

# **Effect of resuscitation strategies on coagulation following haemorrhage and blast exposure**

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## **Abstract**

Approximately one-third of trauma patients are coagulopathic on arrival to the emergency department. Acute traumatic coagulopathy and systemic inflammatory responses are serious secondary consequences of severe trauma and are linked to increased morbidity and mortality. Early tissue hypoxia is a major component in the aetiology of both complications. New resuscitation strategies are aimed at improving tissue oxygenation in the pre-hospital phase, and may attenuate coagulopathy and inflammatory sequelae. This is of particular importance in military personnel who suffer complex injuries, often from blast exposure, and may have extended evacuation times.

This thesis evaluates the effect of a novel hybrid (NH) resuscitation strategy on coagulation and inflammation. Terminally anaesthetised pigs were randomised to one of two injury strands of haemorrhage +/- blast injury; initially resuscitated with 0.9% Saline to a hypotensive systolic blood pressure of 80mmHg for one hour. This was followed by either a return to a normotensive pressure (110mmHg) (NH) or a continuation at the hypotensive level.

Over both injury strands NH significantly reduced Prothrombin Time, PT (mean proportion of baseline:  $1.40 \pm 0.05$  vs.  $1.80 \pm 0.09$ ;  $p=0.001$ ) and interleukin-6 (IL6) levels (mean  $1106 \pm 153$  vs.  $429 \pm 79$  pg/ml;  $p=0.001$ ) compared to the hypotensive groups. PT was positively correlated with IL6 ( $p=0.002$ ) and base deficit ( $p=0.0004$ ). These findings indicate that improving tissue oxygenation reduces the coagulation derangement and the pro-inflammatory response. No difference in coagulopathy was found between injury strands although blast did cause greater inflammation.

Early identification of coagulopathic casualties is essential and a separate feasibility field study was preformed to assess the use of thromboelastometry in a deployed military hospital, evaluating the degree of coagulopathy in battlefield casualties and to monitor the coagulation status during the resuscitation process.

In conclusion, NH attenuated the acute traumatic coagulopathy and inflammatory responses and therefore should be considered when an extended casualty evacuation is enforced.

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## List of Abbreviations

ABE	Arterial Base Excess
ACT	Activated Clotting Time
ACoTS	Acute Coagulopathy of Trauma Shock
ADH	Antidiuretic Hormone
A&E	Accident and Emergency
ADP	Adenosine Diphosphate
ALS	Advanced Life Support
APC	Activated Protein C
APTT	Activated Partial Thromboplastin Time
ARDS	Acute Respiratory Distress Syndrome
AT	Antithrombin
ATC	Acute Traumatic Coagulopathy
ATLS <sup>®</sup>	Advanced Trauma Life Support
ATP	Adenosine Triphosphate
BCSH	British Committee for Standards in Haematology
BD	Base Deficit
BV	Blood Volume
CA	Clot Amplitude
Ca <sup>2+</sup>	Calcium
CAM	Cell Adhesion Molecule
CaO <sub>2</sub>	Arterial Oxygen Content
CFT	Clot Formation Time
CNS	Central Nervous System
CO	Cardiac Output
CO <sub>2</sub>	Carbon Dioxide
CT	Clotting Time
CvO <sub>2</sub>	Venous Oxygen Content
DCR	Damage Control Resuscitation
DO <sub>2</sub>	Delivery of Oxygen
DIC	Disseminated Intravascular Coagulopathy
Dstl	Defence Science Technology Laboratory
EAC	Endogenous Acute Coagulopathy

ED	Emergency Department
EDV	End Diastolic Volume
EICBT	Educational Initiative on Critical Bleeding in Trauma
ELT	Euglobulin Lysis Test
ELISA	Enzyme-linked Immunosorbent Assay
EPCR	Endothelial Protein C Receptor
FI-XIII	Factors I to XIII
Fla-XIIa	Factors I to XIII activated
FBC	Full Blood Count
FDPs	Fibrin Degradation Products
FFP	Fresh Frozen Plasma
FOB	Forward Operating Base
FWB	Fresh Whole Blood
GCS	Glasgow Coma Scale
GP	Glycoprotein
GSW	Gunshot Wound
Hb	Haemoglobin
Hct	Haematocrit
HES	Hydroxyethyl Starch
HMGB	High Mobility Group Box
HSD	Hypertonic Saline Dextran
HTS	Hypertonic Saline
Hypot	Hypotensive
5-HT	5-Hydroxytryptamine
ICU	Intensive Care Unit
IED	Improvised Explosive Device
IL	Interleukin
IV	Intravenous
INR	International Normalised Ratio
ISAF	International Security Assistance Force
ISI	International Sensitivity Index
ISS	Injury Severity Score
JSP	Joint Service Publication
MBP	Mean Blood Pressure

MCF	Maximum Clot Firmness
ML	Maximum Lysis
MOF	Multiple Organ Failure
MP	Microparticle
mRNA	Messenger RNA
MT	Massive Transfusion
MTP	Massive Transfusion Protocol
MW	Molecular Weight
NH	Novel Hybrid
NHS	National Health Service
NICE	National Institute of Clinical Excellence
NO	Nitric Oxide
OCS	Open Canalicular System
OER	Oxygen Extraction Ratio
OR	Operating Room
PAI	Plasminogen Activator Inhibitor
PAR	Population at Risk
PAR1-4	Protease-activated Receptors 1-4
PBI	Primary Blast Injury
PCC	Prothrombin Complex Concentrates
PFA	Platelet Function Analysis
PMNs	Polymorphonuclear Neutrophils
PMPs	Platelet Microparticles
POC	Point-of-Care
PRBC	Packed Red Blood Cells
PS	Phosphatidylserine
PSI	Pounds per Square Inch
PT	Prothrombin Time
RBC	Red Blood Cell
RCTs	Randomised Controlled Trials
RL	Ringer's Lactate
RNA	Ribonucleic Acid
ROTEM®	Rotational Thromboelastometry
RR	Relative Risk

RTA	Road Traffic Accident
SAC	Systemic Acquired Coagulopathy
SaO <sub>2</sub>	Arterial Haemoglobin Oxygen Saturation
SBP	Systolic Blood Pressure
SIMV	Synchronised Intermittent Mandatory Ventilation
SIRS	Systemic Inflammatory Response Syndrome
SV	Stroke Volume
SVR	Systemic Vascular Resistance
TBI	Traumatic Brain Injury
TCT	Thrombin Clotting Time
TEG <sup>®</sup>	Thromboelastography
TEM	Thromboelastometry
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
TGT	Thrombin Generation Tests
TIC	Trauma Induced Coagulopathy
TM	Thrombomodulin
TMR	Tympanic Membrane Rupture
TNF	Tumour Necrosis Factor
tPA	Tissue Plasminogen Activator
tPAI	Tissue Plasminogen Activator Inhibitor
TT	Thrombin Time
TXA	Tranexamic Acid
UK	United Kingdom
uPA	Urokinase Plasminogen Activator
US	United States
vWF	Von Willebrand Factor
WW1	World War 1

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## **Publications, Presentations and Awards arising from this thesis**

### ***Publications***

1. **Doran CM, Doran CA, Woolley T, Carter A, Male K, Midwinter M, Mahoney PF, Watts S, Kirkman E.**  
Targeted Resuscitation improves Coagulation and Outcome.  
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2. **Doran CM, Woolley T, Midwinter M.** (2010) Feasibility of use of Rotational Thromboelastography (ROTEM) to manage the Coagulopathy of Military Trauma in a Deployed Setting.  
*Journal of Trauma – Injury Infection & Critical Care*, 2010;69(1);S40-48.
3. **Doran CM, Midwinter M.** (2009) Dealing with Trauma Coagulopathy on Deployment.  
*Medical Corps International Forum*, 2009;4: 34-36
4. **Tarmey NT, Doran CM, Woolley T, Jansen JO, Easby D, Wood P, Midwinter MJ.**  
Evolution of Coagulopathy Monitoring in Military Damage Control Resuscitation.  
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### ***Abstracts***

1. **Doran CM, Doran CA, Woolley T, Midwinter M, Mahoney PF, Watts S, Kirkman E.** (2011) Novel Hybrid Resuscitation in trauma improves Coagulopathy and Outcome.  
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### ***Academic Presentations***

1. **Military Surgical Conference**, Camberley, April 2009  
Feasibility of the use of ROTEM to manage the coagulopathy of military trauma in a deployed setting
2. **(ATACCC) Advanced Technology Applications for Combat Casualty Care**, Florida, August 2009  
Feasibility of the use of ROTEM to manage the coagulopathy of military trauma in a deployed setting
3. **8<sup>th</sup> NATO Blood Conference**, Portugal, September 2009  
Management of coagulopathy of military trauma in a deployed setting
4. **Royal Society of Medicine**, London, December 2010  
Novel Hybrid Resuscitation in Trauma Improves Coagulopathy and Outcome

5. **Society of Academic Research & Surgery (SARS) Annual Meeting**, Dublin, January 2011  
Novel Hybrid Resuscitation in Trauma Improves Coagulopathy and Outcome
6. **Military Surgical Conference, Association of Surgeons of Great Britain and Ireland**, Bournemouth, May 2011  
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7. **American Association for the Surgery of Trauma**, Chicago, USA, September 2011.  
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1. **Military Surgical Conference, Association of Surgeons of Great Britain and Ireland**, May 2011.  
Novel hybrid resuscitation attenuates systemic inflammatory response after blast injury and shock.

#### ***Awards***

1. **Royal College of Surgeons of England Research Fellowship in Military Surgery**. Awarded May 2009
2. **Rosetrees Essay Prize for Surgical Research**, Royal College of Surgeons, England; Awarded July 2010.
3. **Colt Foundation Prize for Military Research**, Royal Society of Medicine; Awarded December 2010.
4. **Eroll Elridge Prize, Defence Medical Services**. Awarded October 2011.

# Chapter 1: Introduction

## 1.1 The nature of trauma: civilian and military

Trauma accounts for 10% of deaths worldwide (Holcomb, 2004; Murray and Lopez, 1997), being the fourth leading cause of death in western countries and the leading cause of death in the first four decades of life. This prevalence in the young, results in an average of 36 life years being lost per trauma death (Chaira and Cimbanissi, 2003). Furthermore, trauma is also a major cause of debilitating long-term injuries. For each fatality, there are two survivors with serious or permanent disability resulting in trauma being a large socio-economic burden. In 1998, the estimated cost to the NHS of treating all injuries was £1.2 billion per annum. The incidence of severe trauma, defined as an Injury Severity Score (ISS) of 16 or greater, is estimated to be four per million per week (Gorman et al 1995). Given that the UK population in mid-2003 was in the region of 59.5 million, there are approximately 240 severely injured patients in the UK each week (National Confidential Enquiry into Patient Outcome and Death, 2007).

Within the military sphere, the past decade has seen a dramatic change in deaths and injuries occurring in the populations at risk (PAR); this includes both combatants and civilian populations caught up in the conflict. During this period, the UK Defence Forces have been involved in two significant conflicts – OPERATION TELIC in Iraq and OPERATION HERRICK in Afghanistan. In the former, between 1 January 2003 and 31 July 2009, there were 179 deaths and 222 casualties listed as ‘very seriously’ and ‘seriously’ injured (Ministry of Defence, 2009) In the latter ongoing conflict, between 7 October 2001 and 15 March 2011, 359 have been killed and 503 personnel have been ‘very seriously’ and ‘seriously’ injured (Ministry of Defence, 2011). This is in sharp contrast to the conflict in the Balkans in the 1990s, where fatalities were recorded as 72 from 1 January 1993 to March 2006 (Ministry of Defence, 2006).

The death toll of civilians caught in the conflicts has been shown to be significantly higher, although exact figures are difficult to corroborate. Between March 2003 and July 2006, it is estimated that 654,965 excess Iraqi deaths were a consequence of the war. This correlates to a rise in the mortality rate from 5.5 per 1000 people per year pre-invasion to 13.3 per 1000 people per year in the 40 months after; with the majority resulting from gunfire (Roberts et al 2004; Burnham et al, 2006).

One of the main differences between trauma in the civilian world and that in PARs in conflict areas is the mechanism of injury. Blunt trauma accounts for approximately 75% of UK trauma victims, with the main cause being Road Traffic Accidents (RTA) (Brohi, 2003; Peden et al, 2002). This is in contrast to the military statistics where a minority of patients, 7.6% (67 out of 876 patients) in the UK Military Joint Theatre Trauma Registry (1 April 2006 to 30 September 2007, OP HERRICK and OP TELIC only) suffered injuries by a blunt force; 5.1% of this 7.6% resulted from motor vehicle crashes. In that same period, 53.8% of injuries were sustained from blast/ fragmentation and 29.9% from gunshot (Hodgetts et al, 2007). The Improvised Explosive Device (IED) has become the most significant threat to personnel involved in current military UK conflicts (Ramasamy et al., 2008). The US forces recorded that 71% of personnel injured in combat between 2003 and 2006 resulted from explosions (Ritenour et al, 2010).

The risk from blast as a mechanism to cause injury is becoming a threat to civilian populations outside conflict areas as outlined in a systematic review of terrorist bombings during the period of 1966-2004 which identified 29 terrorist bombings around the world in 4 continents, accounting for 903 persons killed and over 7000 injured (Arnold et al, 2004). More recently the reality of bomb threats was brought home to UK citizens with the events of July 7, 2005 where 56 persons were killed and over 700 injured (Bland et al, 2006) in London. Explosive events are not all secondary to terrorist activity; accidental explosions can result from the ignition of gas, dust clouds, vapours, inflammable liquids and chemicals

due to fire or the failure of pressurised containers (Cullis, 2001). A recent case series highlighted this risk in a common workplace by examining the morbidity and mortality of 7 men who had suffered blast injuries from explosions from high pressure tyres (Hefny et al., 2010). It is for all these reasons it is necessary to understand blast; the physics of explosions and the physiological responses to blast.

