesthetists of Great Britain and Ireland through the National Institute of Academic Anaesthesia (2009/2) and the Royal Free Charity (TF35). A manuscript based on the findings of this prospective observational study was accepted for publication in Anaesthesia 2016.

7.1 Introduction

The alterations in coagulation after major liver resection are complex. Contributing factors include pre-existing liver dysfunction, the presence of malignant tumours, extent of intraoperative blood loss, surgical technique and ischaemia–reperfusion injury. A further important factor is the volume of liver remaining following resection, as the majority of coagulation proteins are synthesised within the liver (274, 275). Bleeding and vascular thrombosis are major life threatening complications following liver surgery, and hence assessment of clotting function profile is a significant clinical concern (276). In the early post-operative period, routine coagulation tests show an almost universal increase in prothrombin time (PT) and international normalized ratio (INR) accompanied by a brief fall in platelet and fibrinogen levels. This "coagulopathy" suggests a transient hypocoagulable state; however, in reality, bleeding complications are rare (277). The increase in PT and INR in the early post operative period has traditionally been assumed to represent a potential bleeding risk, and many clinicians would consider administering prophylactic fresh frozen plasma (FFP) to correct the INR to < 1.5 prior to invasive procedures (278, 279). The risk/benefit ratio of such practice is in any case debatable, as the volume of FFP administered rarely corrects the INR to the desired value (280), and can result in adverse effects such as transfusion-related acute lung injury and transfusion-associated circulatory overload (281). In addition, it is also quite common practice to delay removal of epidural catheters used for post operative analgesia until the INR returns to within 'acceptable' values of between 1.3-1.5 (279). Another consequence of the perceived risk of bleeding is that the initiation of pharmacological thromboprophylaxis may be delayed; a recent survey

reported that 35% of centres withhold pharmacological prophylaxis until the INR has returned to within normal range (282).

Although conventional coagulation tests (CCTs), specifically the PT and INR, suggest a hypocoagulable environment early after liver resection (275), these tests are only responsive to procoagulant levels and do not measure the activity of anticoagulant proteins. As has been discussed in earlier chapters, in patients with liver disease there is a decrease in both pro- and anticoagulant levels; and consequently thrombin generation is normal, or even hypercoagulable (7), and is a partial explanation of why PT and INR are such poor predictors of bleeding risk in patients with cirrhosis (45). We hypothesized that a similar situation may exist following liver resection, as it is has been shown that both pro- and anticoagulant levels fall (283), and also that in some patients modified thrombin generation tests show hypercoagulability following major liver resection (284). Global viscoelastic tests of coagulation, including thromboelastography (TEG®) and thromboelastometry (ROTEM®), reflect both pro and anti-coagulant activity; these have been shown to be normal (285, 286) or even hypercoagulable (287) in patients following liver resection.

The risk of thrombotic events following hepatic resection is significant. In a review of over 5500 partial hepatectomies in the National Surgical Quality Improvement Program, the incidence of VTE was 2.88% overall, with much higher rates for right (4.15%) and extended (5.76%) hepatic resections (288). In a prospective study of 410 patients in which patients had protocolised CT scans following liver resection, the incidence of pulmonary embolism (PE) was 6% despite low molecular weight heparin (LMWH) thrombo-prophylaxis (289). Similar rates of VTE have been shown in other series of liver resection (290, 291). Major liver resection was primarily undertaken for metastatic disease and hepatic malignancy but

is increasingly undertaken for live donor liver transplantation as well, and the consequences of VTE are not insignificant for all these groups of patients (292, 293). The extent of liver parenchyma resected appears to be more significant in terms of VTE than any underlying malignant pathology. In a series of 599 patients undergoing liver resection, the incidence of VTE was 4.7% overall with no significant difference for patients with malignant versus benign conditions. It was noted that patients who have a major liver resection were less likely to receive thromboprophylaxis with low molecular weight heparin (LMWH) because of the raised INR, yet the incidence of VTE in this series was 14.3% in patients with a peak postoperative INR ≥ 1.5 compared to 3.6% in patients with peak INR ≤ 1.5 (10). It has also been demonstrated that patients undergoing liver resection have the highest rate of VTE for any surgical procedure (294).

7.2 Aims of this study

The discordance between CCTs and clinical complications merits detailed investigation of post-operative coagulation changes as the results might help inform the necessity (or otherwise) of plasma transfusion and the timing of pharmacological thromboprophylaxis. We undertook this prospective, longitudinal, observational study to document serial changes over time of conventional coagulation tests, coagulation factor levels including procoagulant and endogenous anticoagulant proteins, thrombin generation and thromboelastometry in patients undergoing major hepatic resection.

7.3 Methods

The study was approved by the local research ethics committee (REC no. 10/H0714/12) and all patients gave written informed consent.

7.3.1 Patient selection

Patients referred for major hepatic resection were included in the study. A major hepatic resection was defined as ≥ 30% volume resection judged by CT scan during work-up. Exclusion criteria were evidence of chronic liver disease, anticoagulant or antiplatelet medication in the week preceding operation, oral contraceptive or hormone replacement therapy, history of an inherited or acquired bleeding disorder or previous thromboembolic disease. Patients who had a smaller than planned liver resection, or whose subsequent pathology showed cholestasis or cirrhosis of the liver, were excluded post-hoc. Perioperative care was overseen by a dedicated team of hepatobiliary anaesthetists and surgeons. Intraoperative transfusion of packed red cells was limited to those patients with haemoglobin < 80 $g.l^{-1}$ whilst plasma and platelet transfusions were given at the discretion of the attending clinicians. Post-operative transfusion practice was left to the discretion of the attending surgeons and intensivists. Attending clinicians were blinded to ROTEM® and thrombin generation results although they had access to results of conventional testing.

Thromboprophylaxis followed our standard local protocol. Patients wore thromboembolic stockings unless contraindicated and pneumatic compression devices were used intra-operatively. Prophylactic LMWH was commenced as soon as the INR was ≤ 1.5, and there were no signs of bleeding.

7.3.2 Laboratory assays

Blood sampling was undertaken at five time points: baseline (after insertion of central venous line but before knife-to-skin), end of surgery (at least 30 minutes following removal of liver specimen), post-operative day one, two and five. Samples were drawn either from

central venous catheter (CVC) lines if *in situ* or by atraumatic venepuncture of an antecubital vein using a 21G needle after a 10 ml discard into BD Vacutainer® tubes(Becton, Dickinson and Company, Oxford, England) for study bloods and routine bloods for clinical care. Samples were taken at least 12 h after administration of LMWH in order to minimise any anticoagulant effects on the laboratory assays.

The PT was measured using HemosIL™ PT Fibrinogen HS Plus (Instrumentation Laboratory (IL), Bedford, MA, USA) and INR was calculated using geometric mean PT and the manufacturer's international sensitivity index (1.15). The APTT was obtained using HemosIL™ SynthaSIL (IL, Bedford, MA, USA). The following coagulation factors were analysed on all patients: fibrinogen, factors (F) II, VII, VIII, X, XI, vonWillebrand factor (VWF) antigen, anti-thrombin (AT) levels, protein C activity, Protein free antigen and D-dimers. Blood was collected into BD Vacutainer ® tubes with a blood to citrate ratio of 9:1 (Beckton Dickinson, Oxford, UK). Platelet poor plasma (PPP) was prepared by centrifugation at 2000g for 12 minutes, plasma removed and re-centrifuged at 2000g for 12 minutes. PPP samples were stored in aliquots at -85^{o}C until testing.

Fibrinogen was analysed using Fibrinogen-C reagent on an ACL TOP analyser. Factors VIII, IX, XI and XII were analysed by standard one-stage APTT based assays. Factors II, V and VII were analysed by a one stage clotting PT-based assay on an ACL 3000 (IL, Bedford, MA, USA). Von Willebrand Factor (VWF) antigen was analysed by an in-house ELISA. Protein C activity was tested using HaemosIL chromogenic protein C assay and free protein S using the HaemosIL free protein S assay (both IL). AT activity was measured using Berichrom Antithrombin III assay (Siemens, Germany) on a Cs2000i (Sysmex, Milton Keynes, UK). The

Protein C/ FVIII ratio was calculated at all-time points as a surrogate marker of anti-coagulant and procoagulant balance (157, 262).

Thrombin generation was assessed at all time-points on platelet-poor plasma using the Calibrated Automated Thrombography method as described by Hemker et al (67). Thrombin generation assays were triggered with 5 pM tissue factor reagent containing 4 µM phospholipid (PPP reagent, Thrombinoscope B.V., Maastricht, Netherlands); measurements included lag time (LT), endogenous thrombin potential (ETP) or total thrombin generated, and peak height (PH). All values were normalised and expressed as percent of the normal pooled plasma assayed in parallel in each test run.

A sample of blood was taken in standard Vacutainer tubes containing 0.019 $_{M}$ buffered trisodium citrate for the Protac®-modified thrombin generation assay at baseline and on POD 1 (66). Protac® (Pentapharm, Basel, Switzerland) is a snake venom extract that activates protein C, in a similar manner to thrombomodulin. This assay has been validated in patients with defects of protein C anticoagulant pathways, and has been shown to be sensitive to deficiencies of protein C, protein S and other pro-thrombotic states (295). The endogenous thrombin potential with Protac® (ETP-Protac®) is presented as % of the endogenous thrombin potential without Protac® for that sample.

For the ROTEM analysis, blood was drawn into citrated BD Vacutainer® tubes with blood:citrate equivalent to 9. Analysis was performed on a ROTEM delta analyser (TEM International GmbH, Munich, Germany). Four panels were analysed per timepoint following recalcification with Star-tem reagent (0.2 mol.l^{-1} $CaCl_2$ in HEPES buffer pH 7.4 and 0.1% sodium acid).

ROTEM panels:

EXTEM: recombinant tissue factor and phospholipids – extrinisic pathway activation

INTEM: partial thromboplastin phospholipid and ellagic acid – intrinsic pathway activation

HEPTEM: heparinase I from flavobacteria – intrinsic pathway with exclusion of heparin effect

FIBTEM: cytochalasin D – platelet inactivation, demonstrating contribution of fibrinogen and factor XIII

7.3.3 Statistical analysis:

To assess change over time, repeated measures of analysis of variance was carried out on the levels of each variable assessed at the four postoperative time points, with the baseline values used as covariates. Statistical significance was assessed at the 5% level (P-value < 0.05), and within subject effects were corrected using the conservative Box technique. Variables were assessed for normality using the Shapiro-Wilks test before and after log transformation. Where analyses examining associations between different variables were required, we calculated Pearson correlation coefficients. Analysis was performed using GRAPHPAD® software (San Diego, CA, USA).

