Effects of recombinant activated Factor VII and supplementary oxygen on coagulopathy after trauma

Thesis submitted for the degree of Doctor of Medicine at Newcastle University

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Abstract

Approximately 30 – 40% of severely injured civilian and military casualties develop acute coagulopathy before arrival into hospital, with significant clinical implications such as increased mortality. The aetiology of trauma coagulopathy is multifactorial and thought to include consumption of clotting factors, acidosis and haemodilution. More recently attention has focussed on the role of tissue hypoperfusion and shock in the development of acute trauma coagulopathy. Coagulopathy is currently managed by the early use of blood products with or without adjuncts such as antifibrinolytics, and procoagulant agents. This can be guided by repeated assessment of clotting status to monitor the effects of therapy e.g. using TEG® or ROTEM®. Work presented in this thesis demonstrates the value of using interim values of coagulation, to accelerate the assessment of coagulation. ROTEM® A10 predicted coagulopathy with a sensitivity/specificity of 1.0/0.7, nineteen minutes earlier than running the test to completion.

In a randomised controlled trial in terminally anaesthetised pigs the implications of clinical treatment of coagulopathy were assessed. The implications of administering early oxygen or rFVIIa on coagulation were compared in a model of complex battlefield trauma. The administration of intravenous rFVIIa conferred a “boost” in clotting, however it was followed by an exaggerated deterioration in coagulation. By contrast coagulation was better maintained in the group treated with oxygen. A second, or delayed dose of rFVIIa was simulated in an *in vitro* study where blood from these groups was spiked with rFVIIa. In the animals treated with intravenous rFVIIa, the effect of the second dose was attenuated, while a comparable dose had a greater effect in the oxygen group.

These findings have implications for austere, resource constrained military settings since a boost in clotting might reduce immediate blood loss. However, this needs to be balanced against the loss of responsiveness over time. Limiting shock with oxygen may have a greater long term potential should evacuation be delayed.

Dedications

For Caty, Annnalinn and Christopher

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Publications and presentations related to this thesis

Publications:

1. Utility of interim ROTEM® values of clot strength, A5 and A10, in predicting final assessment of coagulation status in severely injured battle patients. *Injury*, 2012 44(5), pp.593–599. T Woolley, M Midwinter, P Spencer, S Watts, C Doran, and E Kirkman
2. Evolution of coagulopathy monitoring in military damage-control resuscitation. *The journal of trauma and acute care surgery*, 2012, 73(6 Suppl 5), pp.S417–22. N Tarmey, T Woolley, J Jansen, C Doran, D Easby, P Wood, and M Midwinter
3. Resuscitation and coagulation in the severely injured trauma patient. *Philosophical transactions of the Royal Society of London.* 2011, 366(1562), pp.192–203. M Midwinter and T Woolley
4. Haemorrhage and coagulopathy in the Defence Medical Services. *Anaesthesia*, 2013, 68 Suppl 1, pp.49–60. S J Mercer, N Tarmey, T Woolley, P Wood, and P Mahoney
5. The clinical outcome of UK military personnel who received a massive transfusion in Afghanistan during 2009. *Journal of the Royal Army Medical Corps*, 2011, 157(4), pp.365–369. E Allcock, T Woolley, H Doughty, M Midwinter, P Mahoney, and I Mackenzie

Presentations

1. Early determination of hypocoagulopathy based on interim Rotational Thrombolastometry values for clot strength.
    a. International Surgical Congress of the Association of Surgeons of Great Britain and Ireland 2010
    b. Advanced Technology Applications for Combat Casualty Care 2010

Chapter 1. Background

1.1 The nature of civilian and military trauma

Trauma remains the leading cause of death in young adults in the western world (Narayan et al., 2008; Sauaia et al., 1995), however the term trauma is a used to cover a wide variety of injury mechanisms, injuries sustained, and degree of physiological insult. It is therefore important to clarify the predominant type of trauma when comparing published evidence. The traditional UK civilian trauma patient is young, male and suffers blunt trauma, mostly from motor vehicle collision (Spinella et al., 2008; Brohi et al., 2003; London Trauma Office, 2012). When penetrating injuries are present, they are generally simple stab wounds or low velocity gunshots. Civilian trauma in the US has a more evenly distributed ratio of penetrating to blunt injury (Wade et al., 2010; Demetriades et al., 2005; Truitt et al., 2011; Kauvar and Wade, 2005) but remains predominantly young male with blunt trauma from motor vehicle collisions (Hauser et al., 2010; World Health Organization. Injuries Department, 2002).

Military trauma, in contrast, is characterised by blast and penetrating trauma (McMullin et al., 2008; Truitt et al., 2011). The penetrating trauma tends to be secondary to munitions and are therefore complex injuries due to ballistic threats such as fragments from explosive devices or high velocity bullets (Klemcke et al., 2005; Truitt et al., 2011; Jeroukhimov et al., 2002). Military wounds also tend to have a higher level of soft tissue disruption due to the cavitating effects of high velocity projectiles. This makes the wounds more complex with a greater degree of tissue damage than low velocity penetrating trauma seen in civilian practice (Sapsford et al., 2007; Hankin and Jeffery, 2010). Military trauma also differs form civilian trauma in that haemorrhage is the leading cause of death in the battlefield (Sapsford et al., 2007; Champion et al., 2003).

For many years trauma care has been based on the belief that death after trauma follows a trimodal response (Baker et al., 1980) with immediate death at the point of injury, early death within 1 hour and late death after 1 week. Recently this view has been challenged (Meislin et al., 1997; Demetriades et al., 2005) and the late death due to sepsis and multiorgan failure is no longer seen as a third peak. In hospital deaths are more evenly distributed after 1 hour. This is attributed to better trauma care systems. Despite the reduction in late deaths, the leading cause of death in civilian trauma remains head injury (Sauaia et al., 1995; Pfeifer et al., 2009; Kauvar and Wade, 2005), followed closely by haemorrhage which is the most common form of potentially preventable death after trauma.

Recent conflicts have not been conventional, and injury patterns have changed due to an increasing use of improvised explosive devices (IED) (Kamphuisen et al., 2002; Truitt et al., 2011). This has lead to an increase in the number of blast casualties seen (Smith, 2011; Ritenour et al., 2010) in hospital, many with associated blast lung damage. As a response to the change in weapons used against coalition troops, there have been improvements in personal protective equipment, and changes in medical practice. This has returned the focus on the prehospital treatment of catastrophic haemorrhage with direct pressure over bleeding points, and the application of tourniquets with the rapid evacuation of the casualty to a surgical facility (Hodgetts et al., 2006). There is improved medical training for all combat troops and rapid access to combat medical technicians[1] (CMT's) and prehospital care doctors. This allows for potential prehospital therapies to be administered, such as the use of prehospital recombinant factor VIIa (rFVIIa), or oxygen delivered at the point of wounding. There are logistical and safety constraints with some therapies, such as oxygen, which may explode if hit by bullets or shrapnel, therefore their use must be fully evaluated prior to incorporation into protocols, especially in light of new, potentially safer technologies for O_2 delivery

These improvements in medical care have resulted in the early survival of casualties that would previously have died on the battlefield. This cohort of severely injured, hypovolaemic, blast injured patients with catastrophic

[1] Combat medical technicians are military paramedics

extremity injury (Truitt et al., 2011) now enter the medical chain generating new demands on resuscitation strategies. Old resuscitation protocols focused on geographically distinct echelons of care with sequential resuscitation, followed by surgery and finally critical care. Once a casualty arrived in critical care, up to 18% were found to be cold acidotic and coagulopathic (Gonzalez et al., 2007). This set of symptoms, the lethal triad, or bloody vicious cycle (Kashuk et al., 1982), was associated with increased mortality.

1.2 Coagulopathy after trauma

Historically, trauma patients who died in hospital were coagulopathic when they died (Sauaia et al., 1995) and recent changes in resuscitation protocols have focused on arresting this process. Civilian data suggests that approximately 30 – 40% of patients who arrive at hospital after trauma, have an acute coagulopathy on admission (Brohi et al., 2003; Maegele et al., 2007; MacLeod et al., 2003). The proportion of military patients from recent conflicts, who have coagulopathy on admission remains unclear, however several papers have suggested rates around 40 - 50% (Niles et al., 2008; Plotkin et al., 2008; Doran et al., 2010).

Originally coagulopathy after trauma was thought to be due to consumption and dilution of clotting factors. However recent work has suggested the presence of a primary coagulopathy not associated with hypothermia, dilution and consumption at the time of admission to hospital. This coagulopathy, termed acute trauma coagulopathy (ATC) is associated with a significant increase in mortality, injury severity and the presence of shock (Brohi et al., 2003; MacLeod et al., 2003; Maegele et al., 2007).

Treatment options that may target ATC hold a great deal of interest for the military since any potential modulation of this process by simple, therapies without the logistical burden of providing fresh and frozen blood products, may have a profound impact on future conflicts. In order to effectively investigate treatment options, there needs to be a full understanding of the processes involved in normal clotting and where potential deficits might be occurring. It is generally accepted that ATC exists, but the theories behind its pathophysiology

remain divided although microcirculatory shock and subsequent prolonged endothelial activation seems to be an underlying theme (Curry et al., 2012).

Assessment of clotting in blast injured casualties needs to be fully elucidated since there are limitations with the current standard tests of prothrombin time (PT) and activated partial thromboplastin time (aPTT) (Doran et al., 2010). Interest in viscoelastic methodology in elective surgery has translated to renewed interest in the trauma setting (NHS Quality Improvement Scotland, 2008). Viscoelastic methods can potentially diagnose coagulopathy in a more rapid and relevant way than standard tests. One particular device, ROTEM®, has proved to be of use in a deployed military environment, however its usefulness in determining the coagulation profile in blast injured casualties is yet to be fully determined (Doran et al., 2010).

1.3 The purpose of this research

The work in this the thesis forms part of a larger ongoing programme of work aimed at improving combat casualty care. The purpose of this research is to assess the impact of prehospital treatment of casualties with recombinant factor VIIa (rFVIIa) or oxygen as a treatment for blast lung, on their admission coagulation status.

In Chapters 2-4 I will explore and explain the pathophysiology and assessment of coagulation and coagulopathy after trauma, Chapter 5 explains the physics of blast and the effects blast injury has on the body and on coagulation. Chapter 6 deals with the physiological responses to trauma and Chapter 7 the rationale for the use of rFVIIa to treat blast lung and coagulopathy. Chapters 8 to11 provide the details and the results of the experimental work, firstly a clinical assessment of ROTEM® in the diagnosis of acute trauma coagulopathy in Afghanistan and secondly a series of experiments conducted on an animal model of a survivable battlefield injury. In Chapter 12, I will discuss the findings of the experimental work and explore a surprising finding in the assessment of coagulation after administration of rFVIIa. Finally I will discuss the findings of a study designed to assess the optimum time for administration of rFVIIa and the effects of a second dose of rFVIIa after a blast injury and traumatic haemorrhage.

Chapter 2. Normal Coagulation

2.1 Overview of haemostasis

Haemostasis is a physiological process that is initiated when tissue damage occurs. Primary haemostasis occurs in seconds, when sympathetic nerve activity and neurohormonal signalling causes vasoconstriction and a platelet plug forms at the site of injury. Exposed tissue factor (TF) from the subendothelial tissues, activates secondary haemostasis when coagulation enzymes produce thrombin and fibrin. Platelets activated by collagen and thrombin, catalyse further thrombin production at the site of injury and activate inflammatory mediators to promote wound repair (Figure 2-1)

It is important that once the biological process has started, it remains local to the site of injury and does not propagate downstream. Regulation of haemostasis occurs by multiple processes including endothelial suppression of platelet activation, anticoagulant pathways, specific protease inhibitors and fibrinolytic pathways. This process is called localisation and prevents the formation of intravascular thrombus (Gomez et al., 2011). Many of the localisation pathways are also linked to fibrinolysis, the final process in normal haemostasis, and cause the breakdown of the fibrin clot. Haemostasis is therefore a balance between anticoagulation, coagulation, and fibrinolysis.

The ultimate goal of coagulation after stopping bleeding, is to allow wound healing and tissue repair (Gomez et al., 2011; Smith, 2009) and therefore coagulation is intimately linked to inflammation and tissue repair mechanisms.

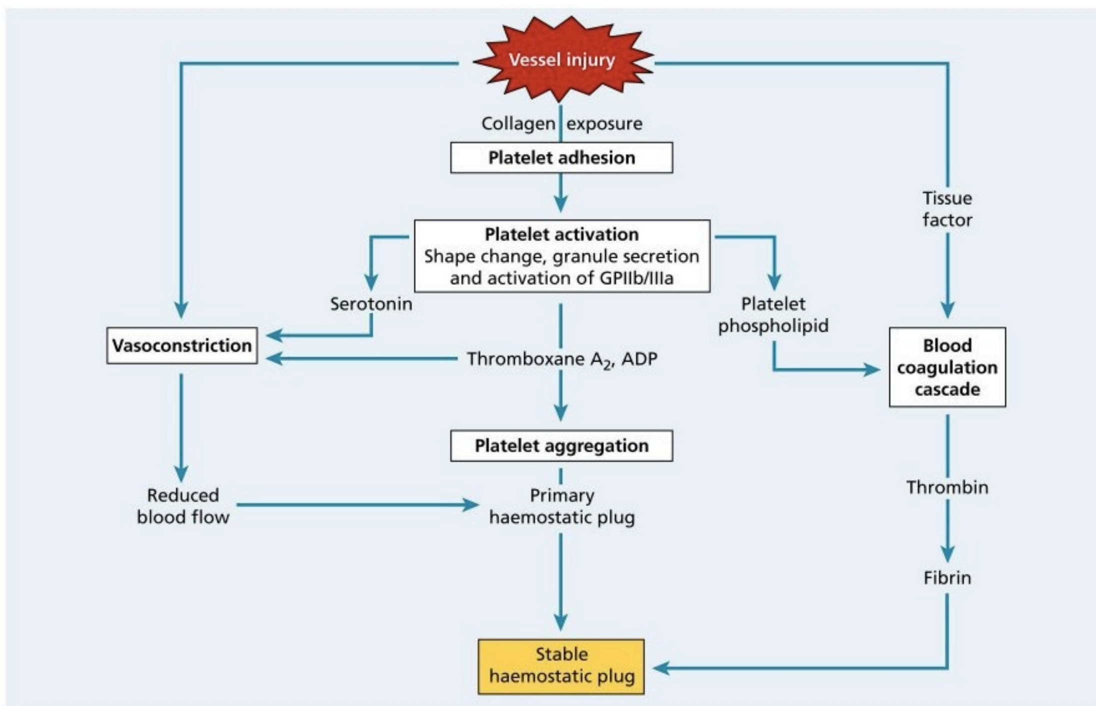

Figure 2-1. Mechanisms involved in haemostasis (From *Essential Haematology* 6th Edn. © 2011 Blackwell Publishing)

2.1 Vasoconstriction

After vessel damage, circular smooth muscle in the vessel wall contracts (spasm) in an attempt to pinch off the vessel and reduce blood loss. Vascular spasm reduces the blood flow to the injured area, reducing downstream flow and increasing the chance of a stable clot forming (Hoffbrand et al., 2006).

2.2 Platelets

Platelets are understood to have a key role in allowing coagulation to occur (Gomez et al., 2011). The platelets first role in coagulation is to form a platelet plug at the site of injury. Once a platelet interacts with the subendothelial matrix after vessel injury, a series of events occur with the platelet adhering to the damaged area (adhesion), release of intracellular enzymes from organelles with expression of procoagulant glycoproteins (activation) and recruitment of other platelets to the damaged area (aggregation) ((Smith, 2009; Levrat et al., 2008; Hoffman and Monroe, 2001; Sawamura et al., 2009; Schöchl et al., 2009; 2010).

Platelets now provide an activated, procoagulant membrane on which clotting factors can assemble allowing efficient thrombin production and ultimately an insoluble fibrin mesh at the site of injury.

2.2.1 Platelet physiology

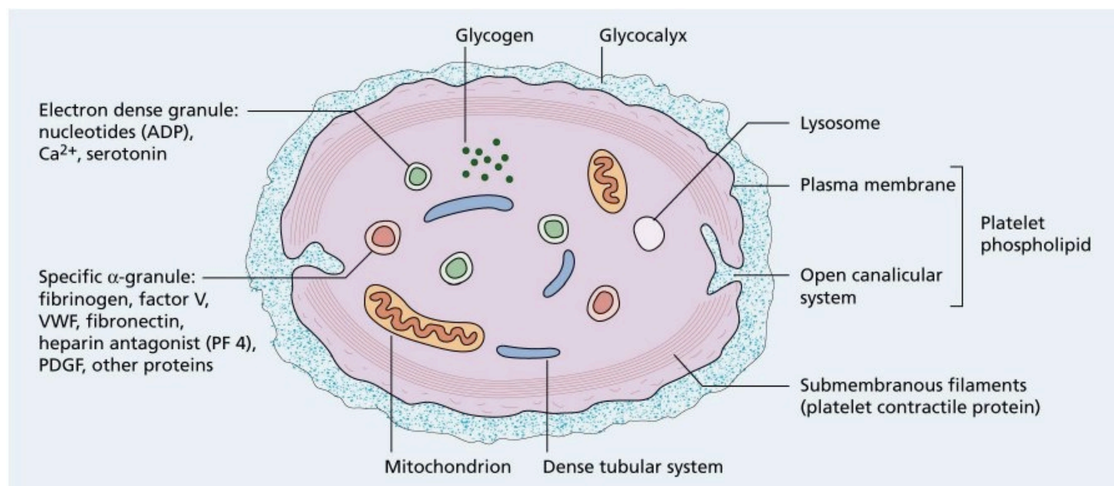

Figure 2-2. Platelet ultrastructure (From *Essential Haematology* 6th Edn. © 2011 Blackwell Publishing)

Platelets are extremely small, discoid cells. Their ultra structure is represented in Figure 2-2 The plasma membrane invaginates into the cell producing canaliculi that give rise to a large surface area to absorb coagulation proteins. The platelet surface is packed with functional glycoprotein (GP) receptors, to facilitate platelet activation and interactions with the subendothelial matrix, other blood cells and other platelets (Hoffbrand et al., 2006).

The platelet also contains several granules that upon activation can fuse with the platelet membrane and thereby release their contents into the surrounding media (Harrison, 2005). The granules are categorized into larger α-granules, the smaller dense granules and lysosomes. (King and Reed, 2002).

2.2.2 *Platelet adhesion*

Following vessel wall injury, platelets adhere to collagen in the subendothelial matrix by synergistic reactions between several receptors on the platelet surface (Figure 2-3)(Furie and Furie, 2008). Local flow conditions can affect platelet function. In low flow areas platelets will tend to marginate increasing the chance of interacting with the damaged endothelium. Margination is therefore increased after vascular spasm. In high flow vessels (such as arterioles) platelet adhesion is augmented by the interaction of von Willebrand Factor (vWF) with platelet glycoproteins.(Hoffbrand et al., 2006)

Figure 2-3. Schematic representation of the platelet and its functional receptors. (From *www.platelets.se)*

2.2.3 *Platelet activation*

Once a platelet adheres to the subendothelial matrix it becomes activated (Furie and Furie, 2008). Platelets become more spherical than discoid and extrude pseudophobia, enhancing vessel wall and inter platelet interactions. Changes in

the cytoskeleton cause the classic “fried egg” appearance of a long, flat platelet with central granules and organelles.

In order for platelet recruitment to continue, the activated state of adhered and aggregated platelets must be transferred to newly arriving platelets. This occurs either due to potent autocrine and paracrine signalling after the release of intracellular granules (degranulation), or directly from thrombin produced via the clotting pathways.

2.2.4 Platelet aggregation

Once a primary layer of platelets has covered the exposed subendothelial matrix, adhesion continues, but in the form of aggregation, when two platelets bind to the same fibrinogen molecule. (Figure 2-3).

2.3 Coagulation

2.3.1 The Clotting cascade

Traditionally clotting was described as a cascade system where activation of one particular clotting factor (serine proteases) led to the activation of another factor and so on. The process was split into two. The intrinsic system, also known as contact activation, has all the proteins required to form clot in the blood. The extrinsic system, in contrast, required the presence of extrinsic tissue factor found in the subendothelial matrix. Finally both the intrinsic and extrinsic pathways came together to form the final “common” pathway, ultimately resulting in the conversion of soluble fibrinogen to insoluble fibrin Figure 2-4).

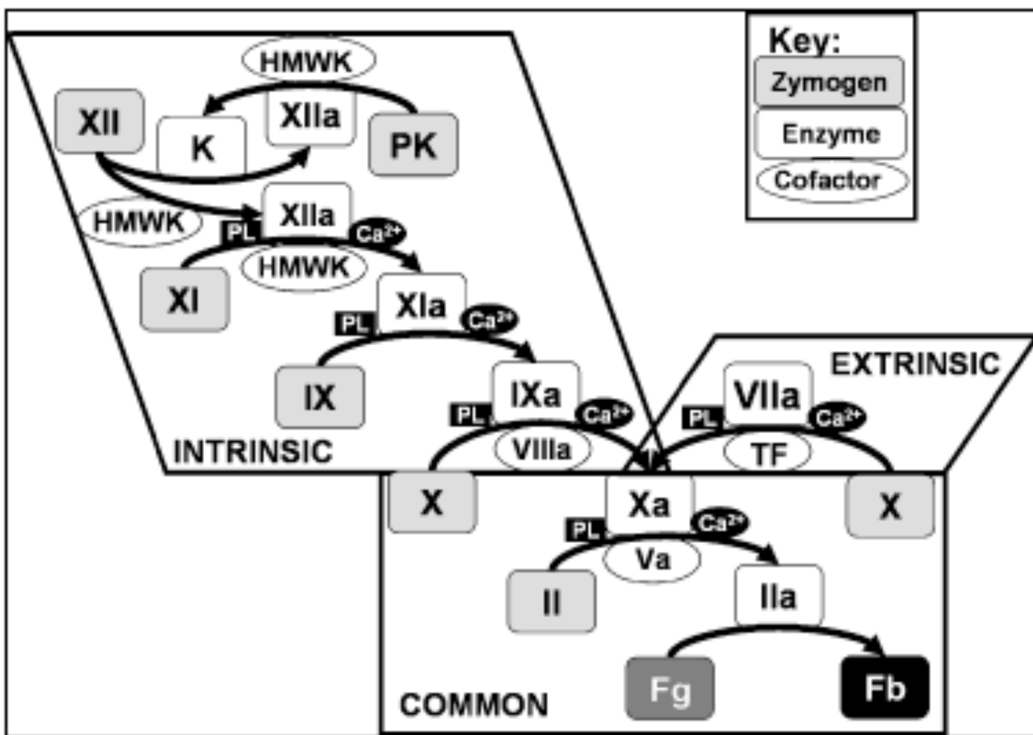

Figure 2-4. The "clotting cascade" (From (Smith, 2009))

This view is now challenged since the cascade system did not explain why deficiency of a certain factors e.g. FXII, may have no effect on clotting, where as deficiency of other factors, e.g. FVIII (haemophilia A) had a profound effect on clotting. Hoffman and Monroe (2001) have subsequently proposed a “cell based theory” of clotting where the role of platelets, in particular, are central to the assembly and function of clotting factors

2.3.2 The role of Thrombin

At the centre of the whole process is thrombin and all aspects of haemostasis feed into the regulation and control of thrombin (Figure 2-5). Thrombin is a procoagulant protein and is produced early on in the clotting process to activate platelets, and later on, in a “thrombin burst”, to cause fibrinogen conversion to insoluble fibrin. Thrombin can also be an anticoagulant protein as it promotes protein C pathways via thrombomodulin (see section 2.6.3). Thrombin also affects clot structure by stabilising clot by the formation of factor XIIIa from FXIII and preventing fibrinolysis by the action of thrombin activated fibrinolysis inhibitor (TAFI) (See section 2.4.4). Thrombin is crucial in the inflammatory response as it activates white cells and the endothelium.

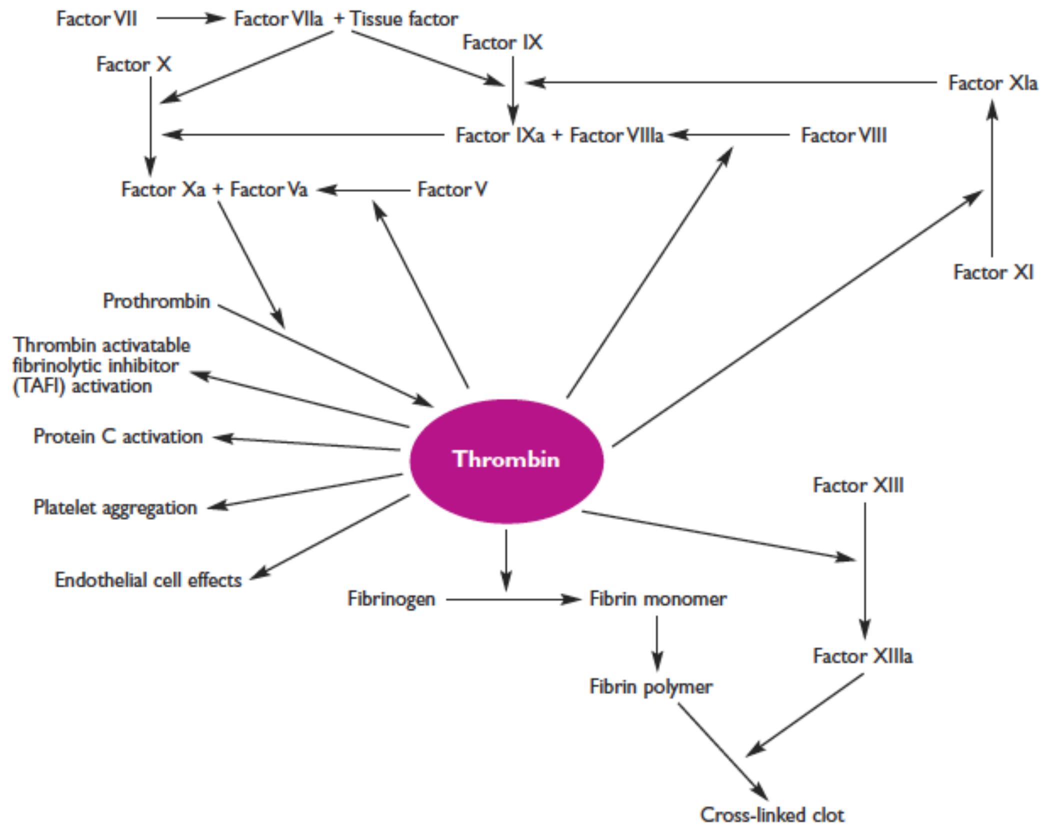

Figure 2-5. The central role of thrombin in coagulation

2.4 Cell based theory of clotting

A more modern theory of coagulation is the cell-based theory, which incorporates the role of cells (platelets) and membrane surfaces in conjunction with the interaction with clotting factors. Platelets are integral to this process as they provide the framework on which all coagulation occurs. The process itself occurs in three distinct phases, initiation, amplification and propagation.

2.4.1 Initiation

It seems that the sole initiator of coagulation is extracellular TF wrapped around the blood vessels, which binds to circulating FVIIa. Factor VII is the only factor that normally exists in its active form in circulating blood (about 1%). Once tissue damage occurs, this FVIIa binds to the exposed TF producing a TF-VIIa complex, which in turn activates further FVII to FVIIa in an autoactivation

process (Figure 2-6). With the interaction of other clotting factors, small amounts of thrombin, FIXa and FXa are produced. FIXa and FXa are required later on in clot propagation unless inactivated by TFPI or anti thrombin (AT) (see section 2.6.2). The thrombin produced in clot initiation is available to directly activate local platelets

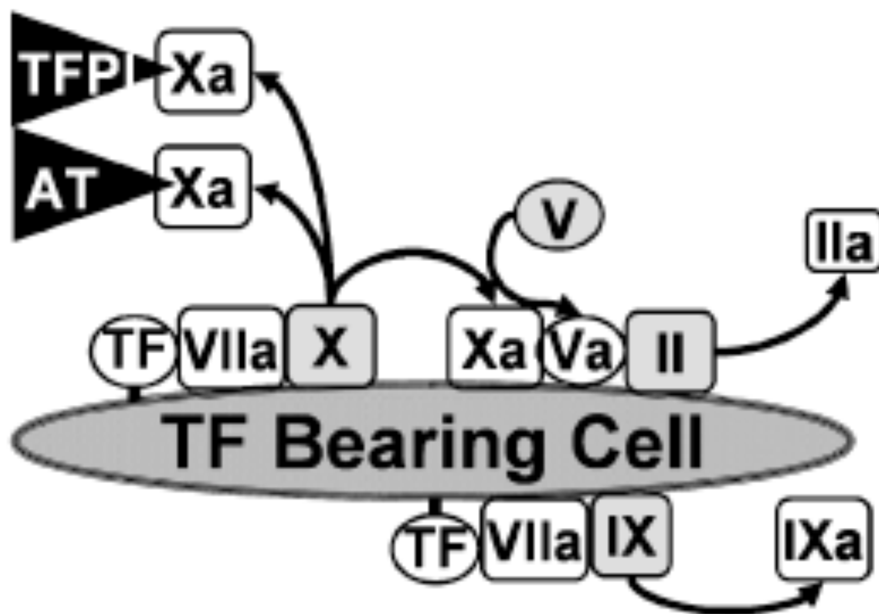

Figure 2-6. Clot initiation (Adapted from (Smith, 2009))

2.4.2 Amplification

The small amount of thrombin generated during initiation diffuses away from the TF bearing cell causing platelet activation. Platelet degranulation occurs with expression of Factor V on the outer surface (Figure 2-7). Thrombin also allows vWF to be realised from FVIII augmenting platelet adhesion.

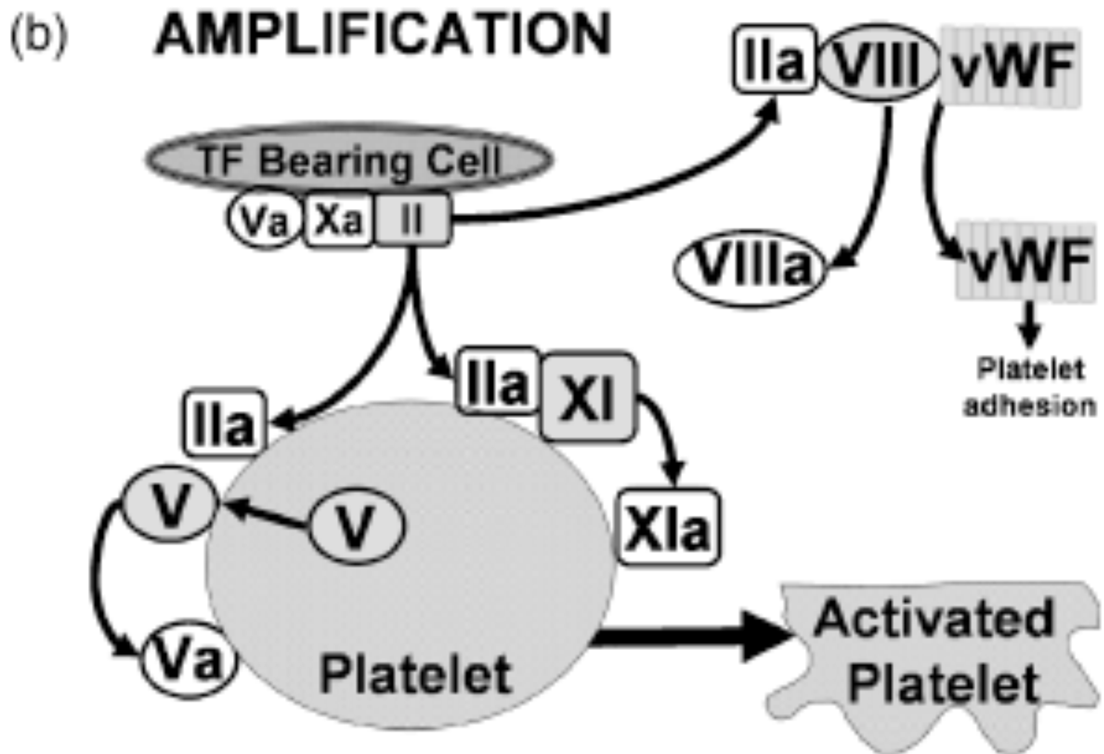

Figure 2-7. Clot amplification (Adapted from (Smith, 2009)

2.4.3 Propagation

Once platelets are adhered to the site of injury and are activated, they provide a procoagulant membrane on which to assemble the clotting factors to efficiently produce a thrombin burst and ultimately convert fibrinogen to insoluble fibrin. This is called propagation (Figure 2-8).

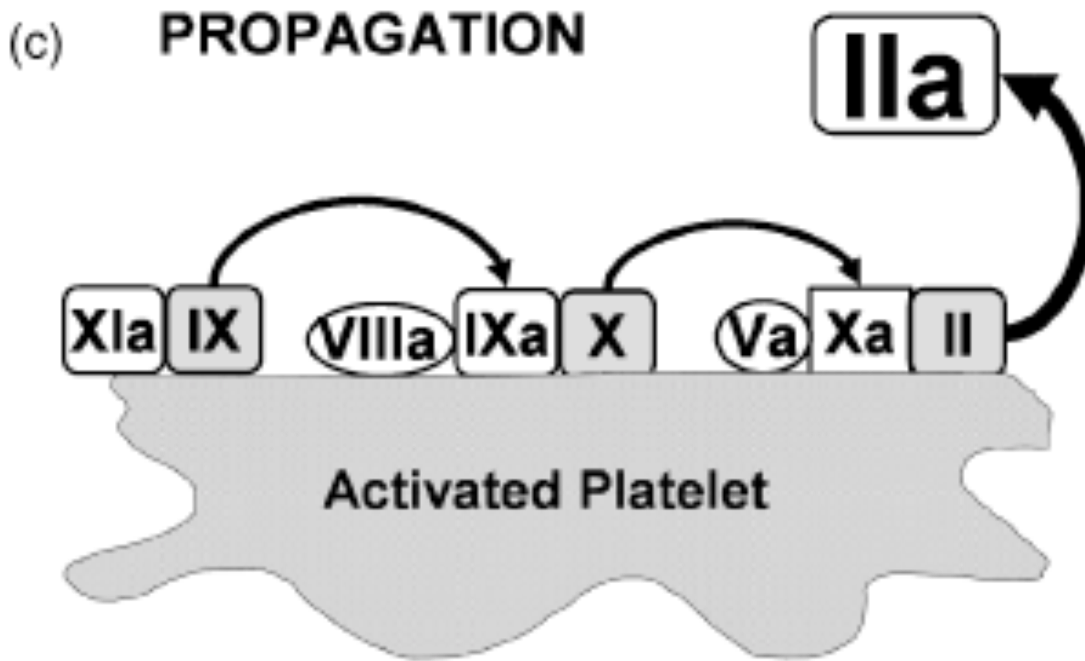

Figure 2-8. Clot propagation (Adapted from (Smith, 2009)

2.4.4 Post clot formation

Thrombin's additional functions after the initial fibrin polymerisation include activation of FXIII to FXIIIa and the activation of thrombin activatable fibrinolysis inhibitor (TAFI).

FXIIIa has important roles in cross-linking fibrin strands improving strength and elasticity of the clot and rendering it more resistant to fibrinolysis. This stability is mediated via the thrombomodulin (TM) bound TAFI. (Cesarman-Maus and Hajjar, 2005). It binds to the same binding sites on fibrin as plasminogen and tissue plasminogen activator (tPA) thereby attenuating their effects.

2.5 The Vascular Endothelium

The resting endothelium provides a physical barrier between blood, extravascular collagen and tissue factor (Figure 2-9) and has an intimate relationship with flowing blood and blood cells. It plays a crucial role in haemostasis, by preventing thrombosis and in linking coagulation to inflammation (Levi et al., 2002).

The endothelium is lined by a glycocalyx of membrane bound macromolecules comprised of sulphated proteoglycans (see section 2.6.2), glycoproteins and plasma proteins. The constituents of the glycocalyx influences anticoagulation, fibrinolysis and inflammatory processes whilst the physical presence of these molecules in the microvascular lumen impacts blood flow, transmission of shear forces and oxygen diffusion (Pries et al., 2000).

The intact endothelium maintains blood in a fluid state by a complex set of interactions. Arguably the most important mechanism is the physical barrier between blood, extravascular collagen and tissue factor (Figure 2-9). Since tissue factor is the sole initiator of coagulation, physical separation provides much of the anticoagulant effect.

2.6 Natural anticoagulation Pathways

2.6.1 Tissue factor pathway inhibitor

Endothelium derived tissue factor pathway inhibitor (TFPI), found mainly in capillaries, bound to the glycocalyx, (Lupu et al., 1995), and reduces the procoagulant effects of the TF-VIIa complex by inhibiting FXa. It inhibits the TF-VIIa complex in a FXA dose dependent manor. Its role is to inactivate FXa that might flow downstream of the injury, thus localising coagulation to the site of injury.

Blood levels of TFPI are known to increase after injection of heparin or low molecular weight heparin (Abildgaard, 1993), although this effect is transient and TFPI is sequestered from the blood as the level of heparin decreases (Lupu et al., 1995).

2.6.2 Antithrombin and heparin sulphated proteoglycans

Antithrombin (also known as antithrombin III) is a potent inhibitor of clotting factors, particularly FXa, thrombin and to a lesser extent FIXa (Zhang et al.,

2005). Unusually it requires activation by heparin or heparan sulfates (Levi and Van Der Poll, 2010) to inhibit its target protein at physiologically significant rates.

Heparan sulfates (HS) are polysaccharides attached to a protein core (proteoglycans) the most common being the syndecans and the glypicans (Bernfield et al., 1999).Syndecan 1 is present on epithelial cells (Tumova et al., 2000) and is therefore the most important proteoglycan involved in coagulation.