Although explosions are the primary mechanism of current battlefield injury, haemorrhage resulting from these injuries is the greatest cause of death on the battlefield. A review of deaths from Vietnam showed that half of all deaths were from haemorrhage and 20% of that was from extremity wounds (Bellamy, 1984). The Israelis have reported that exsanguinations accounted for about 30% of deaths in their recent conflicts (Gofrit et al., 1997). A recent profile of combat injury outlined that over 50% of those who die, did so from exsanguinating haemorrhage. Approximately 80% of these deaths are from uncompressible haemorrhage in the torso, meaning that 20% of such deaths are from areas where the bleeding might be controlled (Champion et al., 2003). This pattern and cause of battlefield fatalities has not changed significantly over the past century of conflicts, and the most recent reviews continue to list haemorrhage as the greatest cause of death (Champion et al., 2009; Ritenour et al., 2010). In civilian trauma, haemorrhage has been reported to be the second cause of death after traumatic brain injury (Suaia et al., 1995).

A final unique consideration of military trauma is the austere, resource and logistically constrained environment in which personnel are injured and the potential for subsequent delayed access to definitive care. In the prospective paper that looked at 1,088 civilian trauma victims brought to the Royal London Hospital between 1993 and 1998 by the helicopter emergency medical service, the median time from injury (estimated as the time from when the emergency services were alerted) to arrival in the emergency department was 73 minutes (Brohi et al., 2003). UK Data from 2007 in current military conflicts has shown that times are a median of 97 minutes but can average up to 174 minutes

(Hodgetts and Mahoney, 2007; Parker, 2007). Transfer timelines shorten as an operation matures but in the early stages these can be significantly prolonged (Bohman et al, 2005). In the first two years of the Afghan Conflict, the US forces reported times of 5 hours (Bilski et al 2003).

In summary, military trauma differs from civilian trauma and therefore with any research in this area, it is essential to consider all these aspects of the mechanism of injury when considering the effects on physiology, resuscitation algorithms and outcomes of trauma victims.

## **1.2 The purpose of this thesis**

The work in this thesis forms part of a larger ongoing programme of work aimed at improving combat casualty care. The purpose of this thesis is to assess the effect of resuscitation strategies used in clinical practice of trauma patients and the secondary consequences of coagulopathy and inflammation following exposure to haemorrhage and blast. The following chapters (Chapters 2-4) explore and explain the physiological responses to trauma, the pathophysiology of coagulation and current resuscitation strategies. Chapters 5 and 6 provide the results of the experimental work, firstly, carried out using an animal model of a survivable battlefield injury and secondly, a clinical feasibility field study assessing measurement of the degree of coagulopathy in battlefield casualties.

## **Chapter 2: The Physiological Responses to Trauma.**

### **2.1 The Physiological Responses to Trauma**

Mortality from trauma has traditionally been described as following a trimodal distribution; immediately, within the first few hours and then much later (days to even weeks) after the injury occurred (Trunkey, 1983). More recent studies have noted a relative skewing of the distribution towards early deaths, with the loss or attenuation of the third peak, resulting in the pattern approximating to a bimodal distribution (Pang et al., 2008, McGwin et al., 2009, Evans et al., 2010, Chalkley et al., 2011).

There are three main physiological systemic responses to trauma: firstly, the cardiovascular response that occurs immediately after the injury; secondly, the immunological responses, which although starts immediately, it may take a few hours to become evident; and finally the metabolic response which are greatest importance in the recovery phase. This chapter will concentrate on the first two responses.

In the physiological sense, trauma is a combination of haemorrhage, tissue injury, pain and fear, but to understand the mechanisms of each response they have often been studied separately, and most of the understanding comes from animal models and human volunteers (Foex, 1999).

#### ***2.1.1 The Cardiovascular Response to Simple Haemorrhage***

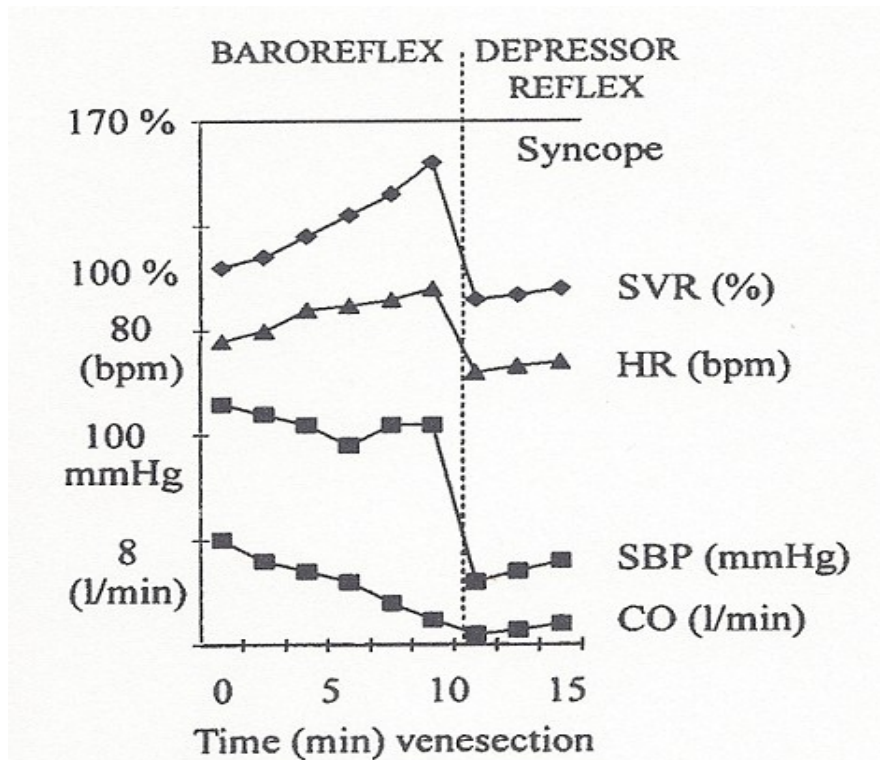
The loss of blood volume from the circulation, either externally or into a body cavity results in the development of hypovolaemia and the patient enters a state of shock; a pathophysiological state in which the circulatory system is unable to adequately perfuse tissues and meet oxygen demand. With the loss of blood, there is a decrease in the volume returning to the heart, and results in a decrease in the pressure of the right atrium. This in turn, results in a drop of the end-

diastolic volume (EDV), i.e. a reduced preload. This drop in preload results in a reduction in stroke volume (SV) – the volume ejected from the ventricle with each contraction. This leads to a decrease in cardiac output (CO; which is the product of SV and the heart rate). Arterial blood pressure is normally maintained until at least 20-30% of circulatory volume is lost, due principally to the effects of the baroreceptor reflex, before there is a drop in arterial blood pressure (see below). The hypotension leads to end-organ hypoperfusion and hypoxia, ultimately causing permanent organ damage and organ failure (Revell et al, 2003). This entire pattern of response is orchestrated by a series of homeostatic mechanisms which are designed to provide short term protection for the most essential organs in the body at the expense of causing a short term reduction in oxygen delivery to organs which are able to withstand this. Serious problems arise when the response is protracted.

#### *The Biphasic Response to Simple Haemorrhage*

The sequence of haemodynamic changes were investigated in detail by Barcroft and his colleagues during the Second World War. These studies investigated the response to venesectioning volunteers (Barcroft et al., 1944) while monitoring heart rate, blood pressure, cardiac output, right atrial pressure, and fore-arm blood flow. It was shown that there was a biphasic response to venesection. As the simple haemorrhage progressed there was a development of a tachycardia while systolic blood pressure (SBP) was maintained. The tachycardia coincided with a rise in the systemic vascular resistance (SVR), which compensated for the drop in CO. These responses are shown in Figure 1. After between 20-30% of the blood volume was lost, there was a second, depressor, phase which comprised of a bradycardia, a reduction in the SVR and a fall in the arterial blood pressure. In Barcroft's volunteers this caused syncope, and there was a profound **increase** in the fore-arm blood flow.





**Figure 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.

The first phase, sometimes called the “compensatory phase”, is due to the action of the ‘Baroreceptor Reflex’ or ‘Baroreflex’ as it is the combined result of the action of the arterial baroreceptors, cardiopulmonary baroreceptors, and arterial chemoreceptors. The stimulation of the arterial baroreceptors, which gives rise to this reflex, is due to a reduction in the arterial pulse pressure. The baroreceptors are stimulated early despite there being no initial fall in mean arterial pressure; this is because they respond to the rate of change of arterial blood pressure and transduce information about the pulse pressure, as well as mean arterial blood pressure (Angell et al., 1971). Consequently there is a reduction in action potential frequency carried in afferent fibres via the vagus nerve and the sinus nerve, branch of glossopharyngeal nerve, to the medulla. The result of this activation of baroreceptors is a reflex withdrawal of cardiac vagal activity and an increase in the sympathetic activity of the heart and vasculature (Spyer et al., 1984; Donoghue et al., 1984). The sympathetic outflow releases noradrenaline which acts upon the cardiac  $\beta$ -adrenoceptors in the heart, increasing the rate and force of contraction. It also acts on the  $\alpha$ -

adrenoceptors in the peripheral vasculature to cause vasoconstriction and the increase in the SVR. This increase in SVR offsets the hypotension that would otherwise accompany the reduced cardiac output which accompanies haemorrhage. The arteriolar constriction also reduces the hydrostatic pressure downstream in the capillaries favouring absorption of fluid from the interstitial space into the circulation. This partially offsets the hypovolaemia which, in combination with the sympathetically induced reduction in volume of the venous reservoir helps promote venous return (McGeown, 1996).

The change in vascular resistance is not uniform throughout all vascular beds in the body. There appears to be a hierarchy with some organs experiencing intense vasoconstriction and large reductions in flow while others are relatively spared. Barcroft noted the increase in SVR in the forearm but also noted that during his experiments in the initial phase there was no change in forearm blood flow suggesting that increase in SVR was caused by vasoconstriction in other vascular beds (Barcroft et al., 1944; Foex, 1999). This possible 'regional flow' change was also described by other investigators: Vatner found that mild haemorrhage in dogs resulted in an increase in mesenteric and iliac vascular resistance but a **reduction** in renal resistance. Furthermore, as blood loss continued, he found there was a greater reduction in mesenteric flow and an increase in coronary blood flow (Vatner, 1974). This was mirrored in human volunteer studies when lower body negative pressure was used to simulate hypovolaemia, where the splanchnic blood flow was more sensitive to the 'hypovolaemia' than renal blood flow (Hirsch et al., 1989). This regional blood flow appears to be ensuring the maintenance in blood flow and thus oxygen delivery to the most immediately vulnerable organs.

As the haemorrhage progresses beyond 20-30% of total blood loss, the second 'depressor' phase becomes evident (Barcroft et al., 1944). This is characterised by profound hypotension which is accompanied by significant bradycardia and a dramatic fall in SVR. This second reflex is vagally-mediated and overrides the baroreflex responsible for the first phase. The bradycardia is mediated by a profound increase in vagal efferent activity which increases the diastolic period resulting in greater cardiac filling and coronary perfusion (both of which occur in

diastole). In addition there is a sympatho-inhibition which reduces systemic vascular resistance (afterload). Thus the reflex appears to reduce the cardiac work at a time when coronary blood flow is compromised (Little and Kirkman, 1997).

The 'depressor phase' is not a terminal event, as Barcroft found this phase was reversible by re-infusion of the venesectioned blood in his volunteers. A further third phase has been described by Jacobson and colleagues before the degree of shock becomes irreversible (Jacobsen et al., 1990; Jacobsen and Secher, 1992). When over 40% of volume has been lost they described a pre-terminal phase of tachycardia and hypotension which appears to be driven by increased sympathetic drive and could be secondary to cerebral hypoperfusion/hypoxia (Foex, 1999).

A host of other reflexes also influence the cardiovascular system during haemorrhagic shock. The peripheral arterial chemoreceptors (which are located in the carotid and aortic bodies) are stimulated by stagnant hypoxia due to poor blood flow and  $H^+$  ions that are rising as a consequence of metabolic acidosis. Stimulation of these receptors drives an increase in respiratory rate and depth of breathing. It also increases the peripheral vasoconstriction and potentiates the baroreceptor reflex when the arterial pressure is low (Acker and O'Regan, 1981).

The efferent pathways mediating these reflexes depend on a host of mediators. The immediate response is due to alterations in sympathetic efferent activity (as described above). The primary transmitters in this case are the catecholamines, both noradrenaline predominantly released from sympathetic nerve terminals and circulating adrenaline principally from the adrenal medulla. Other mediators also play important roles including hormones such as vasopressin/Antidiuretic hormone (ADH) which is both a potent vasoconstrictor and exerts an antidiuretic effect. The sympatho-activation also activates the renin-angiotensin system ultimately resulting in an increase in angiotensin II, which also is a potent vasoconstrictor as well as stimulating the release of aldosterone. The aldosterone in turn has the renin action to conserve sodium and overall a longer

term results in a restoration of the blood volume.