7.4 Results

Sixty patients were recruited, and 45 patients were included in the final analysis as the remainder met exclusion criteria. Patient demographics, type of surgery and histology are shown in Table 7.1. Colorectal cancer metastasis was the most common indication. All patients had normal baseline liver function.

7.4.1 Patient Demographics

Characteristic	
Age	62 (33-85)
Gender	
Male	27 (60%)
Female	18 (40%)
Operative procedure	
Right hepatectomy	23 (51%)
Extended right hepatectomy	4 (9%)
Left hepatectomy	6 (13%)
Extended left hepatectomy	4 (9%)
Multiple non-contiguous segmentectomy	8 (18%)
Histology	
Colorectal carcinoma metastases	35 (78%)
Hepatocellular carcinoma	5 (11%)
Benign adenoma	1 (2%)
Focal Nodular Hyperplasia	1 (2%)
Neuroendocrine tumour metastases	1 (2%)
Other metastases	2 (4%)

Table 7.1. Baseline patient demographics, procedure type and aetiology of hepatic lesions. Values are mean (range) or number (proportion)

7.4.2 Transfusion data

Details of blood product usage are given in Table 7.2. Patients with extended resections received proportionally higher transfusions. One patient died on POD 5 from multi-organ failure. LMWH prophylaxis was administered to 17% of patients on POD1, 48% of patients on POD 2 and 89% of patients by POD 5. In total three patients (6.6%) were diagnosed with DVT and/or PE within one month of surgery (POD 3, 4 and 14). The mean age of these three patients was 76.7 years .The two patients who had VTE on days 3 and 4 had POD 2 INR of 1.7 and 2.3 respectively. The patient with a DVT on day 14 did not start thromboprophylaxis until POD 5.

	Intraoperative		**Post-operative**	
	Units per patient	**Number of patients**	**Units per patient**	**Number of patients**
Red blood cells	0 (0-0)	0	0 (0-2)	18 (40%)
Fresh Frozen Plasma	0 (0-0)	4 (8.9%)	0 (0-0)	7 (15.6%)
Platelets	0 (0-0)	0	0 (0)	3 (6.7%)

Table 7.2 Intra and post-operative transfusion data. Values are for the whole patient group, not just those transfused and presented as median (IQR) or number (percentage).

7.4.3 Changes in blood biochemistry and coagulation

Table 7.3 describes the changes in routine haematology, chemistry and coagulation by POD. The mean drop in haemoglobin in the immediate post- operative period was approximately

20 $g.l^{-1}$, representing a combination of blood loss and haemodilution. Platelet counts decreased reaching a nadir on POD 1, in contrast to fibrinogen, which had the maximum decrease by the end of surgery, and subsequently levels increased above baseline from POD 2. APTT remained within the normal range throughout the study period. The INR was increased from the end of surgery, with the highest values on POD 1 (Median 1.7) and POD 2 (Median 1.6); by POD 5 all but one patient had an INR ≤ 1.5. (Figure 7.1) The number of patients with abnormal conventional coagulation was highest on POD 1 with 30% of patients showing an INR ≥ 2.0.

Parameters; units (Reference range)	**Normal range**	**Baseline**	**End of surgery**	**POD 1**	**POD 2**	**POD 5**
Haemoglobin; $g.l^{-1}$	135-170	118 (19)	95 (18) *	99 (16)*	98 (15)*	102 (14)*
Platelets; $x10^9 .l^{-1}$	140-400	231 (97)	192 (73)	167 (76)*	173 (100)	240 (145)
Albumin; $g.l^{-1}$	35-50	38 (4)	24 (6)*	25 (5)*	27 (4)*	30 (6)
Alanine aminotransferase; $iu.l^{-1}$	<41	38 (41)	335 (197)*	411 (243)*	372 (274)*	149 (86)*
Bilirubin; $\mu mol.l^{-1}$	<21	12 (7)	16 (9)	33 (22)*	27 (24)	31 (32)
Alkaline phosphatase: $iu.l^{-1}$	<129	99 (72)	80 (84)	70 (45)	85 (46)	191 (103)*
Urea; $mmol.l^{-1}$	2.9-8.2	5.1 (1.9)	5.0 (1.8)	5.5 (1.6)	6.2 (2.1)	5.6 (4.1)
Creatinine; $\mu mol.l^{-1}$	66-112	69 (19)	71 (23)	70 (24)	69 (30)	62 (23)
Prothrombin time; secs	9-13.5	13.3 (11.6-14.7)	17* (14.2-19.1)	20.8* (17.0-25.3)	19.8 * (16.6-21.8)	14.3 (12.8-15.5)
Activated partial thromboplastin time; secs	26-34	28 (26.7-29.3)	27.2 (24.6-29.7)	28.4 (27.2-31.9)	27.7 (26.6-30.8)	27.9 (25.7-28.9)
Fibrinogen; $g.l^{-1}$	1.5-4	3.2 (0.7)	2.1 (0.7)*	2.7 (0.8)	3.8 (1.1)	4.5 (1.4)*

Table7.3 Routine haematology, biochemistry and coagulation tests against time. Data are presented as mean (SD) or median (IQR). * P-value < 0.05.

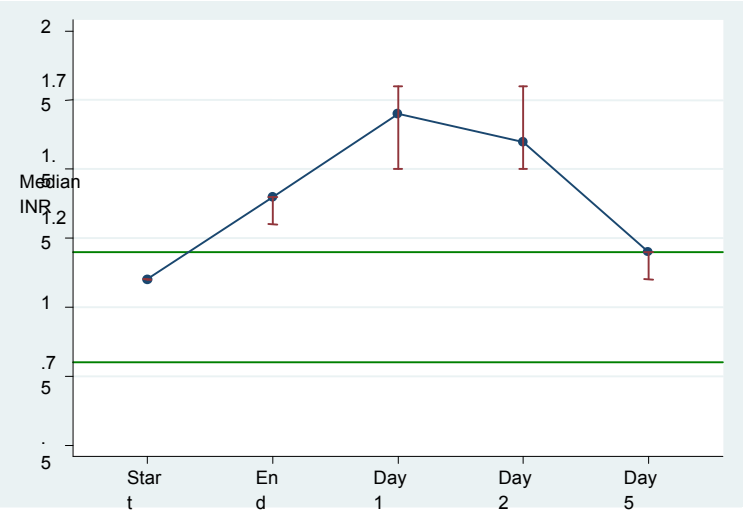
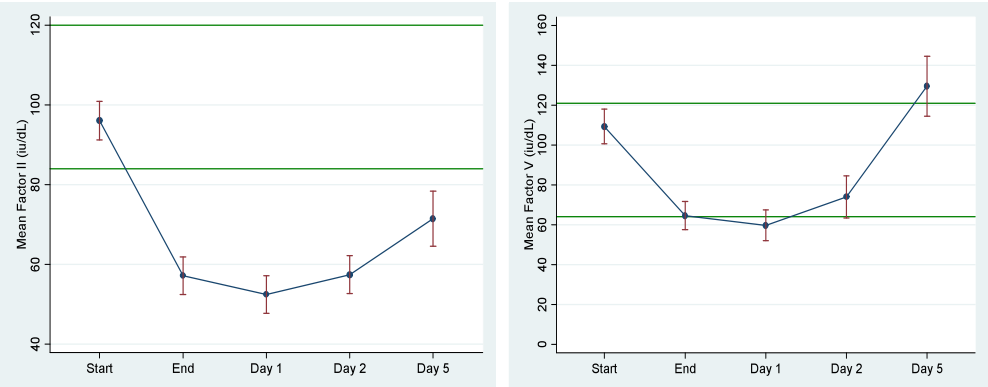


Figure 7.1 : Changes in INR by POD. Variation of INR at post-operative timepoints. Error bars indicate interquartile range. Green lines represent reference ranges (INR: 0.8-1.2)

7.4.4 Changes in pro and anti-coagulant levels

Pro-coagulants: Levels of the procoagulant factors II, V, VII and X all fell postoperatively with the lowest values seen on POD 1. By POD 5 levels were returning towards baseline (Figure 7.2). VWF and factor VIII showed a steady rise from POD 1. (Figure 7.3).



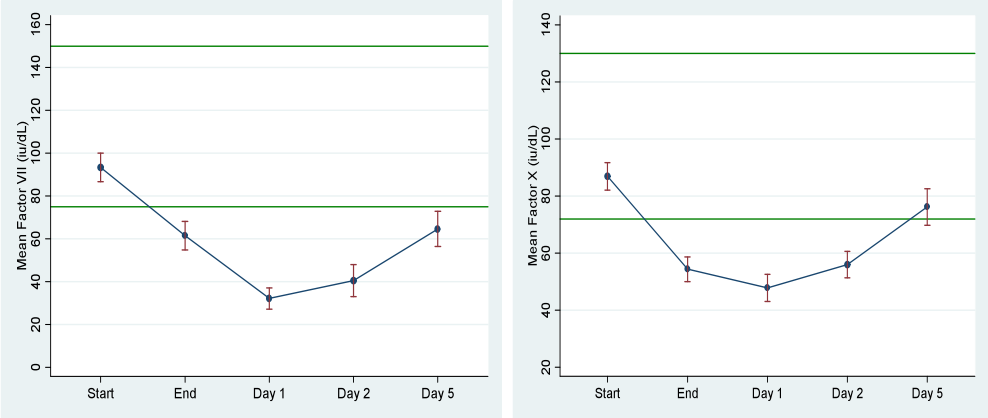


Figure 7.2 Changes in pro-coagulant levels (factors II, V, VII and X) at post operative time points. Error bars indicate 95% confidence intervals. Green lines represent reference ranges (II: 68-144 iu.dl^{-1}, V: 39-129 iu.dl^{-1}, VII: 45-180 iu.dl^{-1}, X: 49-152 iu.dl^{-1})