Heparin is present on mast cells and is commonly isolated to be used as an anticoagulant whilst HS is found on cell surfaces (Sasisekharan and Venkataraman, 2000). Both will bind to AT causing a conformational change and increasing AT's activity up to 1000 fold (Levi and Van Der Poll, 2010). Heparan sulfates therefore function as an activator of AT attached to the vessel wall.

2.6.3 The Protein C pathway

The protein C pathway responds to the presence of thrombin (Esmon, 2003). As the thrombin concentration rises, much of it is bound to thrombomodulin expressed on the endothelial surface. The thrombin-thrombomodulin complex (T-TM) activates protein C (aPC), a process that is enhanced 20 fold if PC is bound to the endothelial protein C receptor (EPCR). Thrombin associated in the T-TM complex is more readily inactivated by AT than free thrombin.

aPC, in conjunction with protein S inactivates factors Va and VIIIa, causing anticoagulation, and inhibits plasminogen activator inhibitor-1 (PAI-1) allowing increased fibrinolysis. TM concentration is 100 fold higher in capillaries than in large vessels, thereby extracting any circulating thrombin. The T-TM complex is also a potent activator of thrombin activatable fibrinolysis inhibitor (TAFI) (see section 2.4.4)

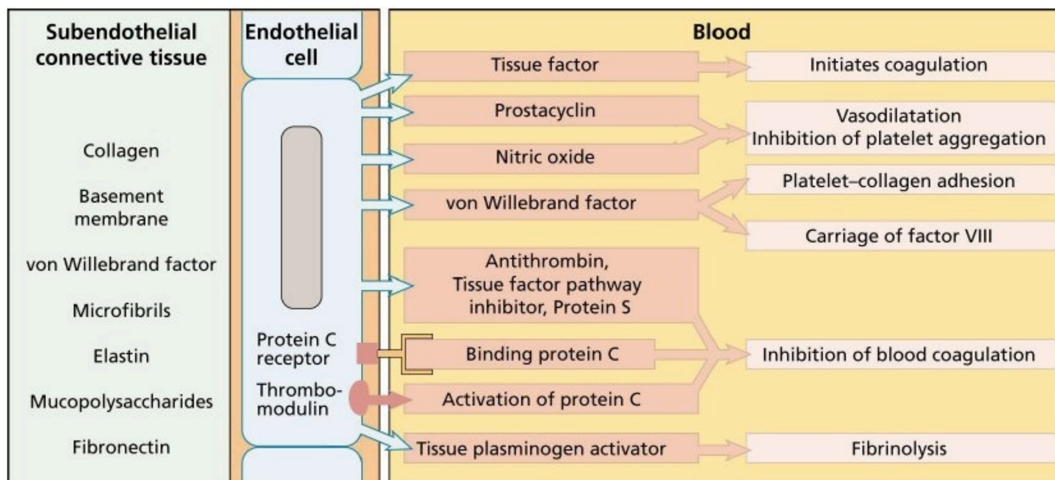

Figure 2-9. The role of the vascular endothelium (From *Essential Haematology* 6th Edn. © 2011 Blackwell Publishing)

2.7 Fibrinolysis

Fibrinolysis occurs to ensure blood fluidity and is tightly controlled by specific activators and inhibitors in conjunction with endothelial receptors (Figure 2-10). Activation of coagulation leads to generation of thrombin and ultimately to fibrin deposition. Plasmin is the main fibrinolytic protease, derived from plasminogen, responsible for cleaving fibrin.

Plasminogen derived from the liver is cleaved to plasmin under the control of tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). tPA is the dominant stimulant and is synthesised from endothelial cells under the influence of many stimuli including thrombin. Its half-life is extremely short and on its own is a poor activator of plasminogen, however its action is increased in the presence of fibrin and strongly localises the plasmin production to the site of the clot. Plasmin binds to terminal lysine residues on fibrin and cleaves off soluble degradation products, and degrades the clot.

Plasmin activity can also be reduced by the presence of thrombin activatable fibrinolysis inhibitor (TAFI) which binds to partially degraded fibrin reducing the efficacy of plasmin and stabilises the fibrin clot (Cesarman-Maus and Hajjar, 2005).

2.7.1 *Inhibitors of fibrinolysis*

Plasmin is inhibited by serpins such as α2-plasmin inhibitor, where as plasminogen is inhibited by the plasminogen activator inhibitors 1 and 2 (PAI-1 and PAI-2). PAI-1 is the most significant of the inhibitors inhibiting both tPA and uPA. The release of PAI-1 is stimulated by various inflammatory and coagulation mediators including thrombin(Cesarman-Maus and Hajjar, 2005).

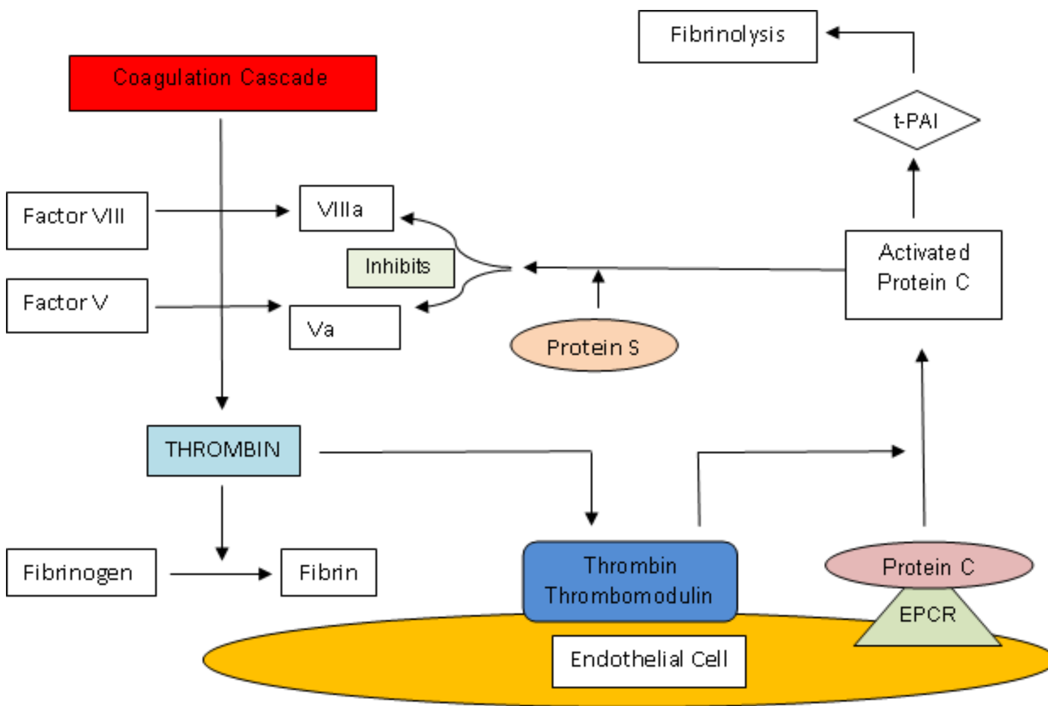

Figure 2-10. Fibrinolytic pathway (Modified from *Essential Haematology* 6th Edn. © 2011 Blackwell Publishing)

2.8 Summary

Coagulation is a complex interaction between coagulation, anticoagulation and fibrinolytic pathways. It is intimately linked with inflammation and its ultimate goal is cessation of bleeding followed by wound repair.

After tissue damage, vasospasm limits blood flow, allowing the interaction of FVIIa and platelets with subendothelial tissue factor and collagen. The ensuing coagulation cascade coupled with activation and aggregation of platelets leads to an insoluble fibrin and platelet clot. Coagulation is localised to the site of injury due to the natural anticoagulant effects of the vascular endothelium and specific factor inhibitors. Finally, fibrinolysis removes any unwanted clot.

Abnormalities in the coagulation system are called coagulopathies and can be congenital, acquired and iatrogenic. The next chapter will discuss the aetiology of coagulopathies, concentrating on coagulopathy after trauma.

Chapter 3. Coagulopathy after Trauma

3.1 Overview of coagulopathy

The term coagulopathy is generally used to describe a deficiency in the clotting system. Technically coagulopathy occurs after a disease state and can cause a decrease in clotting (hypocoagulopathy), or an increase in clotting, (hypercoagulopathy).

Coagulopathies can be inherited or acquired. Acquired coagulopathies can be further divided into iatrogenic, such as warfarin therapy, or pathological, such as disseminated intravascular coagulopathy. Inherited coagulopathies may complicate acquired coagulopathies, however they are not the focus of this discussion. This thesis shall focus on trauma induced coagulopathies and their potential aetiologies. The generally accepted term of “coagulopathy” will be used to describe a deficiency in clotting ability, and “hypercoagulopathy” to describe an increase in clotting ability.

Trauma induced coagulopathies are driven by consumption of clotting factors, hypothermia and acidosis impairing enzymatic and platelet function, dilution of available clotting factors after resuscitation, and hormonal and cytokine changes. Recently a previously unrecognised phenomenon of acute trauma coagulopathy has been described.

Interpretation of the published literature is complicated by the lack of consensus of definitions and terminology of coagulopathy, however these aspects shall all be dealt with in the following chapter.

3.2 Trauma induced Coagulopathy (TIC)

3.2.1 Historical Perspective

Coagulation abnormalities after trauma are not new. In 1985 Hewson et al. (Hewson et al., 1985) described coagulopathy in massively transfused patients after dilution with crystalloid. Associations between shock and clotting abnormalities were noticed during the Vietnam war demonstrating an early hypercoagulopathy and a later coagulopathy (Simmons et al., 1969). Risberg et al. (Risberg et al., 1986) demonstrated that clotting systems were activated after trauma and this was related to a fall in the systolic blood pressure (Bp) below 110 mmHg.

Until a decade ago resuscitation followed Advanced Trauma Life Support (ATLS) principles focussing on the restoration of circulating volume with crystalloid solutions (American College of Surgeons Committee on Trauma, 1993). Anaemia was treated with packed red blood cells (PRBC) whilst fresh frozen plasma (FFP) and other component therapy was only started after a significant number of PRBC’s had been transfused (Curry et al., 2012). This resulted in dilution of available clotting factors leading to a dilutional coagulopathy. Coupled with this, large amounts of clot formed as the body tried to stem bleeding by activating coagulation, leading to a consumptive coagulopathy, and if the bleeding could not be stopped a loss of a clinically significant amount of clotting factors occured. The casualty also became cold due to environmental factors and infusion of cold fluids, and remained acidotic due to inadequate resuscitation, both of which can worsen (or cause) coagulopathy.

This combination of events (hypothermia, acidosis and consumptive/dilutional coagulopathy) is commonly termed the lethal triad or bloody vicious cycle (where each component can augment the others) (Curry et al., 2012; Midwinter and Woolley, 2011; Kashuk et al., 1982) and was originally described after haemorrhage from major abdominal vascular trauma.

3.2.2 Hypothermia

Hypothermia impairs the body's ability to clot, as the temperature falls so protease activity decreases (Reed et al., 1990). Factor VII activity decreases linearly with temperature, only retaining 80% of its activity at 33˚C (Meng et al., 2003). However these effects are minor compared to the effect of hypothermia on platelets. Platelet aggregation and adhesion were decreased by 30 – 40% at 33˚C compared to 37˚C (Wolberg et al., 2004) although in this study the amount of thrombin generated was the same at both temperatures.

A core temperature of 34˚C is a critical point at which a significant alteration in platelet physiology occurs in trauma patients and at which enzyme activity decreases (Watts et al., 1998). In this study the patients had Thromboelastography (TEG®), PT, aPTT and platelet count performed, and patients were stratified according to admission temperatures. The PT and aPTT showed no difference between the temperature groups, however TEG® showed differences in maximum amplitude (MA) only once the temperature dropped to less than 34 degrees. MA is a measure of clot strength, the stronger the clot, the greater the MA, and is determined by the amount of functional fibrinogen and platelets. The change in MA was attributed to platelet function.

Hypothermia is directly correlated to injury severity and is an independent risk factor for mortality, reaching 100% when core temperature is less than 32˚C in patients undergoing surgery (Jurkovich et al., 1987). Hypothermia leads to α-adrenergic stimulation, which in turn leads to, vasoconstriction, exacerbating any organ hypoperfusion, which may be already present secondary to hypotension from the injury. This hypoperfusion leads to worsening acidosis.

3.2.3 Acidosis

Acidosis significantly affects haemostasis. At a pH less than 7.4 a normal platelet will change internal structure and lose the ability to change shape (Djaldetti et al., 1979). At a pH of 7.0 platelets show reduced signs of activation and microparticle formation, compared with a pH of 7.4 (Etulain et al., 2012). Coagulation factors are each affected differently by acidosis, and calcium binding site affinity is decreased. At pH 7.1, thrombin produced in clot

propagation is decreased by 50%, fibrinogen by 35% and platelet count by 50% (Martini and Holcomb, 2007).

3.2.4 Haemodilution

Haemodilution can play a part in (Gando et al., 2002)the development of coagulopathy. Initially there is a shift of extracellular fluid to the intravascular space which will dilute clotting factors (Carey, 1973). Resuscitation with crystalloid has been shown to further dilute factors, and the use of colloids that expand plasma volume to a greater extent can dilute even further, as well as directly inhibiting coagulation pathways and inducing a profibrinolytic state (Bolliger et al., 2010). Resuscitation with component therapy equivalent to whole blood still has a dilution effect since component therapy equivalent to whole blood contains a haematocrit of 29%, Plts 88 $x10^9$/l and 65% coagulation activity (D'Angelo and Dutton, 2010). This dilution, however is less than that produced by the transfusion of red cells with crystalloid alone.

3.2.5 Consumption

Once the coagulation cascade has started there is inevitable consumption of the basic building blocks of a clot, i.e. factors, fibrinogen and platelets. Dunbar and Chandler (Dunbar and Chandler, 2009) demonstrated non wound related thrombin production within an hour of trauma due to the systemically activated coagulation system. This could potentially lead to widespread coagulation activation, which would in turn lead to widespread activation of fibrinolytic pathways and disseminated intravascular coagulation (DIC).

Gando et al (Gando et al., 2011) believe that DIC occurs in response to the massive thrombin surge caused by high amounts of free TF found in blood immediately after trauma. This inevitably leads to widespread coagulation activation and consumption of clotting factors.

The early clinical picture of ATC does look similar to DIC with low platelet counts, low fibrinogen levels, raised PT and high fibrin degradation products

(FDP's). However there is no disseminated coagulation in ATC, only coagulation at the site of injury (Curry et al., 2012). And therefore Gando's opinion of DIC as a cause of coagulopathy after trauma is in contrast to the opinions of others (Lin et al., 2011; Hess et al., 2008) who argue that DIC is not a feature of early coagulopathy after trauma.

Several studies report low levels of clotting factors on admission after traumatic injury, however this is discussed in more detail in section 3.4

3.2.6 Hormone and cytokine changes

Following tissue damage there is release of hormones and cytokines. There are two temporal phases; immediate release of catecholamines and vasopressin and later effects of endothelial cell activation.

Vasopressin stimulates the release of vWF, production of tPA from the endothelium and increased expression of P selectin (Kenet et al., 1999; Hunt and Jurd, 1998).

Release of inflammatory cytokines such as TNF and IL-1 as well as thrombin, cause endothelial cell activation resulting in a change of the endothelial cell phenotype from antithrombotic to pro thrombotic. There is down regulation of fibrinolysis by the increase in PAI-1 levels and reduction in the activation of antithrombin (Hunt and Jurd, 1998; Johansson et al., 2011b).

3.2.7 On-going bleeding

Anaemia has a profound effect on haemostasis. Normal clotting requires platelets to adhere to the endothelial wall. This is augmented by margination of the platelets and increases platelet endothelial interaction. A low haematocrit will reduce this effect (Valeri et al., 2007).

3.3 Acute Trauma Coagulopathy

Recent research has shown that a trauma patient who is coagulopathic on admission to hospital is more likely to require massive blood transfusion, develop multi-organ failure and has up to a fourfold chance of dying (Brohi et al., 2003; Maegele et al., 2007; MacLeod et al., 2003). In 2003, Brohi et al. studied 1088 of 1867 trauma patients admitted to the Royal London Hospital between 1993 and 1998 (Brohi et al., 2003). This was a typical UK civilian major trauma population with 75% blunt trauma and a median injury severity score (ISS) of 20. These authors found that 24.4% of patients had a coagulopathy on admission although this figure rose to 33.1% in those patients who had an ISS>15 (major trauma). Coagulopathy was defined as a prothrombin time (PT) or activated partial thromboplastin time (aPTT) of 1.5 times normal. Median prehospital times were 73 minutes and median prehospital fluid administration was 800mls. Patients with a coagulopathy had a median volume of 700ml prior to admission and those without coagulopathy a median value of 1000ml. The presence of coagulopathy was not found to be related to fluid administration in this study.

As ISS increased, so did the proportion of coagulopathic patients. Age and mechanism of injury had no effect on presence of coagulopathy. The overall mortality rate was 19.5%. However, mortality was associated with the presence of coagulopathy (mortality in coagulopathic patients 46% versus 10.9% non-coagulopathic patients) but statistically independent of ISS.

This important study suggested that there is an acute coagulopathy early in trauma that is unrelated to dilution, but is related to ISS and confers an increased mortality independent of ISS. These findings were confirmed by studies from the USA (MacLeod et al., 2003) and Germany (Maegele et al., 2007).

Macleod and colleagues reviewed prospectively collected data from 20,103 trauma patients admitted to the University of Miami/Jackson Memorial hospital, Florida between 1995 and 2000, of whom 7638 had complete data sets for final analysis. The population was similar to that of the Royal London study

(predominantly male, blunt trauma and 66min (non coagulopathic group) and 74 min (coagulopathic group) prehospital times), although the median ISS and mortality rate were lower with median ISS of 9 and overall mortality of 8.9%. Coagulopathy was defined as those with an abnormal PT or abnormal aPTT. Patients with a normal PT had a mortality of 6.3%, and those with an abnormal PT 19.3%. This study showed that 28% of trauma patients are coagulopathic on admission to hospital and they have an increased risk of early death.

Maegele retrospectively examined 17200 trauma cases from the German trauma registry (Maegele et al., 2007) of which 8724 entries had complete data on coagulopathy. The population was very similar to the London and US populations, although 96% had blunt trauma. He found that 34.2% of patients were coagulopathic. The definition of coagulopathy differed from the previous studies, however, and was defined as a PT<70% and/or a platelet count <100 $x10^9$/l. Using this definition, coagulopathy also increased with ISS and mortality was increased in the coagulopathic group. It was shown that the coagulopathic patient group had a statistically significant increase in multiple organ failure (MOF), ventilator dependant days and ICU length of stay and hospital length of stay. The prehospital fluid administration volume findings were different to those of the two previous studies. The coagulopathic group received a mean of 2198 millilitres, and the non-coagulopathic group 1372 millilitres. Also as the volume of prehospital fluid increased, so did the numbers with coagulopathy, although 10% of coagulopathic patients received less than 500 millilitres. The conclusions from this study were much the same as Brohi's and MacLeod's, namely that coagulopathy on admission increased mortality independent of ISS and as ISS increased so did the presence of coagulopathy and mortality. However, unlike Brohi's findings, the amount of prehospital fluid administration had an effect on presence of coagulopathy. This is a reflection of the prehospital trauma systems where large amounts of crystalloid are often given in Germany, whereas at the Royal London Hospital due to short prehospital times, very little fluid was given. Brohi was therefore unable to comment on the role of crystalloid administration in the development of admission coagulopathy.

Despite the three studies having differing definitions of coagulopathy, they all find that approximately a third of major trauma patients arriving at Emergency

Departments are coagulopathic and that the presence of coagulopathy is associated with increased mortality. As yet there is disagreement on the correct terminology for this condition, however the increasingly accepted term is “Acute Trauma Coagulopathy” (Midwinter and Woolley, 2011; Gruen et al., 2012) and represents another subset of TIC.

3.3.1 Military rates of ATC

The proportion of military patients who have ATC remains unclear, although several papers have suggested rates around 40 - 50% (Niles et al., 2008; Plotkin et al., 2008; Doran et al., 2010). Since the presence of ATC on admission carries a significant increase in mortality (Brohi et al., 2003; MacLeod et al., 2003; Maegele et al., 2007), treatment options that may modulate this process hold a great deal of interest for the military.

3.4 Mechanisms of ATC

The pathophysiology of ATC is incompletely understood but appears to be an excessive stimulation of the normal processes of coagulation and fibrinolysis (Sapsford et al., 2007; Curry et al., 2012). Tissue damage leads to exposure of collagen and TF. Collagen leads directly to platelet activation, and TF stimulates clotting by interactions with FVIIa. Large amounts of thrombin are generated, dependent on the amount of TF exposed (Dunbar and Chandler, 2009). This leads to a consumption coagulopathy. Supporting this is observational data suggesting that those with ATC have high thrombin generating capacity (Sapsford et al., 2007; Dunbar and Chandler, 2009; Chandler, 2010; Woolley et al., 2013) and low clotting factors with reduced protein C levels, platelet counts, fibrinogen and antithrombin (Kamphuisen et al., 2002; Brohi et al., 2007b; Floccard et al., 2012; Jansen et al., 2011; Shaz et al., 2011; Woolley et al., 2013).The lowest factor consistently appears to be FV with levels at 41-46% of normal values (Jansen et al., 2011; Yuan et al., 2007; Bickell et al., 1994; Woolley et al., 2013).

Thrombin generation not only leads to fibrin deposition, but also activates the endothelium, as well as platelets and white cells and causes the release of tPA. tPA release is compounded in the presence of hypoperfusion as is commonly seen after trauma (Liener et al., 2001; Brohi et al., 2008; Hayakawa et al., 2011).

3.4.1 Hypoperfusion

The proposed underlying driving mechanism for early ATC is tissue hypoperfusion leading to inadequate oxygen supply to the microcirculation (microcirculatory shock) (Johansson et al., 2011a; Gruen et al., 2012; Brohi et al., 2003). In normal coagulation, thrombin formation leads to fibrinogen conversion to insoluble fibrin. Fibrin will in turn be broken down to its degradation products by the action of plasmin. Normal localisation pathways include removal of thrombin by thrombomodulin to produce a thrombin-thrombomodulin complex (T-TM), thus stopping the activity of thrombin on fibrinogen. The T-TM complex activates protein C which directly inhibits factors Va and VIIIa on the platelet leading to less thrombin production. T-TM will also increase the effect of plasmin by removing inhibition of tissue plasminogen activator (tPA) on plasminogen (see Figure 3-1). The overall effect is to produce anticoagulation and increased fibrinolysis. It is postulated that ATC is a coagulopathy due to pathologically exaggerated activation of protein C as a consequence of tissue hypoperfusion, rather than simply a factor consumption effect (Brohi et al., 2008).

To investigate this, a blinded study (Brohi et al., 2008) of 208 major trauma patients arriving at a single level 1 trauma centre was conducted. The patients had a mean ISS of 17 and a median time from injury to blood sampling of 32 minutes. There was no vasopressor or colloid use and mean crystalloid administration was 150 millilitres. Platelet counts were normal in all patients. Hypoperfusion was judged to be present if there was a base deficit (BD) of 6 or greater, and coagulopathy to be present if aPTT or PT were 1.5 times normal. As injury severity increased, so did the amount of thrombin generation, but the amount was unchanged as hypoperfusion increased. Coagulopathy only occurred if there was a BD>6. It was found that a BD>6 was associated with a

rise in thrombomodulin levels and a fall in protein C (PC) levels. Activated protein C levels (aPC) could not be measured and the assumption was that a drop in PC levels was due to a rise in conversion of PC to aPC. Thus the hypothesis is that a greater base deficit reflects the tissue hypoperfusion leading to a rise in TM-T complexes, conversion of PC to aPC and therefore anticoagulation via inhibition of factors V and VIII. The fall in PC levels were also associated with fall in plasminogen activator inhibitor one (PAI-1). PAI-1 inhibits tissue plasminogen activator (tPA), so as PAI-1 levels fall, tPA activity increases and hyperfibrinolysis occurs.

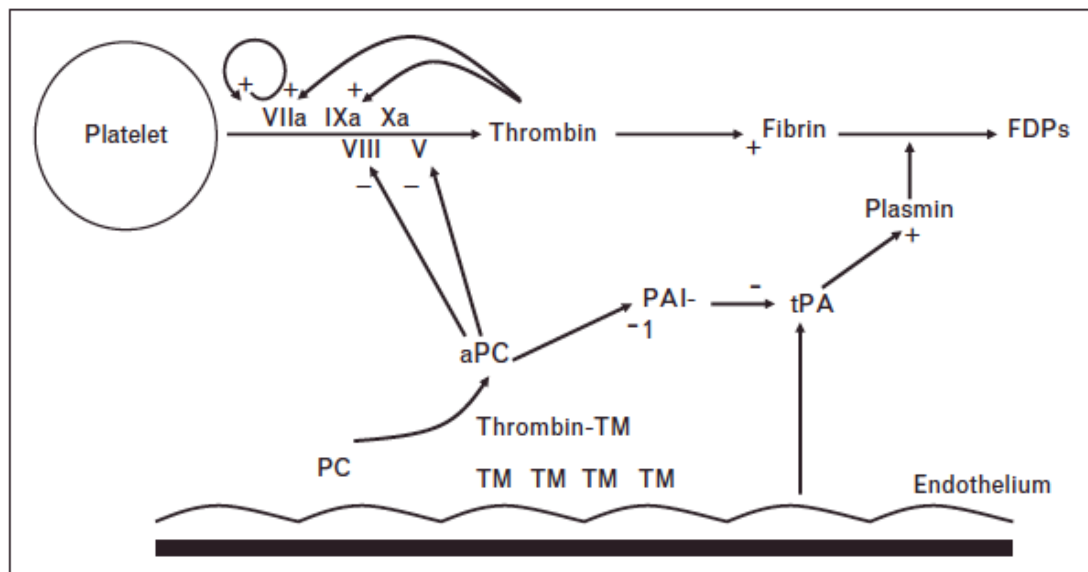

Figure 3-1 Protein C pathway causing anticoagulation (inhibition of FV and VIII, and fibrinolysis (removal of PAI-1 inhibition of tPA on plasminogen) (reproduced from (Brohi et al., 2007a))

Supporting this theory is the fact that FV levels are consistently low in those with ATC representing inactivation by the aPC pathway (van 't Veer and Mann, 1997; Cella et al., 1996; Jansen et al., 2011; Yuan et al., 2007; Haywood-Watson et al., 2011; Woolley et al., 2013). Patient studies (Johansson et al., 2008; Brohi et al., 2003; Frith et al., 2010; Davenport et al., 2011; Jansen et al., 2011; Niles et al., 2008), as well as animal studies (Faller, 1999; Doran, 2012), have demonstrated that as shock worsened, as measured by worsening BD, so the proportion of coagulopathy increased coupled with a fall in clotting factors. It appears that injury severity and hypoperfusion in combination increase the amount of ATC.

3.4.2 Endothelial Cell Activation

Superimposed on these immediate changes are the results of endothelial cell activation (ECA) as described in section 3.2.6. Thrombin and various cytokines cause ECA, an effect that is potentiated by hypoxia and hypoperfusion. Hypoxia causes a fall in cAMP levels with alterations in intracellular signalling. This leads to increases in adhesion molecules with neutrophil recruitment and adherence, exocytosis of Weibel-Palade bodies and reduction in nitric oxide. This results in a proinflammatory, vasoconstricted state (Michiels et al., 2000). This effect is worsened if the hypoxia and hypoperfusion are allowed to continue with inadequate or in appropriate resuscitation. As the endothelial cell changes from antithrombotic to prothrombotic, there is a switch from a net production of tPA, to a net production of PAI-1. This process is effected by Egr-1, a transcription factor present in activated endothelial cells after hypoxia (Ten and Pinsky, 2002). PAI-1 supresses fibrinolysis and, combined with suppression of thrombomodulin, causes a prothrombotic state. The timing of this switch is unclear, it may occur much sooner than previously thought, i.e. in a matter of hours rather than days. It may help to explain the hypercoagulability of patients after trauma as the initial effects of fibrinolytic activation, responding to TXA, are replaced with fibrinolytic shutdown (Curry et al., 2012).

3.4.3 Inflammation, Tissue, endothelial cell and glycocalyx degradation

Traumatic injury is a strong inducer of inflammation and the systemic inflammatory response syndrome (SIRS) is common after significant traumatic injury. SIRS causes endothelial and complement activation (Ganter and Hofer, 2008a; Gando et al., 2002; Lenz et al., 2007). The immune response senses danger through pattern-recognition receptors (PRRs) such as the toll like receptors (TLRs) on immune cells and the endothelium. Recognition of pathogen associated molecular patterns (PAMPs), derived from microorganisms, and damage associated molecular patterns (DAMPs) or alarmins, produced from stressed or damaged cells by TLRs causes upregulation of inflammation. DAMPs are produced in response to trauma, haemorrhagic shock and ischaemia/ reperfusion (Gando et al., 2011). Both

DAMPs and PAMPs have the ability to elicit the inflammatory response, which in turn stimulates coagulation pathways (Levi and Van Der Poll, 2010).

TNFα and IL1b, released as part of the inflammatory response, induces TF expression on microparticles endothelial cells and monocytes (Levi et al., 2002). Pro inflammatory cytokines IL-6, IL-1, IL-12 and TNF all cause an increase in TF expression, increasing thrombin production. Tissue factor in turn can affect the levels of IL-6 and IL-8 whilst thrombin generation will induce monocyte production of TNF-α, IL-1 and IL-6.

High-mobility group box-1 (HMGB1) is a newly recognised "danger signal", that is released after ischaemia reperfusion injuries, by necrotic cells and by activated immune cells. HMGB1 signals via the receptor for advanced glycation end-product (RAGE) and members of the toll-like receptor (TLR) family (Volz et al., 2010).

Recent studies at the Defence Science and Technology Laboratory (DSTL) in a porcine blast/haemorrhage model have demonstrated an increase in HMGB1 expression in animals that were hypotensively resuscitated. It has been suggested that HMGB1 and RAGE may initiate and sustain the inflammatory response (Kirkman et al., 2011). Follow up work also in a pig blast and haemorrhage model investigated the effect of resuscitation strategies on the development of ATC and systemic inflammation (Doran, 2012). They found that animals resuscitated with targeted fluid resuscitation, after 60 mins, to normotension rather than hypotension, had longer survival, lower prothrombin times, less severe base deficit and lower peak levels of IL-6. The suggestion is that reversal of tissue hypoxia and hypoperfusion modulates the inflammatory response after blast and haemorrhage.

Johansson et al looked at the glycocalyx and inflammatory response to trauma in a number of recent studies (Johansson et al., 2011a; 2013; 2012) and linked tissue damage with high catecholamine levels, increased inflammation and increased coagulopathy.

The first study (Johansson et al., 2011a) looked at glycocalyx degradation (syndecan-1), inflammatory markers (IL-6, IL10), DAMPS (HMGB1, histone complexed DNA fragments), natural anticoagulation (TM, PC, TFPI, AT), fibrinolysis (D-Dimer, tPA and PAI-1) and catecholamine levels in 75 patients in three ISS bins (<16, 16-27 and >27) admitted to a level 1 trauma centre. He related these finding to syndecan-1 levels as a marker of glycocalyx degradation and divided the groups into those with high syndecan-1 levels (≥ median) or low syndecan-1 levels (< median). He found that those with high syndecan-1 levels also had higher catecholamines, inflammatory markers, DAMPS, TM, fibrinolysis and a higher mortality (42% vs 14%).

In the second study (Johansson et al., 2012), recruited 80 patients admitted to a level 1 trauma centre as part of the Activation of Coagulation and Inflammation after Trauma 3 (ACIT3) trial between March and November 2010. He looked at link between soluble CD40L (a platelet derived mediator that links inflammation, haemostasis and vascular dysfunction) and similar markers to the first study (endothelial damage, adrenal stimulation, coagulation, inflammation and mortality). He found that when stratified for sCD40L (high sCD40L > median, low sCD40L ≤ median), there is an increase in ISS, tissue and endothelial damage, adrenal stimulation, coagulopathy (reduced thrombin generation, prolonged aPTT), hyperfibrinolysis and inflammation.

The third study (Johansson et al., 2013) looked at the association of tissue damage (increased histone complexed DNA fragments (hcDNA)) to trauma induced coagulopathy, inflammation and endothelial damage. This was performed in 80 patients recruited to the ACIT3 trial between March and November 2010. Patients were stratified to those with high and low hcDNA levels. The results are similar to previous studies in that high hcDNA relates to increased endothelial damage, increased inflammation, coagulopathy and increased glycocalyx degradation. Unlike the previous studies, however, there was an association between high hcDNA and increased TFPI levels. There was also increased platelet activation with an associated reduction in thrombin generation with a prolonged aPTT.

Whilst these three studies broadly say the same things, increased trauma leads to increased tissue damage, increased sympatho-adrenal stimulation, inflammation, coagulopathy, natural anticoagulation and glycocalyx disruption, the third study leads Johansson to speculate that nucleic acids can be potent activators of the contact pathway of coagulation and platelets and affect natural anticoagulant pathways.

3.4.4 Fibrinolysis

Fibrinolysis has a key role in ATC, although opinion remains divided to the pathophysiology behind it. Some clinicians agree with Brohi et al and think that fibrinolysis is driven by an exaggerated aPC pathway mediated by increased TM release from the hypoxic vascular endothelium (Brohi et al., 2008).Others, including Gando, believe that the increase in fibrinolysis is due to DIC. Gando divides fibrinolysis into primary and secondary. Primary fibrinolysis occurs via the aPC pathway without activation of coagulation. Secondary fibrinolysis is the normal response to clot formation and is activated by the presence of thrombin. In DIC the associated increase in fibrinolysis is, in fact, secondary fibrinolysis rather than primary fibrinolysis and so Gando believes that the fibrinolysis seen in ATC is a normal physiological response (Gando et al., 2011).

Regardless of the cause of fibrinolysis, it is well accepted that the presence of fibrinolysis correlates with transfusion requirement and mortality (Kashuk et al., 2010; Levrat et al., 2008; Sawamura et al., 2009; Schöchl et al., 2009; 2010).

A large multinational randomised controlled trial with over 20,000 patients therefore investigated the use of tranexamic acid (a treatment for hyperfibrinolysis) in trauma (CRASH-2 trial). It demonstrated a 9% reduction in mortality in bleeding trauma patients (CRASH-2 trial collaborators et al., 2010). A retrospective study of the use of tranexamic acid in UK Military data also supported this finding (Morrison et al., 2012). However, the effect appears to be greatest when given in the first 3 hours after trauma. After 3 hours there is little benefit and possible harm (CRASH-2 collaborators et al., 2011).

After a period of prolonged endothelial activation, the activated endothelium produces increased amounts of PAI-1, inhibiting tPA action on plasminogen and decreasing fibrinolysis. This is a postulated mechanism for hypercoagulopathy post trauma (Selby et al., 2009; Curry et al., 2012). It is possible that this ”switch” occurs early in trauma; hence TXA administration after 3 hours might be harmful.

3.4.5 Implications for treatment of ATC

The important implication from the Brohi study (Brohi et al., 2008) is that if ATC exists as a result of activation of the thrombomodulin-protein C pathway secondary to tissue hypoperfusion, then there is no failure of thrombin generation. Treatment of ATC would require reversing tissue hypoperfusion rather than simply treatment with blood component therapy. If Gando’s DIC hypothesis is true, (Gando et al., 2011) then early reversal of shock is the key to minimising primary fibrinolysis, minimising the ischaemia reperfusion injury. Treatment of the DIC is by replacing the factors lost during the consumptive phase and supporting the coagulation system as it rebalances anticoagulation, coagulation and fibrinolysis. Importantly both theories presume normal thrombin generation, the coagulopathy is an imbalance in haemostasis rather than a reduced factor activity and failure to produce clot.

The different mechanisms causing TIC have different implications for potential management strategies at different time points during the resuscitation of the trauma patient. Initially it may be that ATC is related to hypoperfusion not abnormal clotting pathways or deficiencies in normal coagulation. Later on the process may be driven by consumption or dilution. This may mean that the treatment goals are different depending on the underlying mechanism involved at that particular time point (Midwinter and Woolley, 2011). Initial resuscitation may need to emphasise tissue oxygenation. It has been shown that early supplementary oxygen after blast injury and haemorrhage improved survival when a hypotensive resuscitation strategy was employed in an animal model (Granville-Chapman et al., 2010). Component therapy in the presence of tissue hypoxia might potentially worsen hyperfibrinolysis by increasing the available thrombin to conjugate with thrombomodulin, driving aPC production (Midwinter

and Woolley, 2011). Therefore initial hypotensive resuscitation, if it leads to significant critical tissue hypoperfusion and hypoxia, could exacerbate ATC.

Once a major trauma casualty arrives in hospital, the requirement for hypotensive resuscitation may no longer be pertinent if systems allow access to the rapid control of major surgical haemorrhage, as the concern over disruption of native clot will no longer pertain (Spahn et al., 2013). Fluid and targeted therapy should be used in order to reverse tissue hypoxia and restore haemostasis. This is not always a trivial objective and requires the ability to closely monitor the patient's tissue oxygenation and coagulation status and changes that occur in these parameters as resuscitation, including surgical manoeuvres, proceed. This requires clinically informed, real-time patient monitoring and evaluation in order to manage the transition through the resuscitation process including surgical haemorrhage control (Gruen et al., 2012).

3.5 Definitions of coagulopathy

Definitions of coagulopathy have been well established in the literature, and include Abnormal PT/ aPTT, PT >18 seconds, aPTT>60 seconds (Hewitt and Machin, 1990; Lundberg, 1994), PT ratios> 1.5 times normal (Hoyt et al., 2008), low platelet count and abnormal INR's (Sauaia et al., 1995). There is no consensus for what criteria should be used in the diagnosis of ATC. Different peer reviewed papers use different definitions. In the pivotal papers describing the presence of ATC, Brohi et al used a definition of PT or aPTT of 1.5 times normal (Brohi et al., 2003). MacLeod's follow up paper from the US used an abnormal PT or aPTT as their definition of coagulopathy (MacLeod et al., 2003), whilst Maegele used a PT<70% normal and a platelet count <100 $x10^9$/l (Maegele et al., 2007).