### ***2.1.2 The cardiovascular response to tissue injury and haemorrhage***

Haemorrhage associated with trauma is often complicated by the effects of musculoskeletal injury, nociception and sometimes pain which in turn modifies the reflex responses to blood loss. Original studies in the 1950's showed that isolated tissue injury caused a 'pressor response' (Howard et al., 1955) where the blood pressure increases with increased sympathetic vasoconstriction and a tachycardia. Furthermore, it was noted that when haemorrhage was combined with soft tissue injury, mortality was increased (Overman and Wang, 1947), thought to be in part due to haemodynamic disturbances associated with the interaction between the cardiovascular reflexes associated with haemorrhage (see above) and those resulting from nociception (Kirkman and Little, 1997). In essence it is thought that the effects of nociception initiates a response akin to the defence reaction (fight or flight response). Little et al (1989) noted in the rodent studies that the 'depressor reflex' that was clearly documented in simple haemorrhage was altered by the presence of tissue injury as the expected bradycardia and hypotension was not seen. Thus the response to injury overrides the simple haemorrhage mechanism (Foex, 1999). However this response is at the expense of enhanced vasoconstriction of vital organs and possibly reduced vasoconstriction in the skeletal muscle (Kirkman et al., 1995). Mackway-Jones et al (1994) studied the effect of haemorrhage in a pig model on the background of a somatic afferent nerve stimulation to simulate tissue injury. After haemorrhage alone (30% blood volume), vascular resistance in the femoral bed was noted to double and there was minimal resistance noted in the splanchnic bed. However, when combined with stimulation of the nerve; the same haemorrhage resulted in a much smaller increase in femoral resistance but a significant increase in the splanchnic resistance.

Whilst this may aid survival for an individual continuing to fight (or fleeing from a fight), it will ultimately reduce survival in the more severely injured as a consequence of reduced blood flow to vital organs. Stimulation of the nociceptive afferent C-fibres to mimic tissue injury demonstrated the same 'modified' haemodynamic responses to haemorrhage indicating that the

conscious patient with fear does not drive these responses alone (Foex et al., 2004).

### ***2.1.3. The Immunological Response to Injury***

The immunological/inflammatory response to injury is initiated by tissue hypoperfusion, ischaemia and subsequent reperfusion during resuscitation. A simple division of the response is to divide it into two components: humoral and cellular. The humoral component involves the up-regulation and down-regulation of many mediators including cytokines, chemokines, the complement system, oxygen radicals and nitric oxide (NO). The cellular response is driven by effector cells including neutrophils, monocytes/macrophages and endothelial cells. All these responses are closely interlinked and lead to the systemic inflammatory response syndrome (SIRS). When this systemic inflammation becomes overwhelming, it can result in a massive systemic immunological activation after severe trauma leading to organ dysfunction and multiple organ failure (MOF) (Schroeder et al., 2009, Tsukamoto et al., 2010).

#### *Humoral Mediators of Response after Trauma*

Cytokines are an important response to injury and is important in the development of SIRS, with the main pro-inflammatory ones being IL-1, IL-6, TNF $\alpha$  and IL-8 (Ayala et al., 1991; Bone, 1996). These initial releases are tempered by the release of the anti-inflammatory cytokines including IL-1Ra, IL-10, IL-11 and IL-13 and the loss of the balance of the pro- and anti-inflammatory cytokines leads to the SIRS and subsequent immunological deterioration (Tsukamoto et al., 2010).

Elective surgery studies have shown that tissue injury caused by surgery will stimulate the production of IL-1 and IL-6 (Di Padova et al., 1991; Baigrie et al., 1992). The production of IL-6 is linked to the severity of the tissue-injury inflicted, and its level appears to be clinically relevant in the estimation of the severity of injury and prognosis after trauma (Svoboda et al., 1994; Biffi et al., 1996; Pape et al., 2007), and was particularly raised in burns (de Bandt et al., 1994). IL-6 levels

have found to be raised on the day of admission of trauma and then gradually decline over the next 48hrs in the patients who make a good recovery. Those patients that went on to develop septic complications found that the cytokine rise was maintained (Svoboda et al., 1994).

The exact role of TNF $\alpha$  in trauma is not clear (Tsukamoto et al., 2010). Rodent models have shown that TNF $\alpha$  was raised in the first 30 minutes after haemorrhage but was undetectable during the resuscitation phase (Ayala et al., 1991; Rhee et al., 1993). In a swine model of blunt trauma, haemorrhage and resuscitation, TNF $\alpha$  was not detectable at any particular level at any stage (Stylianou et al., 1991). This could be a characteristic of swine model rather than a true physiological response in other species. Clinical studies have shown a rise in TNF $\alpha$  in trauma patients (Roumen et al., 1995) but although it was raised in 100% of trauma patients, it was not significantly different compared in healthy controls and there was no correlation with ISS or GCS (Rabinovici et al., 1993).

The anti-inflammatory response is noted with a rise in IL-10 and IL-1ra (interleukin 1- receptor antagonist), both of which inhibit the production of monocytes/macrophage-derived TNF $\alpha$ , IL-6 and IL-8 (Tsukamoto et al., 2010). In animal septic peritonitis models, administration of IL-10 was found to improve the survival rate (Marchant et al., 1994). Pajkrt et al. found that the release of pro-inflammatory cytokines and neutrophils was reduced by the administration of IL-10 to healthy male volunteers (Pajkrt et al., 1997). Clinically, higher IL-10 levels have been measured in those patients that who were hypotensive on admission and found to correlated with the development of sepsis (Sherry et al., 1996). This suggests that levels could be used as an indicator of severity.

Along with the cytokine production, the complement system is also activated which are pro-inflammatory peptides that help with chemotaxis of leucocytes, degranulation of phagocytic cells and an increase vascular permeability. The levels of C3 and C3a (two pro-inflammatory peptides) were found to rise immediately after trauma and the levels were related to severity of injury and mortality after trauma (Sharma et al., 2004).

The humoral modulators appear to play a significant role in the pathophysiology of coagulopathy of trauma (see section 3.7.2).

### *Cellular Effectors of the Response after Trauma*

The cells that act in response to these mediators include the neutrophils, leucocytes, monocytes and macrophages all, of which are activated after trauma (Pillay et al., 2007). Activated polymorphonuclear neutrophils (PMNs) are recruited to the site of injury by IL-8, and this provokes the up-regulation of adhesion molecules on the endothelial and epithelial cells causing damage, leading to permeability of the vessels, cell swelling and dysfunction. The activated PMNs degranulate and release further pro-inflammatory cytokines, oxygen radicals, NO and proteases (Tsukamoto et al., 2010). This heightened status of the inflammatory process is a fine balance between pro- and the anti-inflammatory processes which can be easily destroyed by the onset of a second 'insult' such as an ischaemic/reperfusion injury, undergoing surgery or the onset of infection (Partrick et al., 1996).

An ischaemic/reperfusion injury results in a change from aerobic to anaerobic metabolism at a cellular level and this resultant disturbance of the cell membrane causes an increase in permeability and cell swelling. The reperfusion stage is more important for secondary tissue damage and organ dysfunction as oxygen is reintroduced. The oxygen reacts with hypoxanthine, a product of anaerobic metabolism to create a superoxide anion ( $O_2^-$ ), which then forms hydrogen peroxide and then gives rise to the hydroxyl radicals ( $OH^\cdot$ ). These, along with other reactive oxygen species (ROS) released from the PMNs, cause peroxidation of cellular membranes leading to cellular necrosis and possible cell apoptosis (Nathan and Singer, 2000). All this injury occurring at cellular level leads to cellular swelling and disruption of inter-cellular adhesion. The cellular swelling of the endothelium results in relative narrowing of the capillary lumen and this reduces blood flow. The inter-cellular destruction produces 'leaky capillaries' allowing a net movement of fluid out of the intravascular space into the interstitium taking vital plasma and proteins with it, compounding the insult.

Overall there is a loss of intravascular volume and a drop in the SVR (Tsukamoto et al., 2010).

The antigen-presenting cells (which include the T-cells, neutrophils and macrophages) are activated by endogenous danger signals. These signals are made by damage-associated molecular patterns including the pro-inflammatory cytokine called high mobility group box 1 (HMGB1). Recent studies have shown the importance in HMGB1, especially in the development of sepsis being released by macrophages and damaged cells and also as a mediator of inflammation in trauma/haemorrhagic shock by leading to both epithelial and gut barrier dysfunction (Ombrellino et al., 1999; Goldstein et al., 2006; Wang et al., 2004; Tsukamoto et al., 2010). Antibodies that neutralise HGMB1 actions appear to protect against damage and tissue injury in arthritis and thus future work directed towards the HMGB1 may help alter this crucial step in the inflammatory process (Yang and Tracey, 2010).

The last area to mention when discussing the effect of haemorrhage, tissue injury and inflammation is the gut mucosa. As discussed in section 2.2.1, haemorrhage and tissue injury result in regional diversion of blood flow being mainly diverted away from the splanchnic bed. This reduced gut flow can lead to ischaemia and mucosal damage, with essentially an ischaemic/reperfusion injury (Offner and Moore, 2000). This insult results in an increase in intestinal permeability allowing bacterial overgrowth, and also bacterial translocation; this has been postulated as a common pathway to MOF (Balzan et al., 2007), however there is no clear evidence for this. Deitch (2002) proposed that with the loss of the gut barrier, the intestinal bacteria and endotoxin would cross into the circulation. The majority of these translocating bacteria are phagocytosed by intestinal inflammatory cells but the toxins that are released could contribute to the development of SIRS. Rodent models have shown that this bacterial translocation occurs and is associated with sepsis (Senthil et al., 2007). Immunofluorescence studies were used to study breakdown products of translocated organisms and it was found that bacteria did translocate as the mesenteric lymph nodes were positive; but this may not



correlate with a clinical septic episode (Reed et al., 1994).

The inflammatory responses are an essential part of the response to trauma and establishing an understanding of the both the mechanism and sequelae may help our resuscitation strategies and avoidance of hypoperfusion. Adequate resuscitation goes beyond just restoring blood pressure and urine output and future directions should look at the 'whole' patient (Foex, 1999).

## 2.2 The Fundamentals of Blast.

It is necessary when investigating military trauma to understand the fundamentals of blast. An explosion is a violent phenomenon during which a sudden, large amount of energy is transmitted outwards after detonation. When the explosive detonates it generates an extremely rapid (effectively instantaneous) increase in pressure in the immediate vicinity of the explosion. This energy is transmitted to the surrounding medium (usually air) transferring the high pressure as a wave outwards faster than the speed of sound from the site of the explosion. This rapidly propagating pressure is called the '**blast wave**' and comprises of two components: the '**shock wave**' (or the static overpressure) and the '**blast wind**' (or the dynamic pressure). (Maynard and Cooper, 1988; Stuhmiller et al., 1997; Kirkman et al., 2010).

The **shock wave** initially comprises of a peak, the static overpressure, which lasts a very short time (thousands of a second) and is followed by a rapid, exponential fall back to and below the ambient (atmospheric) pressure. This curve is called the Friedlander wave form. The magnitude of the peak overpressure falls as it travels away from the site of the explosion), initially by an inverse cube relation (doubling the distance reduces the pressure to one-eighth). This is shown schematically in Figure 2 (A and B) below. (Cullis, 2001; Kirkman et al., 2010).

Because the shock wave is a very brief event with conventional explosives it does not cause an object or person to move any great distance (this is not the part of the explosion that 'throws things around'). Fragments (of the munition casing and pre-formed fragments contained within the device) and surrounding debris energized by the explosion are propelled outwards and can collide with the target causing significant injury.

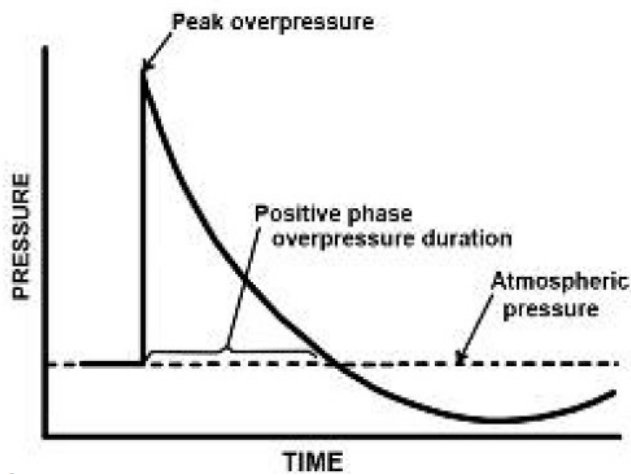


Figure 2 (A): Schematic representation of a shock of pressure magnitude vs time showing the Friedlander Waveform pressure changes in a undisturbed, free field environment.

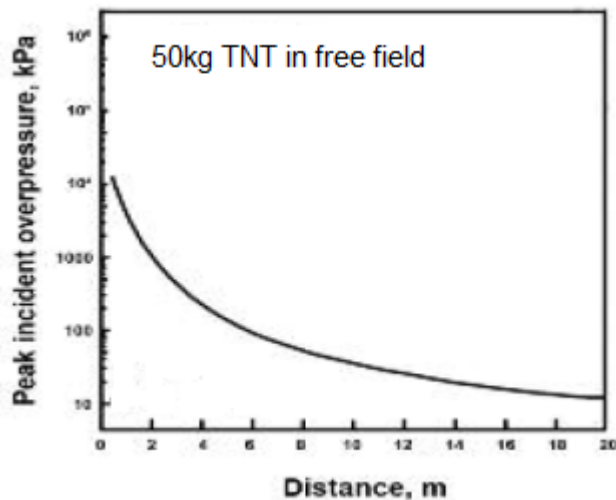


Figure 2 (B) – Schematic graph of pressure vs distance from point of detonation (Reproduced with the permission of Kirkman et al., 2010)

The '**blast wind**' results from the explosion giving rise to large volume of hot gas which is pushed out as a high-speed wind (the dynamic pressure) driving air and debris outwards. This creates more projectile hazard and will physically displace both people and objects.