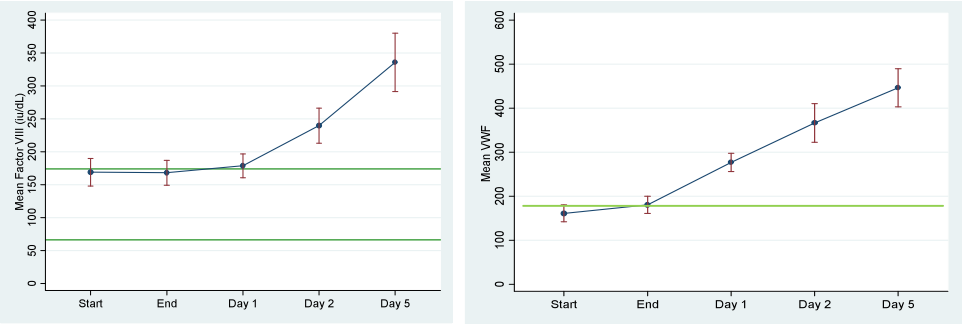
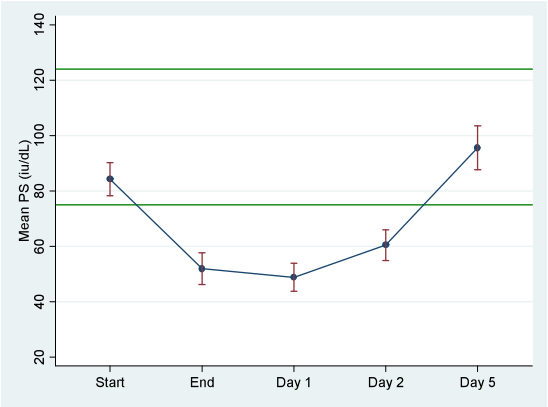


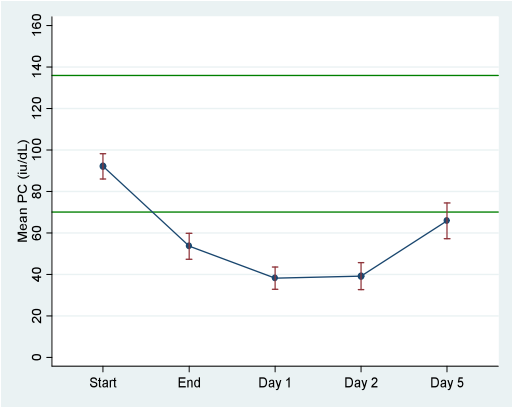
Figure 7.3 Changes in Factor VIII and VWF by POD Variation of von Willebrand Factor (VWF) and factor VIII at post-operative timepoints. Error bars indicate 95% confidence intervals. Green lines represent reference ranges (Factor VIII: 45-169 iu.dl^{-1}, VWF: 45-175 iu.dl^{-1})

Anti-coagulant proteins: Levels of the anticoagulants, AT, protein C and protein S decreased in a similar manner to the procoagulants, but by contrast, by POD 5 AT and protein C remain low at 60 iu.dl^{-1} and 65 iu.dl^{-1} respectively (Figure 7.4). The ratio of the one of most powerful anticoagulant drivers, protein C, to the procoagulant VIII was reduced in all patients from

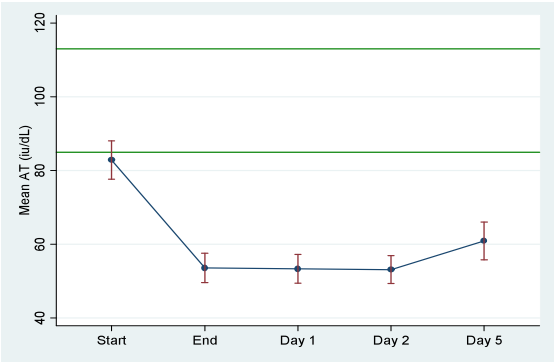
the end of surgery, and this continued to fall and was most pronounced by POD 5. This ratio was lowest in patients with an INR≥2.0 (ratio of 0.09 compared to baseline ratio of 0.69). D-dimers were raised from baseline values (365ng.ml^{-1}) at the end of surgery (2400ng.ml^{-1}) and remained high at POD 5 (3570ng.ml^{-1}).



1: Change in Protein S by POD



2: Change in Protein C by POD



3: Changes in antithrombin by POD

Figure 7.4 Change in anticoagulant levels, protein S (PS), protein C (PC) and antithrombin (AT) by POD. Error bars indicate 95% confidence intervals. Green lines represent reference ranges (Protein C: 70-140 iu.dl^{-1}, Protein S: 60-140 iu.dl^{-1}, Anti-thrombin: 86-114iu.dl^{-1})

Ratio of Protein C to Factor VIII: This ratio steadily decreased from the value at baseline (0.69 ± 0.31)) at all measured time points, and was at its lowest value by POD 5 (0.15± 0.10). Patients with an INR ≤ 1.5 had a PC to VIII ratio of 0.24 ± 0.14 on POD 5, whereas patients with an INR of 1.6 or greater had a ratio of 0.09 ± 0.05.

7.4.5 Changes in thrombin generation parameters

Despite a small numerical reduction in endogenous thrombin potential and peak height initially, these values remained within reference ranges throughout (Figure 7.5). No patient had any thrombin generation parameters suggestive of hypercoagulability, however, ETP-Protac, was higher in the postoperative period (POD 1) compared to the pre-operative period consistent with hypercoagulabilty due to protein C resistance and deficiency. By POD 5, all parameters had returned to a level equivalent to, or prothrombotic with respect to, baseline.

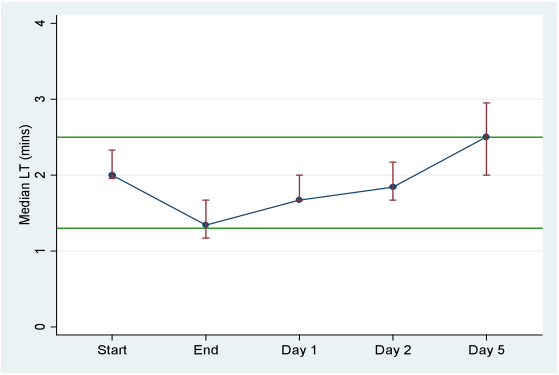
ETP- Protac®% (Derived by dividing ETP+Protac® by ETP without Protac®) demonstrated increased thrombin generation on POD 1 compared to baseline (44% vs 26%). This hypercoagulable profile became more pronounced as the INR increased: ETP-Protac® on POD 1 was 38% when INR ≤ 1.5, 44% when INR 1.6-1.9, and 66% when INR ≥ 2.0.

7.3.6 Changes in ROTEM parameters

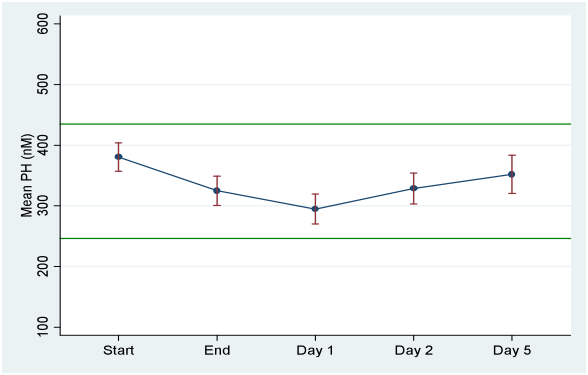
All ROTEM parameters remained within normal range post operatively for the group as a whole, despite a small decrease in values on POD1. The absolute decrease in values are unlikely to represent any clinically significant impairment of coagulation as they remain within normal range (Figure 7.6). In three patients there was a reduction in EXTEM CT, after

the end of surgery, indicative of more rapid clot formation. Very few patients had a CT outside the upper limits of the reference range, and all these had factor II levels less than 30 $iu.dl^{-1}$. EXTEM MCF fell slightly from baseline at end of surgery and on POD 1, but exceeded baseline values by POD 5. FIBTEM levels were lowest immediately post operatively and steadily rose from POD 1, and were outside the upper limit of the reference range by POD 5 in keeping with the increase in fibrinogen levels on days 2 and 5.

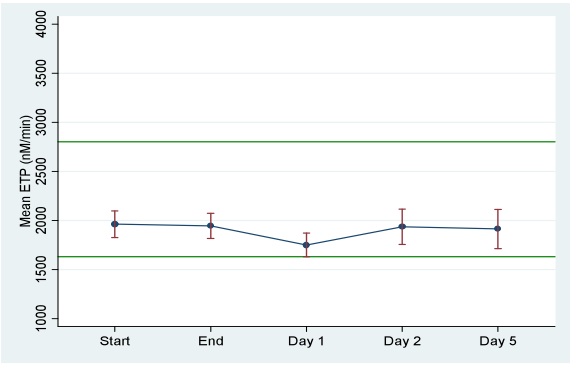
There was minimal correlation between INR and EXTEM CT at each timepoint (r=0.25-0.47, p=<0.001-0.09). EXTEM MCF and platelet count carried a stronger correlation(r=0.61-0.7, p<0.001). FIBTEM MCF and fibrinogen levels also exhibited significant correlation (r=0.56-0.82, p<0.001).



1: Change in Lag Time by POD

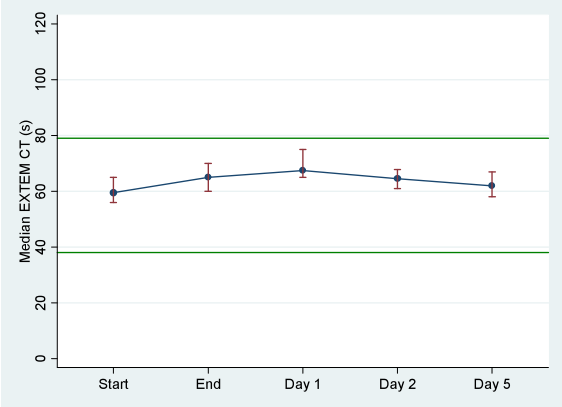


2: Change in Peak Height by POD

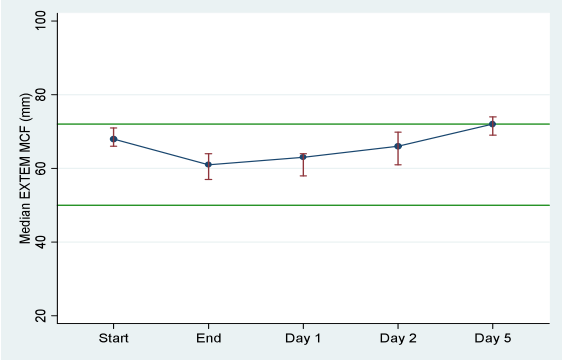


3: Change in endogenous thrombin potential by POD

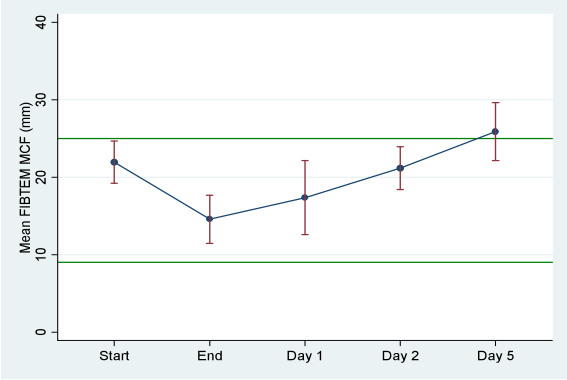
Figure 7.5 Change in thrombin generation parameters by POD Error bars indicate interquartile ranges. Green lines represent reference ranges (LT 1.3-2.5 mins, PH 250-425 nM, ETP 170-280 nMxmin).