Not only do definitions differ, but also the clinical significance of abnormal values. An abnormal number does not necessarily mean a clinical problem. A patient arriving at hospital who was previously well and suffered trauma who has an INR of 1.5 is deemed to be coagulopathic and at increased risk of dying. A patient who is on therapeutic warfarin therapy with an INR of 1.5 is deemed

not coagulopathic enough. Definitions of coagulopathy do not take into account the clinical scenario.

Recently some groups have challenged these definitions of coagulopathy and have tried to relate the abnormal number with a clinical significance (Frith et al., 2010; Davenport et al., 2011). A retrospective multicentre cohort study looked at patients from five trauma registries in London, Germany, Amsterdam, Oslo and San Francisco. Since each centre had different assay methods and reagents for PT, the PT ratio (PTr) was used. If standard definitions of coagulopathy had been used, a PTr of 1.5, then overall 19% of patients were coagulopathic, whereas 36% had a PTr of grater than 1.2. A significant increase in mortality and transfusion requirement was seen at a PTr of 1.2 and the authors suggest a PTr of 1.2 to be diagnostic of ATC (Frith et al., 2010).

3.5.1 Definitions of Coagulopathy with Viscoelastic methods

Accepting that the practical use of PT, aPTT in trauma for clinical decision making is limited (section 4.2.4) some effort has been focussed on using ROTEM®/ TEG® to determine the presence of coagulopathy, and to see if its rapid assessment can aid decision making (Johansson et al., 2009; Doran et al., 2010). Since the use of ROTEM®/TEG® in trauma is relatively recent, there are no formally accepted definitions of coagulopathy using ROTEM®/TEG® parameters (Curry et al., 2012). This makes the incorporation of ROTEM®/TEG® into standard protocols that can be used by the non-expert clinician difficult.

Davenport et al (Davenport et al., 2011) performed a prospective observational study on 300 patients arriving at the Royal London Hospital between Jan 2007 and Jun 2009. Main laboratory and point of care (POC) PT as well as ROTEM® parameters were collected. They adopted the definition of Frith et al (Frith et al., 2010) that a PTr of >1.2 was diagnostic of ATC.

POC PT did not correlate well with lab PT in those cases of ATC and had a 29% false negative rate. ROTEM® amplitude at 5 mins (CA5) of <35mm were more likely to receive red cell and plasma transfusions and had a 77% detection

rate of ATC with a 13% false positive rate. Compared to PT, ROTEM® was better at determining those likely to receive massive transfusion (71% vs 43%). However the positive predictive values for PT and ROTEM were low. The authors concluded that there was a ROTEM® trace characteristic of the presence of ATC.

3.5.2 Military experience of ROTEM®

UK DMS deployed ROTEM® to Bastion for the first time in Jan 2009 and demonstrated that the machine was robust enough withstand field conditions, and detected more abnormalities than standard laboratory testing (Doran et al., 2010). As yet there is no published data on any definition of coagulopathy in military trauma as determined by ROTEM®.

Thus definitions of coagulopathy remain variable with little clinical application in acute haemorrhage. Point of care tests may be able to provide more useful results in the context of acute trauma coagulopathy Their role, however, is likely to be limited to evaluating changes, trends and responses to interventions until exact definitions of ATC can be determined. It is unlikely that any test will replace the clinical assessment of the patient and the clinical scenario at the time of testing.

3.6 Summary

Coagulopathy after trauma is complex, and remains poorly understood. There is little doubt that up to a third of civilian trauma patients arrive in hospital coagulopathic, and their outcomes are worse than those who arrive without coagulopathy. The rates of coagulopathy are likely to be worse in the military environment. The pathophysiology remains uncertain. Fibrinolysis is a key component, however differences in opinion exist as to the role that consumption, DIC and natural anticoagulation have in the development of acute trauma coagulopathy. It is increasingly accepted that the likely driver of the pathophysiological process is microcirculatory shock due to inadequate tissue oxygen delivery, which in turn leads to activation of the vascular endothelium, although its role is yet to be determined fully.

Individualising the treatment of coagulopathy has important implications in resuscitation, appropriate blood use and patient safety. This is even more important in a resource limited military environment. Before we target therapy, we must be able to detect the presence of coagulopathy in clinically relevant time lines. The following chapter will discuss the methods of assessing coagulation in research and clinical settings.

Chapter 4. Assessment of Clotting

Coagulation is a highly complex interaction of enzymes, factors, cells and proteins. Like all biological systems, it is possible to measure all, or most, of these individual factors in isolation. However in a clinical setting, especially in an on-going haemorrhage situation, specific factor assays are not appropriate and global tests of coagulation are required.

4.1 The ideal test

The ideal test would provide an *in vivo* assessment of which component of the clotting process is at fault, i.e. clot initiation, platelet function, factor function or fibrinolysis. It should be reproducible by non-technical persons, rapidly available and be sensitive and specific. Recommended screening tests in trauma (Doran et al., 2010; Rossaint et al., 2010; SGOPL 0809, 2009) include the INR, aPTT, fibrinogen and platelets. European guidelines also suggest the use of thromboelastometry to characterise coagulopathy and to guide haemostatic therapy (Spahn et al., 2013).

No one test, including the recommended tests, fulfils all the required criteria for the ideal test, although in combination a representative clinical picture can be built up.

4.2 Routine laboratory testing.

4.2.1 Activated Partial Thromboplastin Time and Prothrombin time.

Commonly the activated partial thromboplastin time (aPTT) and prothrombin time (PT) are performed together. Both tests were originally designed to aid the diagnosis of inherited bleeding disorders, such as haemophilia, however its use has migrated to that of routine screening. Whilst it is generally accepted that the traditional clotting cascade of the intrinsic and extrinsic pathway is no longer

represents the situation *in vivo*, it is still useful in describing how PT and aPTT detect coagulopathy.

4.2.2 aPTT

The aPTT test activates the intrinsic pathway by using contact activators such as kaolin, silica or ellagic acid. These activators bind directly to FXII resulting in surface activation to FXIIa and on-going clot formation. The aPTT test utilises platelet poor plasma, at 37 degrees with the addition of calcium. The aPTT represents the time taken for clot to start to form, i.e. initiation, and provides no measurement of clot strength or clot stability. It detects deficiencies in the intrinsic pathway, FVIII, FIX, FXI, FXII, and the final common pathway, FII, FV, and FX. Mild deficiencies, up to 40% of factors, will still produce a normal aPTT

4.2.3 PT

The PT test activates clotting via the extrinsic pathway using tissue factor (normally thromboplastin from brain tissue). This binds to factor VII forming a TF-VIIa complex that activates the final common pathway. Like the aPTT test it also uses platelet poor plasma at 37 degrees with the addition of calcium. The PT, like the aPTT, represents the time taken for clot to start to form, i.e. initiation, and similarly provides no measurement of clot strength or clot stability. The test detects deficiencies in the extrinsic pathway, FVII, and the final common pathway, FII, FV, and FX. Mild deficiencies, up to 40% of factors, will still produce a normal PT

In 1977 the Word Health Organisation released a standardised preparation of thromboplastin allowing the PT to be expressed as an International normalised ratio (INR). The INR is the standard test used to measure warfarin therapy.

4.2.4 Limitations of PT and aPTT

It is increasingly accepted that PT and aPTT are not useful in screening for, or detecting clinically significant abnormalities during acute haemorrhage

(Davenport et al., 2011; Johansson et al., 2009). This is for two main reasons, firstly processing up to 45 minutes (Doran et al., 2010), making the result is historic by the time it is available (Brohi, 2009; Segal et al., 2005; Curry et al., 2012; Toulon et al., 2009) and secondly PT and aPTT do not measure the whole clotting process or the elements that are responsible in TIC mechanisms (Rossaint et al., 2010; Brohi, 2009; SGOPL 0809, 2009). Plasma does not contain phospholipid membrane (platelets or microparticles) and the tests do not assess the thrombin potential of the sample.

There are rapid, point of care PT measuring devices. Davenport et al (Solomon et al., 2011; Davenport et al., 2011) compared one such machine, coagulocheck®, to laboratory PT tests in a series of trauma patients attending the Royal London Hospital. He found a good correlation between coagulocheck® and the laboratory for normal samples, however the accuracy of POC testing falls off as the PT prolongs and the haematocrit falls and would be of limited use in the trauma setting.

The PT is run in platelet poor plasma, and thus not physiological, and only gives information of the time taken for clot initiation and does not represent clot strength, clot dynamics (the speed and efficiency at which the clot is developing), clot stability or fibrinolysis. The tests are also designed to detect a fall in coagulation factors to <30 – 40%. In a trauma situation, where trends are important, a clinician needs to know there is a developing problem before levels of clotting factors have fallen to as low as 40% of normal. In this context PT and aPTT are not sensitive enough to give reliable clinical information during acute haemorrhage.

Abnormalities in PT and aPTT are still recognised as one definition of coagulopathy and there is reluctance to fully embrace other coagulation monitors, such as ROTEM® and TEG®, until formal definitions can be produced (De Caterina et al., 1994; Brohi, 2009; Curry et al., 2012). In spite of their clinical limitations, PT and aPTT are still routinely performed in many institution’s trauma blood panels, especially since as a retrospective tool they are still the only formal definitions of coagulopathy that can be used to compare populations.

4.2.5 Platelet count

Platelets are integral to coagulation and for a clot to form a minimum number of functioning platelets is required. New European and Defence Medical Services (DMS) guidelines state that in a bleeding patient platelet numbers should be kept greater than $100x10^9$/l (Spahn et al., 2013; Joint Service Publication 999, 2012). However, the platelet count does not take into account platelet function. Since the DMS started using ROTEM® in Afghanistan there has been some speculative evidence that platelet function may be particularly affected after blast trauma. Recent reports (Doran et al., 2010; Davenport et al., 2011; Jansen et al., 2013) have highlighted abnormalities in platelet activity after trauma in spite of adequate platelet numbers, although the exact mechanism is yet to be determined.

ROTEM® will give an indication of platelet activity and recent interest in the use of multiplate as a measure of platelet aggregation may add an extra dimension to the understanding of platelet activity after trauma (Ganter and Hofer, 2008b; Foex, 1999; Solomon et al., 2011; Hett et al., 1995). We are becoming increasingly aware of the highly influential role platelets and microparticles have in clotting, inflammation and wound healing. Platelet function, rather than absolute number, is key for these processes to occur.

4.3 Whole blood assessment of clotting

Since the cellular components of blood are required for coagulation, it is reasonable to have coagulation tests that assess all the components of blood, namely the clotting factors, and the cells.

4.3.1 The Bleeding Time

Historically clotting has been assessed by the bleeding time (Foex, 1999; Hewson et al., 1985; De Caterina et al., 1994). The bleeding time is an assessment of platelet function. It involves a standard incision to the upper arm below a blood pressure cuff inflated to 40mmHg. Filter paper is used to blot the

edges of the wound every 30s until bleeding stops. Normal values are 2 to 9 minutes. This is an *in vivo* test of the whole process of clot formation, and is no longer routinely practised due to difficulties in standardisation and inter-user variability.

4.3.2 *Activated coagulation time (ACT)*

The ACT is measured by adding fresh whole blood to a test tube containing negatively charged surfaces (celite, kaolin or glass). The test tube is placed into a warmed rotating device. A small magnet is displaced, once enough clot has formed, which activates a proximity switch. Clinically the test is used to assess adequacy of heparin effect, but results may be prolonged due to hypothermia, platelet dysfunction, “intrinsic” factor deficiencies or hypofibrinogenaemia.

Both the bleeding time and the ACT may well give a global picture of clotting function, however any abnormality does not provide information of the specifics of what is wrong. In addition, the bleeding time is impractical during an acute haemorrhage situation.

4.3.3 *Viscoelastic methods*

The viscoelastic method of detecting clot formation is a technique invented in 1948 with the thromboelastography machine, the TEG®. This measures the viscosity of whole blood from clot initiation through to clot lysis. A refinement of this method, rotational thromboelastometry (ROTEM®) is a recent, more modern and robust equivalent. Due to legal issues ROTEM® and TEG® have different terminologies for the same parameters, these are outlined in Table 4-2. The British Military have adopted ROTEM® as its viscoelastic method of choice since it is more robust in an austere environment due to the measurement pin mounted on sapphire ball bearings rather than a freely suspended wire (see section 4.4 and 4.5)

4.4 ROTEM®

The ROTEM® works by measuring the change in torque of a rotating pin as clot forms. A pin mounted on sapphire ball bearings, is inserted into a cuvette of 340µl of blood and the appropriate initiators are added following the on screen instructions on the machine. The blood is maintained at 37°C and the test initiated (TEM, 2010). The pin rotates back and forth and as clot begins to form between the walls of the cuvette and the pin, pin movement is inhibited. Using optical sensors, the degree of pin movement inhibition is graphically displayed and various parameters are calculated.

4.4.1 ROTEM® parameters:

A classical ROTEM® trace (TEMogram) is depicted in Figure 4-1. It is divided in to certain routinely measured parameters

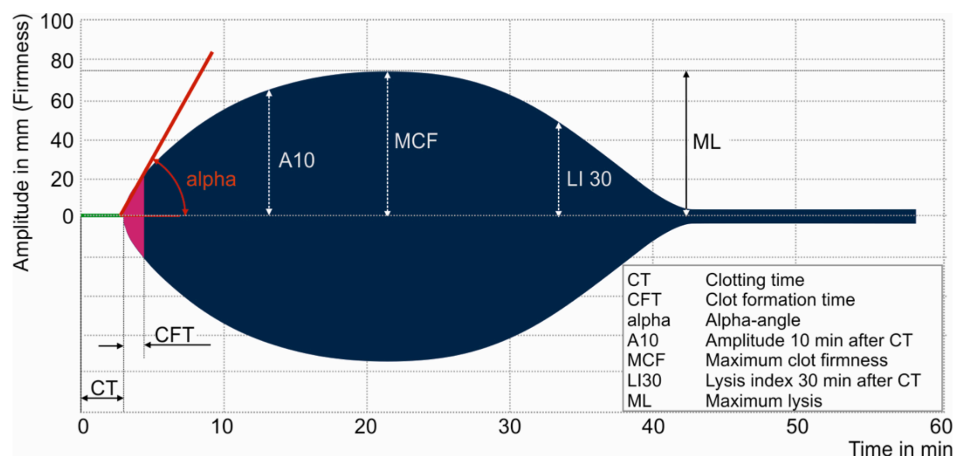

Figure 4-1. Illustration of a TEMogram showing routinely measured parameters. (Figure taken from TEM® training material.)

Clotting Time (CT):

This is the time taken, in seconds, from the start of activation to the initial clot formation once the amplitude reaches 2mm on the trace. This relates to clot initiation and initial thrombin production. Any prolongation of CT is due to factor deficiency (including severe hypofibrinogenaemia) or the presence of anticoagulants.

Clot Formation Time (CFT):
This is the time taken, in seconds, from CT until amplitude of 20mm is reached. This represents a measure of the rate of clot formation, or clot dynamics, and is dependant on fibrinogen, platelet and factor interactions.

α angle: This is the angle from the centre line and a tangent to the curve at the 2mm point. This is another representation of clot dynamics

Maximum Clot Firmness (MCF):
This is a measurement of the maximum amplitude, and therefore maximum strength of the clot. It is determined by the amount of functioning platelets and fibrinogen available for clot formation. Any loss in MCF indicates a deficiency in functional fibrinogen or platelets. Deficiencies in platelets will either be a quantitative or qualitative deficiency. The MCF may well take many minutes to be available. The amplitude at various time points after CT (AX where X = time) are routinely reported. The firmness at A5 (5 mins) and A10 (10 mins) have been reported to be useful in trauma (Davenport et al., 2011).

Maximum Lysis (ML):
Any reduction in clot firmness after MCF is due to loss in clot integrity. The ML is the maximum lysis at the end of the test. If the test is run for 60 mins, then any ML>15% represents hyperfibrinolysis, although this is an arbitrary value (Curry et al., 2012).Since test may be run for variable length of times, it is more usual to use the lysis index. LY30 represents lysis index at 30 mins and is the percentage of clot remaining compared to MCF. An LI30 <94% or an LI60<85% indicates hyperfibrinolysis.

4.4.2 ROTEM® tests

If blood was simply added to the cup and allowed to clot, it would take many hours to produce a result (NATEM). This may well be very sensitive to subtle changes in coagulation, but of limited use clinically. In order to speed up the process, and to allow specific components of the clotting process to be examined, a number of initiators and inhibitors are added to the blood sample. These are summarised in

ROTEM®		Information Provided	TEG®	
Test	Activator/ Inhibitor		Test	Activator/ Inhibitor
EXTEM	Recombinant Tissue Thromboplastin (Tissue Factor)	Assesses factors VII, X, V, II, I, platelets and fibrinolysis		
		Assesses all factors, platelets and fibrinolysis	Rapid TEG	TF- Kaolin
INTEM	Ellagic acid	Assesses factors XII, XI, IX, VIII, X, V, II, I, platelets and fibrinolysis Sensitive to heparin	Kaolin	Kaolin (hydrate aluminium silicate)
FIBTEM	Activated as EXTEM. Platelet function inhibited by Cytochalasin D	Assesses fibrinogen levels and fibrin polymerisation	Functional Fibrinogen TEG	TF + abciximab (ReoPro®)
APTEM	Activated as EXTEM. Aprotinin inhibits fibrinolytic processes	Confirms presence of fibrinolysis		
HEPTEM	Ellagic Acid and Heparinase	Determines if prolonged clot initiation is due to heparin or not.	Heparinase	Kaolin + Heparinase
NATEM	Calcium only added	Allows very sensitive assessment of coagulation activation or inhibition – mainly for in-vitro experimentation		

Table 4-1. Comparison of assessment tests of ROTEM® and TEG®, the activating/inhibiting additive and the information being assessed.

The standard test is the EXTEM, where tissue factor is added to initiate the clot via the extrinsic pathway, the intrinsic equivalent is the INTEM where a contact activator is used (Figure 4-2).

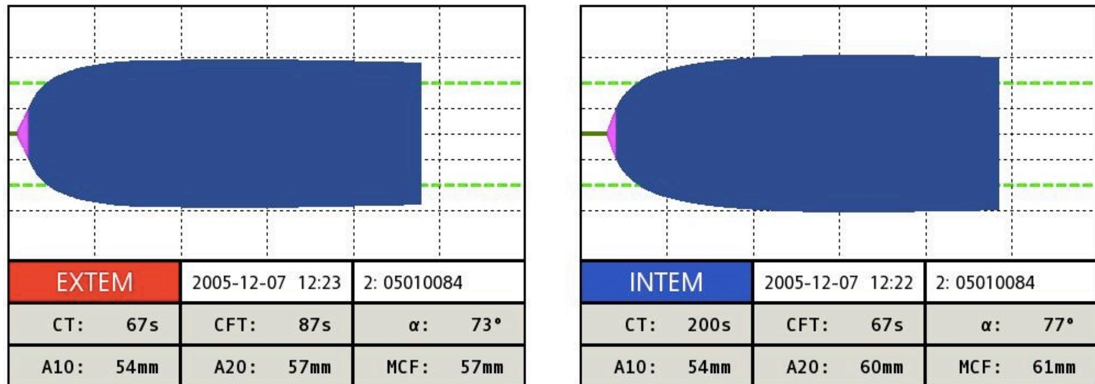

Figure 4-2. Normal EXTEM and INTEM traces

The EXTEM analysis can be used in conjunction with the FIBTEM. The FIBTEM has TF activation in the same way as EXTEM, however with the addition of a platelet inhibitor. Since clot strength (MCF) is determined by functioning platelets and fibrinogen, comparison of EXTEM MCF (PLT and fibrinogen) and FIBTEM MCF (fibrinogen) can give an estimate of the relative contributions to the clot strength of platelets and fibrinogen.

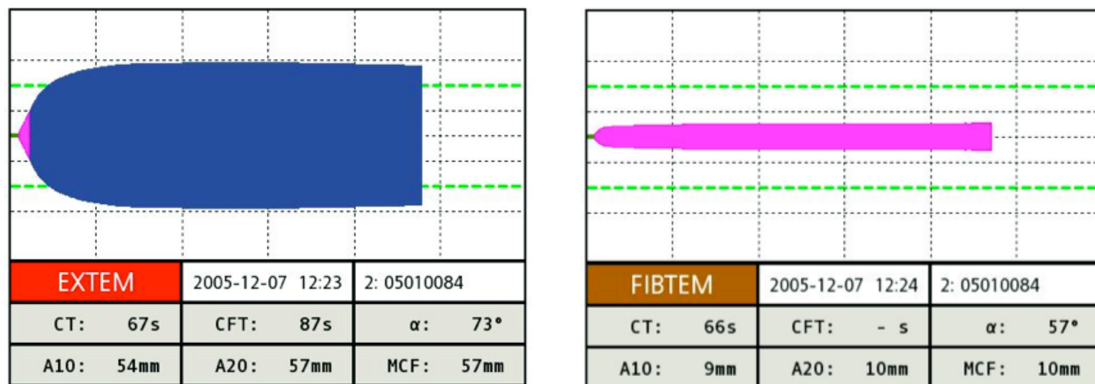

Figure 4-3. Normal EXTEM and FIBTEM traces

In Figure 4-3 the EXTEM MCF is 57mm and in normal limits. The FIBTEM MCF is 10mm and in normal limits. It has been suggested that as an approximate guide the platelet contribution of the clot is EXTEM MCF – FIBTEM MCF = 40mm in this case. This view is unsubstantiated, but as a clinical guide is a useful concept.

The APTEM uses EXTEM activation with the addition of aprotonin. Since aprotonin reverses hyperfibrinolysis, any differences between APTEM and EXTEM are due to hyperfibrinolysis and should be treated if not already done so

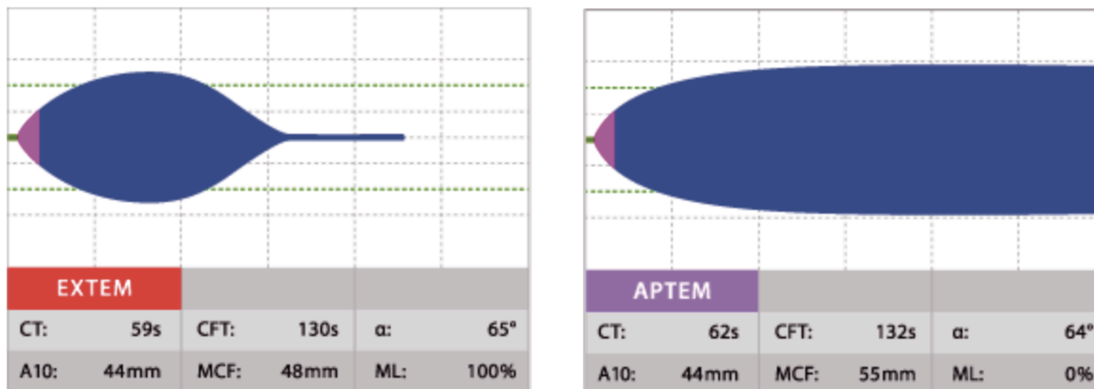

Figure 4-4. EXTEM trace shows loss of clot strength, which is returned in APTEM confirming hyperfibrinolysis

In Figure 4-4.the EXTEM trace has formed a clot with an MCF of 48mm, however after about 20 minutes the clot begins to lose strength and by 30 minutes has an LY30 of 0%. Comparison with the APTEM, which shows a return to normal with no loss of clot strength, confirms that the loss in clot strength is due to hyperfibrinolysis.

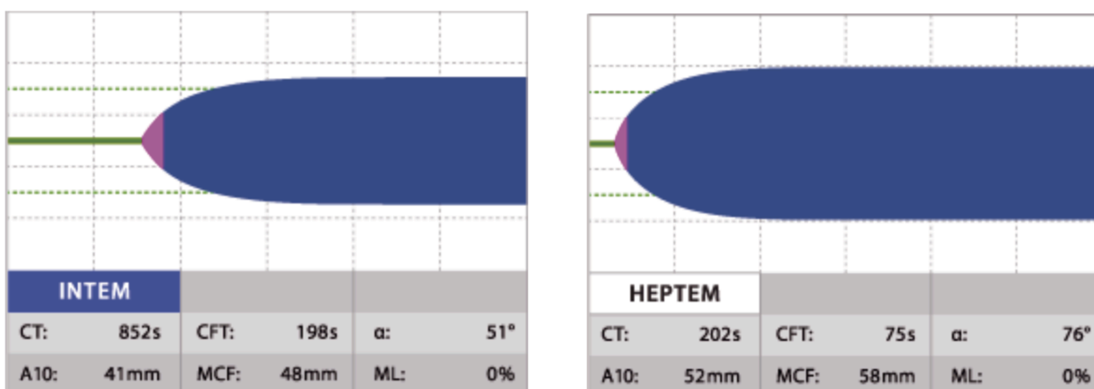

Figure 4-5. INTEM trace shows a long CT, which is reversed in HEPTEM confirming heparin effect

In Figure 4-5 the CT in INTEM is very prolonged. Since this effect is reversed in the HEPTEM, the abnormality is solely due to heparin effect.

In 2009 The DMS underwent an evaluation of ROTEM® in Afghanistan and concluded that ROTEM® was a useful tool (Doran et al., 2010). Since the machine itself has 4 channels, and since INTEM was found to be abnormal only

when EXTEM was abnormal, it was decided that the routine screening test would be EXTEM and FIBTEM. This allowed the second pair of channels to be made available either for a second patient, or a second sample on the same patient.

4.5 TEG®

The TEG® works by measuring the change in tensile strength of the clot as it forms, in a similar way as ROTEM®. The major difference in TEG® is that the cup rotates round a freely suspended torsion wire, rather than the reverse in ROTEM®. This makes the TEG® susceptible to knocks and movements, which tends to cause the test to fail, and as such TEG® is not suitable for use in an austere military environment. As clot forms between the cuvette and the wire, the change in torsion reflects the clot strength.

TEG® analysis is performed in a standard way. 360µL of whole blood is added to TEG® cuvette with the appropriate initiator. The cuvette is then raised in its carrier and the test initiated (Haemoscope, 2007). A classical TEG® trace is depicted in

Figure 4-6

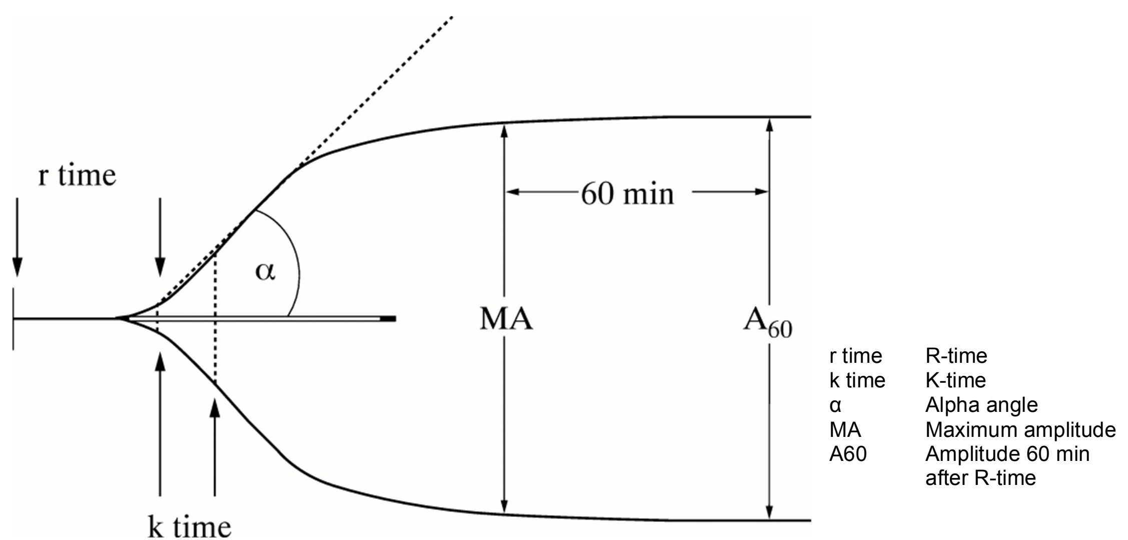

Figure 4-6. Illustration of a routine TEG® trace with routinely measured parameters

4.5.1 TEG® parameters

Terminology is different, although the measured parameters are the same (see Table 4-2). Clot initiation is measured as the r-time, clot strength as maximum amplitude (MA) with amplitudes at timed intervals, commonly CA30 and CA60. The dynamic portion of clot formation is measured by the K-Time and the alpha angle. The clot should remain at, or nearly at full strength for longer than the standard 60 minutes of the test, however any significant loss in strength (reduction in MA) is due to excess clot breakdown, hyperfibrinolysis. Comparison with ROTEM parameters is made in Table 4-1.

TEG	ROTEM	Description of parameter	Parameter interpretation
R Reaction time (sacs)	CT Clotting Time (secs)	Time from start of sample run to appearance of first detectable clot (amplitude of 2mm)	Initiation phase Lag-phase of thrombin generation (dependent on activity of coagulation factors)
K (secs)	CFT Clot Formation Rate (secs)	Time from R or CT until level of clot firmness reaches 20mm	Amplification phase Initial clot strengthening by formation of fibrin fibres
α-angle (degrees)	α-angle (degrees)	Kinetics of clot development - angle between centre line and tangent to the curve through the 2mm amplitude point	Thrombin burst Maximum velocity of clot formation (dependent on platelet function and fibrin polymerisation)
Ax Clot Amplitude (mm)	Ax Clot Amplitude (mm)	Mechanical clot quality at fixed time point e.g. 10 minutes (CA10)	Propagation phase Clot strength at fixed time point determined by platelets, fibrinogen and Factor XIII
MA Maximum Amplitude (mm)	MCF Maximum Clot Firmness (mm)	Mechanical clot quality	Maximal clot strength – determined by platelets, fibrinogen and Factor XIII
CLx (%)	LYx (%)	Ratio of the amplitude to MA/MCF at a given time point after R/CT	Clot termination Percentage clot lysis at fixed time e.g. 30 minutes (CL30, LY30)

Table 4-2. Comparison of ROTEM® and TEG® terminology

4.5.2 TEG® tests

A number of initiators and inhibitors are added to the sample. And are summarised in Table 4-1

The standard TEG® uses kaolin as an initiator and effectively gives an equivalent to INTEM. Rapid TEG uses tissue factor as an initiator and it is possible to use these tests in conjunction with heparinase containing cuvettes, to measure heparin effect. Functional Fibrinogen is measured by the addition of ReoPro® (abciximab) to the sample. This is a platelet inhibitor equivalent to FIBTEM. In essence the TEG® results can be interpreted in exactly the same way as the ROTEM®.

4.6 Alternative initiators for ROTEM® and TEG®.

For research purposes it is possible to use alternative initiators that can potentially provide a more sensitive assessment of clotting in either TEG® or ROTEM®. A commercially available recombinant tissue factor preparation (Innovin®) is used to determine INR and is highly sensitive to deficiencies in factor II, VII, IX and X. It is possible to use dilute Innovin® as a more sensitive initiator and can be compare to standard TEG® or ROTEM®, especially in detecting the effects of rFVIIa (see section 4.7) (Ganter et al., 2008a; Sørensen et al., 2003).

4.7 Monitoring of rFVIIa activity

There is no well accepted monitor of rFVIIa therapy (Lin et al., 2011; Pusateri and Park, 2005). PT and aPTT do not always fully reflect clinical effectiveness. Dutton et al (Kenet et al., 1999; Dutton et al., 2004) noted that 78/78 patients had improved PT after administration of rFVIIa, but not all of them had a clinical improvement in bleeding.

Recently there has been renewed interest in using dilute TF (Innovin) test on ROTEM® or TEG® to monitor rFVIIa therapy. The rationale for the use of dilute

Innovin as a reagent for TEG® analysis comes from the presumption that the increased sensitivity is likely to detect subtle, but important changes not seen in the standard TEG®, ROTEM® or PT.

PT and ROTEM® are designed so that the amount of tissue factor used overwhelmingly activates all the clotting factors present in the sample. *In vivo* however visible fibrin clot develops after only 3-5% of total thrombin is produced, the remaining 95% of thrombin production occurring after clot formation has started (Dutton et al., 2004; Young et al., 2013; O'Neill et al., 2002; Martinowitz et al., 2001).

Sørensen and colleagues tested whole blood and platelet rich plasma with different concentrations of tissue factor on ROTEM®. The authors found that as the concentration of TF decreased, so clotting time and clot dynamics increased, whereas clot strength remained unaffected (Boffard et al., 2005; Sørensen et al., 2010).

In another study Sørensen and colleagues looked at ROTEM® activation using different dilutions of TF (diluted from 1:17 to 1:34 000) (Narayan et al., 2008; Sørensen et al., 2003). He compared haemophiliacs, who clinically had slow, depressed clotting, with normal volunteers. Both groups of patients had identical results when high concentrations of TF were used. However lower concentrations of TF revealed
differences between the groups that were commensurate with the clinical effects of haemophilia. He found the best dilution to be 1:17 000 providing enough initiation to activate the test in a useful time, but sensitive enough to detect differences between groups. This is taken as evidence that the dilute innovin test is more sensitive to subtle, clinically important changes, compared to conventional tests, and is ideal for situations where speed of reporting is not essential.

Ganter et al (Ganter et al., 2008b) recommend that to detect the effects of rFVIIa, only dilute amounts of TF should be used for best sensitivity since high amounts of TF may directly activate coagulation and bypassing the

requirements for FVIIa. The authors recommended a dilution of 1:1000 to effectively monitor rFVIIa therapy.

4.8 Sonoclot

Sonoclot is very similar to TEG® and ROTEM®, however it uses a vertically vibrating probe to detect changes in viscosity, rather than rotation. Is produces a characteristic signature, and determines the time taken for initial fibrin formation, rather than the fibrin/platelet bond which is measured in TEG®/ ROTEM®. Clot initiation is therefore a reflection of fibrin formation, rather than the fibrin/platelet bond. The latter being detected in TEG®/ ROTEM® and more comparable to clot strength. There has been speculation as to the reproducibility of Sonoclot, and its results appear to be influenced by age/ sex and platelet count (Risberg et al., 1986; Ganter and Hofer, 2008a; Hett et al., 1995).

4.9 Individual Factor assays

Individual factor assays are functional measurements of the individual clotting factors. To perform a factor assay, sample plasma is diluted with a reference plasma deficient in a single factor but with normal amounts of all other clotting factors. Mixing the sample plasma with this factor deficient reference plasma will correct all factor deficiencies in the patient's plasma except for the factor being tested. The mixed plasma is then serially diluted and tested using a prothrombin time. The degree of correction in the clotting time is proportional to the amount of factor present in the patient's plasma.

4.10 Summary

There are many ways to detect abnormalities in the coagulation system. Clotting is a delicate balance between procoagulant, anticoagulant and fibrinolytic pathways, and is intimately related to vascular endothelial function. Therefore, any test that is *ex vivo* and does not look at the whole process of

coagulation is limited. In acute haemorrhage situations, results need to be available in a very short time frame so that they are useful clinically.

Currently viscoelastic methods, TEG® and ROTEM®, are the closest to the ideal test, however both have their limitations. In a military clinical setting ROTEM® has proved to be the more useful of these machines principally due to the robustness of the measurement module.

Chapter 5. Blast

Blast as a mechanism of military trauma has increased during the current conflicts in Iraq and Afghanistan. Recent estimates suggest that up two thirds of casualties have exposure to blast (Hodgetts et al., 2007). Blast is also an increasing problem in civilian trauma with recent bombings in London, Madrid and the United States of America.

An understanding of the physics and haemostatic changes after blast are essential if treatments aimed at modulating them are to be investigated.

5.1 Classification of blast injuries

Blast injuries are usually divided into four and occasionally five main categories (Kirkman et al., 2011; Champion et al., 2009) primary, secondary, tertiary, quaternary and quinary

5.1.1 Primary Blast injury

Primary blast injury occurs from the effect of the shock wave depositing energy, mainly at gas liquid interfaces. This particularly affects the alveoli (leading to blast lung), the bowel and the inner ear. Often the injury leads to no external signs. The incidence and extent of injury depends on the duration of exposure and the level of the peak over pressure.

5.1.2 Secondary blast injury

Secondary blast injury occurs from fragmentation of the device. Tissue destruction and loss occur due to the penetrating nature of fragments from the device and other particles, such as stones, surrounding the device.

5.1.3 Tertiary blast Injury

Tertiary Injuries are as a result of the blast wind. This causes a sudden and dramatic movement of air causing the body to be thrown against objects resulting in blunt injury.

5.1.4 Quaternary and quinary injury

Quaternary effects are those of burns or crush resulting from the explosion. Quinary injuries are as a result of the environmental exposure, such as radiation, that may occur following the explosion.

5.2 Physics of Blast Injury

An explosion is a chemical reaction liberating vast amounts of energy in the form of heat, kinetic energy and shock waves. Detonation causes an exponential increase in heat within the explosive increasing the pressure. It rapidly releases energy in the form of heat, gaseous products and a blast wave.

5.2.1 Blast Wave

The blast wave consists of the shock wave and the dynamic overpressure (or blast wind). The shock wave is the front edge and consists of a rim of compressed air surrounding an expanding ball of explosive materiel. Within a few metres of the explosion, the dynamic overpressure slows down, separating from the shock wave, which continues to travel faster than the speed of sound (Figure 5-1).

Figure 5-1.Shock wave depicted from the main weapon of a warship. The shock wave can be seen with the blast wind and finally the flame from the explosion.

If the pressure of the shock wave is measured, there is an almost instantaneous rise in pressure, reaching a peak (overpressure), a drop to subatmospheric pressure followed by a return to atmospheric pressure. This is known as the Friedlander wave Figure 5-2. This wave becomes more complex once inside an enclosed space, such as a building, due to the reflective nature of the walls.

5.2.2 Interaction with the body

The interaction of the shock wave with the human body is complex and not attenuated by the presence of clothing and body armour. At air/liquid interfaces the shock wave will cause differential movement of tissues leading to shear stresses and tearing of delicate structures.