The magnitude of damage due to the blast wave is dependent on the peak of the initial positive pressure wave as this pressure couples with the body. Pressure changes of 60-80 psi are potentially lethal (Born, 2005). Injuries are also dependent on the distance from source of blast and also the degree of openness of the surroundings – the effectiveness of the shock wave to cause injury rapidly

decays in open spaces but not so in 'closed' buildings or underwater where the reflection of the wave off surfaces compounds and enhances the destructive potential (Champion et al, 2009). In a 1996 Israeli study, there was a 8% mortality rate with open-air bombings but this rose to 49% in a closed-space bus environment (Leibovici et al, 2006). The heat released in the explosion will also cause significant injury to those closest to the epicentre.

This understanding of the fundamentals of blast helps explain how the injuries from exposure to an explosion are classified.

### **2.2.1 Classification of Blast Injuries.**

In 1940, Zuckerman classified blast injuries in his paper in the Lancet (Zuckerman, 1940) and the basis of this is still used today (Horrocks, 2001, Champion et al, 2009). He described four classes of blast injury; primary, secondary, tertiary and quaternary and now there is a fifth category to encompass specific additives such as bacteria and radiation (US Department of Defence Directive, 2006). The mechanisms of these injuries in each classification are shown in Table 1.

**Primary Blast Injury (PBI)** occurs when the shock wave interacts with the body and the energy is transferred directly from the transmitting medium (air or water) to the body. Injury occurs in the auditory and gas-containing structures of the respiratory and gastrointestinal tracts injury because energy is predominantly deposited when the shock wave encounters a border between a dense and a less dense medium and it is the deposition of energy that causes tissue damage. Thus, when the shock wave attempts to cross from tissue (which is predominantly water) to pockets of gas such as those found in the alveoli or small airways in the lungs and gas-filled areas in the bowel, energy is deposited and tissue is damaged with resultant contusion and other forms of damage (Guy et al, 1998). Solid organs, including the skin are more resistant to the blast wave and thus patients may display little evidence of trauma (Williams, 1942; Horrocks, 2001). Although currently used body armour protects military personnel from most ballistic projectiles to the torso, it offers little protection against the barotraumas of PBI (Mellor and Cooper, 1989).

<b>Type of Blast Injury</b>	<b>Mechanism of Injury</b>
<i>PRIMARY</i>	Blast overpressure injury resulting in direct tissue damage from the shock wave coupling into the body.
<i>SECONDARY</i>	Injury produced by primary fragments originating from the exploding device (preformed and natural (unformed) casing fragments, and other projectiles deliberately introduced into the device to enhance the fragment threat); and secondary fragments, which are projectiles from the environment (debris, vehicular metal, etc.).
<i>TERTIARY</i>	Physical displacement of the body or part of body by the blast overpressure causing acceleration/deceleration to the body or its parts. Includes structural collapse of buildings.
<i>QUATERNARY</i>	Miscellaneous collection of injuries including burns, inhalational injury and the psychological effects of the explosion.
<i>QUINARY</i>	Clinical consequences of “post detonation environmental contaminants” including bacteria, radiation (dirty bombs).

Table 1: Modified Zuckerman’s Classification of Blast Injury, (Champion 2009)

PBI predominantly affects the air-containing organs; namely the auditory system, the respiratory system, the gut and the eye. The tympanic membrane is structure most frequently injured and at the lowest pressures (Zuckerman, 1940; DePalma et al, 2005). An increase in pressure of as little as 5 psi above atmospheric pressure can rupture the human eardrum (Jensen and Bonding, 1993). A recent study of PBI in US forces between 2003 and 2006 showed that 71% of military injured personnel were injured by an explosion and 9% of those suffered tympanic membrane rupture (TMR) (Ritenour et al., 2010). In reports from explosions in confined spaces, such as the Madrid train bomb, 41% of all victims

suffered TMR with 67% of those being the most critically injured (Gutierrez de Caballos et al, 2005).

The lung is the second most susceptible organ to PBI. "Blast lung" is a clinical diagnosis characterised by dyspnoea, progressive hypoxia, haemoptysis and haemodynamic instability. Associated pathology may include pneumothoraces, haemothoraces, air emboli and pneumomediastinum and the possible later development of subsequent adult respiratory distress syndrome (Coppel, 1976; Cooper et al 1983; Phillips, 1986; Irwin et al., 1997). The transient high intra-thoracic pressure that results when the shock wave hits the chest wall leads to tearing of alveoli septae, stripping of airway epithelium and severe alveolar over-distension with rupture of alveolar spaces, alveolar haemorrhage, oedema and formation of alveolovenous fistulae (Argyros, 1997; Tsokos et al., 2003; Avidan et al, 2005).

PBI results in a characteristic triad cardio-respiratory response of hypotension, bradycardia and apnoea; mediated in large part by the autonomic nervous system (Irwin et al., 1997; Guy et al., 1998). This has been documented in the literature from the first world war through to contemporary events, where a subgroup of survivors from the Oklahoma City bombing in 1995 were noted to be persistently hypotensive, despite having had suffered no other obvious injuries (Irwin et al, 1997). The bradycardia and apnoea that occur immediately after blast result from a vagal stimulation from the pulmonary afferent C-fibres which is activated by an increase in pulmonary interstitial volume or pressure (Daly and Kirkman, 1988; Guy et al., 1998; Ohnishi et al., 2001).

The colon is the abdominal viscera most frequently affected by PBI (Irwin et al, 1999), but mesenteric ischaemia and subsequent infarct can lead to delayed complications. Injuries to the eye include rupture of the globe, serous retinitis and hyphema (DePalma et al, 2005).

**Secondary Blast Injury** is the cause of the majority of casualties in a free field (outdoors) and is caused by the impact of fragments and debris (Wade et al, 2008). The resulting array of both penetrating and non-penetrating injuries will often result in haemorrhage (Cooper et al., 1983). The response to haemorrhage has already been described, but it has been demonstrated that blast augments the second, depressor phase of bradycardia and hypotension. The exact mechanism of this augmentation is not clear, although it has been postulated that it could be that blast inhibits the initial baroreflex or it augments the second hypotensive phase (Sawdon et al., 2002).

**Tertiary Blast Injury** results from the acceleration of the whole body or parts of the body by the blast wave causing translational impacts of the body with the ground or other fixed objects causing classical blunt injuries. In the more severe cases it results in traumatic amputation of body parts and stripping of tissue. This group also encompasses crush injuries that result from the collapse of buildings that occurs from the blast wind.

**Quaternary Blast Injury** is a miscellaneous collection of other injuries that can result from the blast. This includes flash burns, caused by the radiant and convective heat of the explosion and burns caused by the combustion of the environment. It encompasses the effects of noxious gases released in restricted spaces e.g. carbon monoxide or benzenes/potassium perchlorate as documented in WW1 (Laure, 1993). Psychological effects that result from involvement within this group are also grouped under this heading.

**Quinary Blast Injury** describes injuries that are produced when bacteria or radiation are added to explosive device and released on detonation (Champion et al. 2009).

### 2.2.2 *Physiological Sequelae to Blast and Haemorrhage.*

Blast injuries involve both haemorrhage and tissue damage and therefore the reflexes described earlier would explain the expected response to a blast. Initial work in Durham (Sawdon et al., 2002) studied rats that were exposed to blast, followed by a controlled haemorrhage of 40% of their blood volume. The rats that underwent sham blast and haemorrhage displayed the expected biphasic response as described with the initial tachycardia followed by bradycardia. However, the animals exposed to blast followed by the haemorrhage (after 10 minutes) gave a different result profile. The blast resulted in an immediate drop in their heart rates and blood pressures, and after haemorrhage they failed to elicit the compensatory initial tachycardia. Bradycardia ensued from the onset of the haemorrhage and there was no compensatory maintenance of the blood pressure. The authors concluded that coupling of the blast *shock wave* modified the physiological responses to haemorrhage with loss of the initial compensatory phase and augmentation of the second, depressor phase.

Impairment of pulmonary gas exchange is usual after blast (Damon et al, 1971, Avidan et al., 2005). When the blast is combined with haemorrhage (which invariably happens with secondary and tertiary injuries) there is a grossly reduced tissue oxygen delivery resulting in a poor outcome (Parry et al 2005; Garner et al, 2010).

Oxygen delivery is the product of two factors: the oxygen content of arterial blood and the amount of blood perfusing the organs – Equation 1.

$$\text{DO}_2 = \text{CaO}_2 \times \text{CO}$$

**Equation1:** Relationship between whole body oxygen delivery ( $\text{DO}_2$ ), arterial oxygen content ( $\text{CaO}_2$ ) and cardiac output (CO). (Little and Edwards, 1993)

Arterial oxygen content is dependent on both the concentration of haemoglobin in blood and the degree of saturation of haemoglobin with oxygen, as shown in Equation 2.



$$\text{CaO}_2 = [\text{Hb}] \times \text{SaO}_2 \times 1.34$$

**Equation 2:** Relationship between arterial oxygen content (CaO<sub>2</sub>), Haemoglobin concentration [Hb] and arterial saturation with oxygen (SaO<sub>2</sub>). (Little and Edwards, 1993).

As cardiac output declines in haemorrhage, whole body oxygen consumption is maintained despite the reduction in oxygen delivery by an increase in oxygen extraction from the blood. The end result is maintenance of oxygen consumption at the expense of a reduced mixed venous oxygen concentration (Rady et al., 1991).

This situation cannot continue indefinitely and eventually oxygen delivery falls below a level that can be compensated for by an increase in extraction. In blast injury, with lung compromise, a reduction in arterial oxygen content compounds the problem and the victim reaches the point of inadequate oxygen delivery with a smaller reduction in blood flow. Shock states can therefore be more profound with combined blast and haemorrhage. This in turn leads to greater cellular damage, leakage from the capillary bed and a downward spiral would ensue. Therefore during the resuscitation of any blast patient, it becomes even more pertinent to bear all these facts in mind.

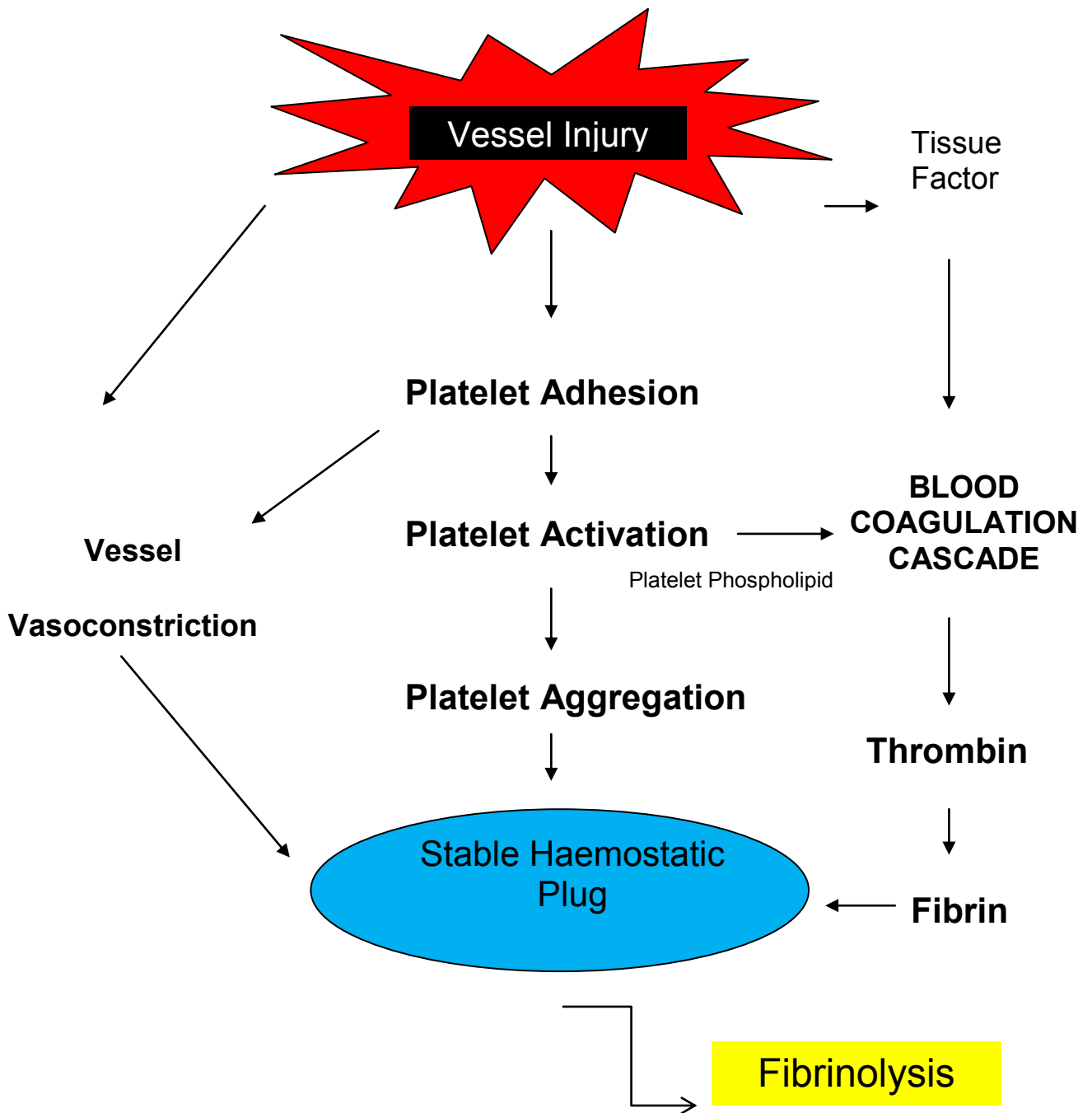
In the medium term, you would expect the sequelae of poor tissue oxygen delivery to impair clotting as has been described in non-blast trauma victims by Brohi et al (2007). However, recent evidence suggests that the immediate effect of blast (probably before the effects of tissue hypoperfusion have had opportunity to manifest) there is evidence of an ultra early hypercoagulable state after blast exposure (Harrison et al, 2008). The aetiology of this hypercoagulation is currently unknown but may be related to blast-induced tissue damage. Chapter 3 will explore the haemostatic process further but it could be postulated from this that blast injured patients may be therefore be more resistant to developing coagulopathy after trauma. The mechanism of haemostasis, the methods employed to test the coagulation status and the understanding behind the coagulopathy associated in trauma is explored in the following chapter.