1: Changes in EXTEM CT by POD



2: Changes in EXTEM MCF by POD



3: Changes in FIBTEM MCF by POD

Figure 7.6 Changes in ROTEM parameters by POD. Error bars indicate interquartile ranges. Green lines represent reference ranges (EXTEM CT: 38-79 seconds, EXTEM CFT: 34-159 seconds, EXTEM MCF: 50-72mm, FIBTEM MCF: 9-25mm)

7.5 Discussion

This study in a large and relatively homogenous group of patients, in which we measured concurrent changes in conventional coagulation tests, pro and anticoagulant levels, thrombin generation and thromboelastometry in the early post operative period, demonstrates that the conventional coagulation tests (PT/INR) need to be interpreted with caution following major liver resection.

The degree of parenchymal loss, and extent of hepatectomy, have a significant effect on the synthesis of liver derived clotting factors, and this is reflected in the fact that most patients following major liver resection have an elevated INR. This has been interpreted as representing a potential bleeding risk and consequently used as a basis for clinical decision-making, including when to start thromboembolic prophylaxis. (296). However, the risk of thromboembolic events in these patients exceeds the bleeding risk, and is increased with the extent of liver volume resected (288). This is becoming of increasing importance because not only is the number and complexity of hepatic resections increasing, but also the age and associated co-morbidity of patients (276).

Our study shows that although procoagulant levels are largely returning to normal by POD 5, levels of the anticoagulant proteins, specifically protein C and AT remain suppressed. The proportional reduction in anticoagulant to procoagulant proteins, as shown by a deranged protein C:FVIII ratio, is more profound in patients with higher values of INR (≥1.5), and this together with the raised levels of Factor VIII and VWF creates a potentially prothrombotic state (157, 297). The situation is therefore far more complex than the commonly held assumption that the post operative increase in INR de facto reflects an increased bleeding risk.

We found that pro-coagulant factor levels were all reduced following liver resection, with the exception of FVIII, which steadily increased [20]. That these changes are specific to hepatic resection, and are not mirrored by other abdominal surgery, such as pancreaticoduodenectomy, has been demonstrated in a number of other studies [21-22, 30-31]. Following other types of major surgery, there is a small (20%) initial reduction in pro and anticoagulant levels up to POD2, and only modest increases in VWF and FVIII (298).

The reduction in coagulation inhibitors persists well beyond the time that INR and fibrinogen levels normalize, suggesting that the haemostatic balance rapidly favors procoagulant rather than anticoagulant mechanisms. We also found a rise in D-dimer levels, consistent with active clot formation and turnover [23-24,32].

Although conventional tests of coagulation (as shown by a significant increase in INR) indicate a hypocoagulable state following liver resection, thromboelastometry and thrombin generation results within our study remained within the reference ranges for the group as a whole, suggesting that haemostatic capacity may be preserved to a greater degree than has previously been thought. These findings are similar to those reported by other groups (284, 287, 299). It is now recognized that conventional coagulation tests have many limitations, in that they are insensitive to endogenous anticoagulant levels, and give no information on the presence or absence of hypercoagulability (300). Viscoelastic coagulation tests, measure the dynamics of clot formation, from initiation, speed of clot strengthening through to clot stabilization and dissolution, and give an integrated assessment of the entire coagulation process. It has been demonstrated that they may give more valuable information about the overall haemostatic status than PT/INR in critically ill patients (301) and in patients with liver disease (135).

Despite an elevated INR, in our series most patients had normal ROTEM parameters, consistent with results shown in other studies. It is of note that where hypercoagulablity has been described in these studies, this relates to either a shortening of the R/CT (clot initiation), usually immediately after surgery, or more frequently, an increase in MA/MCF from POD 5 onwards (283, 287). It is well known that there is poor correlation between the PT/INR and R/CT time of viscoelastic tests (207, 302, 303), and this was also found in our series. The R (CT) time reflects the balance of pro and anticoagulants and is prolonged by excess anticoagulants or low clotting factor levels (≤ 30%), and is shortened by excess tissue factor, high VIII and low protein C (304). An increase in maximum clot firmness or amplitude may be due to a combination of increased fibrinogen levels and also platelet reactivity. It has also been shown there is a moderate to strong correlation of Factor VIII levels to the ROTEM parameters EXTEM MCF and FIBTEM (305). High levels of Factor VIII, together with low levels of its natural inhibitor, protein C, may be a partial explanation of the normal to increased thrombin generation seen in patients following major liver resection and contributes to the normal clot strength seen in these viscoelastic tests (306). Although malignant disease is associated with a pro-thrombotic tendency and increased risk of thromboembolic events, a recent study that monitored patients post operatively with thromboelastography found no difference between patients with either benign or malignant indications for liver resection, and in addition there was no significant difference in patients who had received chemotherapy, with all patients showing TEG parameters within the normal range (307).

Thrombin generation assays, which measure the total amount of thrombin generated in vitro, account for plasma concentrations of both pro and anticoagulants, unlike

conventional coagulation tests that are responsive only to procoagulant levels. In our series no differences were found for parameters of thrombin generation with the exception of peak height, which was lower in patients with an INR ≥ 2.0. Measures of thrombin generation in the first 24 hours suggest an activated coagulation presenting as shortened lag time and time to peak, whereas the lower peak height and slope are consistent with decreased procoagulant factor levels with a relative preservation of the endogenous thrombin potential. Beyond the first 24 hours, thrombin generation parameters return to normal range irrespective of the degree of prolongation of the INR. Simultaneously in the post–operative period ETP-Protac® is increased, and correlated with decreased protein C/VIII ratio, confirming the presence of a prothrombotic environment (157). This ratio has been documented to be associated with an increased thrombotic risk in patients (308). It is of note that the lowest ratios in our series occurred in patients with the most elevated INRs demonstrating dysregulated coagulation. A recent paper also found that although endogenous thrombin potential decreases slightly following liver resection, the addition of thrombomodulin to the assay resulted in increased, and slightly hypercoagulable indices of thrombin generation, indicating that the profound and sustained post operative deficiency in protein C, together with the relative protein C resistance, appears to be mechanistically linked to a post operative pro-thrombotic state (284).

From POD 1 onwards, an INR of ≤ 2.0 s is associated with near normal levels of most procoagulant factors and normal thrombin generation and ROTEM parameters across our study population. Levels of factor VII were ≤ 30 in some patients, irrespective of the INR, but never less than 10%, a level which is considered haemostatic for this factor (309). These findings therefore question the practice of administering prophylactic FFP prior to invasive

procedures purely to reduce a perceived bleeding risk if the INR ≤ 2, or indeed the need to delay removal of epidural catheters until the INR returns to normal range. Although transfusion of FFP will partially correct an elevated INR following liver resection, it has no measurable effect on viscoelastic parameters that are already within normal range (310). Our data supports the need for early initiation of thromboprophylaxis, and this is paradoxically of even more importance in patients with more extensive resections and consequently higher INR values. It is of note that in our own series, using the institutional protocol for initiating pharmacological prophylaxis current at that time, 50% of patients still had not received low molecular weight heparin by POD 2, as their INR was ≥ 1.5. Our study was not powered to detect an association between coagulation profiles and the occurrence of thrombotic events, nor did we prospectively observe for these. However, of the three patients that did develop a VTE, it is of note that they were all relatively elderly, had more extensive resections, and all had a significant delay in initiation of LMWH due to an elevated INR. With the exception of one patient whom subsequently developed multiorgan failure, no patient in this series had any complication related to bleeding.

7.6 Conclusion

We have demonstrated that following major liver resection the post-operative period is characterised by a dynamic dysregulation of coagulation, with initially relatively balanced, but low levels of pro and anticoagulants, with a rapid switch by POD 2 to a prothrombotic environment, with depression of anticoagulant levels, and a decreased protein C/VIII ratio persisting to, and probably beyond, POD5. The persistence of these prothrombotic changes argues for an extended duration of anticoagulant therapy, as has been suggested by others (311).

Although thrombin generation assays give much more representative information of the coagulation status than the INR, as they are sensitive to both pro and anti coagulant proteins, they are moderately complex tests that are not currently routinely available. It is of note that following liver resection, studies that have used global viscoelastic tests (TEG® and ROTEM®) have also demonstrated normal coagulation status, despite a raised INR, and some have shown hypercoagulability after POD 5 (299, 312). It may be that these global tests are more appropriate methods of monitoring the complex changes in coagulation that follow major liver resection. We have shown that thromboelastometry demonstrates normal coagulation in these patients, even when the INR is raised, and this is corroborated by the contemporaneously matched falls in both pro and anticoagulant proteins with preserved thrombin generation. It is important to appreciate that although the two most commonly used viscoelastic tests (TEG, ROTEM) give essentially similar information, the different activators and reagents used in the two technologies can introduce subtle differences, and also standard operating procedures and quality assurance must be robustly maintained when using this equipment (313).

There is likely to be a role for the use of viscoelastic coagulation testing for further characterising patients with deranged conventional tests at the bedside to guide clinical decisions, such as the need for transfusion of potentially hazardous blood products, and to define the relative risk of thrombosis, but large scale clinical outcome studies will be required to test this hypothesis. Crucially, our results show a switch to hypercoagulability after 24-48 hours.

Finally, it is clear, that following major liver resection, an elevated INR should not be taken as evidence of “auto-anticoagulation”. Thromboprophylaxis should always be started as

soon as possible, and certainly when the INR is ≤ 2. The prevalence of VTE in these patients, even in those that receive early chemoprophylaxis, highlights the need for more clinical studies to define the best method of anticoagulating these patients, and to determine how long the period of excess risk persists.