The primary blast wave will commonly affect the ear, rupturing the tympanic membrane, the lungs with the development of blast lung, and the abdominal viscera, most often the colon. There is a well documented physiological

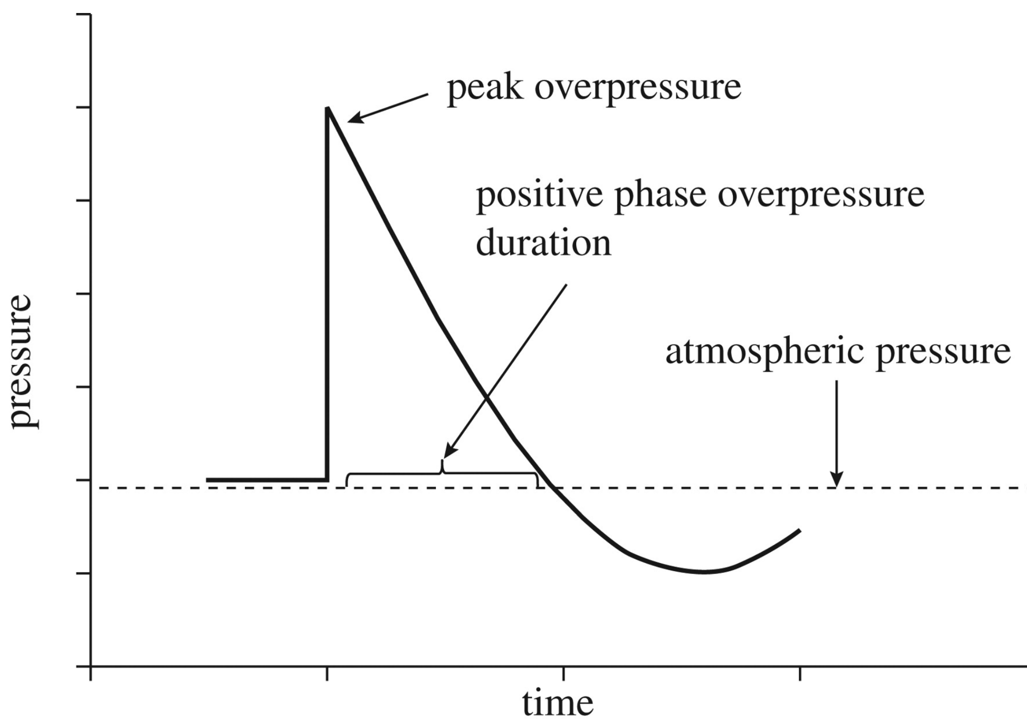

Figure 5-2. Schematic representation of a shock of pressure magnitude *vs* time showing the Friedlander Waveform pressure changes in a undisturbed, free field environment.

response to blast that shows a biphasic response. There is an immediate tachycardia, followed by bradycardia and hypotension (Ohnishi et al., 2001; Irwin et al., 1997) which is presumed to be a vagal response (Sawdon et al., 2002). The bradycardia typically returns to normal after about 15 minutes, although the hypotension may persist. These cardiovascular events are coupled with immediate and delayed respiratory effects.

The immediate respiratory response is apnoea lasting from a few seconds to about a minute, followed by rapid, shallow breathing (Guy et al., 1998). The shock wave causes immediate lung injury with rupture of alveolar capillaries leading to intrapulmonary haemorrhage and oedema (Damon et al., 1971). This will lead to V/Q mismatch and hypoxaemia. This is similar to pulmonary contusion from blunt chest trauma (Gorbunov et al., 1997; Brown et al., 1993). The presence of blood and haemoglobin in the alveoli leads to free radical production and the inflammatory response. There is leucocyte accumulation (within 3 hours) followed by epithelial cell damage at 12- 24 hours. Leading to a picture similar to the adult respiratory distress syndrome (ARDS) (Gorbunov et al., 1997)

5.2.3 Blast and Haemorrhage

Animal studies have demonstrated that blast coupled with haemorrhage (as seen in military casualties) causes a profound, and prolonged hypotension (Sawdon et al., 2002) that coupled with hypoxaemia will result in poor tissue oxygen delivery (Garner et al., 2010).

Rats were exposed to blast or sham blast and underwent a controlled 40% blood volume haemorrhage. There was initial tachycardia followed by bradycardia in the sham blast group However the blast group did not exhibit the initial tachycardia and were immediately bradycardic with no compensatory maintenance of blood pressure. A likely explanation was that the response to blast augmented the depressor response to severe blood loss mediated by the vagal reflexes (Sawdon et al., 2002).

5.2.4 Blast and Haemostasis

Early after blast there appears to be a hypercoagulable state (Granville-Chapman et al., 2010). The cause of this is unknown, but it is postulated that it is due to blast related tissue damage. There is no published evidence on the relationship between blast injury and tissue factor levels. Since blast causes endothelial disruption in the colon and lung, it is likely that there is increased amount of exposure of blood to tissue factor, thereby causing significant activation of coagulation pathways. Coupled with poor tissue oxygen delivery experienced after blast and haemorrhage, military casualties are at high risk for developing ATC.

5.3 Summary

In summary, blast interacts with the body either as a pressure wave disrupting air/ fluid interfaces, or causes trauma via the effects of the body being thrown against objects, or objects being thrown against the body. The effects of blast on the cardiovascular and coagulation systems are well understood and complicate the normal response to trauma. This is discussed in more detail in chapter 6.

Chapter 6. Cardiovascular response to simple haemorrhage

6.1 Biphasic response to haemorrhage

The loss of blood volume from the circulation leads to a development of hypovolaemia and shock. Shock is defined as the body’s inability to adequately perfuse the tissues and meet the oxygen demand.

During the extensive blood donor programme of the second world war, it had been noted that a small percentage of blood donors lost consciousness related to the amount of blood donated (Barcroft and Edholm, 1945). Barcroft et al in 1944 investigated the physiological response to this simple haemorrhage by venesecting volunteers and noting heart rate, blood pressure, cardiac output, right atrial pressure and forearm blood flow.

Barcroft demonstrated a biphasic response after haemorrhage Figure 6-1. Initially there was development of a tachycardia with a compensatory rise in systemic vascular resistance maintaining blood pressure despite a fall in cardiac output. Once 20 – 30 % of blood volume was lost, there was a bradycardia coupled with a loss in systemic vascular resistance and a loss in blood pressure, this coincided with a profound increase in forearm blood flow. This loss of blood pressure was called the decompensation phase.

The biphasic response is mediated by two reflexes.

6.1.1 The arterial baroreceptor reflex.

A small haemorrhage results in unloading of arterial baroreceptors in the aortic arch and carotid sinuses. This causes a reflex reduction in cardiac vagal activity and an increase in sympathetic activity of the heart. At the same time there is an increase in sympathetic vasomotor tone causing an increase in systemic vascular resistance. This change in vascular resistance is not uniform

throughout the body and appears to follow a hierarchy. This will maintain cardiac output to tissues critically dependant on cardiac output (Barcroft and Edholm, 1945; Foex, 1999).

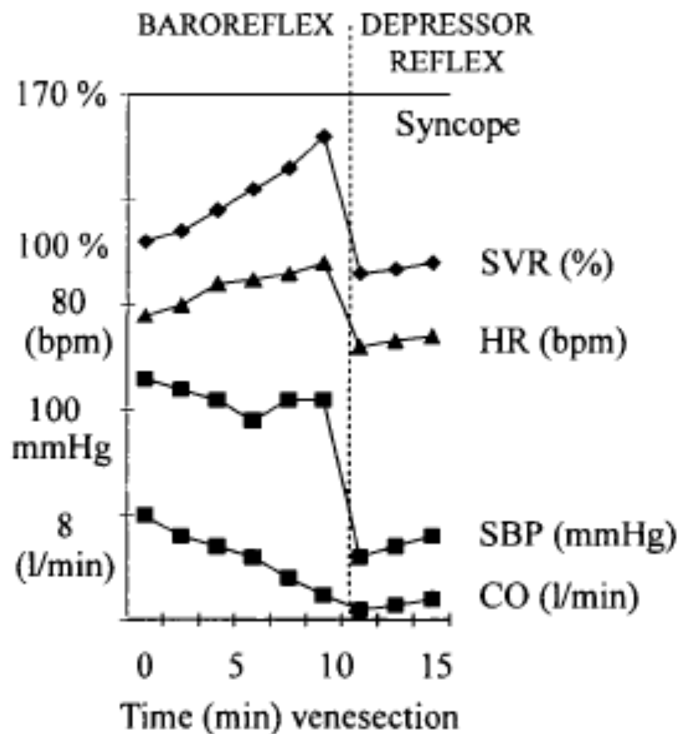

Figure 6-1 Graph showing the biphasic response to haemorrhage with the initial increase in HR, SVR, and maintenance of SBP but drop in CO. Followed by the depressor phase. Adopted from Barcroft et al., 1944

6.1.2 *Depressor reflex*

Once blood loss exceeds a critical volume (around 20% of circulating volume) the “depressor reflex” seems to override the baroreceptor reflex (Foex, 1999). An increase in vagal tone leads to bradycardia, increased diastolic time, with an increase in cardiac filling and coronary blood flow (Little et al., 1989). There is a concurrent inhibition of sympathetic vasomotor tone leading to vasodilatation.

Barcroft found this depressor phase to be reversible by reinfusion of donated blood. However a third, pre terminal, phase has since been described when over 40% blood volume has been lost, consisting of increased sympathetic drive with tachycardia and hypotension.

Other reflexes also occur during haemorrhagic shock. Stagnant blood flow, hypoxia and acidosis stimulate arterial chemoreceptors leading to rapid, deep breathing with increased vasoconstriction potentiating the baroreceptor reflex (Acker and O'Regan, 1981).

6.2 Cardiovascular response to tissue injury and haemorrhage

Haemorrhage associated with trauma is complicated by soft tissue injury, nociception and pain. Isolated tissue injury causes a pressor response with increased blood pressure, tachycardia and increased systemic vascular resistance (Redfern et al., 1984). It was noted that when a minor haemorrhage is coupled with tissue injury, mortality is increased (Overman and Wang, 1947). It is postulated that the sympathetic “fight or flight “ response to pain, overrides the depressor reflex to simple haemorrhage. This occurs at the expense of perfusing the vital organs.

6.3 The inflammatory response to injury

The inflammatory response to injury is caused by tissue hypoperfusion leading to ischaemia and a subsequent reperfusion injury during resuscitation. This effect is mediated by two components, the humoral and the cellular response.

6.3.1 Humoral response to injury

In response to injury both up and down regulation of many mediators occurs, including cytokines, complement, free radicles and nitric oxide. A loss in the balance between pro and anti inflammatory cytokines leads to the systemic inflammatory response syndrome. Activation of complement will cause chemotaxis of leucocytes, degranulation of phagocytic cells and an increase in vascular permeability. These levels are related to the severity of injury and related to mortality (Tsukamoto et al., 2010).

6.3.2 Cellular response to trauma

Neutrophils, leucocytes, monocytes and macrophages all become activated after trauma (Pillay et al., 2007). Upregulation of adhesion molecules causes increased permeability and dysfunction of endothelial cells. Activated polymorphonuclear neutrophils further release proinflammatory cytokines potentiating the humoral response (Tsukamoto et al., 2010).

6.3.3 Ischaemia/reperfusion

Ischaemia sees a switch from aerobic to anaerobic respiration in the cells, which results in increased permeability of cell membranes and cell swelling. If reperfusion occurs, oxygen is reintroduced into the cells creating superoxides and hydroxyl free radicles. This can in turn lead to cellular necrosis and apoptosis (Nathan and Singer, 2000). Cellular swelling in the endothelium results in narrowing of capillary lumens, further reducing blood flow, and loss of intercellular adhesion leading to “leaky” capillaries. This results in loss of plasma and proteins, compounding the hypovolaemia and worsening tissue oxygenation (Tsukamoto et al., 2010).

6.4 Summary

In summary, the normal response to simple haemorrhage is the maintenance of arterial blood pressure via increased systemic vascular resistance and tachycardia. As haemorrhage continues this is followed by a second phase of bradycardia and hypotension, in an effort to preserve blood flow to the vital organs. This second phase may be cardio-protective. If haemorrhage is complicated by trauma, the second phase is overridden by nociceptive responses, which in turn leads to increased mortality.

The inflammatory response to trauma is characterised by an increase in pro-inflammatory processes, which is augmented by an ischaemia reperfusion type injury once resuscitation has started.

Chapter 7. Recombinant factor VIIa

7.1 Mechanisms of action of rFVIIa

Factor VIIa is one of the coagulation factors produced in the liver. It is crucial in the activation of coagulation once it comes into contact with tissue factor (TF). Factor VII is the only factor present in its active form in the normal circulation and the active form represents approximately 1% of the total factor VII. Once it binds to TF it promotes clot initiation at the site of injury.

Recombinant factor VIIa (rFVIIa) was manufactured in the 1980's by Novo Nordisk (Maaloev, Denmark) for the treatment of haemophilia patients with inhibitors of FVIII and FIX. These factors are important in the production of thrombin once coagulation has been activated by the exposure of TF to FVIIa. Haemophilia patients either lack FVIII (haemophilia A) or FIX (haemophilia B) and as such are at risk of life threatening haemorrhage from relatively minor injuries, and are a significant surgical risk. Some Haemophiliacs develop antibodies to FVIII and FIX and so it is not possible to simply treat them with specific factor concentrates.

Administration of rFVIIa will increase the concentration of FVIIa approximately 100 fold (Hauser et al., 2010; Pusateri and Park, 2005), promoting thrombin generation at the site of injury. There appears to be two mechanisms by which rFVIIa exerts its effect. The first is TF-independent and the second TF-dependent (Meng et al., 2003; Pusateri and Park, 2005; Hoffman, 2003; Hedner, 2006).

The TF dependent mechanism occurs in a physiologically normal way, rFVIIa binds to TF at the site of injury causing clot initiation. Under the TF independent mechanism, rFVIIa weakly binds to the activated platelet activating FX to FXa. Although the efficiency of this reaction is low, the concentrations of rFVIIa offset this, and FXa production thus becomes independent of the presence of TF

(Pusateri and Park, 2005; Hoffman, 2003). Both mechanisms cause an increase in the production of FXa and FVa on the surface of activated platelets, thereby converting prothrombin to thrombin. After administration of rFVIIa, the rate of thrombin production is greater resulting in thinner, more tightly packed fibrin strands that are more resistant to fibrinolysis (Dutton et al., 2004; He et al., 2003).

7.2 Clinical use of rFVIIa

rFVIIa license for use is restricted to: congenital haemophilia with inhibitors, acquired haemophilia, Glanzmann's thrombasthenia and congenital FVII deficiency. However in one recent study of 12,644 hospitalisations where rFVIIa was used, 97% were for reasons outside these licensed (off label) indications (Martinowitz et al., 2005; Logan et al., 2011). rFVIIa has therefore been used in a wide range of clinical conditions in an attempt to control bleeding including cardiac and thoracic surgery, post partum haemorrhage and spontaneous intracerebral bleeding.

Lin et all (2011) performed a meta analysis looking at 26 trials where there had been off label use of rFVIIa. There were 14 off label prophylactic trials and 12 off label treatment trials. There was no significant effect on mortality in either group, and a non significant increase in thromboembolic events (Petersen et al., 1999; Lin et al., 2011). Studies had varied dosing regimes (5µg/kg – 360 µg/kg prophylactic group, and 5µg/kg to 1120 µg/Kg therapeutic group). None of the groups included military trauma, and none of them looked at the effect of rFVIIa on blast lung. Four of the studies however (Pusateri and Park, 2005; Rizoli et al., 2006; Boffard et al., 2005) do demonstrate a reduction in ARDS in rFVIIa treated groups, especially after blunt trauma.

The conclusion from this meta analysis was:
"Clinically significant benefits of recombinant factor VIIa as a general haemostatic agent in patients without haemophilia remain unproven. Given its potential risks, such use cannot be recommended, and in most cases, it should be restricted to clinical trials (Pusateri and Park, 2005; Lin et al., 2011).

7.2.1 rFVIIa in trauma

The use of rFVIIa in trauma was first documented in 1999 in a case report of an Israeli solider with uncontrolled haemorrhage from a gunshot wound to the inferior vena cava (Dutton et al., 2004; Kenet et al., 1999). Since then its (off label) use in trauma has become widespread and there have been a some other reports favouring its use in trauma (Young et al., 2013; Dutton et al., 2004; O'Neill et al., 2002; Martinowitz et al., 2001). It would appear to offer a potential increase in clotting ability at the site of injury.

Subsequently Boffard et al (2005) studied patients with penetrating and blunt trauma with administration of rFVIIa or placebo at 1 and 3 hours after transfusion of 8 units of blood (Sørensen et al., 2010; Boffard et al., 2005). There was a significant reduction in blood transfused in the rFVIIa group for blunt trauma, and a non-significant trend in penetrating trauma. There was no difference in hospital length of stay, ICU days, ventilated days or mortality and no difference in the rate of thromboembolic events.

The use of rFVIIa has also been looked at in traumatic intracerebral haemorrhage. Extradural and subdural haemorrhage were excluded due to the surgical treatment required. Patients either received placebo or increasing doses of rFVIIa (40μg/kg to 200 μg/kg). There was no difference in survival, but there was a trend towards reduction in expansion of haematoma, and a trend towards thromboembolic events (Sørensen et al., 2003; Narayan et al., 2008).

In military trauma the only evidence available comes from retrospective analyses (Ganter et al., 2008b; Spinella et al., 2008). Spinella et al looked at those patients in a combat support hospital who had received massive transfusion (>10 units of blood in 24 hours). The rFVIIa group had a lower mortality at 24 hours and 30 days with no increase in thromboembolic events. (Wade et al., 2010) looked at patients between 2003 and 2009 who had received a transfusion and had rFVIIa. There was no difference in survival or adverse events.

7.2.2 CONTROL trial

Following these inconclusive trials, a large multicentre trial (CONTROL) was conducted to look at the role of rFVIIa in traumatic haemorrhage (Pusateri and Park, 2005; Hauser et al., 2010). The aims were to collect 1502 patients with blunt or penetrating trauma with continued bleeding after 4 units of red cell transfusion. Patients received either rFVIIa (200µg/Kg followed by 100µg/Kg at 1 hour and 3 hours) or placebo. The study was powered for a mortality of 27.5% in the placebo group. The study was terminated early after interim analysis due to a lower than expected mortality of 10.8% and thus the study was underpowered. In the patients that were recruited, there was no difference in mortality and no statistically different number of thromboembolic events.

7.3 rFVIIa and physiological abnormalities

Acidosis affects enzymatic activity and platelet function (see section 3.2.3), but it also affects the activity of rFVIIa. A pH of 7.0 will reduce rFVIIa TF independent activity by 90% and TF dependent activity by 60% (Pusateri and Park, 2005; Meng et al., 2003; Hoffman, 2003; Hedner, 2006). However the large pharmacological dose of rFVIIa (a 100 fold increase) may still have enough activity to exert a significant effect (Hoffman, 2003; Pusateri and Park, 2005). Dutton et al. (Dutton et al., 2004) and Martinowitz et al. (Martinowitz et al., 2005) have both suggested that a pH of 7.2 decreases rFVIIa effect. However in Dutton’s study there was an improvement of PT in both responders and non-responders (78/78 patients) in spite of the non-responding group having a pH of 7.02 compared to the responding group having a pH of 7.29. However 6 of the non-responders had a pH of greater than 7.1 and 5 responders had a pH< 7.1. Martinowitz showed a decline in efficacy below 7.2, which only reached significance at a pH of 7.0. In summary the effect of pH on the action of pharmacological doses of rFVIIa remains unclear but the drug is likely to have a reduced efficacy below pH 7.0 and possibly even below a pH of 7.2.

Temperature also affects rFVIIa activity. TF-dependent activity drops by 20% at 33 °C, although interestingly the TF-independent pathway increases as

temperature drops. Previous work by Petersen et al. (Petersen et al., 1999) suggest that FVIIa is only stable in conjunction with its cofactor (TF) and therefore increasing temperature in the absence of TF decreases its stability. It seems therefore that hypothermia has minimal effect on the activity of rFVIIa.

The effect of haemodilution is unclear. Some studies with evidence of haemodilution and or consumption have demonstrated efficacy of rFVIIa, whereas others have suggested that coagulation factors should be at or near normal levels (Rizoli et al., 2006; Pusateri and Park, 2005; Boffard et al., 2005). Most guidelines for the use of rFVIIa suggest that there should be a minimum platelet and fibrinogen level prior to administration.

7.4 Animal studies

Animal studies, conducted mainly on pigs, have produced conflicting evidence. This is in part due to the differing models, end points and doses used. Most studies have looked at the effect of rFVIIa on survival, blood loss or changes in clotting parameters. Only one study looked at the use of rFVIIa with a trauma model (Wade et al., 2010; Howes et al., 2007). Doses ranged from 90µg/kg (McMullin et al., 2008) to 720 µg/Kg (Klemcke et al., 2005; Jeroukhimov et al., 2002), although most studies either use, or include doses of 180µg/Kg. Some studies used blood that developed coagulopathy (Sapsford et al., 2007) or had coagulopathy induced (Klemcke et al., 2005; Schreiber et al., 2002; Lesperance et al., 2012).

The results of these studies are also variable, with some seeing an effect, and some not. In general there was a reduction in bleeding volume from the rFVIIa group, and improvement in clotting indices In one study, rFVIIa was shown to improve survival time in anaesthetised pigs suffering haemorrhage and hypotensive resuscitation (Sapsford et al., 2007). All animals received a 40% blood volume haemorrhage, and infra renal aortotomy. The pigs received either normotensive or hypotensive resuscitation with either rFVIIa or placebo. There was no difference in survival between rFVIIa and placebo in the normotensive group, however there was a significant increase in survival (214 [79-349] vs 35 [19-52] minutes) between rFVIIa and placebo in the hypotensive group. The

conclusions from this study, were that rFVIIa combined with hypotension in a model of arterial haemorrhage improved survival to a clinically relevant time for military trauma.

7.5 rFVIIa in Lung haemorrhage

A case of rFVIIa (two doses of 60µg/Kg) has been reported in a case of a patient with severe traumatic intra thoracic haemorrhage where it appeared to control the bleeding (Kamphuisen et al., 2002). There have also been some reports of its use in the treatment if diffuse intra alveolar haemorrhage (DAH).

DAH is caused by pulmonary microvascular bleeding and is life threatening. It's known causes include connective tissue disorders, cardiac disease, drugs, complications of bone marrow transplantation, pulmonary metastasis and anatomical abnormalities. Treatment is of the underlying cause and reversal of coagulopathies with supportive therapy as required. Several cases of rFVIIa use following failure of conventional therapy have been reported. The doses used vary between 90µg/Kg and 180µg/Kg either in single or multiple intravenous doses (Hicks et al., 2002; Henke et al., 2004).

7.6 rFVIIa use in Blast lung

Blast lung is thought to be as a consequence of intra alveolar haemorrhage similar to DAH. There are no published trials on the use of rFVIIa to treat blast lung, however with increasing numbers of survivors of blast injured patients, the prevalence of blast lung on military intensive care units is increasing. Novel therapies, such as rFVIIa to treat blast lung, require examination.

7.7 Summary

In summary the off label use for rFVIIa has increased dramatically over recent years in an attempt to "boost" coagulation after haemorrhagic shock in trauma patients. There is no strong evidence that the use of rFVIIa leads to a decrease in mortality, and some evidence that its use leads to an increase in

thromboembolic events. However comparison of the studies is limited by the variable doses used, variable end points and, in animal studies, variable models used.

Across many of the studies there appears to be a boost in coagulation after rFVIIa use manifest by a reduction in blood products used and reduction in haematoma sizes. There also is some speculative evidence that rFVIIa use may be of benefit in blast lung.

There is some doubt as to the sensitivity of standard tests of coagulation (PT/aPTT, ROTEM® and TEG®) at monitoring the effects of administered rFVIIa. There is, however, renewed interest in modifying these tests and using dilute tissue factor as an initiator, which appears to provide a more sensitive and realistic assessment of the effects of rFVIIa.

Chapter 8. Aims

8.1 The aims of the Research

1. A human study of a clinical assessment of ROTEM® to assess coagulation status in severely injured military casualties.
2. An *in vivo* animal based study investigating the impact of simulated pre hospital administration of O_2 and rFVIIa on coagulation status in a model of battlefield injury,
3. A laboratory based *in vitro* study to examine the effects of a second dose of rFVIIa on coagulation status rFVIIa compared to buffer.

- To address the first aim a clinical study was performed on severely injured military casualties in Camp Bastion, Afghanistan. The severity and nature of injury may have an effect on coagulopathy. Military trauma is characterised by severely injured casualties who have suffered high velocity penetrating and blast injures. This necessitated the study to be performed in Afghanistan since UK civilian trauma is characterised by low velocity penetrating injuries and blunt trauma.

- The second aim required an animal based study since it would be impossible to conduct a randomised controlled trial to administer, for example, rFVIIa, in a non permissive, military prehospital environment. To achieve this aim I utilised a model of complex battlefield injury, in terminally anaesthetised animals that had been developed, and was being used as part of the on-going work by the combat casualty care team at DSTL, Porton Down

- The third aim was achieved by performing an *in vitro* laboratory based study where blood samples from various time points, taken from the animal study, had either rFVIIa or placebo added to them. The effects of this dose of rFVIIa on coagulation was assessed using TEG®

Chapter 9. Origin of the model used as the basis of the animal work

9.1 Hypotensive resuscitation

A decade ago civilian practise in trauma was based around hypotensive resuscitation in the belief that hypotension allowed better clot formation, and that normotension would disrupt any formed clot, leading to further haemorrhage (Bickell et al., 1994). The Defence Medical Services wanted to determine if this practise was applicable to military trauma.

A large animal model was developed at DSTL, Porton Down (Garner et al., 2009) to investigate the impact of blast injury on the effectiveness of resuscitation after haemorrhagic shock. The study showed that hypotensive resuscitation was associated with poor tissue oxygen delivery, which led to the development of a significant shock state and metabolic acidosis. While survival was good during the first hour of resuscitation, the degree of shock became overwhelming thereafter, when hypovolaemia was combined with blast injury. The problem seemed to be due to reduced tissue blood flow associated with hypotensive resuscitation, especially when combined with blast injury since the improved oxygen transport associated with normotensive resuscitation revearsed the shock and improved survival (Garner et al., 2010). However it should be noted that the study was designed as a physiological investigation of potential problems associated with hypotensive resuscitation and the model, by design, had no element which would lead to re-bleeding during resuscitation (Garner et al., 2010).

9.2 Novel Hybrid resuscitation

A subsequent study looked at the effects of hypotensive resuscitation for the first hour, with a return to normotension by the infusion of 0.9% saline after 60 minutes (novel hybrid resuscitation) (Kirkman and Watts, 2011). The animals were subject to blast or sham blast, a 30% blood volume controlled

haemorrhage followed by a grade 4 liver injury, which was left uncompressed, in order to allow the possibility of a rebleed once the blood pressure was raised. This study demonstrated a significant improvement in survival, reversal of base deficit, reduction in oxygen extraction with no evidence of re-bleeding in the novel hybrid group (Kirkman and Watts, 2011) and an improvement in coagulation associated with the improved tissue perfusion during novel hybrid resuscitation (Doran, 2012).

9.3 Intravenous dose of rFVIIa

There is no consensus regarding the appropriate dose of rFVIIa in trauma or in lung injury. Most trials used a dose between 60 – 120 μg/Kg. Pigs have reduced sensitivity to human rFVIIa, and a dose of 180 μg/kg has been adopted by several investigators as an equivalent clinical dose (Lynn et al., 2002; Sapsford et al., 2007; Schreiber et al., 2002). This dose has been shown to improve survival in pigs with incompressible haemorrhage (aortotomy).

9.4 Prehospital supplemental oxygen and rVIIa for haemorrhagic shock and blast injury

The study which forms the experimental work for this thesis examined changes in clotting status associated with treatment using rFVIIa and oxygen following blast injury, haemorrhagic shock and hypotensive resuscitation.

A strategy was required to target patients at high risk of rebleeding after blast injury where a novel hybrid strategy might be potentially harmful (i.e. injury type had a significant risk of rebleeding). The strategy needed to address shock by increasing oxygen delivery without increasing blood pressure. This can be achieved by administering supplemental oxygen, or by increasing the efficiency of gas exchange in the blast damaged alveolus. rFVIIa was given intravenously in an attempt to decrease alveolar haemorrhage and thereby increasing pulmonary gas exchange.

This model incorporated blast injured pigs who suffered a controlled haemorrhage, a grade 4 liver injury and hypotensive resuscitation. After 30

minutes, simulating the arrival of specialist medical help on the battlefield, the animals were divided into groups that either remained breathing air (control group), were given supplemental oxygen to target SaO_2 95% or given intravenous rFVIIa.

Chapter 10. Utility of interim ROTEM® values of clot strength, A5 and A10, in predicting final assessment of coagulation status in severely injured battle patients.

10.1 Introduction

The effective treatment of trauma induced coagulopathy with massive haemorrhage protocols requires rapid assessment of patient's dynamically changing coagulation status and their response to treatment. As discussed in section 4.2.4, conventional tests (PT, aPTT) take too long to provide a meaningful result, rendering the results historical and of little value in guiding resuscitation.

Viscoelastic methods potentially provide more rapid feedback and could therefore be more useful in determining contemporary coagulation status of the patient. Key parameters include measures of clot initiation time, the dynamics of the clotting process and clot strength. Unfortunately even in viscoelastic methods, it can take up to 45 mins for a full picture to emerge in a coagulopathic patient, although interim values are reported sooner. The purpose of the study presented in this thesis was to evaluate the utility of these interim values in predicting whether the ROTEM® test would indicate coagulopathy when run to completion.

10.2 Aims

The aim of this study is to determine whether measures of clot strength determined by ROTEM® at 5 and 10 min after initiation of clotting (defined by ROTEM® as A5 and A10) provide a reliable indication of coagulopathy when the ROTEM® EXTEM test is run to completion on blood derived from severely injured battlefield casualties.

10.3 Methods

In accordance with standard laboratory practise (TEM, 2010), normal range data was derived from venous samples collected, with informed consent, from a population of 50 healthy volunteers undergoing routine assessment as part of the Emergency Donor Panel (EDP) at the UK DMS deployed Role 3 Field Hospital at Camp Bastion (R3 Bastion), Afghanistan. For the patient population, venous blood samples were collected prospectively, as part of their routine clinical evaluation, on battle patients presenting to R3 Bastion during the study period (21 May 09 – 3 Jul 09). This amounted to 48 seriously injured patients (48:0 male:female, 24[21-26] yr mean age[IQR]). The predominant mechanism of injury involved Improvised Explosive Devices (IED's), with a number of casualties suffering traumatic amputations (48%). The dataset also contains some patients with ballistic injuries (29%) and a few with burns and injuries resulting from road traffic accidents (4% in each). All patients presented in this report were classified as seriously injured patients and had been triaged as T1 or T2. T1 and T2 casualties are those that have immediate or urgent clinical problems requiring full trauma team activation. ROTEM® values from the injured patients were compared to the reference range derived from the uninjured service personnel at Bastion.

Blood samples were collected by assigned nurses or doctors as part of their defined role in the trauma team. Samples were collected from either 16 G peripheral cannulae or 8.5 Fr central venous line (depending on the first vascular access gained). Samples for ROTEM® were analysed within 5 mins by designated operating room staff. Blood samples sent to the laboratory were immediately placed on a standard laboratory tube roller until analysis was performed.

ROTEM® analysis was performed according to the manufacturers instructions for EXTEM and FIBTEM. The following parameters are routinely reported: Clotting time (CT) representing clot initiation, Clot Formation Time (CFT) and angle representing clot dynamics and Maximum Clot Firmness (MCF) representing ultimate clot strength. Amplitude at 5 mins (A5) and 10 mins (A10)

are interim values of clot strength and relate to the amplitude at 5 and 10 mins after CT.

10.3.1 Definition of coagulopathy as determined by ROTEM®.

There are no formal definitions of coagulopathy as determined by viscoelastic methods, including ROTEM® (see section 3.5.1), and no published peer reviewed papers defining actual parameters to establish the presence or absence of coagulopathy. In 2008 the ROTEM® Expert Working Group Meeting (Expert meeting working group, 2008) recommended that an EXTEM MCF of 35 to 45 mm *may* require treatment depending on the clinical condition of the patient. Experimental dilution of blood to model coagulopathies (60% dilution) (Weiss et al., 2010) , and PT of 1.7 times normal (Bolliger et al., 2010) have corresponding EXTEM MCF of 40mm and 39mm. Davenport et al (Davenport et al., 2011) studied 300 patients admitted to the Royal London Hospital and found that an A5 of <36mm identified ATC with a detection rate of 77% and a false positive rate of 13%. We have used a figure of EXTEM MCF <40mm as one definition of hypocoagulation as this represents a risk of bleeding and may require treatment to prevent further deterioration to a clinical coagulopathy.

Using a single parameter such as MCF to diagnose coagulopathy is potentially too simplistic a tool and of no real value unless it is grossly abnormal. It is unlikely that a result 1 or 2 mm outside normal is a clinical problem, unless that subtle abnormality is present in more than one clotting domain. In this case the patient may be at increased risk of a clinical bleeding problem and as such could be assumed to be at increased risk of coagulopathy. As such a subtle abnormality in 2 out of 3 domains should alert the clinician to the possibility that there is a clinical problem, and in the context of the specific circumstances of the patient may represent coagulopathy. We have adopted an alternative definition of coagulopathy; the presence of abnormal values in two of the three domains (initiation (CT), dynamics (CFT or angle) and strength (MCF)) termed the 2/3 rule.

Therefore, in this study two alternative definitions of coagulopathy were used, the first based on the 2/3 rule (using the locally-derived normal values, Table 1)

and the second as an MCF of<40mm. The sensitivity and specificity of an abnormal A5 and A10 in predicting coagulopathy was determined for each definition.

10.3.2 Conventional assessment of clotting using prothrombin time.

Venous blood samples were collected into citrated Vacutainer (9NC 0.105M Vacutainer 367691, Beckton Dickinson, UK), and sent for standard laboratory testing in the hospital laboratory. A coagulopathy based on PT is defined by the British Committee for Standards in Haematology and the American College of Pathologists as a PT greater than 1.5 times normal values (Lang et al., 2005), corresponding to PT > 18s.

10.3.3 Statistical analysis

Data was separated into 'normal' (control) subjects (members of the Emergency Blood Donor Panel) and injured patients. The distribution for each parameter was examined in each group and normalised if necessary by transformation (e.g. log transformation). Summary values for data with a normal distribution (parametric data) are presented as means and 95% confidence intervals while non-parametric data are summarised with medians and inter-quartile ranges.

For control subjects untransformed data were used to produce the normal ranges except for A5 and A10, which were derived from log-transformed data. Parametric tolerance limits were calculated for each parameter (Equation 10.1) This approach allows us to be 95% confident that 99% of the uninjured acclimatised control population are to be found within these limits.

$$\textbf{Tolerance limits} = \textbf{mean} \pm \textbf{KS}$$

Equation 10.1 Calculation of tolerance limits for control (uninjured) acclimatised personnel at R3 Bastion (members of the Emergency Blood Donor Panel). S, standard deviation; K, reliability coefficient taken from tables or calculated from the confidence coefficient, gamma.

The proportion of patient samples falling outside the control range was calculated for each parameter.

A comparison of the statistical distribution of data was made between normal controls and injured patients by informally comparing distribution dot-plots and by formal assessment of normal probability plots.

10.4 Results

10.4.1 Reference ranges based on samples from uninjured volunteers

The reference range derived from 50 uninjured control subjects at R3 Bastion (members of the Emergency Blood Donor Panel) and the published reference ranges based on non-coagulopathic patients and staff at northern European hospitals are shown in Table 10-1, Bastion ranges were not significantly different to TEM's® published ranges (TEM, 2010).

Subjects	CT (s)	CFT (s)	α Angle (°)	A5 (mm)	A10 (mm)	MCF (mm)	CLI30 (%)
EBDP (R3Bastion)	25-90	43-173	58-81	32-71	40-72	44-74	40-102*
Pentapharm	38-79	34-159	63-83		43-65	50-72	94-100

Table 10-1 Reference range EXTEM data from uninjured, non-coagulopathic, subjects (members of the Emergency Blood Donor Panel undergoing routine assessment at R3 Bastion, (EBDP) and published normal range data presented by TEM® based on Lang et al 2005.

10.4.2 Early prediction of coagulopathy based on interim ROTEM® parameters

The age, admission pulse and blood pressure, blood count and clotting indices together with injury severity and blood product use are shown in Table 10-2,108 samples (which included multiple assessments) from 48 trauma patients were used to assess the utility of A5 and A10 to predict hypo-coagulation (attenuated clotting). 58% of these samples were found to be hypo-coagulable based on the 2/3 rule and the remainder were normal (none were hyper-coagulable, i.e.

enhanced clotting, with respect to any parameter). A5 and A10 below their respective reference ranges predicted hypo-coagulation (as defined by the 2/3 rule) in individual samples with respective sensitivities[95%CI] / specificities[95% CI] of 0.97[0.93-1.00] / 0.68[0.54-0.82] (A5) and 0.98[0.95-1.00] / 0.80[0.68-0.92] (A10).

	Mean [95% CI]	Median [IQR]	Range
MBP (mmHg)	92[83-100]		
Heart rate (b/min)	111[102-119]		
Initial [Hb] (g/dl)	10.8[9.8-11.8]		
Plt (10^9/L)		142[107-213]	48-459
Fib (g/dl)		2.9[2.3-3.7]	0.9-5.9
pH		7.30[7.22-7.37]	6.90-7.43
BE (mM)		-4[-8 - -2]	-22 - 2
Age (y)		24[21-26]	18-50
NISS		34[17-43]	5-75
PRBC (units)		10[6-17]	0-39
FFP (units)		10[4-14]	0-90
PLT (units)		0[0-2]	0-10
Cryo (units)		0[0-1]	0-6
Total Blood products (units)		21[11-35]	0-101

Table 10-2 showing baseline blood pressure, heart rate, age, blood results, injury severity and blood product use during initial surgery.