## **Chapter 3: COAGULATION: Mechanism of coagulation, methods of testing and trauma-induced coagulopathy.**

Simmons reported from a US Army Military Hospital in Vietnam that 8% of patients were coagulopathic on arrival and was correlated closely to the degree of shock (Simmons et al., 1969). The link between trauma and coagulopathy has come to the fore over the past decade following a seminal paper from the Royal London Hospital that was published in 2003. Their work found that 24% of trauma patients presenting to the Accident & Emergency (A&E) were coagulopathic on arrival and this group had a significantly higher rate of mortality, 46% vs 11% when compared to those who were not coagulopathic at the same time point (Brohi et al., 2003). There has been a significant amount of research since then into the understanding of the pathophysiology that causes this trauma-induced coagulopathy (TIC), which test to use to evaluate it, and how to manage it. This chapter will discuss the mechanisms of coagulation, examine the clinical tests available to assess the coagulation status in patients and experimental studies and finally, review the evidence of TIC and its pathophysiology.

### **3.1 Mechanism of Coagulation**

The normal haemostatic response to injury that results in vascular damage comprises of a series of complex positive and negative feedback loops between the blood vessel wall, circulating platelets and blood coagulation factors (Kembell-Cook et al., 2005). Simply, haemostasis requires the formation of an impermeable platelet and fibrin plug at the site of injury, alongside which are powerful procoagulant substances working to ensure the process is localised to that site. These interactions are listed in the simplified diagram below (figure 3).



**Figure 3:** Simplified diagram to outline the coagulation process that occurs after vessel injury with the vasoconstriction, platelet activation and activation of the blood coagulation cascade. The platelet activation is the key to the overall process (modified from Hoffbrand et al, 2006).

Each step is crucial and all are interlinked: circulating platelets are recruited to the site of injury and along with the coagulation cascade initiated by tissue factor culminates in the generation of thrombin and fibrin (Furie and Furie, 2008).

The function of platelets, including structure and mechanism of action, tissue factor, the clotting cascade and finally fibrinolysis is described in the sections below.

### **3.2 Platelets**

Alfred Domé in 1842 described the presence of circulating globules viewed under the newly developed microscope (Domé, 1842), however it was Hayem in 1885 who first described the platelets acting as plugs for stemming blood loss after injury to the vessel (Hayem, 1885). The haemopoietic stem cell differentiates into a number of cell-lines including megakaryocytes which fragments to form platelets (each megakaryocyte creates 1-5000 platelets). Development takes approximately 10 days and the average life span is 7-10 days. The normal platelet count is approx  $250 \times 10^9/L$  (range 150-400) (Hoffbrand et al., 2006).

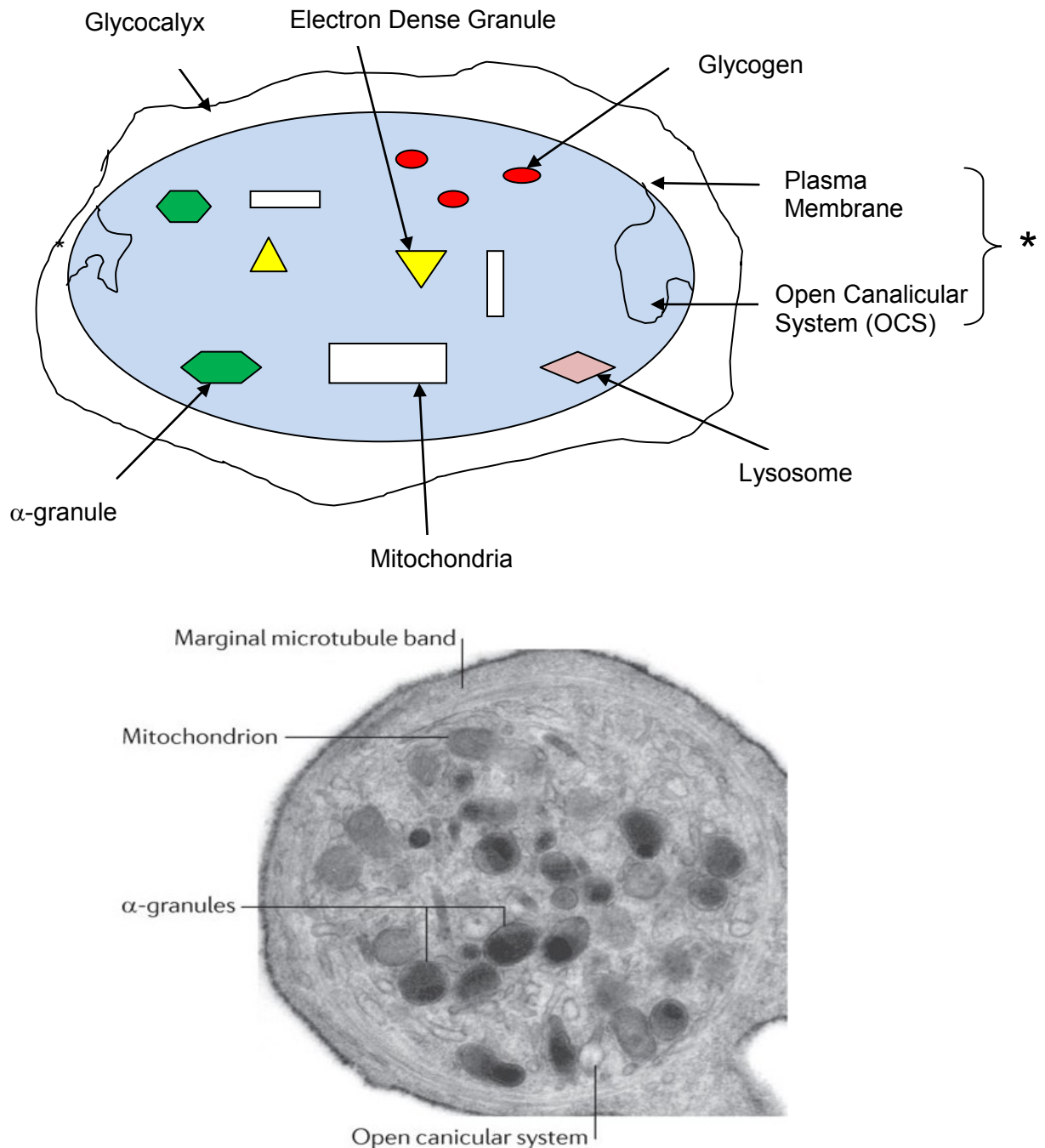
#### *3.2.1 Platelet Structure*

The platelet is 3-5 $\mu$ m and a schematic diagram is shown in Figure 4. The glycocalyx or glycoprotein coat is vital for adhesion and aggregation in the platelet plug. The plasma membrane invaginates to form an open canalicular system (OCS) that provides a large surface area to allow the absorption of the plasma coagulation proteins. The plasma membrane and the OCS combine to form the platelet phospholipid, a platform that is an essential for platelet function. The platelet contains three main storage granules:

1. Electron Dense Granule – contains adenosine diphosphate (ADP)/ adenosine triphosphate (ATP), 5-hydroxytryptamine (5-HT) and calcium ( $Ca^{2+}$ ).
2.  $\alpha$ -Granule – contains fibrinogen, factor V and von-Willebrand factor.
3. Lysosome – contains hydrolytic enzymes and peroxisomes. These contents are discharged into the OCS. There are high levels of calcium within the tubular system in the platelet and it is this  $Ca^{2+}$  that is essential for aggregation and

adhesion (Hoffbrand, 2006).

Von-Willebrand Factor (vWF) is a large multimeric glycoprotein and has a key role in platelet function. It is synthesised by endothelial cells and megakaryocytes and continually released into the blood plasma.



**Figure 4.** Schematic diagram and EM illustration of the ultra-structure of the platelet.

\* The plasma membrane invaginates to form the open canicular system (OCS) and provides the phospholipid surface that is essential for function. (Modified from Semple et al., 2011).

### **3.2.2. Platelet Function**

Platelets have numerous complex functions including haemostasis, thrombosis and inflammation, however in the context of haemostasis, a number of steps occur for platelets to carry out their role. The steps that occur are:

1. Platelet Activation and Adhesion
2. Platelet Aggregation
3. Secretion
4. Clot Retraction

The platelet adhesion and aggregation help establish the haemostatic plug. The secretion phase occurs at the platelet phospholipid membrane to provide the platform for protease activation which leads to the formation of thrombin (Kembell-Cook et al., 2005).

#### *Platelet Activation and Adhesion.*

Following blood vessel damage, the platelets adhere to the exposed subendothelial matrix proteins via adhesive glycoproteins (GP) present on the glycocalyx (Furie and Furie, 2008). Exactly how they adhere is dependent on the rate of shear in the vessel. In arterioles, where shear is intermediate to high, the adhesion is completely dependent on von Willebrand factor and a GP complex. This initiates platelet rolling in the direction of the blood flow with exposed vWF activating other GP receptors, these latter interactions are much stronger. In areas of low shear, as in the venous circulation, adhesion can occur directly to other subendothelial proteins such as collagen and fibrinogen. vWF, present in plasma and in the  $\alpha$ -granules, is exposed on the surface of the thrombus helps recruit further platelets (Savage et al., 1998, Davenport and Brohi, 2009).

During activation, platelets become spherical and extend pseudopodia to allow attachment of other platelets and to the vessel. The "shape change" pushes the granules and organelles into the centre of the platelet body, and allows the granules to be then secreted via the OCS (Hoffbrand et al, 2006). A critical function of platelet activation is to provide a negatively charged phospholipid

surface for the assembly of the protease complexes, 'tenase' and 'prothrombinase', which form a vital part of the coagulation cascade (refer to section 3.4) (Heemskerk et al., 2002).

Referring back to Figure 1, platelet activation in combination with the clotting cascade results in a large amount of thrombin being produced in the vicinity of the platelet surface, which converts fibrinogen to fibrin and further enhances platelet activity. However, the thrombin also diffuses to the intact endothelial cells where it binds to thrombomodulin and activates Protein C (bound to Endothelial Cell Protein C receptor – EPCR). The activated protein C (APC) interacts with a surface protein of the activated platelets to prevent assembly of the above complexes by cleaving FVa and FVIIIa (section 3.5.1). Therefore platelet surface reactions both promote and limit the cascade process.

### *Platelet Aggregation*

This is cross-linking of platelets through binding of fibrinogen and other ligands to the GP coat. This number of active GP increases significantly following fusion of the  $\alpha$ -granules with the plasma membrane. Upon activation, receptor signalling causes the GP to undergo a conformational change that increases its affinity for fibrinogen, VWF and other ligands. A rise in calcium aids this process (Varga-Sazbo D et al, 2008).

### *Secretion*

Primary activation of the platelet leads to the release of its granules. The ADP from the dense granules has an important role in a positive feedback for promotion of platelet activation. Release of the vWF from the  $\alpha$ -granule is critical for normal thrombus formation at intermediate and high flow rates. The  $\alpha$ -granules also contain clotting factors V, VIII, Protein S and plasminogen activator inhibitor (PAI).

Platelet activation also results in the formation of platelet microparticles (PMPs) which are membrane vesicles ranging in size from 0.1 to 1.0 $\mu$ m. These particles were first described by Wolf in 1967 as procoagulant 'dust' found around activated platelets (Wolf, 1967). The exact function of these particles is only now being understood as potent vectors of biological information and protagonists of

an intercellular communication network (Mause and Weber, 2010). The PMPs rely on the anionic phospholipid phosphatidylserine which becomes accessible on the platelet plasma membrane after remodelling that is a result of initial stimulation. In turn, the PMPs promote the assembly of both procoagulant and protein C anticoagulant enzyme complexes acting as a 'checkpoint' between the haemostatic and thrombotic responses (Morel et al., 2008; Davenport and Brohi, 2009). The presence of PMPs, in the presence of activated TF, stimulates the velocity of thrombin generation up 15-fold when compared to synthetic phospholipids (Keuren et al., 2006). P-selectin (a cell adhesion molecule – CAM) acts as the mechanism for transfer of microparticles between platelets and monocytes and appears to contribute to additional localised thrombin production (Breimo and Osterud., 2005).

#### *Clot Retraction*

Platelet activation and aggregation results in a plug that is large enough to cover the area of endothelial injury. The platelets at this stage are completely degranulated and adherent to each other. Retraction occurs by linkage of the GP coat and surface fibrin (Kembell-Cook et al., 2005).