Chapter 8

The efficacy of fresh frozen plasma to reverse coagulopathy following major hepatic resection.

An in vitro study of the efficacy of fresh frozen plasma to correct prolonged INR, and to assess the effect on thromboelastographic parameters following major liver resection published in Transfusion Medicine 2015.

Schofield N, Sugavanam A, Henley M, Thompson K, Riddell A, Mallett SV. An in vitro study comparing two dose regimes of fresh frozen plasma on conventional and thromboelastographic tests of coagulation after major hepatic resection. Transfusion Medicine 2015;25:85-91

8.1 Introduction

Following major liver resection there are frequently significant alterations in the coagulation system. Many factors contribute to this, including pre-existing liver dysfunction, extent of intraoperative blood loss, surgical technique, ischaemia – reperfusion injury, and importantly, the volume of liver remaining following resection, as most coagulation proteins are synthesised in the liver (274, 275). In the early post operative period, conventional laboratory tests of coagulation are often indicative of a temporary hypocoagulable state, however, in reality bleeding complications are rare (277).

Following hepatic resection, although levels of most procoagulants are reduced in the first few days after surgery, leading to an increase in INR, there is also a concomitant decrease in the natural anticoagulants, protein C and antithrombin (AT). Levels of factor VIII are markedly elevated, and at the same time there are increases in von Willebrand's factor (VWF) and reduced levels of its cleavage enzyme ADAMTS-13 (312, 314, 315). The coagulation status following major hepatic resection is therefore extremely complex, and it is known that these patients have a significant risk of developing thromboembolic complications, despite the elevated INR (289). Alternative tests of haemostatic competence may give more meaningful information than conventional coagulation tests (CCT) in these circumstances, and it is of note that global viscoelastic tests (VET) of coagulation (thromboelastograph [TEG®] and thromboelastometry [ROTEM®] , which reflect both pro and anti-coagulant activity have been shown to be within normal ranges (285, 286) or even hypercoaguable (287) in patients following hepatic resection.

Nevertheless, the almost universal increase in prothrombin time (PT) and international normalized ratio (INR) following major liver resection is still generally assumed to represent

a potential bleeding risk. This does, on occasion, lead to the administration of fresh frozen plasma (FFP) to correct the INR to <1.5 prior to invasive procedures (278), or to "cover" epidural catheter removal in these patients ((316) . Although not all institutions would transfuse FFP prior to invasive procedures in patients with an INR >1.5, a significant number would (317). It is known that many clinicians do not routinely repeat the INR after prophylactic transfusion of FFP, despite the fact that the change in INR is frequently minimal (280), and dependant both on the initial INR, and the dose of FFP administered (318).

8.2 Aims

The purpose of this observational *in vitro* study was to determine whether the two most commonly used dose regimes of FFP (7.5ml/kg and 15ml/kg) are effective in decreasing the INR to 1.5 or less, in patients following major hepatic resection who have mild to moderate (1.6 -2.5) prolongation of INR, as this is representative of the majority of these patients that might receive prophylactic FFP transfusion prior to invasive procedures. As a secondary aim, we performed TEG studies on all these patients to determine the global VET profile, and in those whom the INR was >1.5 on POD 2 we determined the effect on the VET parameters of *in vitro* spiking with the same doses of FFP.

8.3 Methods

The study was conducted with approval from the UCL/UCLH Committees on the Ethics of Human Research, and written consent was obtained from all participants between Sept 2011 and June 2012. Patients were screened for eligibility through the hospital theatre booking system. Inclusion criteria included all patients with primary hepatocellular carcinoma or secondary colorectal metastatic disease undergoing major liver resection (at

least 20%, as judged by computed tomography scan during work- up) who had an INR value of greater than 1.5 on postoperative day 2 (POD2) . Patients were excluded if they had an abnormal coagulation screen or platelet count at baseline, were receiving anti-platelet agents within the last 7 days prior to surgery, or refused consent. Citrated whole blood samples were collected at baseline (prior to surgery) and on POD2. Those samples exhibiting an INR > 1.5 were further studied via thromboelastography and repeat INR pre and post FFP spiking in vitro.

8.3.1 Blood Sampling and Testing

Blood was collected from the central venous catheter if in-situ, or by single venepuncture of an antecubital vein using a 21G needle. Two samples were collected, the first 10ml sample was discarded to minimize the effect of tissue thromboplastins and the second sample was used for analysis. Blood was transferred into 2.7ml citrated blood tubes (BD Vacutainer, Franklin lakes, New Jersey,USA), mixed and rested for 30 minutes prior to analysis. All coagulation studies were carried out using an ACL TOP coagulometer (Instrumentation Laboratory, Bedford , MA,USA). Conventional tests of coagulation (INR) was performed using Recombiplastin 2G reagent (Instrumentation Laboratory, the international sensitivity index (ISI) of this was 0.97; and citrated FBC was tested on the Sysmex XS1000i full blood count analyser (Sysmex, Milton Keynes, UK). Viscoelastic tests were performed on citrated blood, which was left for 30 minutes, as per manufacturers instructions, and inverted 5 times prior to analysis to ensure adequate mixing. An unactivated aliquot of 340µl of citrated blood was added to 20µl of calcium chloride (0.2mol/L) using reverse pipetting technique into a cuvette and placed within the analysis well of the 5000 TEG® analyser (Haemonetics, Braintree, MA, USA). Coagulation was then assessed using the TEG 4.2.2

software. A regular two-point quality control (QC) procedure (one sample with normal coagulation parameters and another with hypocoaguable parameters) was performed as per manufacturer instructions.

Throughout this study rather than PT, we have quoted INR, as this is the variable quoted by our laboratory for all routine tests. Given that the reagent ISI is close to unity any differences in the ranges we have measured will in any case be very small.

8.3.2 FFP Spiking

The dosage of FFP used in the study are those commonly used in clinical practice, in keeping with the TOPIC trial, which chose 12ml/kg for the use of FFP in non- bleeding ICU patients (319). FFP from a single donor was obtained from the local blood transfusion laboratory, and the storage time was within the accepted shelf life of this blood product. This was thawed and divided into 2ml aliquots, re-frozen and stored at minus 80°C. Storage at this temperature has been shown to be associated with minimal degradation in clotting factors (320)). The required aliquots were then thawed prior to use. On POD2, whole blood from patients was spiked with FFP *in-vitro*, if the INR was ≥1.5. Blood collected in 2.7ml citrated blood tubes (BD Vacutainer), was mixed and rested for 30 minutes prior to analysis. Using an estimated blood volume of 70ml/kg, a ratio of 0.21ml FFP to 1ml whole blood was used for spiking whole blood with 15ml/kg FFP and 0.11ml FFP to 1ml whole blood was used for spiking with 7.5ml/kg FFP. The spiked samples were then inverted to ensure adequate mixing prior to analysis. The same volume of spiked blood was then analysed using CCT and thromboelastography in the same manner as described above. Whole citrated blood, which was not spiked, was used as a control. TEG® parameters were collected at all time points.

8.3.3 Statistical Analysis

During the study period, 47 patients were eligible for inclusion and entered the selection process. Twenty patients (42.5%) had an INR > 1.5 on POD2 and were eligible for further analysis following *in vitro* spiking with FFP.

Data from the CCT and TEG analysis were tested for normality and then reported as mean +/- 1 standard deviation for the group. Analysis was performed using Graphpad® software (San Diago, USA), and a students t-test to compare the means of two groups. CCTs and TEG® values were compared between baseline and POD2 as well as pre- and post- FFP spiking on POD2. A p- value of <0.05 was accepted as statistically significant.

8.4 Results

8.4.1 Conventional and VET coagulation tests at baseline and POD2

Post operative changes in coagulation for patients with INR ≥ 1.5 on POD 2, and for patients with INR ≤ 1.5 are shown in Table 9.1. For the group in which INR was ≥ 1.5, there was a significant increase in INR from 1.15 at baseline to 1.95 on POD 2 (p=0.0001), which was associated with a significant decrease in platelet count from 166 x 10^9 L^{-1} to 95 x10^9 (p=0.0001). The TEG parameters were unchanged, except for MA which was significantly reduced from 66.91mm to 54.03 mm (p=0.001). For the group with an INR ≤ 1.5 on POD 2, there was a significant increase in INR from 1.07 at baseline to 1.27 (p=0.0001) and a decrease in platelet count from 188 x 10^9 to 120 x 10^9 (p=0.005). All the TEG values remained unchanged.

8.4.2 Changes in coagulation with FFP spiking in patients with INR > 1.5

Changes in coagulation with FFP spiking are shown in Table 8.1. There was a significant reduction in the INR from 1.94 (SD 0.59) to 1.46 (SD 0.27 p=0.005) and 1.36 (SD 0.18 p=0.0007) with FFP 7.5 ml/kg or 15ml/kg respectively. Haemoglobin also dropped with spiking from 91.7 g/l (SD 21.2) to 80.1 g/l (SD 12.7 p=0.45) and 72.3 g/l (SD 12.3 p=0.002) with FFP 7.5 ml/kg or 15ml/kg respectively. There was no significant change in platelet count with FFP.

FFP spiking had no significant effect on any TEG parameter (Figure 8.1). The R-time remained below the normal range after FFP spiking, suggesting a hypercoagulable state. The MA remained within the normal range before and after FFP spiking. At the higher doses there was a slight lengthening of the R time, the percentage of patients and delta change in INR after FFP spiking are shown in Table 8.3, and the percentage of patients in which the INR corrected to < 1.5, and the mean change in INR is shown in Table 8.4.