51% of samples were found to be hypocoagulable according to the MCF < 40mm rule. In this case A5 and A10 (below their respective reference ranges) predicted hypocoagulation in individual samples with sensitivities / specificities of 0.96[0.91-1.00] / 0.58[0.45-0.71] (A5) and 1.00 / 0.70[0.58-0.82] (A10) respectively.

10.4.3 Incidence of hypo-coagulation in admission samples at R3 Bastion

Blood samples from 31 of the severely injured patients were taken on arrival at R3 Bastion or soon as possible (within approximately 30 min) thereafter. The proportion of patients showing abnormal EXTEM parameters indicative of hypo-coagulation on admission to R3 Bastion is shown in Table 10-3. Approximately

the same proportion of patients had abnormalities in all 3 domains of clotting (initiation, dynamics and strength). No patient showed abnormalities indicative of hyper-coagulation. Prior to the first sample being taken for ROTEM® analysis 19 patients had received no or minimal blood products, the remainder had preceding blood products. 39% (21%-59%, 95%CI) of patients were found to be coagulopathic as defined by the 2/3 rule, and 40% (23%- 59%, 95% CI) as defined by MCF < 40mm rule on admission.

Reference Range	CT	CFT	α Angle	MCF
EBDP (R3 Bastion)	0.29	0.33	0.31	0.43
Pentapharm[+]	0.39	0.41	0.45	0.63

Table 10-3 Proportion of 31 severely injured patients showing abnormal values on EXTEM compared to the EBDP and Pentapharm reference ranges on presentation at R3 Bastion.

10.4.4 Comparison of ROTEM® and prothrombin time (PT)

Of the EXTEM ROTEM® samples analysed in the entire dataset, 40 samples from 30 patients were also subjected to conventional laboratory testing. There was agreement between conventional testing and ROTEM® for only 24/40 (60%) of these samples (5/24 coagulopathic and 19/24 normal clotting samples). Of the remaining 16 samples 8 were abnormal by ROTEM® only and the remaining 8 abnormal by conventional testing only. Conventional and ROTEM® assessment therefore does not appear to provide good agreement on coagulopathic samples.

10.4.5 Statistical comparison of ROTEM® parameter distributions between volunteer and admission samples from trauma patients

A detailed comparison of the distribution of EXTEM data from patient and uninjured volunteer groups revealed differences between various ROTEM® parameters. Parameters relating to clot initiation (CT) and dynamics (CFT and Angle) showed similar distributions between patient and control groups, with a significant number of outliers in the patient groups Figure 10-1.

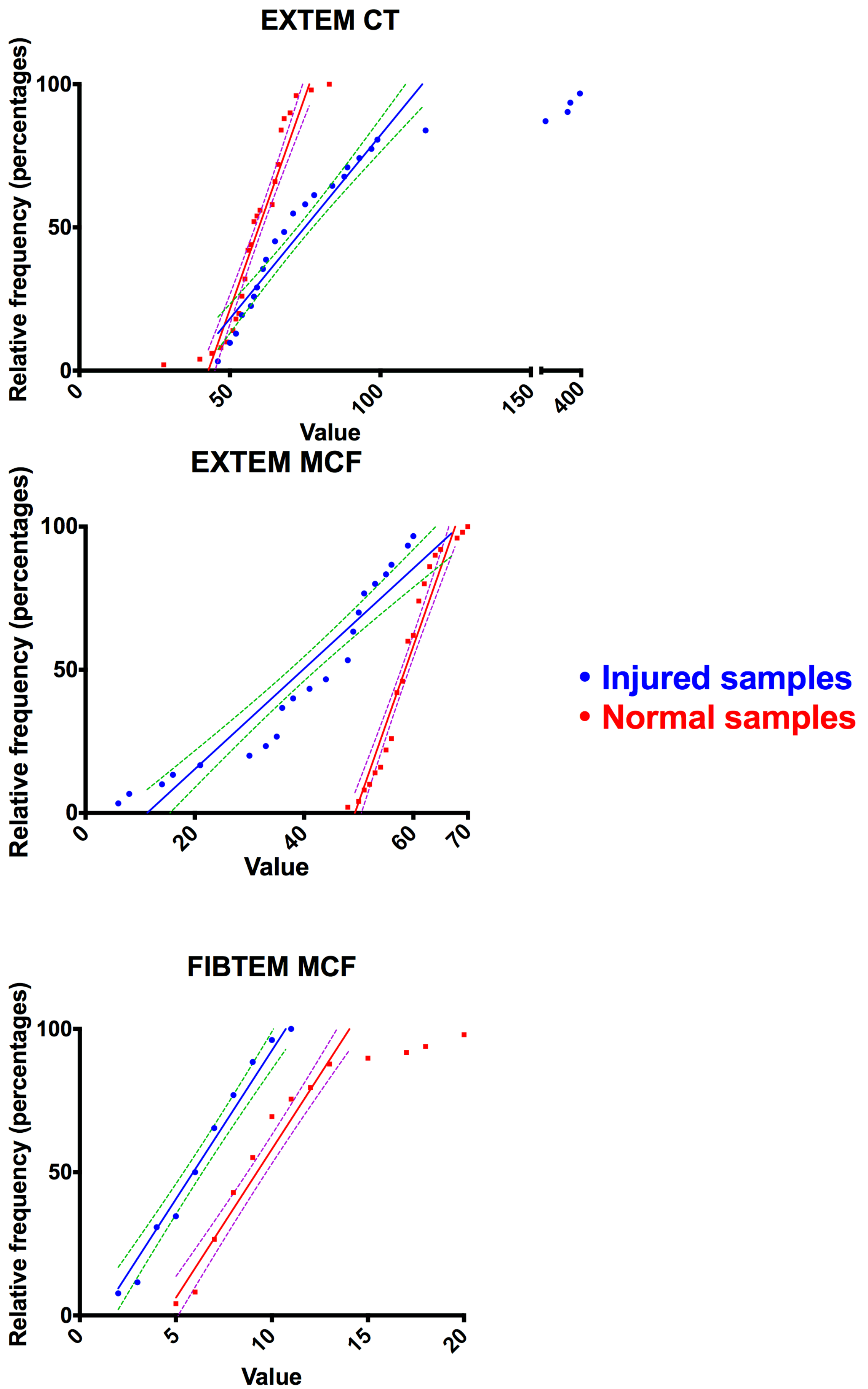

Figure 10-1. Normal probability plot of EXTEM CT, EXTEM MCF and FIBTEM MCF on normal volunteers (Red) and admission samples from the injured patients (Blue) groups

By contrast, parameters relating to clot strength (MCF) showed a fundamental difference in distribution between the volunteer and patient groups Figure 10-1. However, when the MCF data derived from the FIBTEM assay were compared, the differences in distribution between patient and volunteer groups were much less marked Figure 10-1.

Platelet counts were available for 23/31 of the patient admission samples and showed a mean value of 142 x 10^9 $litre^{-1}$ [107 - 213 x 10^9, IQR]. The difference in distribution of MCF values for EXTEM was therefore unlikely to be due to a gross abnormality in platelet numbers since the values were broadly within the normal range.

10.5 Discussion

The principal finding of this study is that a value of ROTEM® A5 and in particular A10 below the reference range predicts a final conclusion of coagulopathy with a high degree of sensitivity and specificity. This is true for either the 2/3 rule or the MCF<40mm rule.

ROTEM® values are known to be influenced by age and sex (Lang et al., 2005) and there is no data to determine whether the effects of acclimatisation to an environment such as that found in Afghanistan (compared to northern Europe, which forms the basis of the published ranges) has any influence on ROTEM® parameters. Consequently the reference range for Bastion was viewed as the most applicable to the patient population of this study. This approach is also consistent with the advice that each hospital should derive its own reference range as a basis for assessing their patients (TEM, 2010).

Complex trauma coagulopathies are not simply a failure of clot initiation. They represent an imbalance between clot formation, clot breakdown and innate anticoagulation with dimensions of clot dynamics and ultimate clot strength. Additionally there are complex interactions between factors and cellular elements (platelets) of clotting. In our injured population approximately 40% of patients had admission coagulopathy. Since prehospital times are short and

standard operating procedures limit the use of crystalloid resuscitation, it is unlikely that dilution has any significant effect on admission coagulopathy. Approximately the same proportion of patients had abnormalities in each of the three domains of clotting implying that abnormalities affecting initiation, dynamics and strength should all be interpreted when diagnosing trauma induced coagulopathy.

Definitions, and therefore diagnosis of coagulopathy do not refer to viscoelastic methodology (Lundberg, 1994) and using ROTEM® to diagnose coagulopathy is therefore impossible until there are accepted definitions. We accept that a clinical decision can be reliably made on a grossly abnormal value in any single parameter, however it is not clear what weight can be put on a mildly abnormal value. Current UK Defence Medical Services policy on the treatment of trauma induced coagulopathy (SGOPL 0809, 2009) is directed at preventing deterioration in coagulation status.

In order to use any test as a diagnostic tool, there needs to be definitions adopted to allow clinicians to follow protocols. We have therefore adopted (non validated) definitions to allow ROTEM® use in military protocols whilst formal definitions are approved. Expert opinion seems to suggest that an MCF of 40mm represents coagulopathy, and this is one definition used. In this study it is proposed that a single abnormality just below the normal range does not, on its own, indicate failure of the coagulation system, however if there are abnormalities below the normal range in two or more of the three domains of the clotting process (initiation, dynamics and clot strength), then there is potential failure of coagulation or coagulopathy (the 2/3 rule). Since the 2/3 rule incorporates more than simply clot strength, it is potentially a better definition. The 2/3 rule is defined as:

“Any values outside the normal range in two out of the three domains of clotting (initiation, dynamics and strength)”

In the profoundly coagulopathic patient it takes a long time to reach CFT and MCF (MCF is reached in 30 mins [IQR 15 – 45 mins]). Therfore A clinician will use an interim value of A5 or A10 as predictor of final MCF. This represents a

time saving of approximately 19 minutes compared to running the ROTEM® test until MCF is achieved, since A10 was reported within 11 min (CT plus 10 minutes) of initiating the test. This time saving is of considerable benefit during the initial process of damage control resuscitation as the results are contemporaneous enough to allow clinical decisions to be made. However this study shows that A10 is not simply a predictor of final MCF, since it also predicts coagulopathy, as defined by the 2/3 rule, with a high degree of sensitivity and specificity.

Since ROTEM® is a near patient test there is the added advantage of the trace developing in real time on the screen, thus anaesthetists and surgeons are able to look at the developing trace and make judgments based on the shape of the curve produced (Luddington, 2005). The rapidity of obtaining and interpreting ROTEM® results makes it a useful tool in guiding trauma resuscitation once resuscitation has started, rather than as a means to initiate haemostatic resuscitation in severely injured patients. It is likely that the initial in hospital resuscitation will follow predefined massive transfusion protocols that are empiric to all trauma (SGOPL 0809, 2009). Whilst massive transfusion protocols are essential, a one-size fits all approach is not necessarily correct for all trauma. It is not clear what the optimum ratio of transfused PRBC:FFP (Hess and Holcomb, 2008; Duchesne et al., 2008; Midwinter and Woolley, 2011; Maegele et al., 2008; Kashuk et al., 2008) should be, and as soon as possible a patient should have timely, directed therapy in order to ensure that the right product is given to the right patient at the right time. Since A10 is a reliable predictor of coagulopathy as defined by the 2/3 rule, an abnormal A10 can alert the clinician to the real possibility of the presence of trauma induced coagulopathy, or the timely monitoring of efficacy of treatment allowing an informed decision to be made on the immediate management of the patient.

This study was not designed to compare incidences of coagulopathy defined by viscoelastic tests on whole blood versus plasma-based clot initiation tests such as prothrombin time, both of which are established methods of assessing clotting problems. However, it was of interest to note that, in the small sample where data was available from both forms of testing, agreement was good for non-coagulopathic blood. Conversely, agreement appeared poor in blood that

was found to be coagulopathic by either one test or the other. This conclusion has been borne out in other studies (Davenport et al., 2011) which also states there is poor correlation between PT testing and ROTEM®. The number of samples investigated here are far too small to make any definitive conclusions but clearly a larger study is necessary to underpin clinical standards for trauma patients where there are no universally agreed techniques for assessing coagulopathy.

Finally, a statistical comparison of the distribution of values representing clot initiation, dynamics and strength between normal volunteers and injured patients revealed some potentially interesting similarities and differences. The distribution of values representing clot initiation and dynamics (based on the EXTEM) between the normal and injured group, suggested a fundamentally similar population (with outliers in the trauma population). However the distribution for clot strength was fundamentally different between normal and trauma group.

It is unlikely that this difference between clot initiation/dynamics and strength is a quirk of the statistics since similar findings of a fundamentally different distribution between control and injured groups were found for the other parameters relating to clot strength (A5 and A10). This difference was significantly reduced when the normal/trauma comparison was made for FIBTEM data where the platelets were disabled as a part of the test. Again, the findings relating to final clot strength in FIBTEM were corroborated by similar findings for FIBTEM A5 and A10.

Interpreting the physiological significance of these initial findings is highly speculative. However, one important difference between EXTEM clot strength parameters and measures of clot initiation and dynamics, is that the clot strength parameters depend on platelet and fibrinogen function whereas clotting factors play the pivotal role in the clot initiation and dynamic parameters. By contrast, in the FIBTEM test the platelets are pharmacologically disabled, leaving only the clotting factors and fibrinogen . Since platelet numbers are above recommended platelet targets ($100x10^9$ $litre^{-1}$) (SGOPL 0809, 2009; Rossaint et al., 2010), it is possible that these findings, taken collectively,

indicate that the initial clot development is similar between control and injured groups, but that there is a difference clot strength relating to platelet function early after injury. This conclusion is at present speculative but the data suggest that there may be some merit in a further detailed investigation of platelet function early after injury as this may help clarify mechanistic aspects of trauma coagulopathy and possibly provide a focus for developing targeted treatments.

10.6 Conclusions

Trauma coagulopathy is a significant problem in severely injured patients and requires prompt, targeted resuscitation in order to reverse coagulopathy and restore the patient's physiology. During the dynamic phase of early resuscitation rapid assessment of the injured casualty is viewed as being of paramount importance. Interim ROTEM® parameters A5 and particularly A10 were found to accurately predict the outcome of the ROTEM® EXTEM test, and can be used to determine coagulopathy by either predicting a low MCF, or a positive 2/3 rule. In spite of these definitions not being validated, A5 or A10 can be used clinically to evaluate whole blood clotting in patients.

Chapter 11. Effects of simulated pre-hospital treatment with oxygen or rVIIa: Methods

The underlying survival study that provided the basis for this research was conducted on 18 terminally anaesthetised Large White pigs, weighing 51.8 ± 2.2 [48-56] Kg (mean+/-SD [range]). A power analysis based on an increase from 0.1 to 0.8 in the proportion of animals surviving to 8 hours from the onset of resuscitation indicated that 7 animals would be required in each of the hypotensive and new treatment groups (Power 0.8, Alpha 0.05, Chi Squared test), with a total of 21 pigs. However an interim analysis by a statistician (independent of the study team) was planned and performed when n=6 had been attained in each group which revealed that there was a clear statistically and clinically significant increase in survival associated with supplementary oxygen and therefore the study was terminated with a final sample size of 6 animals in each group.

The study was ethically reviewed and conducted in accordance with the Animals (Scientific Procedures) Act 1986.

Prior to the study the animals were housed indoors and fed on a complete wheat-soya based ration at 1.5–1.7 kg per day. They were fed and allowed water *ad libitum*. Feeding was discontinued 18 hours before planned induction of anaesthesia and the animals allowed continuous access to water.

11.1 Induction and maintenance of anaesthesia

All animals premedicated with midazolam (0.1mg/Kg), anaesthesia was induced by inhalation of Isoflurane, O_2 and N_2O (1:1 ratio) and the animal intubated. Initially anaesthesia was maintained with isoflurane, O_2 and N_2O and the animal ventilated with a Manley Ventilator for the duration of the surgery. Once vascular access had been obtained (see section 11.2), anaesthesia was converted to intravenous anaesthesia using alfaxalone (Alfaxan, Vetoquinol UK

Ltd, Buckingham UK) for the remainder of the study. Initial monitoring consisted of end-tidal CO_2, pulse oximetry and ECG monitoring. The animals were humanely killed at the end by a lethal overdose of phenobarbitone.

11.2 Surgical preparation

The skin was prepared with povidone-iodine solution. The left carotid artery, left internal jugular vein, left femoral artery and vein were all cannulated (Portex 8FG, Hythe, UK). A background infusion of 0.9% Saline at 10 ml/kg/hr was given during surgery to cover insensible losses. A balloon tipped pulmonary artery flotation catheter (744MF75 Swann-Ganz, Edwards Life Sciences Ltd, Newbury, UK) was advanced through an introducer sheath (Desivalve Catheter Introducer, Vygon, Cirencester UK) in the right internal jugular vein. Pulmonary arterial placement was confirmed by pressure wave monitoring (Propaq 106 EL, Dräger Medical AG &Co. Lübeck, Germany).

A midline laparotomy was performed and the spleen contracted by topical application of adrenaline (1.0 to 1.5 ml of a 1mg/ml solution), vascularly isolated and then removed. A surgical snare was placed around the left medial lobe of the liver and exteriorised for later induction of a grade IV liver injury as a source of incompressible haemorrhage.

The bladder was cannulated suprapubicaly (14 G Foley catheter), and the catheter secured using a purse string suture. The bladder was emptied. Once all surgical procedures were complete the surgical wounds were sutured.

The animals were weaned from the ventilator and continued to breathe air spontaneously for the remainder of the experiment unless the study protocol dictated otherwise (see section 11.3, O_2 group). If there were clinical signs of respiratory depression (RR<10, $PaCO_2$ >6KPa, PaO_2 <7 KPa synchronised intermittent mandatory ventilation was instituted. The animals recovered for 1 hour from surgery before baseline measurements were made, during which time they were transported to the blast arena.

11.3 Protocol

11.3.1 Overview

The animals were randomly allocated to one of three groups corresponding to treatment during resuscitation;

- Control (treated with placebo, breathing air throughout)
- Oxygen (treated with placebo, supplementary oxygen during resuscitation)
- FVIIa (treated with ***intravenous*** rFVIIa during resuscitation, breathing air throughout).

All animals received a standardised injury comprising of blast exposure, haemorrhage, haemorrhagic shock followed by hypotensive resuscitation. The protocol is summarised in Figure 11.1.

11.4 Blast exposure

After baseline measurements were made, animals were placed supine on a trolley and covered with a Kevlar blanket to protect them from minor debris during the blast exposure. Physiological monitoring during this phase consisted of respiratory rate and ECG. The animals were placed 2.1m from a standard 2.2Kg charge of Octol, which was detonated remotely. The animals were then removed to the physiological monitoring suite for the remainder of the study.

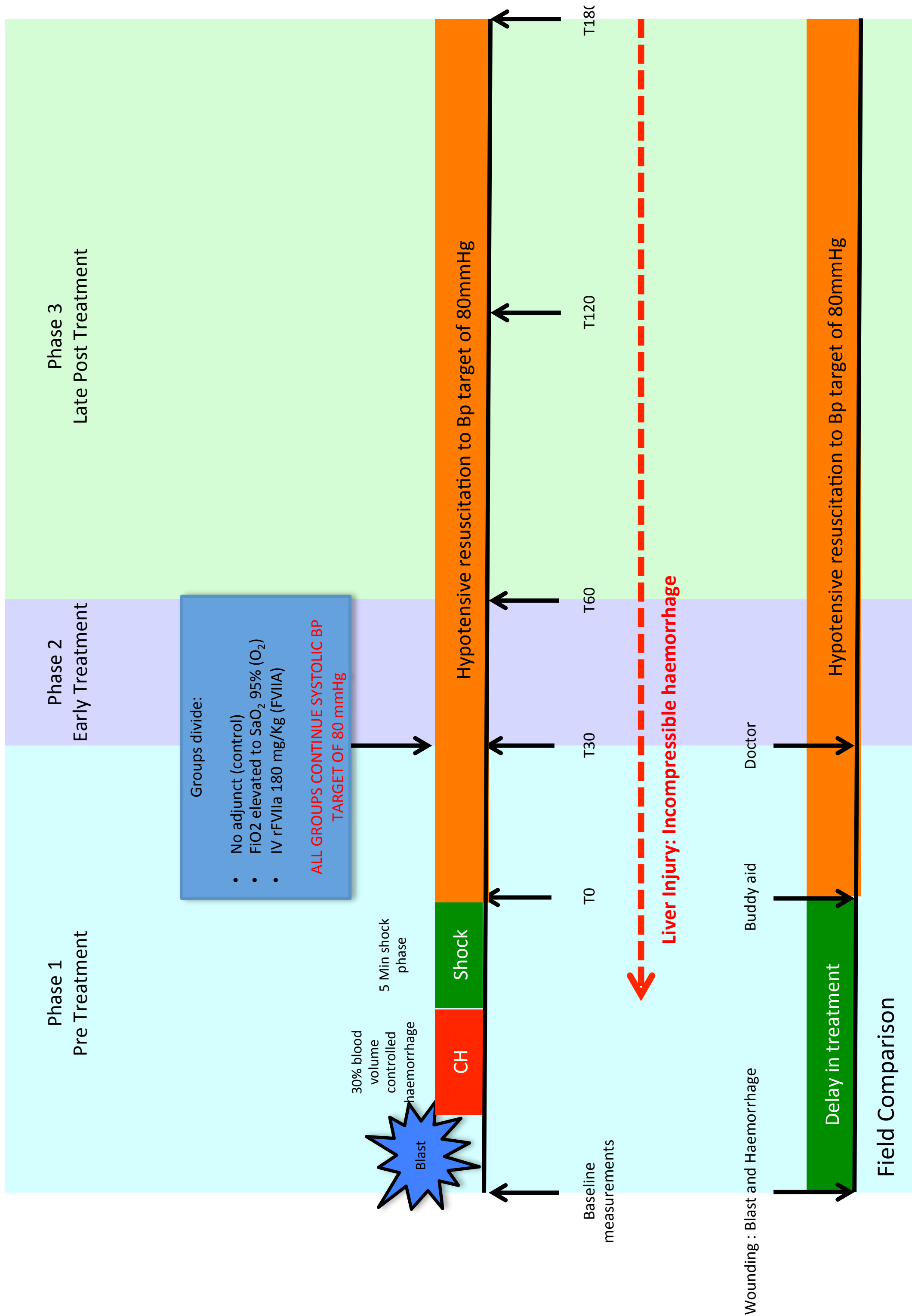

Figure 11.1: Experimental protocol commencing approximately 60 minutes after the end of surgery

11.4.1 Haemorrhage

Fifteen minutes after blast exposure all animals received a controlled haemorrhage of 30% total blood volume via the femoral artery catheter. The blood volume was estimated according to Equation 11.1. Blood was removed using a computer controlled pump (MasterFlex® LIS® Computerised Drive Pump, Cole-Palmer Instrument Co. Il, USA). The bleed occurred over 5.3 minutes, with a progressively decreasing flow rate (Equation 11.2) designed to reflect the reducing flow over time from a real arterial injury (Stern et al., 1993). By monitoring the weight of removed blood, the accuracy of exsanguination volume was confirmed.

$$\textbf{Total Blood Volume (B0) (ml/kg)} = \mathbf{161.4751(Wt^{-0.2197})}$$

Equation 11.1 Blood volume by swine body weight

$$\textbf{Arterial Rate of Exsanguination (V)} = \mathbf{B0(1-e^{-0.04t})}$$

Equation 11.2 Haemorrhage rates in arterial exsanguination (V=the total blood loss (ml/Kg) and the per cent time until death)

Immediately after completion of the controlled haemorrhage, an incompressible haemorrhage was simulated by amputating a section of the liver by traction on the pre-implanted liver snare (Figure 11.2). This was left uncompressed for the remainder of the study and injury confirmed on post mortem analysis. The liver injury represents approximately 6% (by weight) of the liver and does not transect major blood vessels.

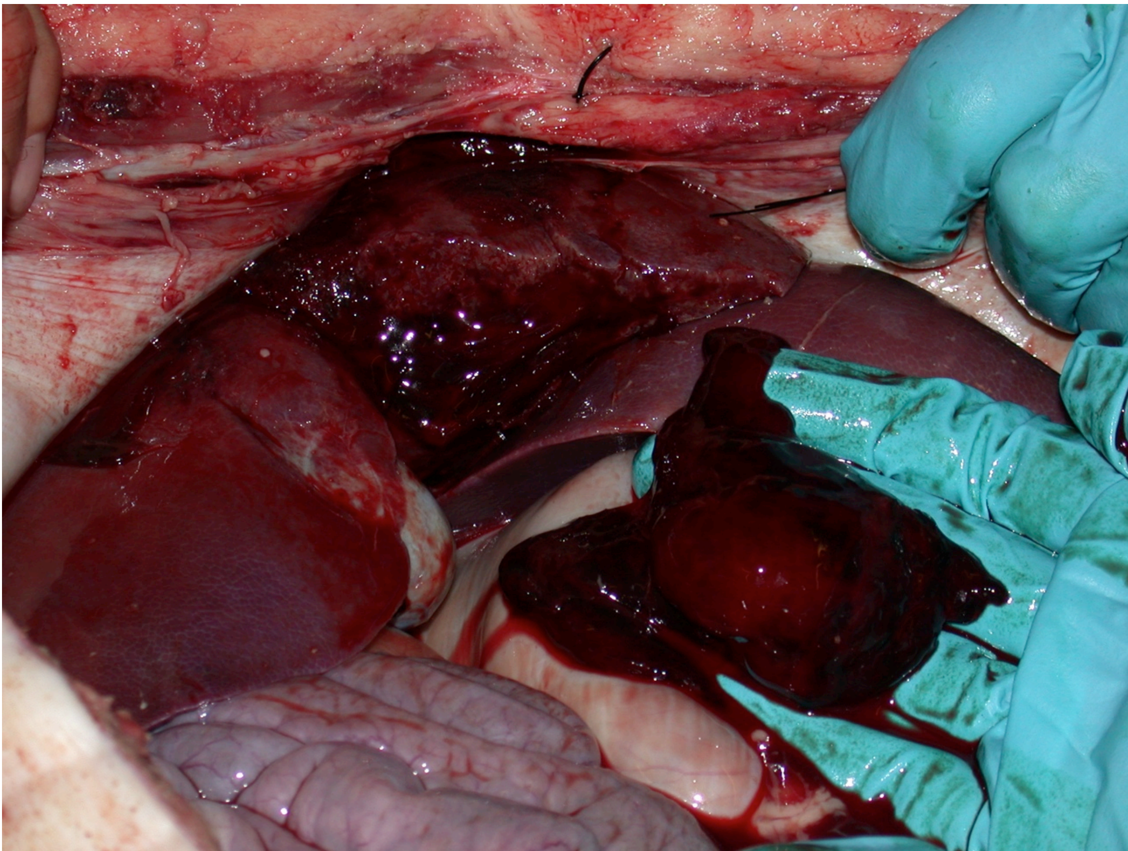

Figure 11.2: Amputated section of liver at post mortem demonstrating the size (approx 6%) of the amputated section and surface clot on remaining liver

11.4.2 Haemorrhagic shock resuscitation

After completion of the haemorrhage, there was a 5 minute shock phase, during which no treatment was given, designed to reflect a realistic minimum time interval before resuscitation could commence in a military environment. Time '0' was designated as the onset of resuscitation (end of the shock phase). Warmed 0.9% saline was infused into the femoral vein, at a rate of 3ml/kg per minute to attain a target systolic blood pressure (SBP) of 80 mmHg. Further aliquots were given as necessary to maintain the target SBP (80mmHg) during the remainder of the experiment (up to 480 minutes after the onset of resuscitation unless death occurred sooner).

11.4.3 Treatment Groups

All groups were given fluid resuscitation as described in section 11.4.2. The three treatment groups diverged 30 minutes after the onset of resuscitation.

Control group: Placebo (0.18 ml/kg) was administered 30 minutes after the onset of resuscitation. Animals breathed air throughout.
Oxygen group: Placebo (0.18 ml/kg) was administered 30 minutes after the onset of resuscitation. FiO_2 elevated to attain a SaO_2 of 95%. SaO_2 was monitored continuously and FiO_2 adjusted to maintain the target for the remainder of the study.
rFVIIa group: **Intravenous** rFVIIa (180 µg/kg, 0.18 ml/kg) was administered 30 minutes after the onset of resuscitation. Animals breathed air throughout.

11.4.4 Physiological monitoring

Cardiovascular variables were recorded continuously (except for the period immediately surrounding the blast exposure) using a computerised data acquisition system (Maclab 8/s, AD Instruments, UK) and associated software (Chart v4.2.3, AD Instruments, UK). Arterial blood pressure, pulmonary arterial and central venous pressures were recorded using a strain gauge manometer (Sensonor 840, SensoNor a.s., Norway). Zero pressure for all transducers was set at heart level. Continuous cardiac output was monitored as a 6 minute rolling average using a cardiac output monitor (Vigilance™ Volumetrics CEDV, Edwards Lifesciences™, USA).

Paired arterial and mixed venous blood samples were taken anaerobically into heparinised syringes from the carotid and pulmonary artery catheters respectively for blood gas, base excess and lactate analysis (Gem Premier 3000 Blood Gas Analyzer, Instrumentation Laboratories, Warrington, UK).

Body temperature was maintained at approximately 38°C using external heating/cooling and blankets as appropriate. The bladder was drained at hourly intervals.

11.4.5 Haematology

Arterial blood samples taken for assessment of haematocrit (by centrifugation, HaematoSpin 1300, Hawksley, UK) platelet count (details) and Clauss

Fibrinogen (details). Arterial blood samples were also taken for coagulation assessment as described in section 11.5

11.5 Assessment of coagulation

11.5.1 PT, aPTT and ROTEM® analyses

Arterial blood samples were taken into citrated vacutainers (9NC 0.105M Vacutainer 367691, Beckton Dickinson, UK), centrifuged at 1500 x g for 10 min and the plasma separated and stored at -80°C for determination of PT and aPTT using the ACL Elite (Beckman Coulter, UK). Further citrated samples were immediately subjected to standard ROTEM® analysis (EXTEM, FIBTEM) (section 4.4). Finally fresh (uncitrated) blood samples were processed immediately for TEG® analysis as described in section 14.2.2

11.5.2 Individual factor assays

Citrated plasma samples were stored at -80°C for batched analysis. This aspect of the study was conducted as a supplementary rather than as a pre-planned analysis (see section 13.9).

Factor II, V, VII and X are PT based assays (see section 4.9) and were performed using standard factor deficient plasma kits (HemosiL, Instrumentation Laboratory, Warrington, Cheshire). TFPI, syndecans, and heparans were analysed by ELISA respectively using Imubind TFPI ELISA, Sekisui, Invitech Ltd, Huntingdon, Cambridge, Diaclone Human sCD138 (syndecan-1) ELISA kit 2B Scientific Ltd, Upper Heyford, Oxfordshire ; General Heparan sulphate ELISA kit, AMS Biotechnology (Europe) Limited, Abingdon, Oxfordshire).

The analysis described in this sub-section were all performed in collaboration with Professor Beverley Hunt, Director of the Thrombosis and Haemostasis research Unit, Guys and St Thomas's hospital, London.

11.6 Exclusion of data points during extreme dilution

In this current study, the protocol required infusion of 0.9% saline to keep the systolic blood pressure at 80mmHg. For the majority of the time during the resuscitation phase, while the animals were relatively stable, target blood pressure was maintained with repeated small aliquots of 0.9% saline. This resulted in a gradual decline in haematocrit, and consequently haemodilution in all groups. However when animals started to decompensate the protocol demanded that a continuous infusion of saline was given in an attempt to maintain blood pressure. However during this brief terminal phase this was ineffective, resulting in a relatively large amount of 0.9% saline being given over a short period of time as the animal slipped towards death. Therefore during this terminal phase there was a very marked haemodilution that was not representative of the remainder of the study. Since such haemodilution would be expected to have a profound effect on clotting, which is of little interest in the context of this thesis since it is dilutional, all data relating to that terminal time point where haematocrit had fallen to <15%, was excluded from analysis.

11.7 Statistical analysis

11.7.1 Phases of treatment

Phase 1 of the study encompasses all the pre-treatment time points and thus includes baseline, blast injury, haemorrhagic insult, shock phase and initial hypotensive resuscitation (target SBP 80mmHg). There were no injury or treatment differences between groups during this phase therefore the only anticipated differences were those resulting from the injurious insult and the magnitude of differences should be the same between groups.
Phase 2 refers to the time period T30 to T60. Since treatment with either O_2 or rFVIIa was initiated immediately after the T30 time point, this is the time in which the effects of rFVIIa are likely to be the greatest and is considered in the early treatment phase. Within this period, the T30 time point represents a pre-treatment time point. However it is considered in the early treatment phase

since it is the last time point prior to treatment and thus could be considered the baseline measurement for this early treatment phase.

Phase 3, is the late treatment phase, T60 to T180. There were insufficient animals in the control group to perform meaningful analysis between either the O_2 group and control groups, or the IV rFVIIa group and control groups. Therefore for this phase of the study, the statistical comparison could only be made between the O_2 group and the rFVIIa groups.

11.7.2 Statistics

All data are presented as mean +/- s.e.mean unless indicated otherwise. Survival times in were compared using Kaplan-Meier analysis (Mantel-Cox log rank test) using SPSS v10. Data from animals still alive after 8 hours were treated as right-censored. Cardiovascular, blood gas and chemistry data were compared using two-way analysis of variance (ANOVA) with repeated measures over time. Single time-point analyses were made using 2 way and 1 way ANOVA as appropriate. Where data was found to be non-normal a non-parametric equivalent was used as indicated in the text. In all cases a significance level of $P \leq 0.05$ (two tailed) was used.

Chapter 12. Results

12.1 Survival

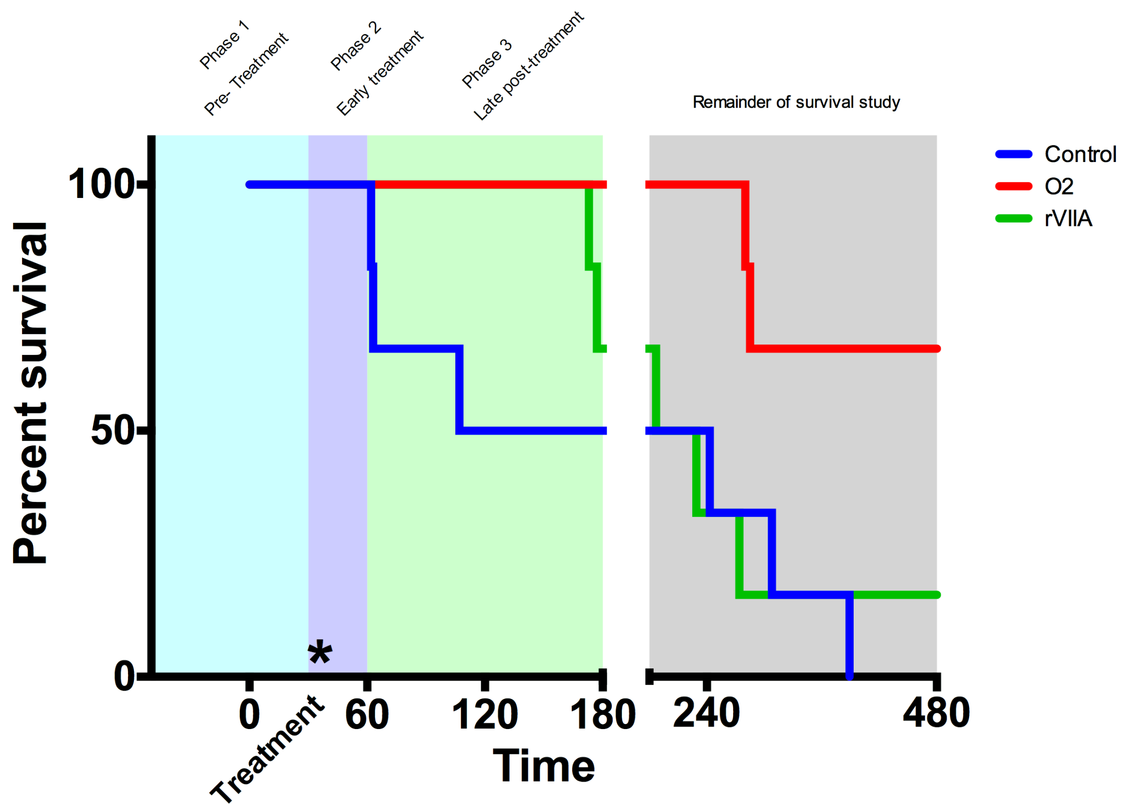

Figure 12-1. Kaplan-Meier survival graph for the three study groups. Phase 1 (Pre treatment phase, blue), phase 2 (Early treatment phase, purple) and Phase 3 (late post treatment phase, green). The data analysed in this thesis was taken from the first three phases of resuscitation (coloured area on the graph). The survival study (not the subject of this thesis) extends to 8 hours of resuscitation (grey area of the graph) during which physiological and coagulation data were not analysed. The asterix denotes treatment with either O_2 or FVIIa

Survival times in the group treated with rFVIIa were not significantly different from those seen in the placebo-treated animals (P = 0.649, Figure 12-1) By contrast, survival times in the group treated with supplementary oxygen was significantly better than those seen in both rFVIIa and placebo-treated animals (P=0.020 and P=0.014 respectively, (Figure 12-1)

It is possible that rFVIIa conferred a small survival advantage compared to placebo, but that the study did not have sufficient statistical power to detect it. A *post hoc* power calculation (Power 0.80, alpha 0.05, Log rank test) indicated that 23 animals per group would be required to determine whether the small difference in survival between rFVIIa and placebo shown in Figure 12-1 was statistically significant. Since oxygen showed a far superior effect it was decided that pursuit of this relatively small effect of rFVIIa did not justify the use of additional animals.