### **3.3 Tissue Factor**

Tissue factor (TF) is a membrane-bound glycoprotein, predominantly present in the subendothelial tissue that is expressed or exposed at sites of vascular injury and is essential for haemostasis (Kretz et al., 2010). TF is also found in the  $\alpha$ -granules and the open canalicular system of resting platelets and is expressed in PMPs, when the platelet is activated. A small amount of TF is expressed on the plasma membrane of many cells that are not exposed to blood and acts as a receptor for Factor VII and Factor VIIa and is required for the initiation of blood coagulation. It has been shown that the inactivation of the mouse TF gene resulted in embryonic lethality (Carmeliet et al., 1996; Toomey et al., 1996). TF exerts its effects when the integrity of the vessel is disrupted and when an adequate TF challenge is presented, a full coagulant response follows; if the TF challenge is insufficient, the procoagulant response is arrested by the synergistic activities of the TF pathway inhibitor (TFPI), Antithrombin and the protein C pathway (Butenas et al., 2009). TF bearing cells, that present the TF that becomes activated, includes endothelial cells, smooth muscle cells,



fibroblasts, leucocytes and macrophages (Ovanesov et al., 2005). A small reservoir of circulating or 'blood-borne' TF has been detected, disseminated by vascular cells and monocytes derived microparticles (Giesen et al., 1999). An accepted understanding is that this circulating TF is incorporated into thrombotic clots at the periphery and participates in its growth (Hathcock et al., 2004; Hoffman et al., 2006). Nemerson and colleagues showed that the 'blood-borne' or 'circulating' TF existed in healthy individuals that was found to enhance thrombosis in an ex-vivo model (Giesen et al., 1999). However, other work has shown that circulating TF in healthy individuals is extremely low and unlikely to contribute to clotting although it is felt that future work with increasing validity of assays would provide more answers (Butenas et al., 2009).

### **3.3.1 Microparticles**

Microparticles (MPs) are present in the blood of healthy individuals and are increased in various diseases, including cardiovascular disease, sepsis and cancer (Morel et al., 2006; Burnier et al., 2009). These are small membrane 'blebs' or vesicles that are released from cell surfaces by proteolytic cleavage of the cytoskeleton. They have a number of functions including mediation of cell-cell communication by transferring a cargo of cell surface receptors, mRNAs and microRNAs, from the cell of origin to target cells (Mause and Weber, 2010). They are also procoagulant by providing a surface for assembly of components of the coagulation protease cascade. Their procoagulant activity is increased by presence of two phospholipids, phosphatidylserine (PS) and TF. In cancer, PS+, TF+ MPs are derived from tumours and may be a useful biomarker to identify patients at risk for venous thrombosis (Owens and Mackman, 2011). This is an area of research in both healthy and disease states.

## **3.4 The Coagulation Cascade**

The historical "waterfall or cascade" model of coagulation, proposed by McFarland, Davie and Ratnoff in 1964 (Davie, 2003) has been superseded by a cell-based system where platelets amplify the haemostatic process allowing the propagation of coagulation to occur effectively (Hoffman, 2001; Monroe et al., 2006). The cascade model described each clotting factor as proenzymes (and

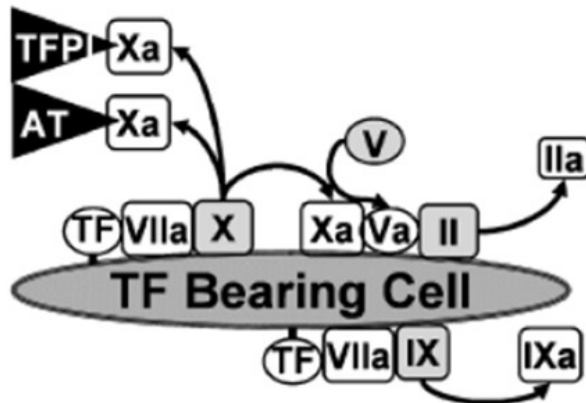
cofactors), which were converted to an active enzyme in a series of sequential steps. Whilst separating the process into the two classical pathways of intrinsic and extrinsic, this only related to the clotting factors present in plasma and did not adequately refer to the haemostatic process with cellular interaction that occurs *in vivo* (Hoffman et al., 2001 & 2005; Becker, 2005; Furie and Furie, 2008). The cascade model could not explain why if one arm (for example the intrinsic) pathway were intact, why did a deficiency of FVIII cause a bleeding tendency, but those with FXII deficiency did not (Hoffman 2005) and why clots don't constantly form from the circulating factors? The cell based theory was proposed by Hoffman et al (2001) and has gained acceptance as it addresses the aspects of the complete haemostatic process that were unexplained by the cascade model. The cell based theory incorporates the role of cells, and in particular platelets, integrated into the dynamic vascular system where haemostasis occurs with activated components, and TF is crucial (Smith, 2009). This can be visualised by referring back to the simplistic diagram (Figure 3) that highlights that both the humoral and cellular components of coagulation are required.

The complex interactions that lead to the formation of thrombin occurs in distinct but overlapping steps: *INITIATION* for generation of small amounts of thrombin (picomolar concentrations); *AMPLIFICATION* where the thrombin promotes activation of the platelets and coagulation factors V, VIII and FXI (Oliver et al., 2002); and finally *PROPAGATION* which produces a burst of thrombin and fibrin formation that generates the clot (Monroe and Hoffman, 2006).

### **3.4.1 Initiation**

The vessel wall is lined with endothelium and when the wall is breached, TF is exposed. The TF binds with the circulating plasma factor VIIa (approximately 1-2% of circulating FVII is active but only expresses proteolytic ability when it binds to TF). As illustrated in figure 5, this complex then activates small amounts of factors X and IX. The activated factor X, Xa, in turn activates the cofactor V to form Va, a prothrombinase assembly (Monroe et al., 1996). This generates the formation of Thrombin (Factor IIa) from Prothrombin (Factor II). This stage has also been called the TENASE stage (Heemskerk et al., 2002).

When Xa dissociates from the TF-bearing cell, it is rapidly inhibited by two inhibitors: TF pathway inhibitor (TFPI) or antithrombin (AT) (Kembell-Cook et al., 2005). This inhibition of Xa localises its action on the tissue bearing cells at this stage in the clotting process since appreciable amounts cannot diffuse away from the tissue factor bearing cell to influence neighbouring cells and platelets.

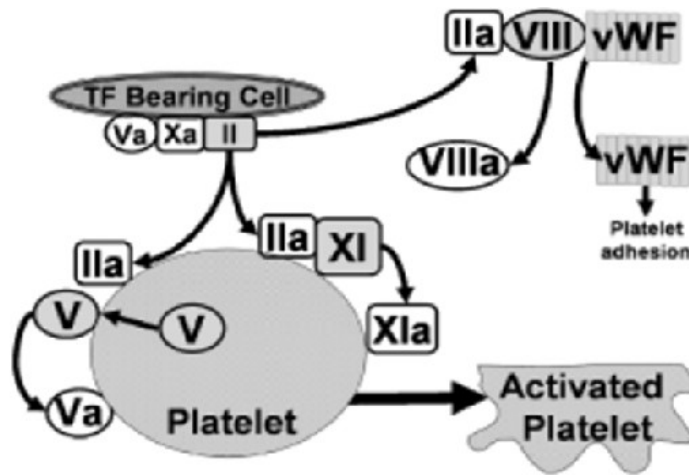


**Figure 5:** Schematic diagram of the initiation phase (TENASE stage) which occurs when Tissue Factor (TF) is exposed. The initiation phase is quickly turned off by two inhibitors: TFPI (Tissue Factor Pathway Inhibitor) and AT (Antithrombin). Part of the process to prevent unchecked thrombosis (diagram modified from Smith, 2009).

### 3.4.2 Amplification

The small amount of thrombin generated on the TF-bearing cells has two functions; firstly to activate the platelets and secondly to further activate cofactors V and VIII (Alberio and Dale, 1999). Platelet activation by thrombin results in shape change of the platelet allowing the formation of the platelet phospholipid creating the procoagulant membrane surface. The activation also releases the platelet  $\alpha$ - and dense-granules allowing the contents necessary for thrombus formation. The activated platelet releases factor V onto the surface and this in turn is activated by the thrombin. Factor XI and VIII are also activated (XIa and VIIIa respectively). The FVIIIa cleaves vWF which mediates additional platelet adhesion and aggregation at the site of the injury. This process is amplified by the presence of  $Ca^{2+}$  (Furie and Furie, 2008; Smith, 2009). This has been described as the PROTHROMBINASE stage and the

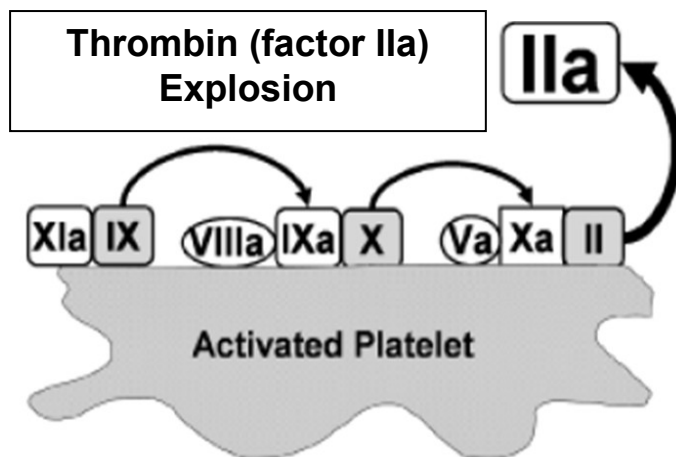
further activation of platelets allows an increase in platelet adhesion. Amplification is illustrated in figure 6.



**Figure 6:** Schematic diagram of amplification showing how Thrombin (IIa) activates the platelet, factor V, VIII and XI. The Prothrombinase stage. (diagram modified from Smith, 2009)

### 3.4.3 Propagation

This final stage occurs on the surface of the activated platelet and results in an explosion of thrombin. Factor IXa, which was activated in the initiation phase by the TF:FVIIa complex, binds with VIIIa on the platelet surface, and this complex in turn binds with X on the activated platelet to form Xa (Monroe and Hoffman, 2006). Additional IXa can be activated on the surface of the platelet by the action of platelet-bound XIa. This Xa binds with Va and  $Ca^{2+}$  on the platelet and this in turn cleaves prothrombin (II) to thrombin (IIa), as shown in figure 7. This prothrombinase activity results in a burst of thrombin generation. The thrombin hydrolyses fibrinogen into two fibrin monomers, which links with Hydrogen bonds (H-bonds) to form an insoluble fibrin polymer. Activated factor XIII stabilises the fibrin polymers (Lorand, 2001).



**Figure 7:** Schematic diagram of Propagation - Thrombin explosion that occurs when FXa and Va convert Prothrombin (II) to Thrombin (IIa) (diagram modified from Smith, 2009).

### 3.5 Physiological Limitations of Blood Coagulation

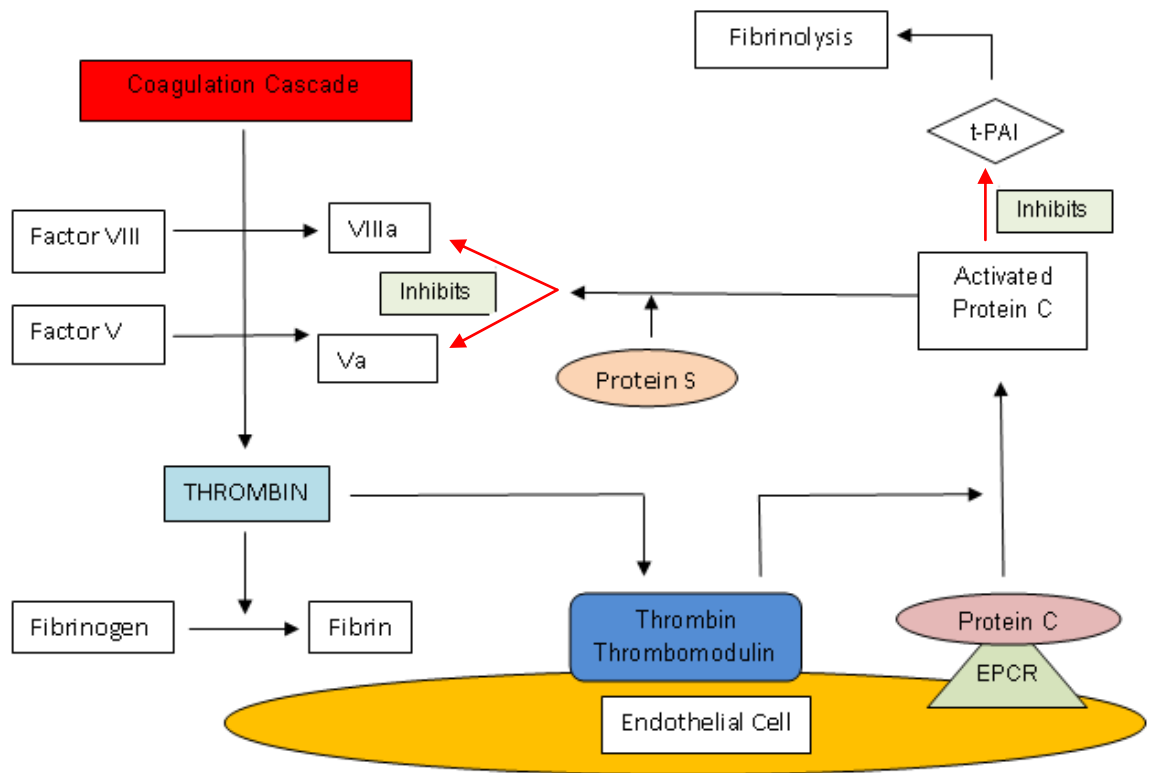
A delicate balance must be kept after injury to ensure that an efficient clot is formed but to prevent this extending that could lead to complete vascular occlusion. Two main mechanisms exist to help localise the clot: plasma protease inhibitors and a pathway to destroy activated factors, namely FVa and FVIIIa (Hoffbrand et al., 2006).