	INR < 1.5 on POD 2			INR > 1.5 on POD 2		
	Baseline Mean (SD)	POD 2 Mean (SD)	P-value	Baseline Mean (SD)	POD 2 Mean (SD)	P-value
Conventional tests (Normal range)						
INR (0.9 -1.2)	1.07 (0.08)	1.27 (0.17)	0.0001*	1.15 (0.15)	1.95 (0.59)	0.0001*
Haemoglobin (130-160 g/l)	110 (20.4)	100 (13.7)	0.2	97.9 (13.0)	91.7 (21.2)	0.3
Platelets (140-400 x 10^9)	188 (58)	120 (43)	0.005*	166 (47)	95 (38)	0.0001*
Thromboelastography (Normal range)						
R time (9-27 min)	5.8 (1.36)	4.6 (1.85)	0.3	6.17 (2.25)	6.7 (3.75)	0.6
Alpha angle (22-58)	67.71 (8.11)	60.8 (10.75)	0.3	66.51 (8.06))	58.7 (12.69)	0.06
MA (44-64 mm)	56.60 (21.98)	47.80 (9.62)	0.6	66.91 (9.28)	54.03 (10.57)	0.001*

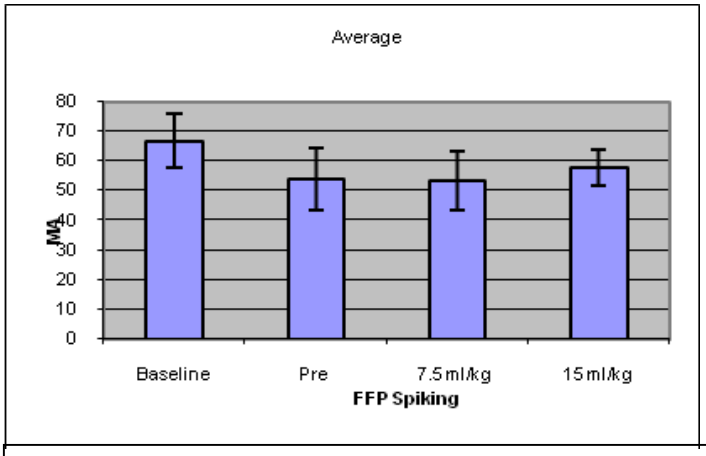
Table 8.1 Changes in coagulation variables between baseline and POD 2 in patients with INR < 1.5 (n=27), and in patients with INR > 1.5 (n=20)

* Denotes significance

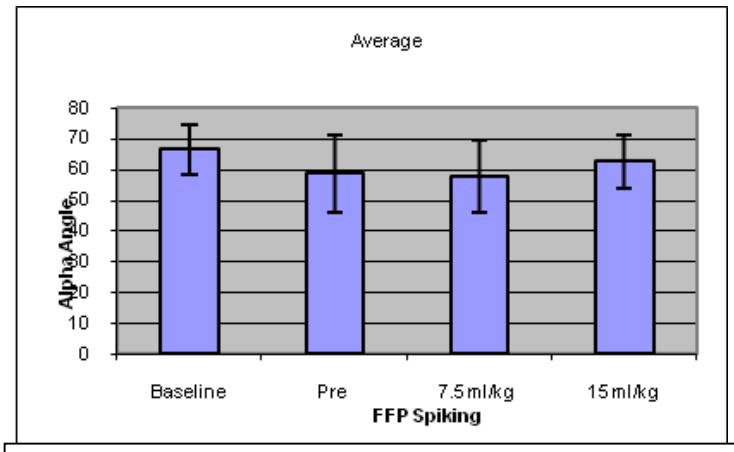
	POD 2 Mean (SD)	FFP 7.5 Mean (SD)	FFP 15 Mean (SD)	FFP 7.5 P-Value	FFP 15 P-Value
Conventional tests (Normal range)					
INR (0.9 -1.2)	1.94 (0.59)	1.46 (0.27)	1.36 (0.18)	0.0048*	0.0007*
Haemoglobin (130-160 g/l)	91.7 (21.2))	80.1 (12.7)	72.3 (12.3)	0.0446*	0.0016*
Platelets (140-400 x 10^9)	95 (38)	83 (34)	88 (30)	0.3052)	0.5185
Thromboelastography (Normal range)					
R time (9-27 min)	6.7 (3.75)	7.02 (2.42)	7.13 (3.02)	0.7669	0.7082
Alpha angle (22-58)	58.71 (12.69)	57.74 (11.89)	62.41 (8.8)	0.8240	0.3360
MA (44-64 mm)	54.04 (10.57)	53.3 (9.82)	57.81 (6.1)	0.8349	0.2031

Table 8.2 Changes in coagulation variables with FFP spiking in patients with INR > 1.5

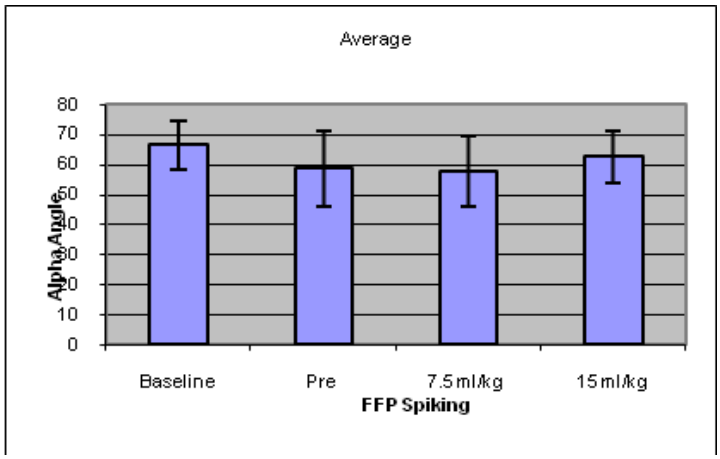
*Denotes significance



(a) Change in TEG MA (mean (SD)) from baseline to POD2, and after FFP spiking with 7.5ml/kg and 15ml/kg FFP. (Normal range 44-64)



(b) Change in TEG R-time (mean (SD)) from baseline to POD2, and after FFP spiking with 7.5ml/kg and 15ml/kg FFP. (Normal range 9-27)



(c) Change in TEG Alpha-angle (mean (SD)) from baseline to POD2, and after FFP spiking with 7.5ml/kg and 15ml/kg FFP. (Normal range 22-58)

to

Figure 8.1 Change in TEG parameters from baseline to POD 2

Change in INR	7.5 ml/kg	15 ml/kg
- 0.1 to 0.2	28.6%	14.3%
- 0.3 to 0.4	42.9%	50%
- 0.5 to 0.9	14.3%	21.4%
- >1.0	14.3%	14.3%

Table 8.3 Percentage of patients and extent of reduction in INR after FFP spiking

Initial INR	FFP 7.5mls/kg Correcting to INR <1.5	FFP 15 mls/kg Correcting to INR <1.5	FFP 7.5 mls/kg Delta change in INR (mean)	FFP 15 mls/kg Delta change in INR (mean)
>2.0	0%	33.3%	1.01	1.28
1.7-1.9	66.6%	100%	0.28	0.40
1.5-1.6	100%	100%	0.27	0.33

Table 8.4 Percentage of patients in which INR corrected below 1.5 after FFP spiking based on POD2, and mean delta change in INR after FFP spiking

8.5 Discussion

This is the first study, to our knowledge, investigating the effects of *in vitro* FFP spiking of whole blood from patients undergoing major liver resection with elevated INR values. A total of 42.5% of patients following major liver resection had an INR ≥1.5 on POD2, a value which is often taken as the threshold for transfusing pre-procedural prophylactic FFP, or for withholding pharmacological thromboprophlaxis therapy. There was a dose related reduction in INR after spiking with FFP and the degree of correction was dependent on both the initial INR value and the volume of FFP used. All patients showed a reduction in INR in

response to FFP, and this response was more pronounced with the larger (15mls/Kg) dose. This higher dose also led to more pronounced dilutional effects, with the haemoglobin falling by approximately 20g/l. It must be considered that *in vivo* this effect would likely be modified by many factors, and the fall may well not be as pronounced as in the *in vitro* model, but it is nevertheless a real issue when transfusing large volumes of FFP, and could result in transfusion of red blood cells to maintain the haemoglobin above a pre-determined level. There is also the associated risk of transfusion related acute lung injury (TRALI) and transfusion associated circulatory overload (TACO).

The findings from this *in vitro* study are in agreement with previous observational studies in patients, which demonstrate when the INR is only mildly to moderately prolonged, changes in INR following FFP administration are small, inconsistent, and even at 15ml/Kg, correction is not guaranteed (280, 321).

At baseline, all our patients had normal or hypercoagulable TEG parameters in keeping with findings from other published studies. Despite the commonly described fall in platelet count found postoperatively after liver resection (315)the MA remained within the normal range. Although the INR was elevated, none of these patients had an R-time that was prolonged, and most had values below the normal range (hypercoagulable). It has consistently been shown that there is very poor correlation of R-time to PT/INR (97, 98, 201), and the fact that the R-time was relatively short, suggests that these viscoelastic tests may give information which is not adequately reflected by the INR alone (322, 323).

The R time (time to clot initiation) is a reflection of the balance of both pro and anticoagulants, and in general, is prolonged by excess anticoagulant or low clotting factor levels, and is shortened in the presence of excess tissue factor, high factor VIII levels and

low protein C levels (304)). The changes in coagulation that occur following liver resection (reduction in both pro and anticoagulant factors and increased factor VIII and VWF) are not adequately reflected by the INR value, which is only sensitive to procoagulants, especially factor VII. This explains why the INR alone may not be a useful indicator of the potential risk of bleeding following liver resection, and why VET may give more clinically relevant information (324). Our finding that FFP partially corrects a moderately prolonged INR without leading to any change in viscoelastic parameters is similar to results found in an *in vitro* study of FFP in patients with cirrhosis: FFP provides both pro and anticoagulant factors, and where both are simultaneously reduced, the addition of FFP will leave the balance of coagulation unaltered and thrombus generation unchanged (325).

There are of course limitations extrapolating *in vitro* studies to a clinical scenario: the contribution of endothelial and vascular factors, buffering, pH control, metabolic derangement and electrolyte environment are lacking in these studies. In addition, it is possible that thrombomodulin (a natural activator of protein C) is needed to activate the natural anticoagulants and assess their contribution to clot formation. Despite these limitations, spiking with FFP has been shown to be a good model for studying the effects that transfusion of such products has on coagulation (325, 326), and VET analysis is increasingly used to determine the *in vitro* effect of procoagulant and other haemostatic therapies.(304, 327). Indeed, *in vivo* trials using FFP to treat prolonged INR have been notoriously difficult to conduct, and a recent study in non bleeding ICU patients (TOPIC trial) had to be stopped early due to poor recruitment. The trial organisers suggested that there was still a general lack of knowledge about FFP , and that there are very entrenched

personal beliefs about the preferable transfusion strategy, which contributed to the very slow recruitment to this study (328).