12.2 Oxygen Saturation

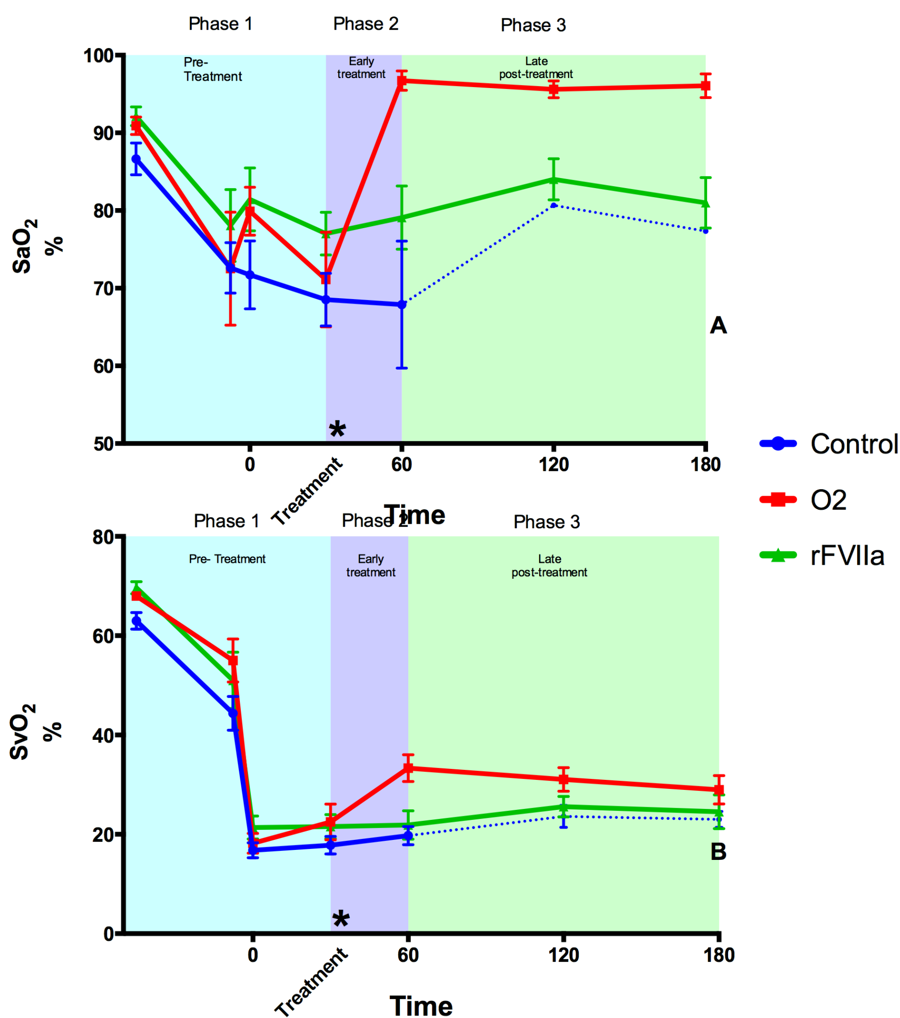

Figure 12-2 O_2 Arterial oxygen saturations (A) and venous oxygen saturations (B) over time in each group. Phase 1 (Pre treatment phase, blue), phase 2 (Early treatment phase, purple) and Phase 3 (late post treatment phase, green). The asterix denotes treatment with either O_2 or rFVIIa. Dotted line in control group denotes insufficient number of animals to allow meaningful statistical comparisons. Mean values +/- SEM

12.2.1 Arterial oxygen saturation

Assessment of arterial oxygen saturation (SaO_2) revealed a significant difference between groups in the pre-treatment phase of the experiment, phase 1 ($P=0.0373$) and a significant effect over time ($P=0.0003$) (Figure 12-2 A). The difference between groups was in the control group at baseline prior to any blast, haemorrhage or treatment. Since the magnitude of the difference was small, and both values lie within the normal range, this difference is not clinically significant. The effect over time occurred around the blast injury, haemorrhage and shock period and was a rapid drop in arterial O_2 saturations in all groups.

In the early treatment phase, there was a significant effect between groups ($P=0.0003$) and a significant effect over time ($P=0.0053$) due to the administration of O_2 to the O_2 group at T30, the beginning of phase 2 and represented a higher SaO_2 in the O_2 group by T60.

In phase 3 there remained a significant effect between groups ($P<0.0001$), however there is no significant effect over time ($P=0.6938$). The higher SaO_2 was in the O_2 group due to the administration of O_2 throughout this phase of the experiment.

12.2.2 Mixed venous oxygen saturations

Assessment of venous oxygen saturations (SvO_2) in the pre-treatment phase of the study showed a significant difference between groups ($P=0.0136$) and a significant effect over time ($P<0.0001$) (Figure 12-2 B). This difference occurred in the control group at the baseline measurement prior to any blast, haemorrhage or treatment. Since the magnitude of the difference was small, and both values lie within the normal range, this difference is not clinically significant.

In the early treatment phase, T30 to T60, there was a significant difference between the O_2 group, who had O_2 administered at T30 ($P=0.0057$) and the control and rFVIIa groups, however there was no significant effect over time ($P=0.506$). The level of SvO_2 in the O_2 group by T60 is 33.31% (+/- 2.72)

compared with the rFVIIa group level of 21.83% (+/- 2.89), or the control group 19.72% (+/- 1.85). Therefore, in spite of the statistically significant difference between the groups, the values are so low compared to the normal range of 60-80%, that this is unlikely to represent a clinically significant difference

The difference between the O_2 and rFVIIa groups remained significant in phase 3 of the study (P=0.0025), however the effect over time is not significant (P=0.6587). The higher SvO_2 was in the O_2 group due to the administration of O_2 throughout this phase of the experiment.

In summary there was a difference in arterial oxygen saturation in the O_2 group after treatment with O_2 at T30, however this effect does not result in a clinically significant difference in venous oxygen saturation even in the O_2 group after treatment with O_2 at T30.

12.2.3 Oxygen extraction ratio

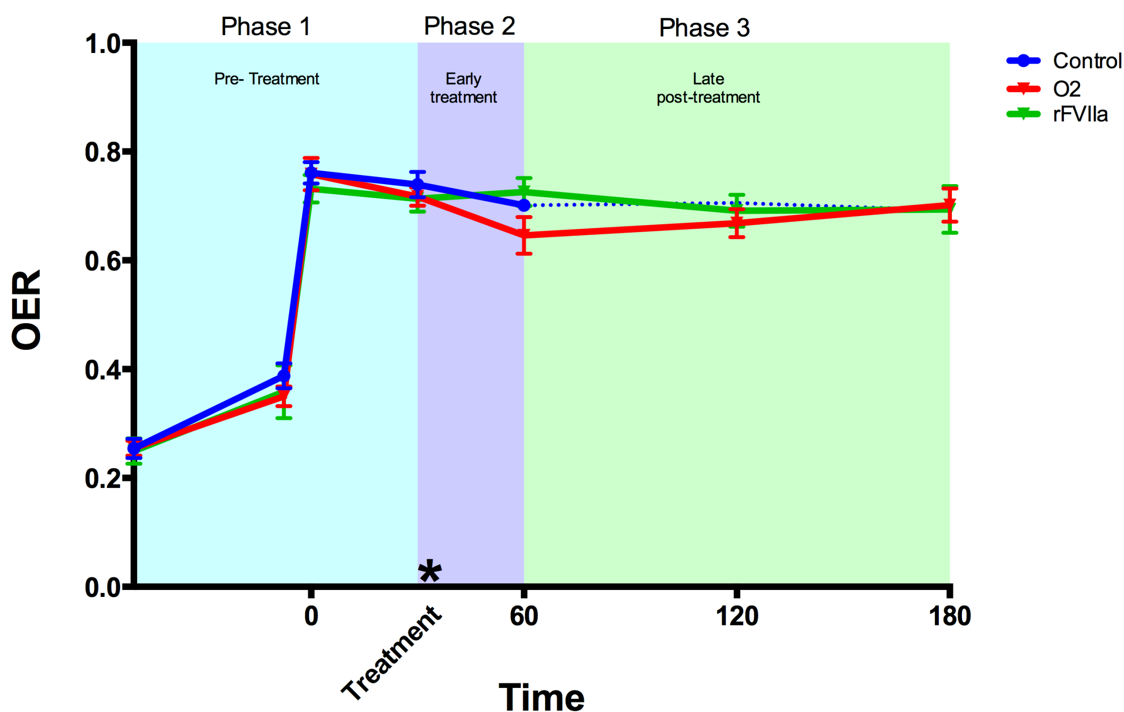

Figure 12-3 Oxygen extraction ratio over time in each group. Phase 1 (Pre treatment phase, blue), phase 2 (Early treatment phase, purple) and Phase 3 (late post treatment phase, green). The asterix denotes treatment with either O_2 or rFVIIa. Dotted line in control group denotes insufficient number of animals to allow meaningful statistical comparisons. Mean values +/- SEM

Assessment of oxygen extraction ratio (OER) (Figure 12-3) during phase 1 showed a significant change over time (P<0.0001) as OER increased during shock, although there is no difference between groups (P=0.1097).

During phase 2 there is no further change in OER over time (P=0.8885) and no difference between groups (P=0.1368). During the third phase a small difference between groups became apparent (P0.0475), although post hoc analysis could not specify a difference between any two groups. There was no overall significant difference over time during phase 3 (P=0.9171)

12.3 Assessment of shock

12.3.1 Arterial Base Excess

Assessment of arterial base excess in the pre-treatment phase of the study showed no significant effect between groups (P=0.0595) however there was a significant effect over time (P<0.0001) (Figure 12-4 A), due to a progressive worsening of base excess due to the onset of tissue shock over the blast and haemorrhage phase of the study.

During the early treatment phase of the study, in spite of a trend for a deteriorating ABE in the control and rFVIIa groups, there was no significant difference between groups (P=0.1796) and no significant effect of time (P=0.0588).

Likewise in the late post-treatment phase of the study, the significant effect of group remained (P=0.0025), and there remained no differences over time (P=0.9677). The value of ABE at T180 in the O_2 group was -12.27 mM (+/- 3.03), and the value of the rFVIIa group was -18.7 mM (+/- 1.34).

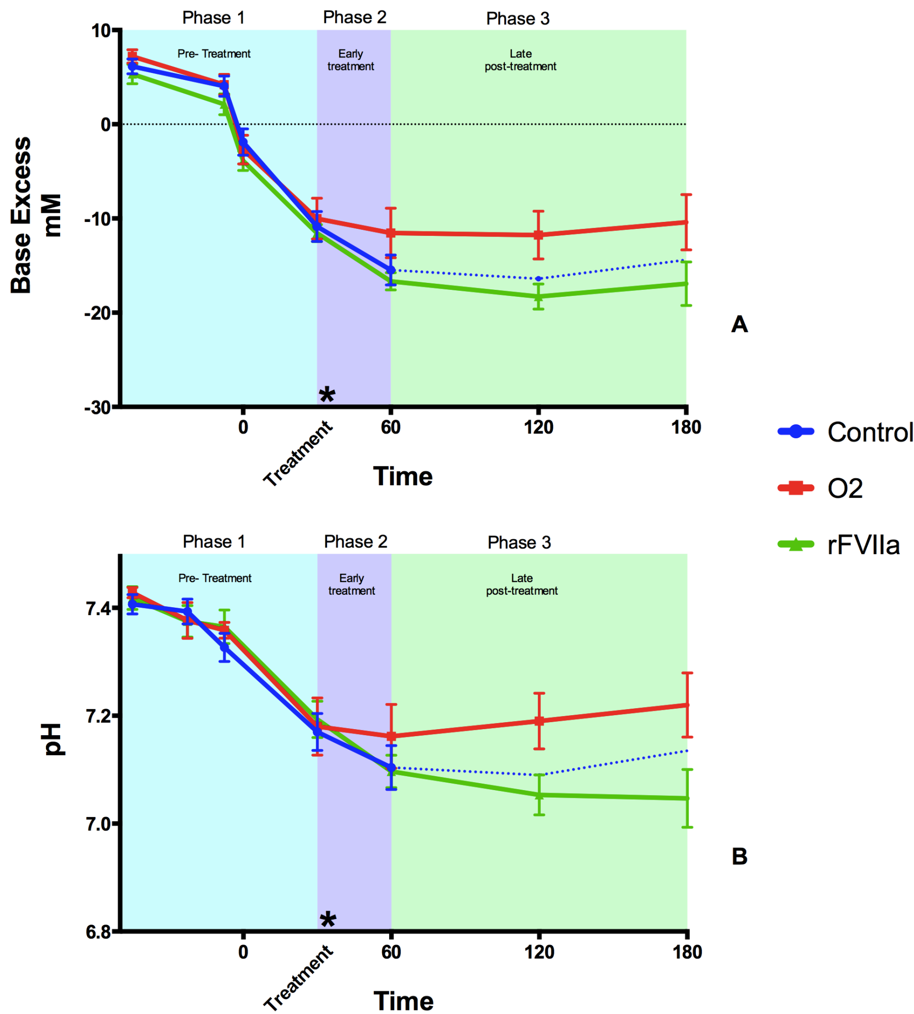

Figure 12-4. Measurement of arterial base excess (A) and pH (B) in all three groups. Mean +/- SEM. (For further details see Figure 12-2)

12.3.2 pH

Assessment of pH in the pre-treatment phase of the study revealed no significant differences between groups (P=0.6371), however there was a significant effect overtime (P<0.0001) (Figure 12-4 B) due to a falling pH during the blast, haemorrhage and shock phase of the experiment.

During the early treatment phase, phase 2, there was no significant difference between groups (P=0.7616) and no significant effect over time (P=0.2916)

However by the late post-treatment phase, there was a significantly higher pH in the O_2 group (P=0.0068), however there was no significant effect over time (P=0.8619). The pH in the O_2 group at T180 was 7.22 (+/- 0.06) compared to the rFVIIa group, which was 7.05 +/- 0.05.

In summary the fall in both ABE and pH in the pre-treatment phase of the study represented the physiological insult of the blast, haemorrhage and shock period. In the late post-treatment phase there was a difference in the O_2 and rFVIIa groups with the O_2 groups having a higher (better) pH and ABE compared with the rFVIIa group. The difference between groups may well be of clinical significance (pH 7.22 vs 7.05, ABE -12.27mM vs -18.7mM), however both values fall in the range of values define shock (definition of shock commonly pH <7.2 and ABE >-6.0 mM) and therefore both groups have a clinically significant degree of shock.

12.4 Prothrombin time and activated partial thromboplastin time

12.4.1 Prothrombin time

Clotting assessment using prothrombin time revealed no difference between groups (P=0.239) during the pre-treatment phase (phase 1) of the study protocol. However during phase 1 there is a significant change over time (P=0.0079) with an increase in PT during the short period immediately before the onset of resuscitation at T0 in all groups. The magnitude of this change is small and unlikely to be of clinical significance (Figure 12-5 A).

During the early treatment phase (phase 2), 30 to 60 minutes after the onset of resuscitation, there was a significant difference between groups (P=0.0003) and change over time (P=0.0264) due to shortening of PT (enhancement of clot initiation) in the rFVIIa group.

Thereafter in the late post-treatment phase (phase 3) analysis of the rFVIIa and O_2 groups showed a significant difference between groups (P=0.0095) and a significant change over time (P=0.0002) The difference is due a progressive increase in PT in the rFVIIa group after an initially shortened PT.

In Summary the main difference in PT was seen as a shortening of PT in the rFVIIa group at T60 with a subsequent increase in the rFVIIa group over late phase of the study.

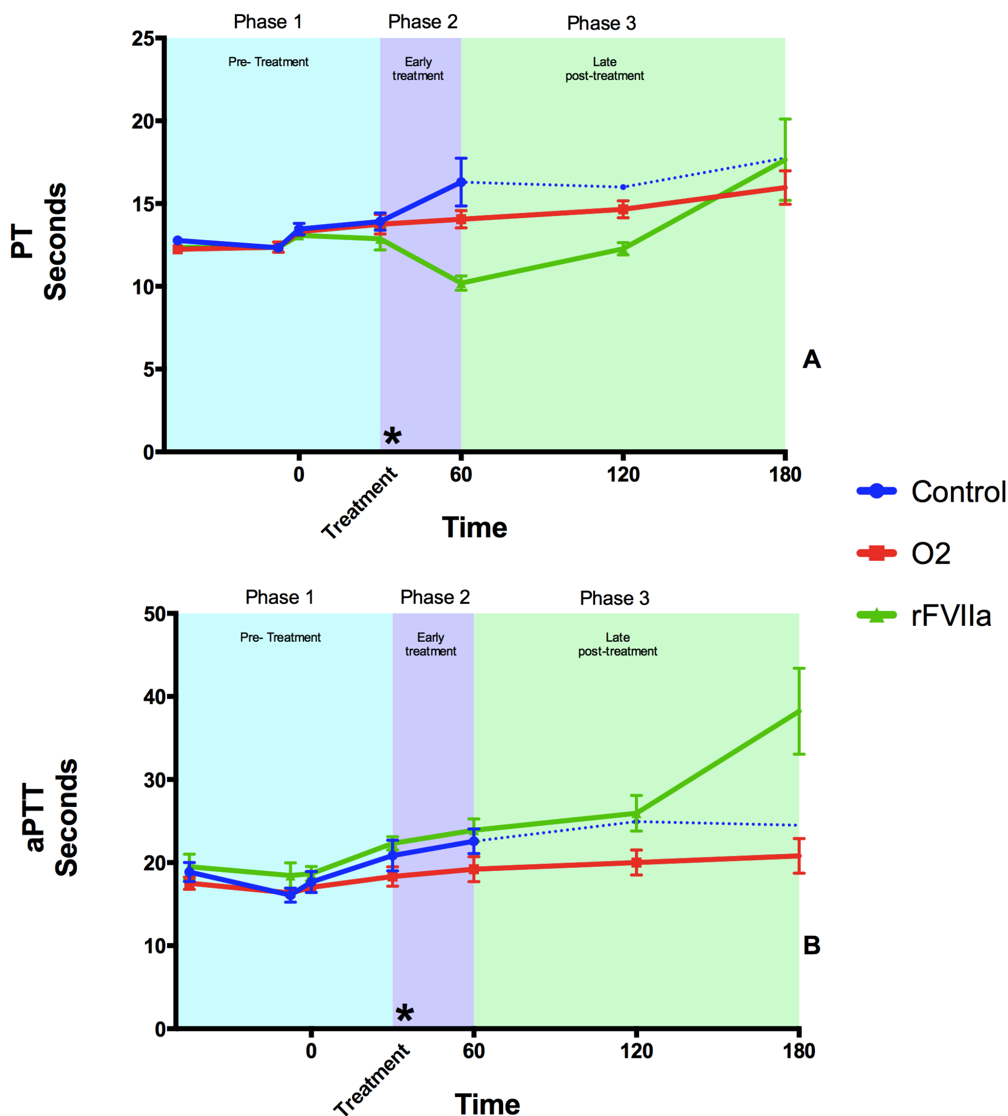

Figure 12-5. Measurement of prothrombin time (A) and activated partial thromboplastin time (B) in all three groups. Mean +/- SEM. (For further details see Figure 12-2)

12.4.2 Activated partial thromboplastin time

Activated partial thromboplastin time in the pre treatment phase of the study showed a significant difference between groups (P=0.0145) and a significant

change over time (P=0.0499). The differences are clinically insignificant (Figure 12-5 B).

A significant difference over time was apparent between groups (P=0.0137), with the rFVIIa group displaying the longest aPTT in the early treatment phase. However there is no significant change over time in this phase (P=0.6636).

In the late post-treatment phase of the study, a comparison of the O_2 and rFVIIa groups revealed a significant difference between groups (P=0.0003) and a significant change over time (P=0.0182). This is due to a prolongation of aPTT the rFVIIa group whilst the O_2 group remained unchanged.

In summary the main differences in aPTT show a progressive lengthening of aPTT over time, especially in rFVIIa group in the late phase of the study.

12.5 Clotting assessment by ROTEM® and TEG®

12.5.1 Clot Initiation

There was no significant difference in the ROTEM® clotting time (CT) between groups (P=0.0524) or over time (P=0.3028) during the baseline, immediate post injury and pre haemorrhage periods. (Figure 12-6 A)

Thereafter there was a statistically significant, clinically insignificant reduction in CT in the rFVIIa group only, between pre-haemorrhage and 30 minutes after the onset of resuscitation (P=0.0295). This resulted in a statistically significant difference between groups at 30 minutes after the onset of resuscitation, with CT being highest in the O_2 groups and lower in the rFVIIa and control groups.

Treatment with either rFVIIa or O_2 was given immediately after the T30 blood sample was taken, and the next time (T60) shows a significant difference between groups (P=0.0031), although there was no significant change over time (P=0.319).

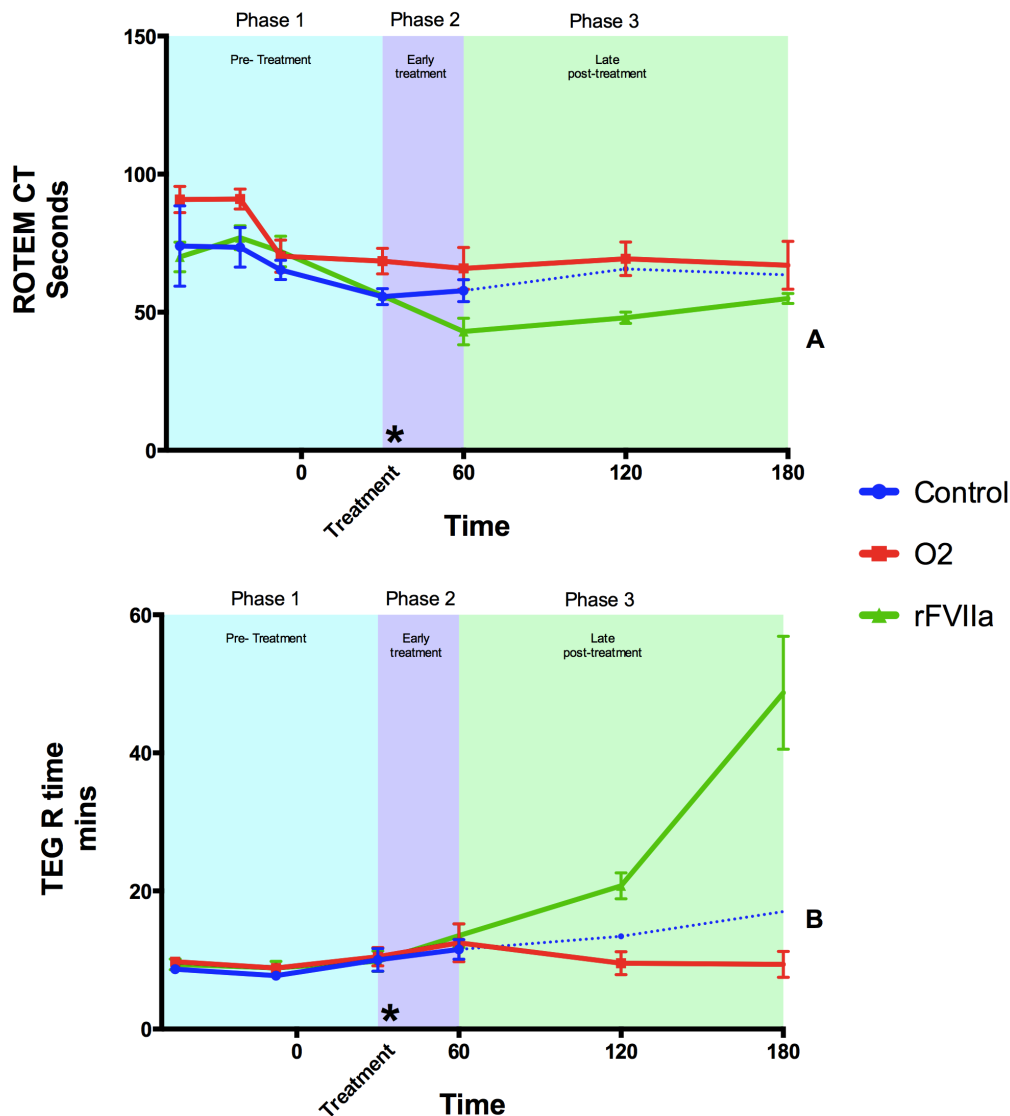

Figure 12-6. Measurement of clot initiation in all three groups using standard ROTEM® clotting time (A) and dilute innovin TEG® R time (B). Mean +/- SEM. (For further details see Figure 12-2)

Beyond T60 there was an insufficient number of survivors in the control group to allow meaningful statistical analysis, limiting the comparison to the rFVIIa and O_2 groups. In the period T60 to T180 there was a significant difference between groups (P=0.0006), with the rFVIIa being lowest, but no significant change over time (P = 0.7975).

Assessing clot initiation by TEG® (R-time) revealed a different pattern. There were no significant differences between groups (P=0.6058), or changes over time (P=0.6752) during the early part of the study (pre rFVIIa or O_2 treatment) (Figure 12-6 B)

Furthermore, administration of rFVIIa or O_2 did not cause a significant change in R-time (P=0.3516) and there were no significant differences between O_2, rFVIIa or control groups by T60 (P=0.853)

However, thereafter there was a significant difference between rFVIIa and O_2 groups (P<0.0001), together with a significant change over time (P < 0.0001), due to an increase in R-time in the rFVIIa group, while O_2 group remained unchanged throughout this period.

In summary the main change in clot initiation was seen in the rFVIIa group during the period T60 - T180 minutes after the onset of resuscitation; ROTEM® indicating an enhancement in clot initiation (a significant reduction in CT), whilst TEG® showed the opposite (significant increase in R-time). No change was detected in clot initiation in the O_2 group by either method.

12.5.2 Clot dynamics

There were no significant differences between groups (P=0.1082) or changes over time (P=0.2647) in clot dynamics assessed by ROTEM® (CFT) during the initial phases of the study (up to T30, Figure 12-7 A).

Furthermore, administration of rFVIIa or O_2 immediately after T30 did not cause a significant change in CFT (P=0.8131), so that at T60 there were no significant differences between groups (P=0.9381).

Again, because of loss of animals in the control groups, further comparisons could only be made between the rFVIIa and O_2 groups. During the period T60 – T180 there was a significant change in CFT over time (P<0.0001) and between groups (P=0.032) as CFT increased in the rFVIIa group but remained unchanged in the O_2 group.

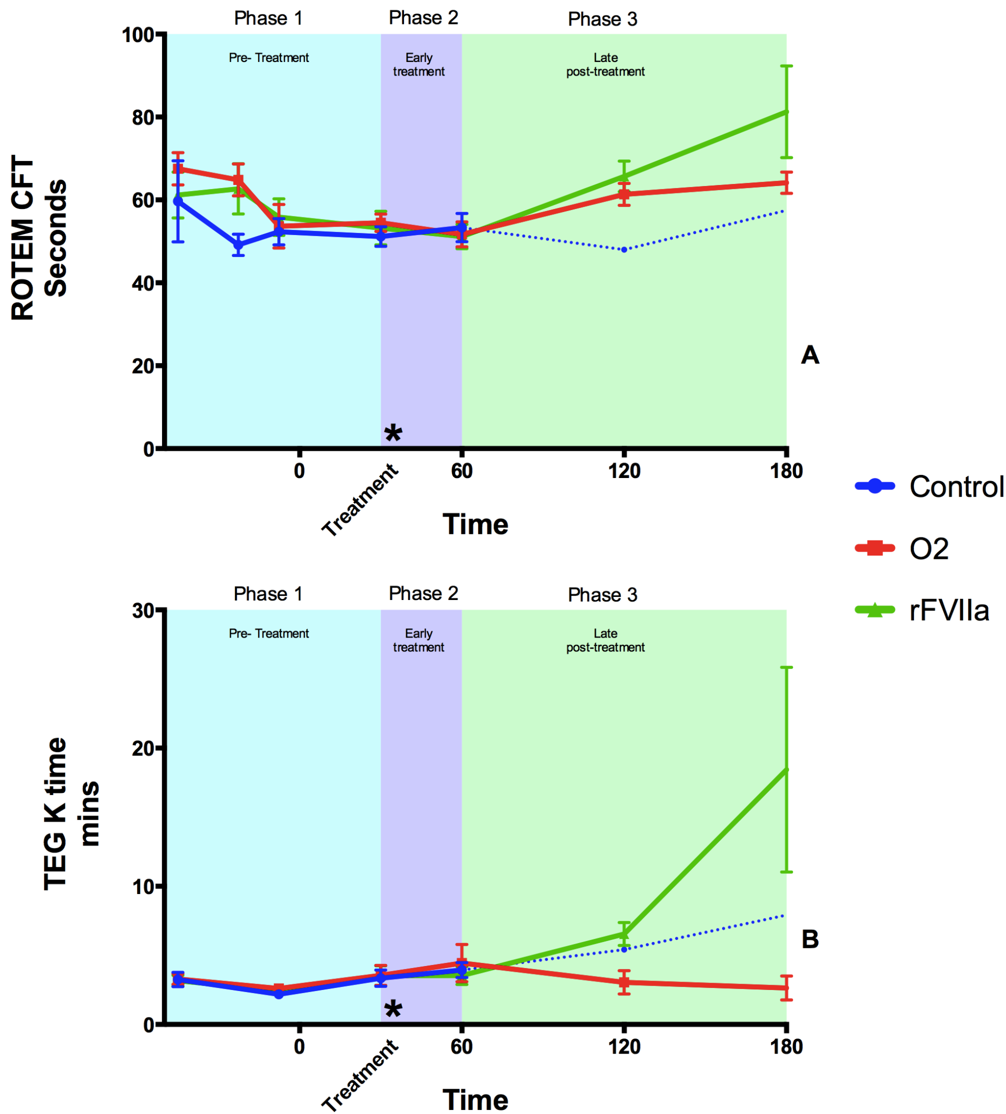

Figure 12-7. Measurement of clot dynamics in all three groups using standard ROTEM ® clot formation time (A) and dilute innovin TEG® (B). Mean +/- SEM. (For further details see Figure 12-2)

A similar picture was seen when clot dynamics was assessed using TEG® (K-time). There were no significant differences over time (P=0.6216) or between groups up to T30 (P=0.8599) (Figure 12-7 B). Administration of rFVIIa and O_2 did not cause a significant change in K- time (P=0.8262) so that there were no differences between the control, rFVIIa and O_2 groups at T60 (P=0.8324).

Thereafter analysis of the rFVIIa and O_2 groups revealed a significant difference between groups during the period T60 – T180 (P=0.0001) and a significant change over this time period (P=0.0001). As with the ROTEM® analysis the

change during T60 to T180 was due to a progressive increase in K-time in the rFVIIa group, whilst the O_2 group did not change.

In summary the main difference in clot dynamics was seen with both techniques and represented a reduction in clot dynamics (increase in CFT and K-time) in the rFVIIa group during the period T60 – T180, whilst the O_2 group remained unchanged.

12.6 Clot Strength

Clot strength assessed using ROTEM® (MCF) revealed no difference between control, rFVIIa and O2 groups (P=0.6587) or over time (P=0.1304) during the initial period up to T30 (Figure 12-8 A).

Furthermore immediately after administration of rFVIIa (between T30 and T60) there is no difference in clot strength between the groups (P=0.1203) or over time (P=0.4744).

Thereafter comparison could only be made between rFVIIa and O_2 groups in the period T60 – T180. Here there was a significant difference between groups (P=0.0105) and over time (P=0.0048). This difference was due to a fall in MCF in the rFVIIa group whilst it remained unchanged in the O_2 group. Due to loss of animals and failure to reach MA in a number of samples when assessed using TEG®, meaningful statistical analysis could not be performed for clot strength data using this technique. However a qualitatively similar pattern can be seen in TEG® derived data as in ROTEM® derived data (Figure 12-8 B).

In summary the change in clot strength was seen in the period T60 – T180 and represents a reduction in clot strength in the rFVIIa group whilst it remained unchanged in the O_2 group.

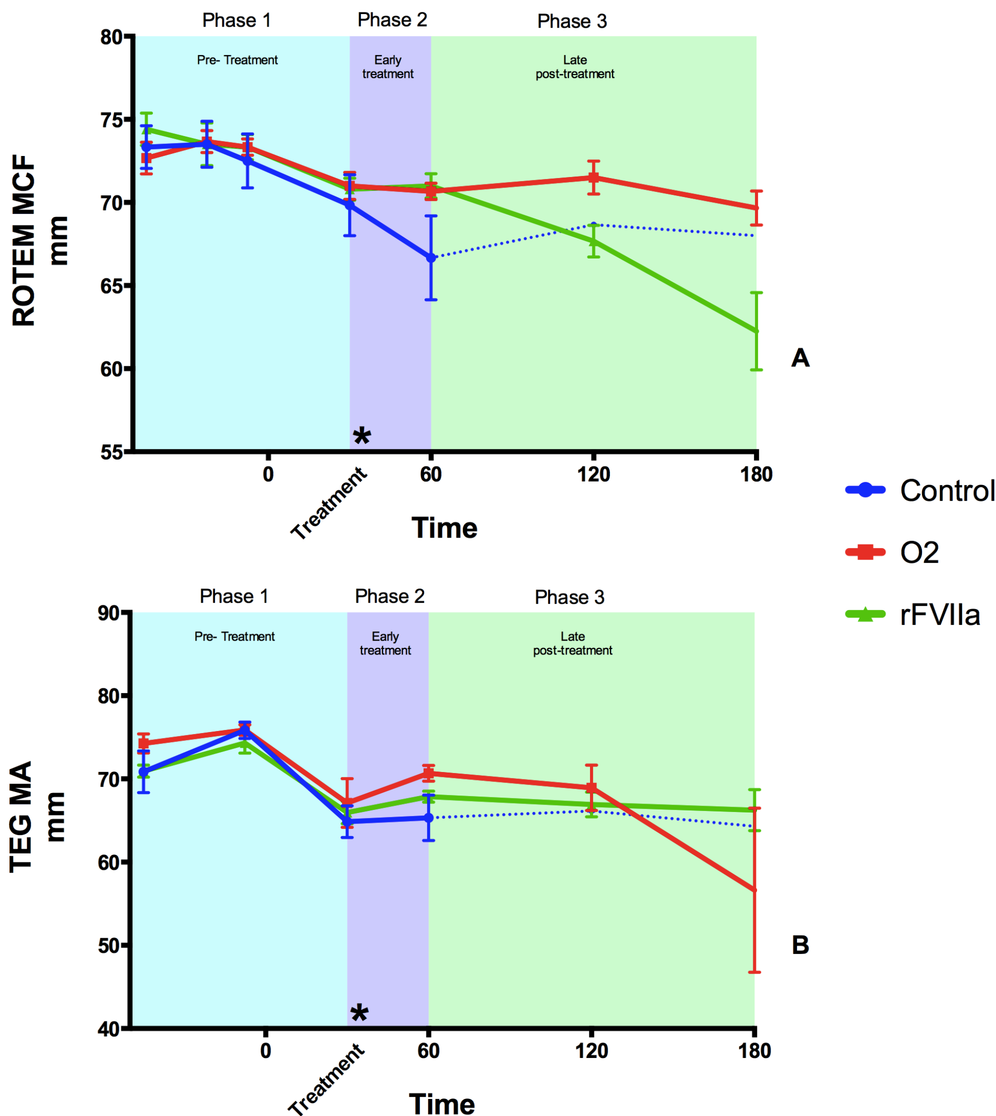

Figure 12-8. Measurement of clot strength in all three groups using ROTEM® maximum clot firmness (A) and TEG® maximum amplitude (B). Mean +/- SEM. (For further details see Figure 12-2)

12.7 Fibrinogen

12.7.1 Clauss fibrinogen

Assessment of fibrinogen levels with Clauss fibrinogen measurements during the initial phases of the study, showed no significant differences between groups (P = 0.1689), however there was a significant effect of time (P= <0.0001). This effect of time is immediately prior to onset of resuscitation at T0 and reflects the early effects of blast and haemorrhage on Clauss fibrinogen levels (Figure 12.9A).

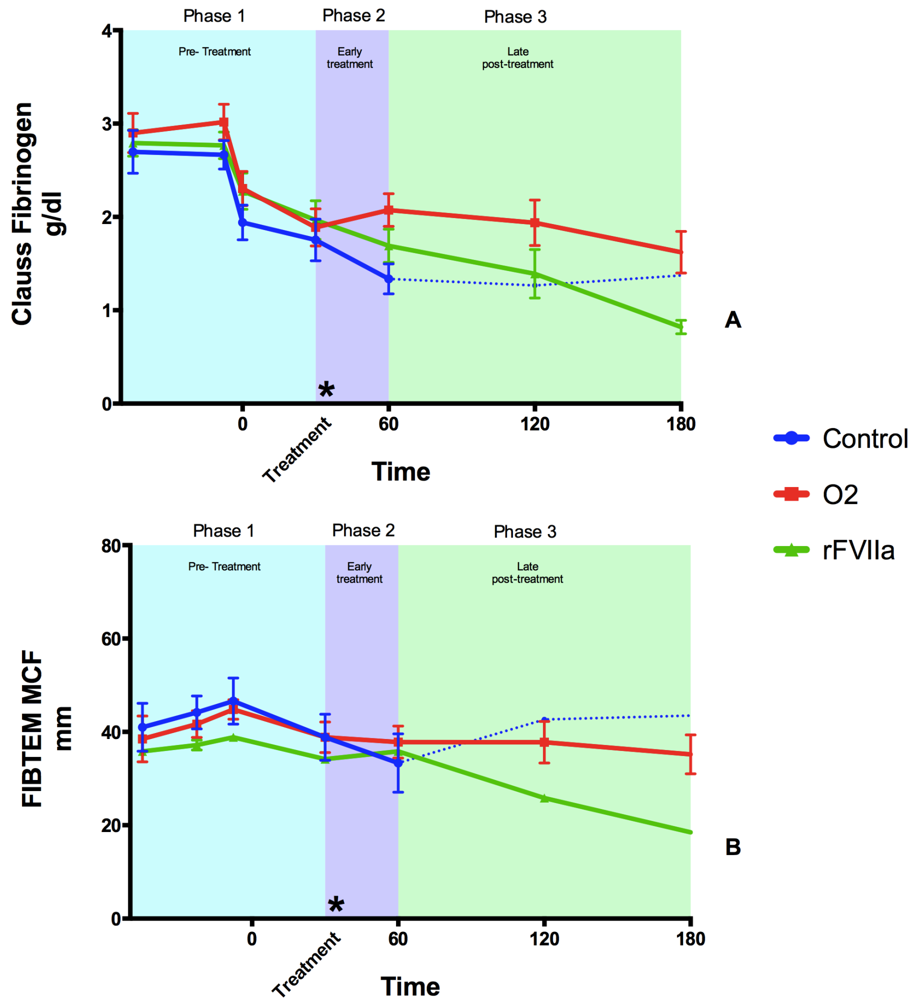

Figure 12-9. Measurement of fibrinogen levels in all three groups using Clauss fibrinogen (A) and FIBTEM maximum clot firmness (B). Mean +/- SEM. (For further details see Figure 12-2)

In the time period T30 to T60 there are no differences between groups (P=0.0853) and no effect of time (P=0.2962)

Comparing O_2 and rFVIIa groups only in the latter phase reveals a difference between groups (P=0.0057), however there is no difference over time (P=0.0653)

In summary there was a significant drop in CF levels after blast and haemorrhage in all groups reflecting activation of coagulation. Thereafter there was a progressive difference between O_2 and rFVIIa groups over time, particularly in the late phase of the study. During this time there was no

significant change over time, but there was a trend towards a decrease in CF levels.