Two of the first group of plasma protease inhibitors are Tissue Factor Pathway Inhibitor (TFPI) and Antithrombin (AT) which stop the initiation phase, thus preventing the continuous stimulation of the amplification phase, as described previously. (Figure 5). Any factor that diffuses away from the area of cellular damage is rapidly inhibited (Monroe and Hoffman, 2006).

The second mechanism, the protein C pathway, prevents propagation of the coagulation on healthy intact endothelium and is described below.

#### 3.5.1 The Protein C Pathway

This is the pathway designed to prevent FVa and FVIIIa from continually driving the formation of thrombin. The mechanism is outlined in Figure 8.



**Figure 8:** Schematic diagram of formation of the Thrombin/Thrombomodulin (TM) complex and its subsequent activation of Protein C. The TM complex promotes the EPCR (Endothelial Protein C Receptor) which presents the Protein C. This stimulates Tissue Plasminogen Activator Inhibitor (t-PAI) which inhibits fibrinolysis (section 3.5.2) Red arrows indicate inhibition. (Modified from Hoffbrand et al., 2006).

Protein C is a Vitamin-K dependent glycol-protein and plays a regulating role in coagulation, inflammation, cell death and vessel permeability (Mosnier et al., 2007). The thrombin formed by the coagulation cascade binds with thrombomodulin (TM) which is an integral transmembrane receptor on the endothelial cell and is present in all body tissues. The binding of thrombin helps 'mop up' excess thrombin and therefore is no longer available to cleave fibrinogen. This TM-thrombin complex also activates the Protein C that is localised on the surface, held there by its receptor - Endothelial Protein C Receptor (EPCR). This Activated Protein C (APC) destroys the active factors VIII and V by protein cleavage (Jakubowski and Owen, 1989). This process is enhanced by Protein S, a cofactor to Protein C by helping to bind the APC to the platelet, and Protein S is thought to act synergistically with Protein C (Hoffman et al 2003). Although it is widely believed that the activated protein C (APC) terminates thrombin generation on the activated platelets and endothelial

site of action may be more important under normal circumstances since APC/Protein S is much more efficient in inactivating factor Va on the surface of endothelial cells than on the surface of platelets (Oliver et al 1999). Therefore, some authorities believe that the primary role of APC/Protein S is to prevent the generation of thrombin on healthy endothelial cells, thereby contributing to the restriction of thrombin generation to the immediate area of an injury (Hoffman et al 2003). While this is likely to be true under conditions of normal clotting it is also possible that under the extreme conditions seen in the very severely injured, a role of APC/Protein S acting via platelets may become important.

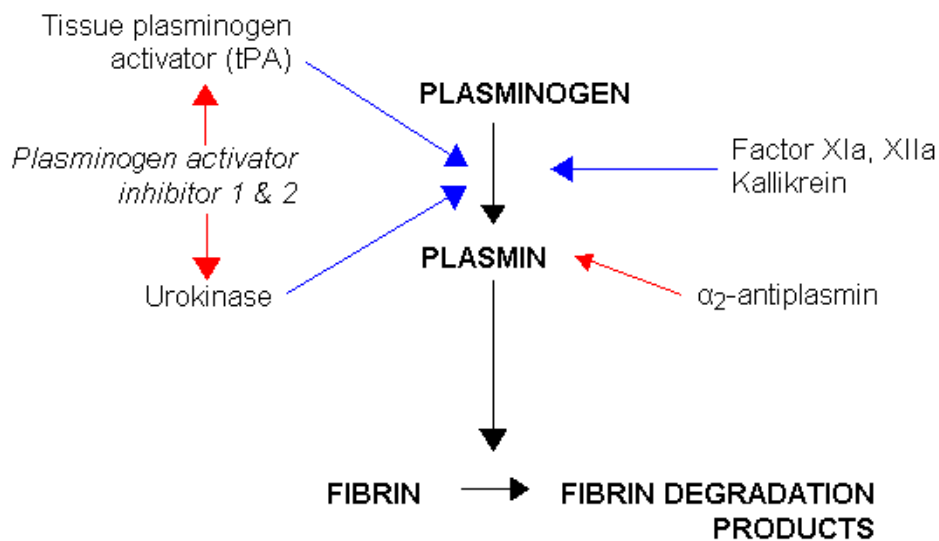
In addition to anticoagulation, APC also plays an important role in clot breakdown (fibrinolysis) by inactivating the inhibitor Tissue Plasminogen Activator Inhibitor (t-PAI or PAI-1). This allows the enhancement of fibrinolysis with acceleration of plasmin production, which in turn breaks down fibrin (Rezaie, 2001).

### **3.5.2 Fibrinolysis**

This is the natural progression of the coagulation process, to ensure that the clot is destroyed as part of repair of the tissue. The main enzyme in this stage is Plasminogen which is converted to Plasmin by the action of two activators: tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). Plasmin then degrades the cross-linked fibrin into fibrin-degradation products (FDPs) (See figure 9). Other factors from the vessel walls; factors XIa, XIIa and kallikrein also activate plasminogen, but tPA is the dominant stimulant. The tPA also binds to the clot fibrin, which enhances the capacity for the conversion of thrombus-bound plasminogen into plasmin. This dependence of fibrin by tPA strongly localises the plasmin formation to the clot (Hoffbrand et al., 2006). FDPs, namely D-dimers, can be measured to indicate thrombolysis.

Fibrinolytic agents are widely used in clinical practice. Recombinant tissue plasminogen activator (rtPA) is used to thrombolyse clots in many diseases

including myocardial infarction, major vessel thrombosis and acute ischaemic strokes. A second commonly used fibrinolytic agent is Tranexamic Acid (TXA), a lysine analogue that occupies binding sites for the activators on the plasminogen molecule, thus preventing the conversion to plasmin and inhibiting fibrinolysis (Levy et al., 2010). This primary effect of inhibition of clot breakdown portends a favourable effect on patients with haemorrhage from vascular disruption (Shakur et al., 2010; Morrison et al., 2011). The use of TXA in the treatment of traumatic coagulopathy will be discussed later in chapter 4.



**Figure 9:** The Fibrinolytic system, Blue arrows indicate activation and red arrows indicate inhibition. Tissue Plasminogen Activator (tPA) is the main activator. Plasminogen activator inhibitors 1&2 (PAI-1 and PAI-2) inhibit the action of the activators.

### *Hyperfibrinolysis*

Hyperfibrinolysis occurs when the fibrinolytic activity is potentially greater than fibrin formation such that clot integrity is threatened. This can result in a pronounced coagulopathy and sometimes fatal bleeding (Hunt and Segal, 1997). Terms such as hyperfibrinolysis and fulminant hyperfibrinolysis are widely used but poorly and arbitrarily defined in the literature (Levrat et al., 2008; Schochl et al., 2009; Kashuk et al., 2010; Schochl et al., 2012). There is little information that can provide a true definition of how to exactly quantify hyperfibrinolysis. A variety of assays have been developed to detect



hyperfibrinolysis but most of these tests lack practicability and reliability, making them unsuitable for rapid diagnosis of hyperfibrinolysis (Eeckhoudt et al., 2009). Specific tests determining t-PA activity, plasminogen activator inhibitor-1 (PAI-1) activity,  $\alpha$ 2-antiplasmin or plasmin-antiplasmin-complexes, euglobulin lysis test (ELT) are time consuming and are not routinely available in most trauma centres. Assays measuring fibrin/fibrinogen degradation products and D-dimers lack sensitivity and specificity, as these markers are elevated in most trauma patients (Lang et al., 2006). Practically, viscoelastic tests such as thromboelastometry (ROTEM<sup>®</sup>) or thromboelastography (TEG) are currently considered to be the most appropriate tools to detect hyperfibrinolysis (Luddington, 2005) and this is explored further in section 3.6.2.

With this understanding of the process of coagulation, the next section of the thesis will examine the methods available to test the haemostatic function that may have relevance in the clinical assessment of patients.

### 3.6 Tests of Haemostatic Function

A number of tests are available to assess the coagulation process by assessing platelets, the vessel wall and coagulation components of haemostasis. Testing provides a risk assessment for potential bleeding tendencies and can offer insight into the pathophysiology of the clinical bleeding problem (Davie, 2003). An ideal test would be one that gives an accurate, reproducible real time reflection of the *in vivo* clotting status that can be carried out by a non-technically skilled person. Interpretation of the results of such a test should indicate which component of the clotting process is at fault and thus allow correction of the clotting to be targeted. Currently no one test allows this, but current testing is by standard laboratory tests of haemostasis and when combined with more novel point-of-care tests, a wider picture of a patient's haemostatic status can be obtained.

Historic tests included the Bleeding Time, where a cut, of standardised width and depth, is made in a patient's forearm (a proximal sphygmomanometer is placed initially to maintain a constant pressure). The time taken for bleeding to stop was measured giving an indication of platelet function by how long the platelet plug takes to form. Although this was a whole blood *in vivo* test, numerous disease processes affect the result and is therefore rarely used clinically (Kembell-Cook et al., 2005).

The following sections describe some of the clinical tests in current use. Specialised laboratory assays are used to look at thrombin generation tests (TGTs) by measuring the quantity of thrombin produced (van Veen et al., 2008). There is also a wide range of other tests that are used in the research environment, mainly with ELISA to allow measurement of certain components of the coagulation process. Examples include prothrombin fragments, thrombomodulin, thrombin-antithrombin complex, Activated Protein C, and plasmin activator inhibitor. Measurement of these has provided the evidence to suggest the pathophysiology of the coagulopathy of trauma (Brohi et al., 2007).

### 3.6.1 Routine Laboratory Tests

A number of tests can be carried out to assess the function of the coagulation system. The routine standard tests are listed in Table 2.

#### *Prothrombin Time (PT)*

This is an *ex-vivo* coagulation assay performed by adding a commercial source of tissue factor (usually thromboplastin from brain tissue) and calcium to citrate-anti-coagulated plasma. The time to clot (12–18s) reflects the activity of the coagulation factors II, V, VII, X and fibrinogen – all involved in the classical ‘extrinsic’ and ‘common’ pathway.

Thromboplastin is a phospholipid-protein extract that can vary in its response to anticoagulant. In 1977, WHO released a standardised preparation that allows the PT to be reported as the International Normalised Ratio (INR). It is calculated by equation 3:

$$\text{INR} = (\text{Patient PT} / \text{Mean Patient PT})^{\text{ISI}}$$

**Equation 3:** Calculation of INR.

ISI is the International Sensitivity Index for the thromboplastin used to perform the PT measurement at any given laboratory (Davie, 2003). This is the standard test to measure the degree of coagulopathy induced by taking Warfarin.

#### *Activated Partial Thromboplastin Time (APTT)*

This estimates the activity of the of the coagulation factor proteins involved in the classical ‘common’ and ‘intrinsic’ pathways – factors II, V, VIII, IX, X, XI, XII, fibrinogen, prekallikrein and high molecular weight kininogen. During testing, phospholipid, a phospholipid surface activator (e.g. kaolin) and calcium are added. The normal APTT is 30-40s and if prolonged, it indicates that a reduction of at least 40% of the normal clotting factor activity levels has occurred (Kessler et al., 2007).

<b>TEST</b>	<b>Function of Test</b>
Full Blood Count ( <b>FBC</b> )	Measures platelet count and mean platelet volume.
Prothrombin Time ( <b>PT</b> )	Measures factors II, V, VII, X and fibrinogen. Warfarin affects result. Can be expressed as the international normalised ratio (INR)
Activated Partial Thromboplastin Time ( <b>APTT</b> )	Measures factors II, V, VIII, IX, X, XI, XII and fibrinogen.
Fibrinogen Level	Tested by Clauss assay or by PT-derived fibrinogen level (PT-Fg).
Fibrinogen Quantitation / Fibrinogen degradation Products( <b>FDPs</b> ) / D-Dimers	Abnormal results from fibrinogen deficiency or abnormal break down of coagulation process
Blood Film	Allows erythrocyte/ platelet morphology be examined.
Thrombin Time ( <b>TT</b> ) or Thrombin Clotting Time ( <b>TCT</b> )	This is the rate of clot formation. Is sensitive to fibrinogen deficiency / inhibition of thrombin.
Platelet Function Analysis ( <b>PFA</b> )	PFA-100 measures platelet aggregation. Replaced the historic bleeding time.

**Table 2:** Routinely used laboratory tests for coagulation.

Many of the techniques listed above are taken in a sample tube that contains sodium citrate as an anticoagulant. It is vital for correct sampling technique is followed to prevent erroneous results that can occur as a result of over- or under filling of the sample tube. The correct ratio is 1:9 of dilution of the anticoagulant. If there is decreased plasma volume, then the anticoagulant is more concentrated. The citrate present cannot neutralise the level of calcium that is added with the standard recalcification agents that allow coagulation to occur in vitro.