8.6 Conclusions

There is a surprising lack of evidence of any known efficacy of FFP transfusion in a variety of clinical situations for which it is commonly prescribed, particularly when the INR is <2. (2, 321, 329, 330). Although up to 50% of FFP is transfused prophylactically, there are no trials that demonstrate a benefit (331). Nevertheless, the practice of prophylactic transfusion of FFP still continues, driven largely by historical precedent that it is a "good thing to do", and the desire to cover all eventualities, including litigation, should excessive bleeding occur. Increasingly, clinicians are recognising that a substantial amount of FFP is transfused without clinical benefit, is wasteful of a valuable resource, and can cause unintentional morbidity, and even mortality, in recipients (332). Most FFP transfusions in non bleeding patients are performed to correct an abnormal INR. Recently, some institutions have adopted a restrictive transfusion policy for FFP, and demonstrated significant cost savings without any detrimental effect to patients.(318, 333).

This study reinforces the fact that INR alone is a poor indicator of overall coagulation following hepatic resection surgery. Moderate elevations of INR suggesting hypocoagulability were not reflected by viscoelastic coagulation parameters, This study goes some way to highlighting that in patients who have had hepatic resection surgery, the practice of transfusing FFP prophylactically in order to correct mild to moderately elevated INR values is unlikely to achieve the desired reduction in INR. We also have shown that TEG parameters remain essentially unchanged by *in vitro* FFP administration, and consequently, further question the clinical value of administering prophylactic FFP to patients solely on

the basis of the INR, as this practice is almost certainly unnecessary, potentially wasteful, and associated with patient risk. This *in vitro* data is only a preliminary step, but it highlights the need to design clinical trials that are based not on INR, but on alternative tests of haemostasis, in order to stratify and manage bleeding risk in these patients.

Chapter 9

Thesis discussion, conclusions and future directions

The hypothesis presented in this thesis is that global viscoelastic tests of coagulation provide more clinically relevant information than conventional laboratory tests in patients with liver disease, and also in patients who have had major liver surgery. As the liver is the major site for synthesis of coagulation proteins, an elevation of the PT/INR is a good indicator of liver synthetic function, and consequently is used in a number of scoring systems, such as MELD and UKELD. However, an abnormal PT/INR is also perceived as an index of bleeding risk in these patients, and consequently used as a trigger threshold for transfusing pre-procedural plasma, or as a basis for withholding chemical thromboprophylaxis in the belief that these patients are "auto-anticoagulated". This is despite the fact that numerous studies have demonstrated that these tests have a very poor predictive value for determining an increased risk of bleeding (2), and also that many of these patients are in fact at an increased risk of thromboembolic events compared to the general population (70). There is a discrepancy between the information from clotting tests that have routinely been used in clinical practice and the evidence regarding thrombotic and bleeding events (334).

In patients with liver disease, haemorrhagic complications, such as variceal bleeds or procedural bleeds are common, and frequently associated with abnormalities of standard coagulation tests, and consequently a direct cause-effect relationship has been assumed. It has therefore been common practice to treat or prevent bleeding with blood products such as fresh frozen plasma, platelets and also factor concentrates. In general the ability of these prohaemostatic interventions to prevent or stop pre-procedural bleeding has not been validated in randomised clinical trials, but is based on their capacity to improve or correct

the abnormalities of the coagulation tests. As a consequence of an elevated INR, an inordinate amount FFP is administered in patients with cirrhosis for prophylaxis without evidence of utility (335). In stable cirrhosis, many patients are relatively hypercoagulable, however in decompensated patients bleeding is relatively common (40%), and relates to raised portal pressure, and also possibly to systemic endogenous heparinoids released from the vascular endothelium in response to stress due to sepsis or hypoxia. In order to improve the management of these patients it is clear that better ways of assessing the coagulation status are required to refine clinical practice in this setting.

To establish the basis of this thesis, in chapter 1, traditional (cascade) and newer (cell based) models of haemostasis are reviewed and then discussed in relation to the limitations of conventional coagulation tests to patients with liver disease. These tests are performed using platelet poor plasma, and therefore cannot account for thrombin generation that is mediated by the presence of platelets (39). In addition, these tests conclude at the point that fibrin strands start to form, and when only a tiny fraction of total thrombin generation has occurred, so giving no information on overall clot strength and stability. They inform on the initiation of clotting but not the haemostatic capacity in terms of clot formation and maximal thrombin generation. The majority of coagulation tests assess pro-coagulant capacity in isolation, and give no information about the integrated effect of pro and anti-coagulants, platelets and fibrinogen, nor do they reveal the effect of potential compensatory mechanisms within the haemostatic system such as elevated Von Willebrand's factor.

It therefore seems prudent to explore if global viscoelastic tests using whole blood can provide more relevant information. In chapter 2, the principles and limitations of VET are

discussed. The fact that they can detect hypercoagulability and also fibrinolysis, neither of which can be determined using conventional tests, is highlighted as a potential advantage in patients with liver disease.

In chapter 3, a review of the current literature that has referenced VET in patients with liver disease is presented. Although these are mainly observational trials, they do illustrate the potential of these tests to give more clinically useful information. Coagulation analysis with TEG and ROTEM has corroborated the concept of re-balanced haemostasis in liver disease as proposed by Tripodi, and also by Lisman (7, 241). The majority of stable patients with chronic liver disease have normal viscoelastic tests, and this calls into question the practice of giving prophylactic treatment purely to correct an elevated INR value or a moderately reduced platelet count (141, 325). A recent randomised trial of pre-procedural plasma and/or platelets in cirrhotic patients based on conventional coagulation tests (INR ≥1.8 or platelet count ≤ 50,000 x 10^9) or thromboelastometry parameters of R time and MA, demonstrated highly significant reductions in transfusion in the TEG group without any increase in bleeding complications (336).

In chapter 4, the use of VET in guiding haemostatic interventions during liver transplantation is discussed. Their potential use is twofold; firstly they prevent the unnecessary and inappropriate transfusion of blood products purely to correct abnormal laboratory tests when the TEG/ROTEM results are within normal range (337), and secondly they facilitate targeted, goal directed therapy of specific haemostatic defects during active bleeding, resulting in more rapid control of coagulopathy and ultimately less transfusions (120).

Whether fibrinolysis is a significant factor in producing excess bleeding during liver transplantation, and how it should be managed, has always been somewhat contentious.

This is now an even more relevant issue as the number of patients that can undergo liver transplantation without the need for blood or blood product transfusion is increasing every year due to the adoption of more aggressive fluid restriction, and other patient blood management techniques (338). The risk benefit of using anti-fibrinolytic drugs has shifted as blood loss has decreased and the risk of thrombosis is increasingly recognised (227). In chapter 4, a retrospective review of TEG samples in patients undergoing liver transplantation demonstrates that the prevalence of fibrinolysis is actually quite low (<25%), and throws doubt on the value of giving prophylactic anti-fibrinolytic therapy to these patients. It is also clear that the timing of the appearance of fibrinolysis is important. It is most common immediately after reperfusion of the liver graft, and will usually spontaneously disappear without treatment in the presence of good donor graft function. Conversely, fibrinolysis appearing earlier, during the dissection or anhepatic stage, will usually progress and become more severe if anti-fibrinolytic treatment is not administered.

It is now recognised that a raised INR does not preclude the possibility that some patients may be prothrombotic, as demonstrated by enhanced thrombin generation and high factor VIII/protein C ratios (66, 297). In addition, it is known that these patients have increased levels of circulating microparticle Tissue Factor activity that also contributes to the activation of coagulation and thrombosis in these patients (339). Knowledge of individual coagulation components does not allow a comprehensive estimation of overall haemostatic/thrombotic risk. In general this risk results from a combination of changes in several components of the haemostatic system (340). There are unmet needs with regard to identifying patients at risk of thrombosis. A possible solution is the use of global coagulation tests that reflect the major physiological aspects of the haemostatic process in vitro (341).

There does appear to be a correlation between hypercoagulability detected on VET and thromboembolic complications in patients undergoing major surgery (342). In Chapter 6, we describe the prevalence of hypercoagulability in patients undergoing liver transplantation. There appears to be a different distribution of hypercoagulability according to disease aetiology, increased clot strength (platelet-fibrinogen interaction) is increased in patients with cholestatic liver disease, and also in some patients with acute liver failure, whilst evidence of plasmatic hypercoagulability (short R time) is more common in patients with alcoholic and viral liver disease. In this cohort of 100 patients, the incidence of thrombotic complications was too low to demonstrate a definite association, but it is clear than an elevated INR in no way precludes the possibility of hypercoagulability.

Following major hepatic resection it is known that the risk of pulmonary embolism exceeds that of bleeding, and that this risk increases with the volume of liver parenchyma resected. It appears paradoxical that patients with INR values > 1.5 are more likely to have thromboembolic complications than those with an INR <1.5. In chapter 7, we undertook a prospective evaluation of coagulation changes in patients following major hepatectomy. We found that the proportional reduction in anticoagulant to procoagulant proteins, as shown by a deranged protein C:FVIII ratio, is more profound in patients with higher values of INR (≥1.5), and this together with the raised levels of Factor VIII and VWF could create a potentially prothrombotic state. The situation is therefore far more complex than the commonly held assumption that the post operative increase in INR de facto reflects an increased bleeding risk. Despite the elevated INR, viscoelastic tests did not indicate hypocoagulability at any point, and were borderline hypercoagulable by post operative day 5. This study demonstrates some of mechanistic reasons for the prothrombotic state, and

also that viscoelastic tests provide a more realistic assessment of underlying haemostasis in this setting than do conventional coagulation tests.

As it is still common practice for clinicians to use INR as the basis for prescribing pre-procedural FFP, in chapter 8 we evaluated the *in vitro* effect of administering FFP to patients with an elevated INR following major liver resection. Although there was some degree of correction of the INR, which was dose dependant, there was no significant change in thromboelastographic variables, which remained within normal range. This emphasises that INR is only responsive to pro-coagulant levels, and is not a good indicator of thrombin generation or haemostatic capacity.

Overall we have demonstrated throughout these studies that viscoelastic tests of coagulation give more clinically relevant information than conventional tests. No test can predict with absolute certainty that a patient with liver disease will bleed during a procedure or will definitely develop a thrombotic complication, however the global, integrated nature of VET gives insight into the underlying haemostatic status, and could potentially be used to provide a method of risk stratifying these patients. Studies are starting to be published that demonstrate that if VET parameters are normal, it is possible to avoid unnecessary transfusion with plasma and platelets even though conventional tests would suggest otherwise (336, 337, 343). In addition, as VETs are often normal, or even hypercoagulable in many of these patients, despite an elevated INR, this may encourage earlier use of pharmacological thromboproplylaxis. The various activators used to initiate these tests need to be considered, as they will lead to different interpretations (97, 103), and there is a need to standardise the methodology used. There is a clear need for future prospective outcome studies to determine how these tests can be integrated into clinical

practice to stratify a given patients risk of bleeding and/or thrombosis. It is to be hoped that this thesis provides some of the preliminary background for such studies, and the impetus to undertake further work in this field.