12.7.2 FIBTEM MCF

Assessment of functional fibrinogen using FIBTEM MCF revealed no difference between groups in the pre-treatment phase of the study (P=0.646), and no significant change between baseline and 30 mins after the onset of resuscitation (p=0.8137) (Figure 12.9 B).

During phase 2 of the study there was also no significant difference between groups (P=0.6998) and no change during the first 30 mins after rFVIIa/ O_2 treatment (P=0.7864).

However in phase 3 comparing, O_2 and rFVIIa only, there was a significant difference between groups (P=0.0017) and a significant change over time (P=0.0213). This is characterised by a marked drop in FIBTEM MCF by T180 in the rFVIIa group, but not in the O_2 treatment group

In summary there is a significant fall in FIBTEM MCF in the rFVIIa group late after administration of rFVIIa.

12.8 Platelet count

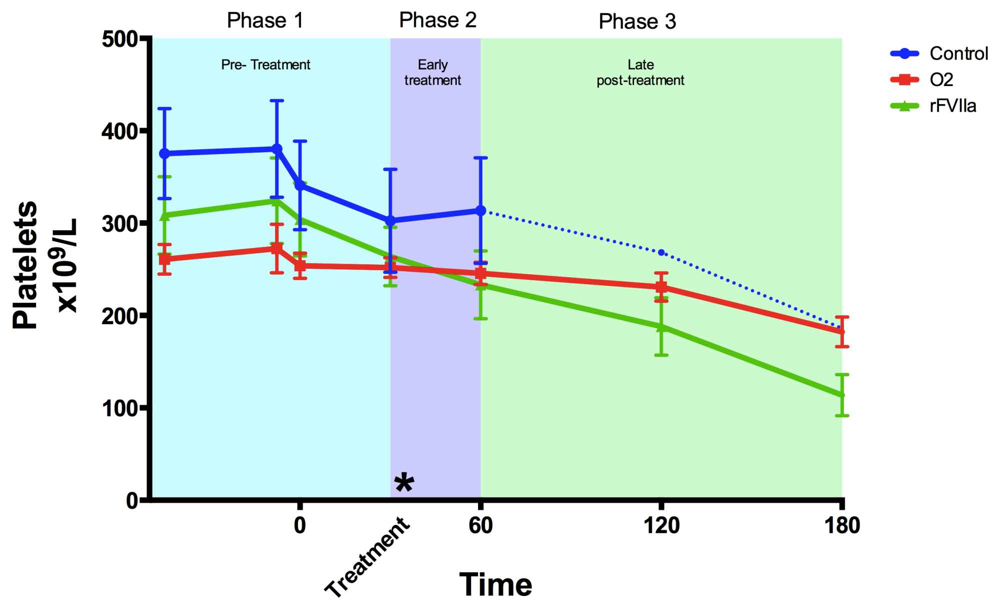

Figure 12-10. Platelet count in all three groups. Mean +/- SEM. (For further details see Figure 12-2)

There was a significant difference in platelet count during phase 1 (P=0.0105), but not in phase 2 (P=0.2086). This difference was due to a higher value in the control group compared to the O_2. Overall there were no changes in time during the initial phases (P=0.894 and 0.9222 respectively) (Figure 12.10).

In the late phase, comparing O_2 and rFVIIa groups only. There was no difference in groups (P=0.0663) however there is a significant effect over time (P=0.0099).

In summary the main changes in platelet count is a progressive reduction in platelet numbers over time but no difference between groups.

12.9 Hct

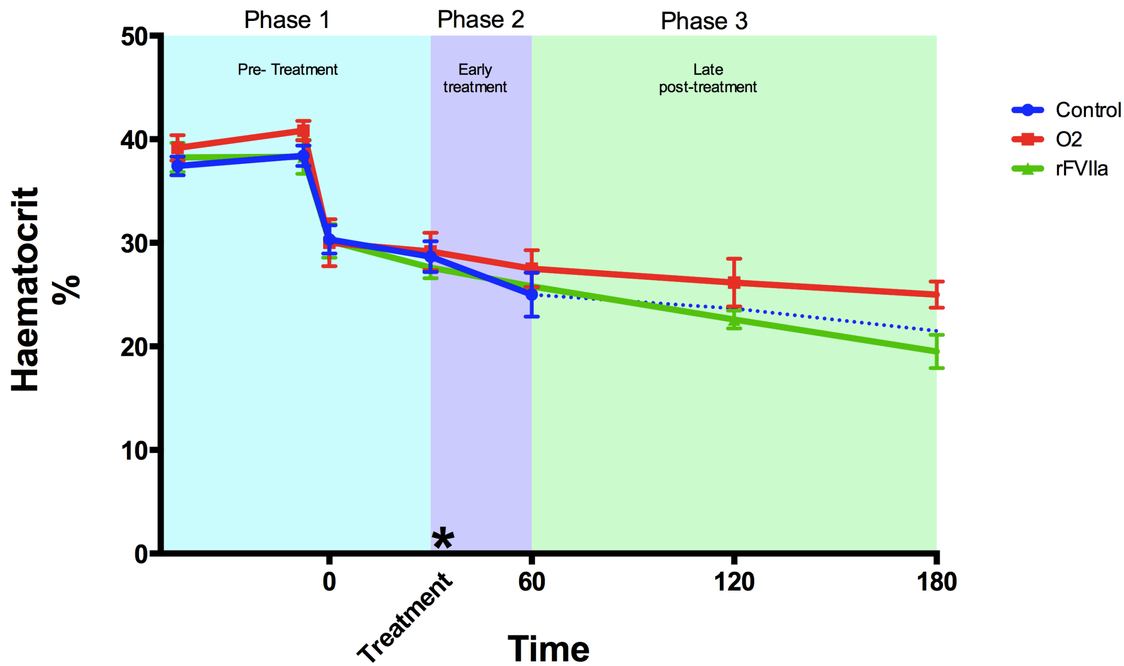

Figure 12-11. Measurement of haematocrit in all three groups. Mean +/- SEM. (For further details see Figure 12-2)

There were no significant differences between groups in haematocrit during Phase 1 (P=0.4537), however there were significant changes over time in this Phase (P<0.0001), with a sharp fall in haematocrit in all groups associated with haemorrhage and the shock phase (Figure 12.11).

It is of note that the greater part of the fall in haematocrit had occurred before the onset of resuscitation at time 0. Thereafter there was a continued trend of a fall in haematocrit during Phase 2 in all groups, although this did not attain statistical significance (P=0.2891) and, again, there were no significant differences between groups (P=0.5128). Comparing the O_2 and rFVIIa groups only during Phase 3 there was no significant difference between the two groups (P=0.0544), although there was a significant change over time during this late post-treatment period (P=0.0384).

12.10 Follow up analysis of Individual clotting factors and natural anticoagulation

Due to the numbers of samples available for individual factor assays it is only possible to report results in the following way.

All groups (Control, O_2, rFVIIa) were assessed at the experimental baseline and T60 (30 mins after treatment with O_2 or rFVIIa). The remaining comparison was only able to made between the O_2 and rFVIIa for the pre-treatment phase, (baseline to T30), the early treatment phase (T30 to T60) and late post-treatment phase (T60 to T120) only. This is due to the amount of stored plasma available for the follow on analysis and is discussed in the methods (section 13.9).

12.10.1 FX

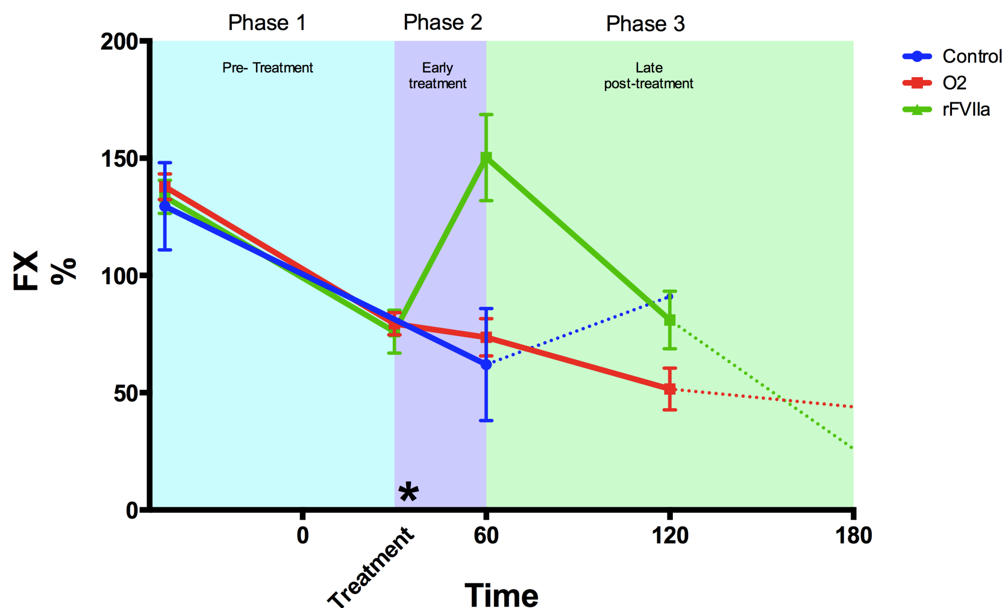

Figure 12-12. FX assay in all three groups. Special considerations in interpreting rVIIa group must be considered when intepreting the results. (see section 13.10) Mean +/- SEM. (For further details see Figure 12-2)

Looking at the pre-treatment phase and the early treatment phase (baseline and T60) in all 3 groups (control, O_2 and rFVIIa), there was a significant difference

between groups (P=0.0128) with a significant effect of time (P=0.0014) (Figure 12.2). The difference lies at T60 in the rFVIIa group.

Looking at the rFVIIa and O_2 only, in the pre-treatment phase (baseline to T30) there was no difference between groups (P= 0.5648), however the effect of time was significant (P<0.0001). This was due to a drop in FX levels following the blast injury and controlled haemorrhage and the initial 30 minutes of hypotensive resuscitation, and may reflect a consumption of FX due to the activation of coagulation.

In the early treatment phase, T30 to T60, there was a significant effect of group (P=0.0035) and a significant effect of time (P=0.0011). This was due to a difference in the rFVIIa group at T60

Thereafter from T60 to T120 there remained a significant difference between groups (P=0.0036) and a significant effect of time (P=0.0005). This was due to a difference in the rFVIIa group, which was declining rapidly over time so that by T120 there was no difference between the groups.

In summary FX levels were significantly higher in the IV rFVIIa treatment group compared to the other two groups at T60. Thereafter there was a rapid decline in FX levels in the rFVIIa group between T60 and T120 so that by T120 the levels in the rFVIIa and O_2 groups were not significantly different. It should be noted that there are special considerations which must be made when interpreting the results in animals given a therapeutic dose if rFVIIa which are discussed in section 13.10).

12.10.2 FV

Looking first of all at all 3 groups at the experimental baseline and T60 (30 mins after treatment with IV rFVIIa or O_2), there was no significant difference between groups (P=0.2824), however there is an effect of time (P=0.0127) (Figure 12.13). The lack of plasma available for analysis is probably the reason for the lack of statistical significance in this phase. FV levels did appear to be

high at T60 in the rFVIIa treatment group. Lack of significance between groups may be due to the small number of plasma samples available for assay.

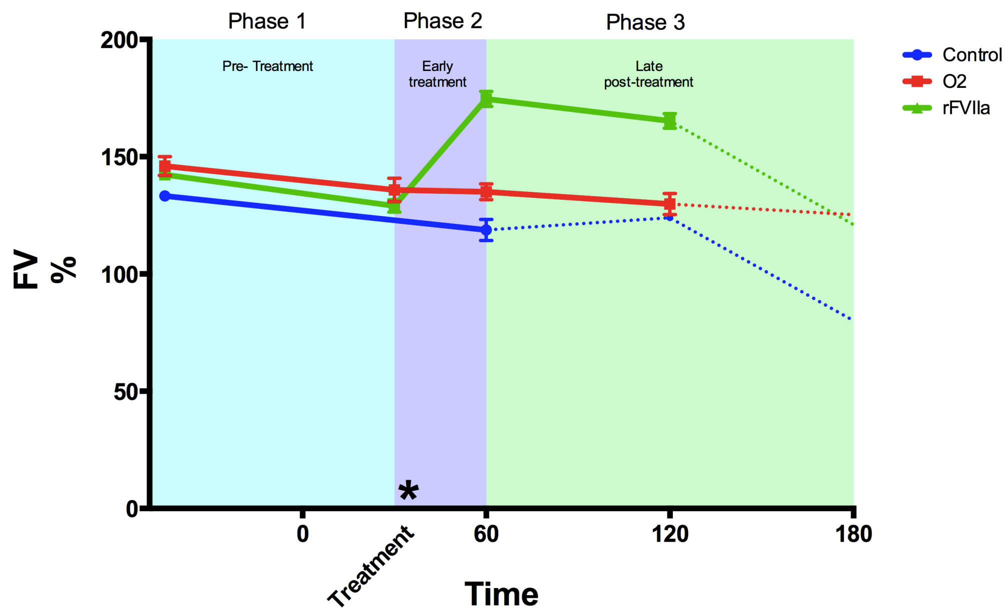

Figure 12-13. FV assay in all three groups. Special considerations in interpreting rVIIa group must be considered when intepreting the results. (see section 13.10). Mean +/- SEM. (For further details see Figure 12-2)

Looking at the O_2 and rFVIIa groups only in the pre-treatment phase, there was no difference between groups (P=0.1508) and no effect of time (P=0.6499)

In phase 2, however, there was a significant effect of group (P=0.0066) and a significant effect of time (P<0.0001) due to a rise in FV levels in the rFVIIa group.

Finally in the late post-treatment phase (T60 to T120) there remained a significant difference between groups (P<0.0001) and a significant effect of time (P<0.0001) and a rapid decline of FV levels in the late post-treatment phase.

12.10.3 FII

Looking at al 3 groups at baseline and T60, there was a significant difference between groups (P<0.0001) and a significant effect of time (P<0.0001) (Figure 12-14). Similar to FX and FV assays, the difference is due to the high levels of FII in the rFVIIa treatment group at T60.

Looking at the rFVIIa and O_2 only: in the pre-treatment phase, there was no significant effect between groups (P=0.8133), however there is a significant effect of time (P<0.0001). This is due to a drop in FII levels following the blast injury and controlled haemorrhage and the initial 30 minutes of hypotensive resuscitation, and may reflect a consumption of FII due to the activation of coagulation.

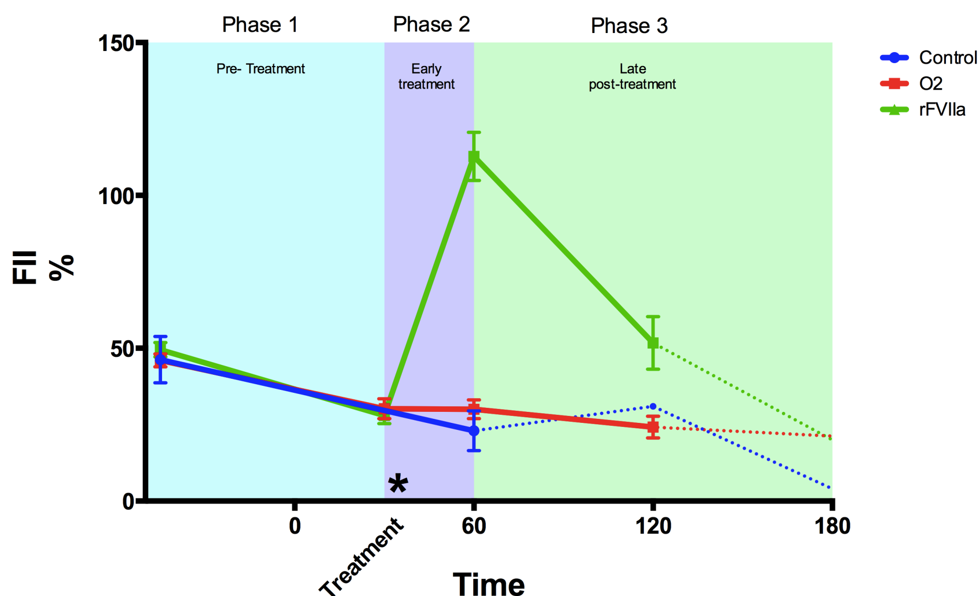

Figure 12-14. FII assay in all three groups. Special considerations in interpreting rVIIa group must be considered when intepreting the results. (see section 13.10). Mean +/- SEM. (For further details see Figure 12-2)

In the early treatment phase there was a significant difference between groups (P<0.0001) and a significant effect of time (P<0.0001). The early treatment phase likewise showed significant difference between groups (P<0.0001) and a significant effect of time (P<0.0001), which is the same for the late post-treatment phase, a significant difference between groups (P<0.0001) and a significant effect of time (P<0.0001). Similar to the other factor assays the

difference is due to a rise of FII at T60 in the rFVIIa treatment group, followed by a rapid decline to T120.

In summary the assessment of factor levels is complicated due to the administration of rFVIIa at T30 in the rFVIIa group. This means that the assay is performed with an unknown concentration of the factor in question, and an unknown concentration of the rFVIIa administered at T30. Thus the levels in the rFVIIa group qualitatively reflect the action of rFVIIa and the factor in question, rather than simply a quantitative assessment of the factor itself. This does not allow meaningful comparison of absolute levels between groups, but does reflect the effect that the rFVIIa has on the coagulation system *in vivo*. The only conclusions that can be drawn from these results are that the action of rFVIIa causes a “boost” in activity beyond T30, which declines rapidly by T120.

12.10.4 FVII:

There was not enough plasma to obtain enough results to allow statistical comparison and so no comparisons were made

12.10.5 HS, TFPI, Syndecans

For all of these assays the results fell well below the lower limit of the detection range for each assay. Since the assays are research assays for human plasma, it is possible that these assays are not suitable for porcine blood. The results have therefore not been analysed

Chapter 13. Discussion

13.1 Introduction

The principal findings of this study were that blast and haemorrhage in a hypotensively resuscitated pig model leads to acute trauma coagulopathy characterised by a progressive deterioration in coagulation parameters. As expected, the administration of intravenous (IV) rFVIIa confers a short “boost” in clotting, however it was followed by an exaggerated deterioration in coagulation. This exaggerated deterioration, superficially, is a surprising result but has potentially important implications for military resuscitation. In a military context with limited logistical support and finite amounts of blood products, there is a potential role for the use of rFVIIa as a resuscitation adjunct to “boost” clotting and reduce blood loss (see section 7.2.1). If the administration of rFVIIa causes a late deterioration in clotting, albeit when no further treatment was given in the current study, then the use of rFVIIa may be contraindicated. Potential causes of the deterioration in the rFVIIa group are consumption of clotting factors, increased expression of natural anticoagulation or the presence of acidosis. The limitations of this study make it impossible to categorically differentiate between these factors as the underlying cause, although consumption appears to be most likely.

In the current study, coagulation deteriorated over time, implying that prolonged hypotension is detrimental to clotting. In a clinical setting, therefore, the ongoing presence of coagulopathy may be symptomatic of ongoing microcirculatory shock caused by under resuscitation, even if appropriate blood product replacement has occurred using local transfusion policies. If a clinician is able to rapidly assess coagulation and assess the results together with other clinical information, the presence of ongoing coagulopathy may point towards an under resuscitated patient.

The following discussion will lay out the evidence for these conclusion, address the limitations of this study and outline future work to expand and corroborate the findings.

13.2 Survival

This study formed part of a survival study based on the investigation of O_2 or rFVIIa as a treatment of blast lung. The survival in the O_2 was significantly higher that in either the rFVIIa or control group rendering comparisons between groups in the late phases of the study more difficult. This represents one of the limitations of this study.

13.3 The injury Model

The injury model was successful in producing representative aspects of a battlefield injury, incorporating blast injury and haemorrhagic shock with the ensuing trauma induced coagulopathy.

The blast loading was of sufficient magnitude to produce significant lung contusions and a clinically significant fall in PaO_2. The presence of blast lung was confirmed at post mortem. At the same time, the blast loading was not overwhelming and compatible with survival of the animals.

The combination of the blast and the haemorrhage produced significant changes in arterial oxygen saturation (SaO_2), venous oxygen saturation (SvO_2) and in arterial base excess (ABE). The low SvO_2 and ABE confirmed the presence of shock in the animals. In this model the severity of the shock is of sufficient magnitude to cause acute trauma coagulopathy.

The liver snare was deployed successfully and amputated the desired section of the liver. This was confirmed at post mortem. The amputated section was a “clean” wound with relatively little tissue damage. There was a robust clot on the remaining liver, also confirmed at post mortem... Since the amputated section of the liver amounted approximately 6% of liver mass, it is unlikely to consume a

significant amount of clotting factors. Also the liver injury was performed in all groups prior to treatment with either O2 or rFVIIa and in any case a well developed clot would have formed within the 30 minutes between the deployment of the liver snare and the administration of either O2 or rFVIIa. The clot size and characteristics were the same between all groups with no evidence of on-going bleeding in any group and is therefore unlikely to be the cause of any differences between groups.

13.4 Injury Burden

Shock is defined as the inability of whole body oxygen delivery to meet the metabolic demands of the tissues. After trauma, shock can occur due to inadequate perfusion of the tissues due to a fall in cardiac output after haemorrhage, or inadequate oxygenation due to altered gas exchange in the lungs. In this model the shock occurs due to both the effects hypovolaemia after the 30% blood volume haemorrhage and the effects of blast lung decreasing oxygen diffusion in the alveoli.

As oxygen delivery to the tissue falls, the body tries to compensate by extracting more O_2 from the blood resulting in less O_2 at the venous end of the circulation measured by the SvO_2. When oxygen supply does not meet the demands of the tissue the body is unable to produce energy via aerobic respiration, and relies instead on anaerobic respiration producing lactic acid as a byproduct. This metabolic acidosis is reflected as a fall in base excess and a fall in pH.

13.4.1 Acute Trauma coagulopathy in relation to oxygen delivery and shock.

In the current study there was a noticeable drop in the SaO_2 around the time of the blast and the haemorrhage. The SaO_2 then remained at low levels throughout the remainder of the experiment in the rFVIIa and control group (around 80%), however they were returned to normal levels in the O_2 group once supplementary oxygen was given. This is a clinically significant difference.

SaO_2 determines the oxygen saturation of the inflow to the microcirculation whereas SvO_2 reflects the oxygen saturation of blood leaving the microcirculation and therefore represents the oxygen “left over” after the tissues have extracted all they need. SvO_2 reflects oxygen delivery and oxygen uptake; if SvO_2 is low, in spite of a high SaO_2, then the oxygen supply is still unable to meet the oxygen requirement of the tissues and in the presence of a simultaneous metabolic acidosis, microcirculatory shock is present.

During the late phase of this study the rFVIIa group had SvO_2 levels around 25%, and the O_2 group had levels around 30 %. In humans, in the intensive care unit, SvO_2 rarely falls below 60% and sustained low levels are associated with increased mortality, with levels around 50% deemed critical (Rivers et al., 2001). In both the O_2 and the rFVIIa group, the SvO_2 levels are significantly lower than 50% and are so low that microcirculatory shock is present in both groups. Microcirculatory shock is thought to be the driver for ATC (Gruen et al., 2012; Curry et al., 2012) (Section 3.4.1). Since the difference in SvO_2 between the O_2 and rFVIIa groups is likely to be clinically insignificant as far as the effects on the microcirculation is concerned, it is unlikely that the difference in SaO_2 is responsible for any differences in coagulation between groups. This view is confirmed by a change in OER to maximum levels during shock, with little difference between groups during subsequent treatment. This means that the tissues are extracting maximal oxygen from the blood, which is unable to meet the metabolic demands of the tissues (confirming shock) and is the same in all groups in spite of the O_2 group receiving oxygen.

However, in spite of low SvO_2 confirming the presence of microcirculatory shock in both the O_2 and the rFVIIa groups, the O_2 group does have a clinically significant increase in SaO_2. This leads to an improved survival in the O_2 group and is therefore exerting a clinically significant effect at a metabolic level. It is therefore not possible to exclude the administration of O_2 after T30 as a potential reason for any improvement in clotting indices compared to the rFVIIa group.

Most of the studies that examine the aetiology of ATC do not relate either SaO_2 or SvO_2 to the development of ATC (Brohi et al., 2008; Curry et al., 2012; Gruen

et al., 2012). The studies discuss poor tissue perfusion and tissue hypoxia as a potential driver for ATC and appear to use these terms interchangeably. A low SaO_2 or low SvO_2 confirms poor tissue oxygen delivery, which leads to anaerobic respiration, and subsequently to acidosis with a low pH or a low ABE. These studies therefore use ABE as a marker of tissue shock.

13.4.2 Arterial Base excess

Base deficit or negative arterial base excess or are the most described parameters when relating trauma induced coagulopathy with evidence of tissue shock. An ABE <-6mM is generally accepted as a definition of hypoperfusion (Brohi et al., 2007b; Curry et al., 2012) and linked to the development of ATC. Brohi et al demonstrated that as ABE worsened, so PT and aPTT increased, as did levels of enzymes involved in the activated protein C pathway. He did not further categorise data beyond an ABE of -7.7mM and therefore it is impossible to determine how coagulation changes as ABE worsens beyond 6mM.

Other studies have attempted to stratify the degree of hypoperfusion based on ABE. Jansen et al (Jansen et al., 2011) further subdivided patients in ABE groups of normal perfusion (ABE 0 to-6mM), moderate hypoperfusion (ABE -7 to -12mM) and severe hypoperfusion (ABE <-12mM). Whilst there were significant differences between groups in factor levels of FI, VII, IX, X and XI the majority of values were in the normal range. In addition, it appears that the difference is between the severe hypoperfusion group and normal group. This study concluded that there was a relationship between ABE and factor levels, however the association is only moderate and the effect on coagulation tests such as PT or aPTT was not reported. It therefore remains uncertain what effect a progressively worsening base deficit has on coagulation levels beyond a base deficit of 6mM.

In the current study ABE levels are significantly different between groups after T30 reflecting administration of O_2 treatment, The Control and rFVIIa groups have ABE of -14 to -18 in phase 2 and 3, whereas the O_2 group had a ABE of -10 to -12. Whilst the difference may be statistically and may even be clinically significant, all 3 groups fall in the category of “hypoperfusion’ or “severe

hypoperfusion" as defined by Brohi and other authors. Mutshler et al (Mutschler et al., 2013) performed a retrospective analysis of the German trauma registry and determined that as ABE worsened so mortality increased. He demonstrated that an ABE between -6 and -10 mM had a mortality of 23.9%, whereas an ABE <-10 mM had a mortality of 51.5%. It is unknown what ABE is the threshold for hypoperfusion in pigs, however Garner et al (Garner et al., 2010) in previous work at DSTL demonstrated that prolonged hypotension and worsening ABE was associated with an increased mortality. He didn't directly relate ABE to mortality, however interpreting the presented results suggests that an ABE of around -10mM in Garner's study was associated with a mortality of around 50%, comparable with humans. The similarities between pigs and humans in terms of mortality, would suggest that it would not be unreasonable to assume an ABE <-6 in pig represents the same degree of hypoperfusion as seen in humans. In the current study, the ABE was well below -6mM and therefore represents hypoperfusion in all groups.

13.4.3 pH

Acidosis is known to effect clotting and the activity of rFVIIa (See section 7.3) on phospholipid membranes, however the exact level at which pH has an effect is uncertain. Decreased thrombin generation has been shown to occur at a pH of 7.1 in pigs (Curry et al., 2012; Martini and Holcomb, 2007), however does not appear to affect clot initiation measured either by TEG® or PT (Martini et al., 2005; Viuff et al., 2008). In Martini's studies the pH is induced to 7.1 with an infusion of HCl and is not physiological acidosis, and Vuiff's study with an acidic buffer. Other authors suggest problems occur at a pH of only 7.2 (Gruen et al., 2012; Meng et al., 2003). What is well accepted, however, is that a reduction of pH will affect platelet activity (Djaldetti et al., 1979; Etulain et al., 2012), microparticle formation (Etulain et al., 2012) and coagulation factor activity (Curry et al., 2012). The efficacy of rFVIIa at a pH <7.2 has been questioned (Meng et al., 2003; Knudson et al., 2011) and recent clinical recommendations suggesting restoring pH to at least 7.2 to ensure efficacy of rFVIIa action (Spahn et al., 2013). However other experimental work has suggested that that rFVIIa will work even in the presence of a physiological lactic acidosis in a pig

model (pH 7.14) (Lesperance et al., 2012) or an *in vitro* induced pH of 6.8 (Viuff et al., 2008).

In the current study the pH is between 7.05 and 7.22 in phase 2 and 3 in all groups. There is a statistical difference between the rFVIIa group (being lower) and the O_2 group (being higher). The question is "does that difference in pH make a clinical difference in coagulation?" Both groups fall into the pH range (7.0 to 7.2) where uncertainty exists as to the exact effect on coagulation. Therefore, because of this uncertainty, it remains possible that the difference in pH seen between the O_2 and the rFVIIa groups might explain any differences seen in coagulation between the groups.

In summary, SaO_2 is significantly different between groups in statistical and clinical terms, which has resulted in improved survival in the O_2 group. The SvO_2, is also significantly different between the groups although the level has fallen so low that the difference between groups is unlikely to be of clinical significance. There is a statistical difference in the ABE and pH between groups, which is likely to be the consequence of the increased arterial oxygen content/ delivery in the O_2. This difference may well be clinically significant, although its effect on clotting is not clear. It is therefore not possible to rule out the effects of acidosis as an explanation for the differences seen in clotting between the O_2 and rFVIIa groups.

13.5 The effect of dilution on clotting

Dilution of clotting factors with either infused fluids, or movement of fluid from the extracellular to intravascular space, is known to affect clotting. Maegele et al (Maegele et al., 2007) noted that the incidence of coagulopathy after trauma increased with the volume of fluid administered. However the coagulopathic group also had significantly higher injury severity scores. The incidence of coagulopathy increases as injury severity score increases (Brohi et al., 2003) and so it is not possible to rule out the effect of worse injury as the cause of the increased coagulopathy in Maegele's study.

Bolliger et al (Bolliger et al., 2009) studied the effects of dilution on thrombin generation. He diluted clotting factors to about 20% of baseline, although he maintained haematocrit at 20 – 23%. He noted that peak thrombin generation was reduced by about 50% after dilution, however the lag time remained unchanged. Based on these findings it would be expected that dilution would effect clot dynamics and/or clot strength, but would not affect clot initiation.

In the present study, there is no difference in Hct between groups, although there is a difference over time. Some very dilute samples, due to the protocol driven increase in fluid administration in the terminal phase of an individual animal, were excluded as these data point were not representative of the study as a whole (see section 11.6)

In phase 1 of the experiment there is a significant effect over time in all groups. This difference is, prior to treatment with either O_2 or rFVIIa, was as a consequence of the blast and haemorrhage and would be expected.

The effects of dilution would not simply affect the Hct, but also the factor levels including the fibrinogen. An estimate of the degree of haemodilution at any timepoint can be made from the proportional fall in haematocrit from baseline in each animal. This figure can then be applied to each animal's baseline fibrinogen level to predict the fibrinogen level that would be seen at each timepoint simply as a result of haemodilution. If this predicted figure is subtracted from the actual (observed) fibrinogen level an evaluation can be made regarding the impact of haemodilution on fibrinogen level. A difference of zero would imply that the observed fibrinogen level is likely to be due simply to haemodilution, while a negative difference would indicate that the observed value is lower than would be predicted simply by the effects of dilution i.e. an additional process such as consumption may also be playing a part. These data are plotted in Figure 13-1.

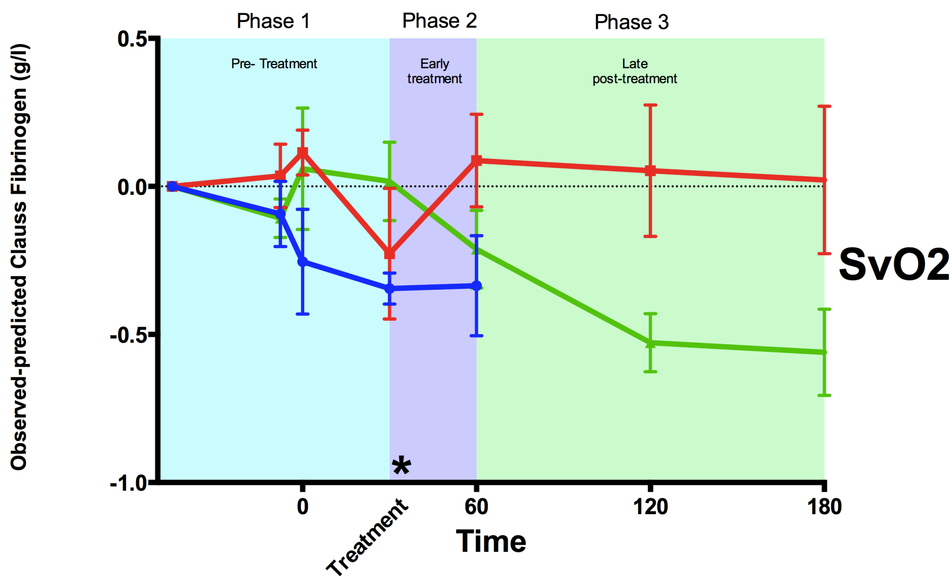

Figure 13-1. Arithemetic differences between observed Clauss fibrinogen levels and values predicted based upon resuscitation-induced haemodilution (assessed by fall in haematocrit). Mean +/- SEM.

During Phase 1 and Phase 2 there was no significant difference between groups (P=0.1118), nor any significant difference from zero or change over time (P=0.4808), suggesting that the fall in fibrinogen levels seen during this period is principally the effects of haemodilution in all groups. In Phase 3 it is only possible to compare the IV rFVIIa and O_2 groups up to T120, due to loss of data points in the Control group and at T180 in the IV rFVIIa group. During this period there is a significant difference between the IV rFVIIa and O_2 groups (P=0.0176) due to the IV rFVIIa group falling significantly below zero at T120 (P=0.0058) while the O_2 group does not (P=0.8198). The implication from these findings is that the fall in fibrinogen levels in the IV rFVIIa group during Phase 3 (Figure 12.9 A) is due to some process(es) in addition to simple haemodilution, while the fall in fibrinogen in the O_2 group can be accounted for by dilution alone. Caution must always be attached to analyses such as this one where group sizes are small, which renders statistical analysis and interpretation difficult. These data would, however, be consistent with the suggestion that there is additional consumption in the IV rFVIIa group during Phase 3, which is not occurring in the O_2 group, although a firm conclusion could only be drawn with a study over an extended timeline with greater numbers. Since we do not have a sufficient dataset in the Control group it is impossible to ascertain whether it is the IV rFVIIa that has caused accelerated consumption or O_2 that

has attenuated consumption. However, it is likely that O_2 is the better treatment compared to IV rFVIIa with respect to fibrinogen levels in Phase 3.

In summary dilution does not appear to explain the difference between the IV rFVIIa and oxygen groups in the late past-treatment phase. Any dilution that did occur would be expected to affect clot dynamics and would not explain any differences seen in clot initiation between groups. Comparing fibrinogen levels with estimated dilution, suggests that dilution alone does not explain the fall in fibrinogen levels.

13.6 The rationale for using dilute Innovin to measuring clotting

The rationale for the use of dilute Innovin as a reagent for TEG® analysis comes from the presumption that the increased sensitivity is likely to detect changes not seen in the PT or ROTEM®.

PT and ROTEM® are designed so that the amount of tissue factor used overwhelmingly activates all the clotting factors present in the sample. However visible fibrin clot develops *in vivo* after only 3-5% of total thrombin is produced, the remaining 95% of thrombin production occurring after clot formation has started (Young et al., 2013).

Sørensen et al looked at ROTEM® activation using different dilutions of TF (diluted from 1:17 to 1:34 000) (Sørensen et al., 2003). Sørensen and colleagues compared blood from haemophiliacs, who clinically had slow, depressed clotting, with that from normal volunteers. Both groups of patients had identical results when high concentrations of TF were used. However lower concentrations of TF revealed differences between the groups that were commensurate with the clinical effects of haemophilia. He found the best dilution to be 1:17 000 providing enough initiation to activate the test in a useful time, but sensitive enough to detect differences between groups. He concluded, therefore, that ROTEM® initiated with dilute TF may reflect better the haemostatic potential in patients with suspected impaired haemostasis.

Ganter et al (Ganter et al., 2008a) investigated the optimum way to monitor the effects of rFVIIa. He recommend that to detect the effects of rFVIIa, only dilute amounts of TF should be used for best sensitivity since high amounts of TF may directly activate coagulation and bypassing the requirements for FVIIa. He recommended a dilution of 1:1000 to effectively monitor rFVIIa therapy. When Comparing Sørensen's dilution and Ganter's dilution, it should be remembered that Sørensen described a final concentration of 1:17 000 . Ganter added 20µl of 1:1000 TF to 340µl of blood giving a final concentration of 1:17 000. In this current study the final dilute innovin concentration was 1:50 000, comparable in magnitude to Sørensen and Ganter and therefore is likely to be effective at monitoring rFVIIa therapy.

13.7 Assessment of clotting

In this section I shall deal with all the aspects of clotting (initiation, dynamics and strength) in turn before considering the process as a whole, which is often an easier way to conceptualise the process.

13.7.1 Clot initiation

The changes in PT and ROTEM® CT are broadly the same, particularly in the early phases of the experiment. They demonstrate no real clinically significant difference between the groups up until T30. Thereafter the IV rFVIIa group shows a marked reduction in the time taken for clot initiation. This effect would be expected since rFVIIa works by increasing thrombin generation and decreasing the lag time for thrombin generation, either via its TF dependent pathway, initiating clot via the TF-VIIa pathway, or by its TF independent pathway directly producing FXa from the activated platelet (Grounds et al., 2006).

In the late phase of the study observed from T60 through to T180 the O_2 and rFVIIa groups only, show differences between the PT and ROTEM®. PT shows a marked deterioration in the rFVIIa group over time. ROTEM® however, showed no difference over time in the rFVIIa group.

PT and ROTEM® differ in that PT is performed in platelet poor plasma. ROTEM®, by contrast, uses whole blood and therefore contains platelets as well as other cellular components. Since rFVIIa works, in part, by releasing thrombin from the activated platelet, it is possible that the difference in the PT and ROTEM® trend over this time is due to the ongoing, albeit increasingly weak, stimulation of thrombin release from the platelets in the ROTEM® sample.

In contrast, the dilute Innovin TEG® shows a different finding from PT and ROTEM®. The dilute Innovin TEG® was performed as it is known to have an increased sensitivity to coagulation changes, particularly in abnormal blood (See section 13.6). The initial assumption was that the dilute Innovin TEG® would mirror ROTEM® results, although the changes might be detected earlier in the experiment, however the results showed a surprising, opposite effect.

Initially, up to T60, there were no differences between groups, or over time, and the Innovin TEG® results mirrored ROTEM® and PT. However in phase 3 of the study (T60 – T180) TEG® R time increased significantly (demonstrating a deterioration in clot initiation). This result is, on the face of it, different from ROTEM® and PT.

A possible explanation is that the amount of TF present in PT and ROTEM® is sufficient to maximally stimulate all the available clotting factors in the sample. It may well be that the dilute Innovin TEG® is detecting a change masked by the concentration of TF in the ROTEM® and PT. The increased sensitivity of the dilute Innovin TEG® might therefore be performing as was originally intended, and picking up changes in the coagulation system earlier than ROTEM® or PT.

A final consideration for the difference between the TEG® results and ROTEM® is also due to the increased sensitivity of the dilute Innovin TEG®. Whilst the

pathophysiology of ATC remains unclear, microcirculatory shock and the hypoxic endothelium as a driver for the altered balance between coagulation, natural anticoagulation and fibrinolysis is increasingly accepted as the likely cause. It is plausible, therefore, that the dilute innovin TEG® is detecting the effects of the natural anticoagulants such as tissue factor pathway inhibitor (TFPI), anti thrombin (AT) and heparans.

It is possible that the dilute innovin TEG® is sensitive to the effects of TFPI. TFPI acts by complexing with FXa and inhibits the TF-VIIa complex in a FXa dependent manner (Lupu et al., 1995) (see section 2.6.1) It is therefore possible that the extra FXa, produced after the administration of rFVIIa, complexes with the increased TFPI produced from the activated endothelium, which may exert an exaggerated natural anticoagulant effect causing delayed clot initiation and a prolonged R -time. This is highly speculative.

Increased TFPI levels have been shown to affect clot initiation and dynamics. Audu et al. (Audu et al., 2006) investigated the effects of TFPI on TEG® kinetics and found that addition of TFPI (in large doses, twice normal concentration) delayed clot initiation (prolonged R time). Dahm et al. (Dahm et al., 2005) looked at the difference between standard TFPI assays (ELISA) and the dilute prothrombin assay using dilute tissue factor. He found that the dilute prothrombin assay was sensitive to the effects of full-length TFPI chains that are released from the endothelium after stimulation by thrombin.

Prolongation of R time is also a feature of heparin administration, which acts in conjunction with AT. Whist none of these animals received heparin, there is a possibility that they have increased heparans produced after endothelial damage and activation following trauma and microcirculatory shock.

Heparans act in a similar way to heparin and also act in association with AT and are also a potent inhibitor of clotting. It may be that the dilute innovin TEG® is detecting the heparin like effect of heparans produced in ATC.

Liener et al. (Liener et al., 2001) looked at AT activity after trauma and found an initial drop in AT activity in blood samples drawn immediately at the site in

injury, followed by a rapid rise in activity after admission to hospital, despite no administration of AT. This effect was most significant in the more severely injured group and the rise in activity to near normal levels occurred within the first 2 hours. He speculated that the initial drop is due to consumption, in response to activation of coagulation, and the rapid return to normal levels is due to upregulation of AT production.

Heparan levels have not been reported after trauma, however are attached to the endothelium via syndecan-1. Syndecan-1 levels as a marker of endothelial cell wellbeing have been looked at in recent work by Johansson et al. (Johansson et al., 2011a; 2013). They demonstrated that trauma increases the degradation of the endothelial glycocalyx (raised syndecan-1 levels), with associated increases in TFPI and DAMPS. Haywood-Watson et al (Haywood-Watson et al., 2011) have also demonstrated an increase in syndecan -1 levels after trauma which improved after resuscitation. Raised Syndecan-1 levels imply endothelial damage and poor endothelial health. Raised syndecan-1 levels may not themselves exert an anticoagulant effect, however they are attached to the heparans, which may exert an anticoagulant effect, and are a marker of endothelial activation, the driver behind ATC.

The evidence for natural anticoagulants affecting coagulation, or being detected by viscoelastic methods remains patchy and warrants further investigation. However it appears that TFPI levels increase after trauma, that glycocalyx degradation, with presumed associated rises in syndecans and heparans increases after trauma and that AT levels fall due to their increased requirement to provide natural anticoagulation protection to the increased activity of clotting pathways. It is therefore possible that the increased sensitivity of the dilute Innovin TEG® can detect the imbalance of these natural anticoagulants after trauma.

13.7.2 Clot dynamics

Assessment of clot dynamics in both ROTEM® and TEG® followed the same pattern, There were no differences between groups over time in the first 2 phases of the study up to T60. Between T60 and T180, however, there is a

significant deterioration in clot dynamics in the IV rFVIIa group. The dynamic phase of clot formation represents the interaction between platelets, fibrinogen and clotting factors. The dynamics will be affected by absolute amounts of these factors, but also their activity.

This deterioration in dynamics can be explained by decreased factor, specifically fibrinogen levels, in the rFVIIa group, the presence of increased natural anticoagulation due to ATC, or simply due to the increased acidosis in the rFVIIa groups inhibiting enzyme function.

Low fibrinogen levels and low platelet levels are known to affect clot dynamics. Fenger-Erikson et al. (Fenger-Eriksen et al., 2009) examined the effect of dilution on clot dynamics, and how the addition of fibrinogen altered these parameters. He found that the addition of fibrinogen improved clot dynamics and clot strength, but not clot initiation. Fibrinogen levels are significantly lower in the rFVIIa group and discussed in more detail in the section on clot strength (section 13.7.3). These low levels may explain the deterioration on clot dynamics seen, particularly in the rFVIIa group.

Similarly to the role of anticoagulants in clot initiation, the presence of inhibitors such as TFPI may have an effect on clot dynamics. Audu et al. (Audu et al., 2006) demonstrated that that addition of TFPI (in large doses, twice normal concentration) reduced clot kinetics (decreased angle) as well as delayed clot initiation (prolonged R time). However the ROTEM® is insensitive to the effect of natural anticoagulation, due to the large dose of TF used as an initiator, therefore the presence of natural anticoagulation cannot explain the differences seen.

Enzyme function is impaired by acidosis. The rFVIIa group is significantly more acidotic than the O_2 group, although it is unclear the effect that acidosis has on rFVIIa activity (Lesperance et al., 2012).This is discussed in more detail in section 13.4.3 but remains a possibility to explain the effects in clot dynamics seen in the rFVIIa group.

13.7.3 Clot strength

Clot strength remains largely unchanged in the first two phases of the study in both ROTEM® and TEG®. When assessing clot strength using ROTEM®, there is a statistically significant fall in clot strength over time in the rFVIIa group, such that by T180 there is a statistically significant difference between the rFVIIa group and the O_2 group. Failure to reach MA in the TEG® assessment means that it was impossible to perform meaningful analysis of the TEG® data, however qualitatively it agreed with the ROTEM® results.

Clot strength is determined by the availability of functional platelets and fibrinogen. Examining the platelet count over the same time periods demonstrated a significant effect over time in both the O_2 and rFVIIa groups with a drop in platelet count over this period. This represents ongoing consumption of platelets due to activation of clotting and would be a normal effect of prolonged activation of coagulation.

However the platelet count does not qualitatively assess the function of platelets. Both ROTEM® and TEG® are influenced by the qualitative function. It is speculated that there is a platelet dysfunction early after trauma (see section 3.2.5) (Jansen et al., 2013) and thus a loss of functional platelets can reduce clot strength in spite of a high platelet count

Assessment of Clauss fibrinogen levels shows a reduction in fibrinogen levels in the rFVIIa group over the latter phases of the study. Examining the time point T180 between the O_2 and the rFVIIa group there was no statistical difference between groups, however the trend over time is such that the final fibrinogen level in the rFVIIa group is 0.796 g/dl (+/- 0.565) compared with a level of 1.62g/dl (+/- 0.223) in the O_2 group. It is unclear what a clinically significant low fibrinogen level is in a pig, however this value would be well below the 1.5 to 2.0 g/dl target for a human (Spahn et al., 2013) and may therefore represent a critically low fibrinogen level in the pig. This level could significantly impact the final clot strength.

FIBTEM MCF, which assesses functional fibrinogen levels, demonstrated a statistically significant difference between the O_2 and rFVIIa groups at T180, and a statistically significant fall in MCF over time thus confirming a clinical significant low fibrinogen level in the rFVIIa group.

It is plausible therefore that the loss in clot strength is caused by a progressive consumption of fibrinogen and/or functional platelets in the rFVIIa group.

13.8 Overall assessment of clotting

Whilst the ability to differentiate the phases of clotting is useful in targeting therapy, it is often easier to look at the overall picture when describing the whole process.

The clotting, as assessed by ROTEM® suggests that the addition of rFVIIa causes a short term "boost" in clotting manifest by a shortened clot initiation, although there is no effect on clot strength or clot dynamics. After T60, 30 minutes after the administration of IV rFVIIa, the effect changes. There is a gradual, progressive deterioration in clot initiation, clot dynamics and clot strength. This results in a coagulation taking a long time to produce a poor quality clot.

The clotting as assessed by TEG® has a slightly different result. The initial phases are the same as for ROTEM®, however the addition of rFVIIa at T30 presents a different picture and does not demonstrate a short term "boost". By T60 there is a rapid worsening of clot initiation with a rapid, progressive worsening of clot dynamics and clot strength. This picture is not dissimilar qualitatively to ROTEM, but is different quantitatively.

The causes for these changes are still unclear. There is the possibility that the rFVIIa follows the normal pattern, but the administration of O_2 at T30 provides enough O_2 to the inflow of the microcirculation (raised SaO_2) that it has a beneficial effect on clotting. This is less likely than some of the other potential causes since the SvO_2, representing O_2 uptake is so low. There is the potential action of the natural anticoagulants, particularly TFPI and heparans that may

have an effect that can be measured using the dilute innovin TEG®. The rFVIIa group has a worse acid base status, which will affect clotting, and may well be clinically significant. Finally, as an additional alternative mechanism, there is evidence of consumption of both platelets and particularly fibrinogen in the rFVIIa group.

13.9 The evidence for consumption and inhibitors

The original study was not intended to perform any assessment of coagulation other than PT, ROTEM® and TEG®. However the possibility that increased consumption after rFVIIa may cause a progressively worse coagulopathy, and the possibility that the natural anticoagulant pathways may have a more significant role in the development of acute trauma coagulopathy prompted a further set of investigations.

These investigations were done on completion of all the *in vivo* experiments and analysis of clotting parameters (PT, ROTEM®, TEG®) and so the opportunity to collect plasma specifically for clotting factors and inhibitors had passed. There were small amounts of plasma from a limited number of animals and time points available, so the supplementary analysis was considered worthwhile, however the assays required prioritisation as discussed below.

The original data set included the ROTEM® and TEG® results which implied consumption due the gradual deterioration over time of clot initiation, clot dynamics and cot strength, which was worse in the rFVIIa group. Some of these effects could be attributed to the presence of inhibitors as described in section 13.7.1 However combining the gradual decline in platelet numbers, fibrinogen /FIBTEM levels particularly in the rFVIIa group, and the suggestion that the administration of rFVIIa accelerates fibrinogen consumption (see section 13.5), then the consumption theory is perhaps more plausible than the very speculative inhibitor theory. This view was supported by personal communication with Professor Beverley Hunt. The effect of acidosis always exists, as the possibility for the differences between the rFVIIa and O_2 groups, however the effects of acidosis cannot be investigated by further plasma analysis.

It was therefore decided to prioritise the investigation towards consumption over inhibitor, consequently further assays were performed where possible outlined in section 13.10.

13.10 Supplementary analysis of clotting factors

The final pathway of coagulation involves the production of FXa and the production of thrombin. It is the production of FXa and FIIa that are key, whether produced via the normal clotting process initiated by the interaction between FVIIa and TF, or whether produced from the activated platelet stimulated by the administration of rFVIIa.

With the limited plasma available, it was decided that FII, FV, FVII and FX were the main extra coagulation factor assays of interest. Assays for TFPI and heparans were performed to assess the contribution of the natural anticoagulant pathways and the presence of syndecan-1 as a marker of endothelial cell degradation.

Previous work from DSTL has demonstrated that PT based human factor assays (FII, FV, FVII and FX) can be applied to pig blood. However the measurement of TFPI, heparans and syndecans have not been reported on pig blood before and so it was unknown if the assays would be effective.

The factor assays showed interesting findings. FII, FV and FX all follow the same pattern. There is a significant difference in the measurement of the individual factors at T60 in the rFVIIa group, followed by a sharp decline. The other groups (control and O_2) show a gradual and progressive decrease in activity down to critically low levels. Fibrinogen, platelets, and factors II, V and X all fall throughout the course of the study. The longer the experiment lasts for, the longer there is activation of clotting. It would be expected, therefore, that over time there would be a progressive consumption of clotting factor levels, which would lead to a progressive deterioration in clotting factors over time. This may be compounded by the presence of ATC with an increase in natural anticoagulation and fibrinolytic pathways.

Interpretation of the factor assays in the rFVIIa group is difficult due to the unknown levels of rFVIIa in the sample. I shall use the FX assay to explain how the results of the PT based assay in this study should be interpreted, and this can be applied to the FII and FV results. The treatment with rFVIIa at T30 means the FX assay is not simply a quantitative assessment of FX. The PT assay utilises a maximal dose of tissue factor as an initiator in order to fully activate all the clotting factors in that sample. It then measures the time for clot initiation in this fully activated plasma. If there are low levels of FX or FXa present then the PT will be prolonged. This prolonged time is compared to a reference plasma with dilutions of 25, 50 and 100%, and the results are given as a percentage, with normal values of 50 – 150% (in human blood).

In this study I would hypothesise the following scenario: FX levels are likely to be the same in all the groups in phase 1 of the study, since up to this time there has been no difference in treatment. In phase 2, the time when the IV rFVIIa would be having its greatest effect, as plasma concentrations are highest, there should be increased FXa, compared to the O_2 group, and therefore decreased FX. This boost in conversion of FX to FXa would extend throughout phase 3 so that by the end of phase 3 there would be reduced amounts of FX in the rFVIIa group. To measure this we need a pure FX and FXa assays, however there is no FXa assay available and it is therefore only possible to measure the effects indirectly.

At first glance the FX assay looks like there is an increased amount of FX produced in the rFVIIa group. As discussed above, the FX assay is a PT based assay and assumes an unknown concentration of FX in the sample plasma on a background of a known concentration of all other factors. Therefore any differences in PT are solely due to the amount of FX in the sample plasma. In our study there is also the added complication of the rFVIIa in the IV rFVIIa group. Therefore the assay is measuring not only the amount of FX in the sample, but the effect of a raised FVIIa as well. Whilst this measurement cannot quantify the amount of FX in the sample, it does reflect the *in vivo* situation where we have artificially raised the amount of VIIa available to activate coagulation.

Therefore the results indicate that qualitatively there is a boost in clotting due to the effects of the IV rFVIIa. This activity rapidly declined, such that by T120 there was no difference between groups. The effects of rFVIIa would be wearing off by this time due to its half-life of around 2 hours and so it is not clear whether the lack of difference between groups at T120 is due to the reduced amounts of rFVIIa, or due to the accelerated consumption of factor X caused by the “boost” in clotting.

The FII analysis follows the same pattern as the FX assay, which is not a surprise since FII is converted to FIIa (fibrin) due to the action of FXa. FV levels, however, are slightly different although this may be due to the small numbers of samples available for assay.

The results for TFPI and heparans were all below the minimum detection range for the assay, and the syndecan value was 0 in all samples. It is therefore clear that the assays for TFPI, heparans and syndecans were not effective in these samples and so no further analysis has been performed on these results. It has therefore not been possible to comment on the potential role of natural anticoagulants in the development of ATC. FVII analysis was not possible due to limited plasma available for analysis.

In summary the PT based factor assay indirectly measures the effect of rFVIIa on FX, FV and FII activity, which showed a “boost” after treatment with rFVIIa at T30. Unfortunately there was limited plasma available for assay at the later time points and so it was not possible to determine if an increase in consumption of clotting factors occurred in the rFVIIa group. The assay for natural anticoagulants failed to produce a result.

Chapter 14. An *in* vitro assessment of the effects of rFVIIa on coagulation

14.1 Rationale

It is well understood that treatment with rFVIIa will have an effect on clotting which may lead to a reduction in blood product use but may or may not have an effect on clotting (See section 7.1). However what is less well understood is what happens if a second dose of rVIIa is given. The military clinical scenario is the following:

> A soldier is injured by a blast device and exhibits clinical signs of respiratory distress. 30 minutes after injury he is administered rFVIIa as a potential treatment for blast lung (See section 7.6). He is hypotensively resuscitated as per UK military battlefield resuscitation guidelines(Joint Service Publication 999, 2012). On arrival in hospital he is found to be coagulopathic. What effect will a second dose of rFVIIa, as a treatment for coagulopathy, have, and when is the best time to administer this second dose?

Preliminary investigations into the optimal timing of drug administration can be conducted *in vitro* whereby rFVIIa is added to appropriate samples of blood from the animal model. The impact of the added rFVIIa on clotting can be assessed using TEG® or ROTEM®. These types of studies are often referred to colloquially in the published literature as “spiking” studies since the sample of blood is “spiked “ with the drug in question. Careful application of this approach allows a significant amount of progress to be made without the need for large, multiple groups of animals.

14.2 Methods

14.2.1 Blood samples

Blood samples drawn form the pigs from the animal study described in Chapter 11 were analysed, at the same time points as the coagulation assessment, with the administration of an *in vitro* spike of rfviia. To avoid confusion, rfviia refers to the spiking dose of rfviia, whereas rFVIIa refers to the ***intravenous*** dose used in the animals.

14.2.2 TEG® analysis and in vitro administration of rFVIIa

Fresh arterial blood was divided into two 942μl aliquots. One aliquot was treated with 29μL of rFVIIa (final concentration of 2.6μg/ml equivalent to a clinical dose of 180 μg/kg). The second aliquot was treated with 29μl of buffer (the solvent for rFVIIa, in order to match the dilution as control). These are known as the rfviia spike and the buffer spike. Both aliquots had 29μl of dilute tissue factor (Innovin, Dade Behring; marketed by System UK Ltd, Milton Keynes, UK) added to make a final volume of 1000μl, with a final innovin dilution of 1:50 000. TEG® analysis was performed in the standard way (see section 4.5). All TEG® analyses were performed in triplicate.

14.2.3 Statistical analysis

The phases of treatment were the same for the spiking study as for the animal study (see section 11.7.1) and consisted of phase 1 (baseline to T30), phase 2 (T30 to T60) and phase 3 (T60 to T180)

Paired assessment of buffer and rfviia spiked blood samples were performed to determine the effects of a rfviia spike on clot initiation, dynamics and strength (TEG R time, K time and MA). The data are presented as the difference between the rfviia and buffer spiked samples: a positive difference indicating a larger value in the rfviia spiked sample and a negative difference representing a smaller value in the rfviia spiked sample compared to the buffer spiked sample.

Time series data were compared using two-way analysis of variance (ANOVA) with repeated measures over time. Single time-point analyses were made using 2 way and 1 way ANOVA as appropriate. Where data was found to be non-normal a non-parametric equivalent was used as indicated in the text. In all cases a significance level of $P \leq 0.05$ (two tailed) was used

14.3 Results

14.3.1 R Time

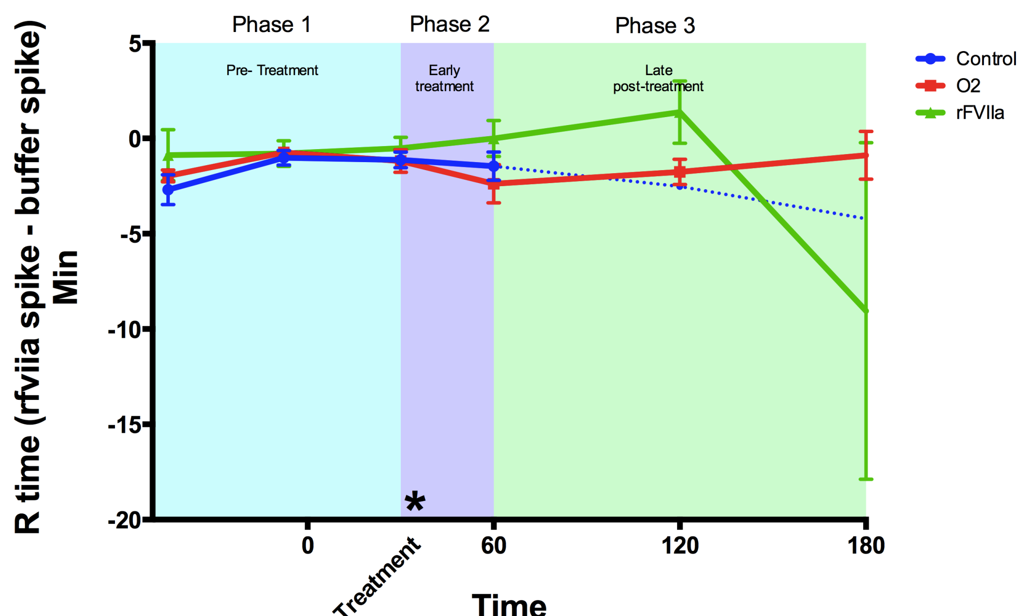

Figure 14-1. Effect of spiking study on R time. Values plotted are paired analysis and the difference between rfviia spike and buffer spike. Mean +/- SEM. (For further details see Figure 12-2)

During the pre-treatment phase a rfviia spike led to a significantly shorter R time compared to buffer spike (negative value rfviia spike - buffer spike), this was significantly different from 0 (P=0.0261). During phase 1 there was no difference between groups (P=0.2867) or over time (P=0.5167) (Figure 14-1)

Again, during phase 2 there were no differences between groups (P=0.1334) or over time (P=0.06819).

Comparing the O_2 and ***IV*** rFVIIa groups in phase 3 (T60 and T120 only due to loss of data points at T180) there was a difference between groups (P=0.0363), with the ***IV*** rFVIIa group being more positive than the O_2 group, but no difference over time (P=0.6529).

In summary prior to initiation of treatment at T30, the rfviia spike caused a shortening of R time (enhancement of clotting) in all groups. During the late post –treatment phase there was a difference between the ***IV*** rFVIIa group and the O_2 group, the ***IV*** rFVIIa group being more positive, meaning that there was a reduced effect of the rfviia spike.

14.3.2 K time

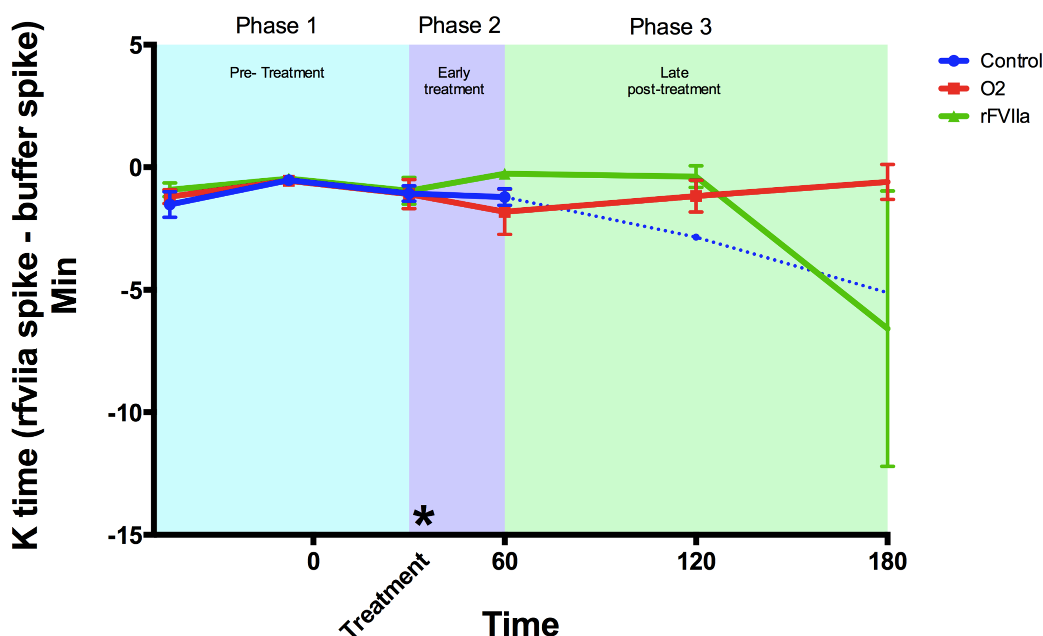

Figure 14-2. Effect of spiking study on K time. Values plotted are paired analysis and the difference between rfviia spike and buffer spike. Mean +/- SEM. (For further details see Figure 12-2)

In phase 1 rfviia spike led to a significantly shorter K time compared to buffer spike (P=0.0442). The difference between rfviia spike and buffer spike was not different between groups (P=0.6877) and did not change significantly over time (P=0.4165) (Figure 14-2).

Similarly, during phase 2 there is no difference between groups (P=0.2597) and no difference over time (P=0.5881).

During phase 3 (O_2 and ***IV*** rFVIIa groups only at T60 and T120) there were no significant differences between groups although there was a trend for the ***IV*** rFVIIa group to be less negative (P=0.0582). There were no significant changes over time during this period (P=0.7441).

In summary prior to initiation of treatment at T30, the rfviia spike caused a shortening of K time (enhancement of clotting) in all groups. During the late post –treatment phase there was a trend for the rfviia group being more positive, meaning that there was a reduced effect of the rfviia spike

14.3.3 MA

Due to failure to reach MA in a number of samples, sample size was too small to draw meaningful inference, however the trends suggest that during phase 1, rfviia spike enhanced clot strength, but there was a trend for this effect of rfviia spike to be lost in the ***IV*** rFVIIa group, while it persisted in the O_2 group during phases 2 and 3 (Figure 14-3). This is the same pattern as in the R time and the K time where the effect of the rfviia spike seems diminished over time in the ***IV*** rFVIIa group.

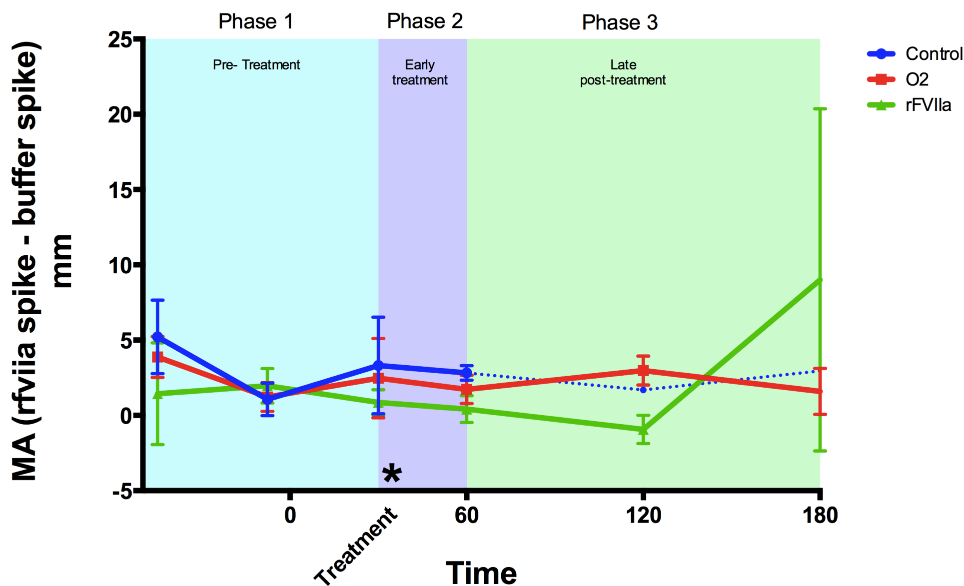

Figure 14-3. Effect of spiking study on MA. Values plotted are paired analysis and the difference between rfviia spike and buffer spike. Mean +/- SEM. (For further details see Figure 12-2)

14.4 Discussion

The spiking study was performed to investigate the efficacy of a dose of rfviia on coagulation as measured by dilute innovin TEG. The spiking was performed on all three groups, however due to loss of animals by T120, the late phase of the comparison could only be made between the O_2 and rFVIIa groups. To avoid confusion when discussing the spiking study the term "***IV*** rFVIIa" group refers to the animals treated with ***IV*** rFVIIa at T30, whilst the term "rfviia" refers to the rfviia used in the spike. To take advantage of the paired nature of the samples, the results are presented as the difference between the rfviia spike and the buffer spike (rfviia – buffer). Thus a negative result is a smaller number in the rfviia group, whilst a positive result is a larger number in the rfviia group. Therefore when assessing R time or K time, a smaller number in the rfviia group, leading to a negative difference, means a shorter time for clot initiation and clot dynamics and therefore an improvement in clotting. In relation to MA, a smaller number in the rfviia group means a smaller amplitude and therefore a weaker clot.

14.4.1 The effect of the rfviia spike on clot initiation

In the early pre-treatment phase of the study, the effect of the rfviia spike was to produce a shorter R time compared to the buffer, with no difference between groups. This is to be expected since at this stage all the groups have undergone the same procedures and have not been treated with either O_2 or ***IV*** rFVIIa. The implication is that at this stage a dose of rfviia would improve (shorten) the time taken for clot initiation. This effect was maintained throughout phase 2. At this early stage of the study, therefore, a dose of rfviia would be effective in shortening the time taken for clot initiation. The mechanisms are the same as the mechanisms whereby the ***IV*** rFVIIa dose had an effect on clot initiation beyond T30 (See section 13.7.1), that is a boost in the production of thrombin due to the actions of rfviia on activated platelets and the TF-FVIIa activated pathway.

This effect of the rfviia spike diminishes however in the late post-treatment phase of the study when comparing the O_2 and ***IV*** rFVIIa groups only. During this phase the rfviia spike has less effect in the ***IV*** rFVIIa group. The potential causes of this are the same as discussed in section 13.8 and remain consumption (most likely), natural anticoagulation and acidosis.

14.4.2 The effect of the rfviia spike on clot dynamics

The results of the rfviia spike on the clot dynamics broadly follow the same pattern as the results on the clot initiation. There is an improvement in clot dynamics after rfviia spike compared to buffer spike (negative difference) in the pre-treatment phase and in the early treatment phase. In phase 3, the trend was for a reduction in the efficacy of the rfviia spike compared to buffer spike in the same manner as the clot initiation, however this did not reach statistical significance.

14.4.3 The effect of the rfviia spike on clot strength.

Unfortunately it has been impossible to run comparisons between the groups using MA due to the number of samples failing to reach MA. This has been discussed in more detail in section 13.7.3 and is due to the run time of the TEG being inadequate for the relatively mild initiation of clotting with dilute innovin thereby taking a long time to reach MA. However the pattern broadly follows that of the spiking study pertaining to clot initiation and dynamics. Clot strength is improved after the rfviia spike in the early phases of the study, but the effect diminishes in the ***IV*** rFVIIa group in the late phase of the study

14.4.4 Summary of the spiking study

The three phases of coagulation (initiation, dynamics and strength) assessed in the spiking study with dilute innovin TEG® broadly follow the same pattern. A spike of rfviia is effective early, but the effect diminishes over time in the ***IV*** rFVIIa group.

The exact reason for this can not be distinguished between consumption, natural anticoagulation and acidosis.

Using the clinical scenario describe in section 14.1, where a hypotensively resuscitated soldier, injured by a blast device with concomitant haemorrhage, arrives at hospital and is administered rFVIIa either as a first dose (O2 and control group) or as a second dose (***IV*** rVIIa group). The results show that over time, administration of rFVIIa is increasing less effective, and this is worse for a second dose of rFVIIa. Therefore the clinical analogy is that if rFVIIa is to be used, it is best used early, and if a second dose is to be used it will be increasingly less effective over time.

Chapter 15. Overarching discussion and future directions

15.1 Overall summary

The interpretation of the results from the animal experiments are made difficult due to the lack of a control group out to T180. Late comparisons can only be made, therefore, between O_2 and rFVIIa groups, which have a significant difference in arterial oxygen levels, and acid base status. The difference in SaO_2 seems to have an overall clinical effect, as survival in the O_2 group is significantly higher than either the Control or rFVIIa groups, and may well affect clotting. However the presence of very low levels of SvO_2 in all groups, including the O_2 group, may mean that the effect of the oxygen on clotting is mild. This is because as the blood traverses the microcirculatory vasculature, from the arterial to the venous side, the overall elevation in oxygen seen by the endothelium in the O_2 group is modest which is reflected in the significant oxygen extraction by the tissues. Conversely the acid base status might have had a clinical effect contributing to the differences seen between the O_2 and rFVIIa groups late in the study. The underlying picture is that hypotension and blast injury cause a deterioration of coagulation measured by either PT, aPTT, ROTEM® or TEG® characterised by a progressive consumption of clotting factors, particularly fibrinogen. It is impossible to ignore the potential effect that acidosis may have on the coagulation system that may well explain the "slowing down" of clot initiation and dynamics, but would not explain the loss in clot strength or the difference in fibrinogen levels. Finally there is the potential that as ATC develops, so the natural anticoagulant pathways become more important. Whilst the natural anticoagulant pathways are less likely to explain these results, since ROTEM® and PT are insensitive to these pathways, it has been impossible to exclude them as a potential explanation.

The spiking study showed that the efficacy of rfviia administration in the ***IV*** rFVIIa group declined over time, however it is impossible to determine if this is due to the diminished effects of rfviia in the presence of low fibrinogen and low platelet activity or the effects of acidosis impairing enzyme function. It is also

unclear how much effect acidosis has on the efficacy of spiked rfviia since in recent studies, (Lesperance et al., 2012; Viuff et al., 2008) rfviia spikes have been shown to be effective in acidotic blood.

Finally, the premise of the study was to determine the effect of O_2 and rFVIIa on clotting in a model that represented a military casualty who received only minimal treatment of O_2 or rFVIIa soon after injury, and remained hypotensive thereafter. The results show that O_2 administration increases survival and has a less detrimental effect on clotting than the administration of rFVIIa.

It is therefore impossible to recommend the use of rFVIIa as a treatment aimed at improving coagulation unless there is concomitant resuscitation aimed at restoring oxygen delivery to the microcirculation.

15.2 Future directions

Military operations dictate that advanced medical care must be administered to very severely injured casualties in a resource constrained environment. In this context it is imperative to investigate the possible use of medical adjuncts that may improve the clinical situation, in this case coagulation, which have less of a logistical requirement than fresh and frozen products such as blood and FFP. rFVIIa is stable at room temperature, has a long self life and is reconstituted with sterile water.

Administration of rFVIIa does cause a short term “boost” in clotting and has been shown to decrease blood transfusion (see section 7.2.1). If this is combined with resuscitation, there may be a place for the use of rFVIIa in resource limited environments, specifically if used in conjunction with other medical therapies and used to target coagulation specifically (Gruen et al., 2012).

A follow up study should be performed to investigate the effects of rFVIIa and adjunct therapy on the coagulation system. Since consumption of fibrinogen particularly, seems important, the combination of rFVIIa and fibrinogen should be compared to fibrinogen or rFVIIa alone. This should be performed in animals

undergoing concomitant resuscitation following current resuscitation protocols using early blood products and limited crystalloid. This future study should utilise dilute innovin TEG® and dilute innovin ROTEM® as well as formally collecting plasma for specific factor activity assays rather than simply a PT based factor assay. This would require some model development in order to ensure that the assays are suitable for porcine blood. The study itself should not be a survival study (i.e. although a severe, representative insult is utilised the treatment should be sufficient to ensure survival of all/most subjects to a predefined time), ensuring enough control animals for comparison at later phases of the study.

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