TEG/ROTEM Studies in Liver Disease and Liver Surgery	Study type	Comparative group and /or tests	Additional clinical information
Liver Disease			
Ben –Ari et al **J Hepatol 1997**	Observational 40 cholestatic cirrhotic & 40 non cholestatic cirrhotic patients	Control healthy volunteers TEG, SCT	Hypercoagulability (reduced R time, increased α angle and MA) common in patients with cholestatic liver disease
Chau et al **Gut 1998**	Prospective observational 20 cirrhotic patients with active bleeding	TEG, SCT Serial measurements over 7 days	Prolonged R and K time, and reduced α angle in group that bled. No difference in SCT
Papatheodorisidis GV et al **Hepatology 1999**	Prospective observation 84 cirrhotic patients	Native TEG, SCT Admission day 5	Deterioration in TEG parameters (R,K,α,MA) in all patients with confirmed infection
Mancuso A et al **Blood Coagul Fibrinolysis 2003**	Observational in 30 cirrhotic patients	Native whole blood versus citrated blood	Good correlation. Citrated whole blood allows delay between sampling and test run
Zambruni A et al **Scand J Gastroenterol 2004**	Prospective observational study in 30 cirrhotic patients	Paired native & heparinase TEGs, SCT	HLE in patients with infection. 60% also had elevated anti-factor Xa levels
Viera da Rocha EC et al **Clin Gastroenterol Hepatol 2009**	Prospective observational 92 cirrhotic patients post variceal band ligation	TEG (Kaolin), SCT, clotting factor levels & vWF	5 re-bleeds, no difference in any measured coagulation tests
Thalheimer U et al. **Scand J Gastroenterol 2009**	Prospective observational 10 cirrhotic patients pre and post TIPS	Native and heparinase TEGs	8/10 developed HLE post TIPS insertion. Persisted for 24-48 hrs.

Tripodi et al. **Thromb Research 2009**	Observational study in 51 cirrhotic patients & 58 healthy volunteers	ROTEM (EXTEM, INTEM & FIBTEM) & SCT	ROTEM CFT & MCF differentiates between health & cirrhosis & correlates with MELD. No correlation between PT/INR and CT (r =0.26)
Cakir T et al **J Gastroenterol Hepatol 2009**	Prospective observational 23 patients with obstructive jaundice	TEG, SCT, PFA-100	80% patients hypercoagulable on TEG. Correlation between MA and bilirubin concentration
Hickman et al **Ann Hepatol 2009**	Prospective observational in 28 patients with NAFLD	TEG, SCT 22 healthy controls	Clot kinetics altered in NAFLD : Increased clot strength and decreased susceptibility to lysis
Stravitz RT et al **J Hepatol 2011**	Prospective observational in 51 patients with ALF	TEG, CCT, pro and anticoagulant factor levels	Despite elevated INR (3.8) normal coagulation on 5 TEG parameters (mean values). Hypocoagulability and hypercoagulability observed in some patients. Thrombotic > bleeding complications
Agarwal B et al **J.Hepatol 2012**	Prospective observational 20 patients ALF admitted to ICU	Paired native & heparinase TEGs CCT, factor levels, vWF	Mean INR 4.2 No bleeding complications HLE present TEG normal in 45% & hypercoagulable in 35%
Tripodi A. et al **Liver International 2013**	Prospective study 26 cirrhotic thrombocytopenic patients	CCT, ROTEM and thrombin generation	Assessed pre and post transfusion one pool of platelets. Poor increment in platelet count, and global tests did not reach normal values.
De Pietri **Hepatology 2015**	RCT in 60 cirrhotic patients pre-procedure	Baseline TEG and CCT.	TEG group received significantly fewer transfusions (16% versus 100%) than conventional group. 1/60 had a bleeding complication (SOC group).

Liver Transplantation and resection			
Kang YG et al **Anesth Analg 1985**	Prospective observational 66 OLT patients	Historical cohort managed with SCT	33% reduction in total transfusion Goal directed treatment with TEG
McNicol PL et al **Anaesthesia Int Care1994**	Observational study of 75 OLT patients	TEG & SCT	TEG facilitates selective use of blood component therapy. Targeted treatment of fibrinolysis
Harding S et al **Br J Anaesthesia 1997**	Prospective observational 55 OLT patients	Paired native and heparinase TEGs	Allows assessment of heparin like effect Rationalises blood component therapy
Hendriks et al **Blood Coagul Fibrinolysis 2002**	Prospective observational 6 OLT patients	Administration of 80mcg/Kg rVIIA: SCT & TEG	rVIIA shortens PT and R value and also increases α angle & MA. Speed of clot formation increased and physical properties of clot altered
Cerruti et al. **Liver Transplant 2004**	Prospective observational in 10 Liver related liver donors	TEG, SCT	Normal coagulation on TEG despite increase in INR 50% hypercoagulable by day 5
Lerner et al. **Anesth Analg 2005**	Retrospective review of case reports of intraoperative Cardio-pulmonary thrombi	Comparison of TEG and SCT	SCT all hypocoagulable Majority of TEG hypercoagulable at time of event.
Coakley et al **J Cardiothorac Vasc Anesth 2006**	Prospective observational 20 OLT patients	Comparision of TEG,ROTEM and standard lab tests	Transfusion practice likely to differ according to method of monitoring FIBTEM useful for haemostatic management
Gorlinger K **Hamostaseologie 2006**	Retrospective observational in 642 OLT patients	ROTEM	Goal directed transfusion algorithms Targeted treatment of fibrinolysis

Gorlinger K **Hamostaseologie 2006**	Retrospective observational in 642 OLT patients	ROTEM	Goal directed transfusion algorithms Targeted treatment of fibrinolysis
Agarwal S et al **Liver Transplantation 2009**	Retrospective observational study in 211 OLT patients	Paired native & heparinase TEGS	At baseline HLE more prevalent in ALF (45.8%) than CLF (29%) HLE at reperfusion universal. Resolves spontaneously by end of case in 50% , persistence may indicate marginal graft function
Senzolo M et al **J Hepatol 2009**	Observational 20 OLT patients	10 ALF 10 Cirrhotics Paired native & heparinase TEGs	HLE at baseline in 50% ALF No difference in TEG derived thrombin generation ALF vs CLF
Hebstreit F et al **Anaesthesia 2010**	Prospective observational 20 OLT patients	ROTEM, SCT and point of care PT/PTT	ROTEM gives useful information on fibrinogen and platelets No correlation of CT and PT r=0.2
Roullett S et al **Br J Anaesthesia 2010**	Prospective observational 23 OLT patients	ROTEM & SCT	Detects thrombocytopenia & hypofibrinoginaemia
Noval-Padillo JA **Transplant Proc 2010**	Prospective observational 20 OLT patients	Historical cohort of 59 OLT patients	Increased use of fibrinogen concentrate with ROTEM Sig. reduction in overall transfusion
Gouvea et al **Liver Transplant 2010**	Prospective observational 16 Live related liver donors	ROTEM & SCT	Increase in INR post operatively but coagulation assessed by ROTEM remained normal
Trzebicki J et al **Ann Transplant 2010**	Prospective observational 39 OLT patients	Historical cohort of 39 patients	Targeted treatment of fibrinolysis with ROTEM. Total transfusions decreased
Wang et al	Prospective RCT 38	TEG versus	Decrease in FFP transfusion

De Pietri et al. **Eur J Anaesthesiol 2010**	Prospective observational 38 liver resections and 18 pancreatic	TEG, CCT	Post operatively TEG remained within normal range in liver resection patients, transient hypocoagulability in pancreatic patients.
Stancheva A et al **Clin Lab 2011**	Prospective observational 30 OLT patients	ROTEM vs standard coagulation tests	POC with ROTEM more information than SCT
Blasi A et al **Transfusion 2012**	Prospective observational in 236 OLT	ROTEM & CCT	ROTEM results very reliable (negative predictive accuracy 95%) in ruling out the need for transfusion of platelets and fibrinogen
Wang et al **Liver Transplant 2012**	Retrospective observational 77 OLT	TEG 2 different transfusion thresholds	>35% outside normal range versus just outside normal range, no difference in blood loss, but significantly decreased FFP and platelets at higher threshold.
Barton L et al. **HPB 2013**	Prospective observational 40 liver resections	TEG, CCT, Clotting factor assays	Increase in PT/INR post operatively. TEG transiently hypercoagulable (short R), then all values within normal range.
Alamo JM et al **Trans Proc 2013**	Retrospective 330 OLT case controlled study	ROTEM or standard of care	Use of intraoperative ROTEM decreased transfusion of blood and blood products if MELD>21, difficult surgery, or significant bleeding. Reduced post operative complications, including re-bleeding
Yang L et al **Liver Transplant 2014**	Prospective observational 27 OLT	Assessment of Rapid TEG and functional fibrinogen	Rapid TEG facilitates early assessment of MA. Functional fibrinogen over estimates Claus fibrinogen if < 1g/l at reperfusion. Good correlation at baseline.

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1**: Mallett SV**, Chowdary P, Burroughs AK. Clinical utility of viscoelastic tests of coagulation in patients with liver disease. Liver international 2013;33(7):961-74.

2: Krzanicki D, Sugavanam A, **Mallett SV.** Intraoperative hypercoagulability during liver transplantation as demonstrated by thromboelastography. Liver transplantation 2013;19(8):852-61.

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6: **Mallett SV**, Sugavanam A, Krzanicki DA, Patel S, Broomhead RH, Davidson BR, Riddell A, Gatt A, Chowdary A. Alterations in coagulation following major liver resection. Anaesthesia 2016 *in press*

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1: Clevenger B, **Mallett SV.** Transfusion and coagulation management in liver transplantation. World J Gastroenterology : WJG. 2014;20(20):6146-58.

2: Donohue C, **Mallett SV**. Reducing transfusion requirements in liver transplantation. World Journal of Transplantation: World J Transplant. 2015;5:165-82

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