Using citrated plasma which is spun down to produce 'platelet-poor' plasma and testing can take variable length of times to return from the laboratory to the clinician (from 30 to 99 minutes) (Jeger et al., 2009; Singer et al., 2008). The use of dedicated laboratory for emergency department patients reduced times to as quickly as 30 mins (Craig et al, 2008; Singer et al., 2008) and in a deployed military hospital where the laboratories are next to the emergency department, the average time for return of coagulation results was 45 minutes (Doran et al., 2010). In the situation of a dynamic trauma resuscitation, coagulopathies could develop in the time for the results to return from the lab and thus not be detected or monitored in a timely manner that would allow management decisions based on current patient coagulation status. For this reason point of care testing has increased significantly over the past number of years and the using whole blood assays (Kaufmann et al., 1997; Johansson et al., 2009).

### **3.6.2 Point-of-Care (POC) Testing.**

The advantage of the method of testing beside the patient is having the results which rapidly assess the coagulation status at hand as they are produced. The first bedside system developed was activated clotting time (ACT) described by Hattersley in 1966 (Hattersley, 1966) and was used in to assess coagulation in cardio-pulmonary bypass. Instruments have developed since then and one widely used is the 'i-stat' analyser (Abbott Laboratories, East Winsor, NJ) which was designed for whole-blood-based testing, initially for blood gas and electrolyte analysis (Jacobs et al., 1993). These machines have been used in cardiac surgery setting and emergency departments, but there were difficulties associated with their use in the extreme conditions that is the military experience, possibly because of alterations in temperature changes (M Midwinter, personal communications). Recent work in a Level 1 Trauma Centre used a simple point of care device called Coaguchek<sup>®</sup> (Davenport et al., 2011) and compared the results with standard laboratory tests. The Coaguchek<sup>®</sup> (Roche Diagnostics Ltd, Burgess Hill, UK) is a portable, easy to use, commercial device that measures PT and INR. Overall a good agreement was documented between the Coaguchek<sup>®</sup> and the laboratory

results, but it was confined only to patients without coagulopathy, when there was 99% of the CoaguChek<sup>®</sup> results were within 95% confidence levels. However, a low haematocrit was associated with larger discrepancies between this point-of-care device and the laboratory results. This would not give reliable results in the trauma patient setting.

The most significant development over the past two decades has been in systems that provide a global assessment of blood clotting and platelet function by use the thromboelastography (TEG) / thromboelastometry (TEM).

### *Thromboelastography / Thromboelastometry*

TEG was first developed in 1948 by Hartert in Germany, measuring the viscoelastic changes that occur in blood providing information on coagulation initiation, propagation kinetics, fibrin-platelet interaction, clot firmness and fibrinolysis (Hartert, 1948). It remained largely as a research tool until the start of the 1980's when the technique of evaluating whole blood during the perioperative period began to have resurgence (Kang et al., 1986, Spiess, 1995, Shore-Lesserson et al., 1999) – mainly in cardiac and liver transplant surgery. In 2008, a health technology assessment report for the NHS was published looking at the clinical and cost effectiveness of TEG / TEM (Craig et al., 2008). This concluded that the technique was both a clinical and cost effective intervention by reducing the need for inappropriate transfusions and decreasing blood product requirement, therefore improving the transfusion in cardiac and liver transplant operations. At the time of publication, the authors commented that was no robust, controlled clinical data to support the use of TEG/TEM in other major operations associated with major blood loss, including trauma, although published observational evidence did support its use (Kaufmann et al., 1997; Schreiber MA et al., 2005; Rugeri et al., 2007).

With the acceptance that effective and rapid management of acute bleeding in the trauma patient is of the utmost importance, a multidisciplinary task force for Advanced Bleeding Care in Trauma was formed in 2005. They published a guideline for the management of bleeding following severe injury in 2007 (Spahn et al.) and this was updated and published in 2010 (Rossaint et al.).

Key changes in the updated version included new recommendations on coagulation support and the appropriate use of local haemostatic measures such as tourniquets. Recommendation no 12 highlighted that routine tests, such as INR and APTT, fibrinogen and platelets, should be used to detect post-traumatic coagulopathy but they should not be used in isolation to guide haemostatic therapy. Their recommendation was to use TEG/TEM in characterising the coagulopathy and in guiding haemostatic therapy (Rossaint et al., 2010).

The test is carried out by placing a whole blood sample in a cuvette or cup into which a wire is suspended. The cuvette is oscillated through 4°75' and as the clot begins to form, the change in viscosity and subsequent shear is transmitted through pin. This change is translated into a characteristic graph known as the thromboelastograph (Mallett and Cox., 1992). A practical limitation of Hartert's classical thromboelastographic test was the long observation time when coagulation is not activated by biochemical agonists, therefore the modern tests employ various activators and inhibitors with the aim of accelerating the test times and getting differential diagnostic information while maintaining the principal detection method (Neilson VG et al., 2000; Lang et al., 2004).

### *TEG vs. TEM*

Two commercial instruments are available for carrying out TEG/TEM: ROTEM<sup>®</sup> (Tem Innovations [Pentapharm] GmbH, Munich, Germany), a self-contained, touch-screen that is analogous with Thromboelastograph - TEG<sup>®</sup> (Haemoscope Cooperation, Niles, IL). Both instruments are shown in figure 8. Although the nomenclature differs slightly between TEM and TEG the essential difference between them is a discrepancy in the actual mechanism of detection; table 3 outlines the main features between the two machines.

For the purposes of this thesis, TEM will be used as the term meaning either TEG or TEM and the focus will be placed on the mechanism and interpretation of ROTEM<sup>®</sup>. TEG<sup>®</sup> is used when the actual machine is being referred to.



**Figure 10:** Pictures showing TEG<sup>®</sup> on the left and ROTEM<sup>®</sup> on the right.

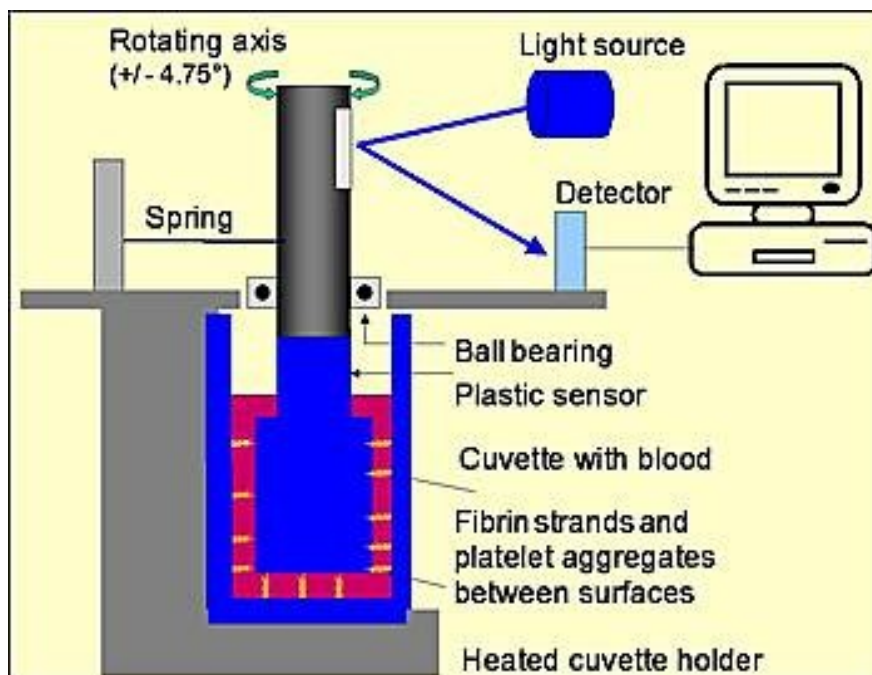
<b>TEG<sup>®</sup></b>	<b>ROTEM<sup>®</sup></b>
Two independent channels per analyzer Connected to separate computer	Four independent channels per machine with integral computer and touch-screen
Size: 25 x 26 x 29 cm	Size: 57 x 37 x 57 cm
Requires electrical point and stable, non- vibrating surface. Works with Windows XP	Requires electrical point. Runs in an integrated Linux program
Manual pipette	Automated pipette
Cup rotates 4°45' around fixed pin transduction system	Pin transduction system rotates on axis 4°75' around fixed cup
Daily quality control checks	Weekly quality control checks
Tests run are standard, heparinase, functional fibrinogen, platelet mapping. Adjustable measurement temperature	Tests run are EXTEM, INTEM (looking at extrinsic and intrinsic pathways), fibrinogen (FIBTEM), hyperfibrinolysis (APTEM), heparinase (HEPTEM). Adjustable measurement temperature
Sample must be tested within 4 minutes of taking (wait 30 min if using citrated sample; add calcium)	Citrated sample can be used immediately, stable up to 6 hr
Results exportable in Excel. Via USB or network	Results exportable in Excel, txt, and JPEG format. Via USB port or networked

**Table 3:** Features of TEG<sup>®</sup> / ROTEM<sup>®</sup> systems.



## ROTEM®

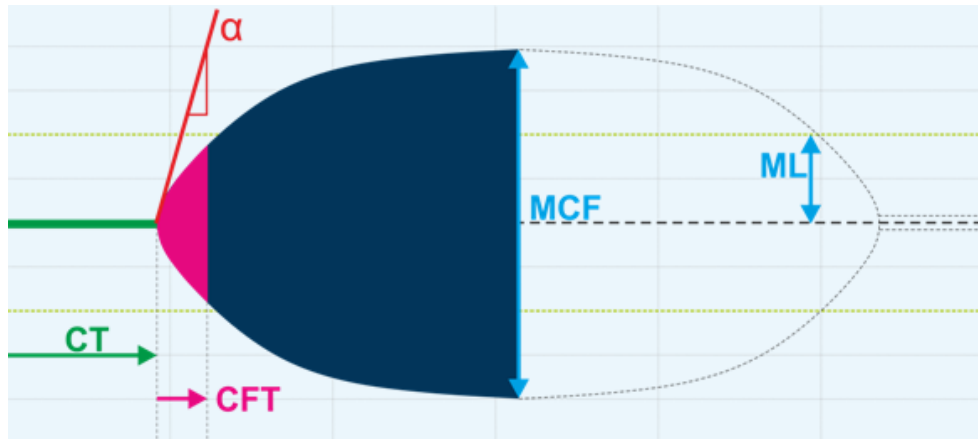
This system was developed in Munich, Germany between 1995 -1997 (Calatazis et al., 2002). The detection method is outline in figure 11. The blood sample is placed in cuvette, held stationary in a warmed holder (at 37°C), whilst the pin sensor is attached to the rotating axis. All pipetting steps of blood and reagents are performed in a standardised way by following the integral automated electronic pipette programme. As the clot forms it restricts the rotation of the pin inversely proportional to the overall clot firmness. The degree of rotation is detected optically and translated into the curve, figure 12. Hartert's original design and that of TEG®, the pin is fixed and it is the cuvette that is rotated. This makes the instrument susceptible to vibration and mechanical shocks, which is of relevance when considering use in a deployed military setting.



**Figure 11:** Schematic diagram showing the detection of the degree of rotation of the axis by an optical detector (reproduced by permission of Tem Innovations GmbH).

### *Result Analysis*

Each TEM trace provides the following trace, which is explained in figure 12.



**Figure 12:** TEM trace providing the following parameters: CT- clotting time; CFT – clot formation time;  $\alpha$ -angle - Speed of clot formation / function of rate of polymerisation; MCF – maximum clot firmness; ML – maximum lysis. (Produced by kind permission of Tem Innovations GmbH, Germany).

The trace provides the following parameters:

**Clotting time (CT)** – the time in seconds from the start of the activation process to the initial clot formation when the amplitude reaches 2mm on the trace. This equates to the initiation of clotting and thrombin formation. A prolongation occurs in factor deficiency and use of anticoagulants, or severe hypofibrinogenaemia (Nielsen et al., 2005)

**Clot Formation Time (CFT)** – time taken from clot initiation until a clot firmness of 20mm is reached. This equates to fibrin polymerisation and stabilisation of the clot with platelets and Factor XIII, representing the clot formation dynamics. This time is shortened by an increase in fibrinogen and platelet function. A prolonged CFT indicates a failure for the clot to form and is also prolonged by anticoagulants such as heparin.

**$\alpha$ -Angle** – this is given by the angle between the centre line and a tangent to the curve through the 2mm point. This is also giving the kinetics of clot formation, ie the smaller the angle the weaker the clot formation is happening (Lang et al., 2005) and decreased values would be seen with hypofibrinogenaemia and thrombocytopenia (Mallet and Cox, 1992).

**Maximum Clot Formation (MCF)** – this is a measurement of the maximum firmness and is a reflection of the absolute strength of the fibrin clot equating to

the maximum dynamic properties of fibrin and platelets. A reduction in the MCF indicates a deficiency of clottable substrate – either platelet or fibrinogen (Luddington, 2005). Platelet abnormalities, whether they are qualitative or quantitative in nature, substantially disturb the MCF (Mallet and Cox, 1992). The firmness at 5 and 10mins following the CT are recorded as CA5 and CA10, which can give early indications of abnormal results (Davenport et al., 2011).

**Maximum Lysis (ML)** – this is a reduction in the clot firmness after MCF in relation to MCF. It is a function of time and reflects loss of clot integrity as a result of lysis. The lysis is often recorded as Ly 30 and Ly 60 which is the percentage of lysis that has occurred 30 and 60 mins after MCF. Normal value is <15% lysis in 60 mins. This percentage of lysis increases in hyperfibrinolysis.

#### *TEM and the cell-based model of haemostasis*

In section 3.4, the cell-based model of haemostasis was described in three phases: Initiation, amplification and propagation. These phases are reflected in the TEM parameters. Recent work that has looked at the morphology of the clot during thromboelastography by electron microscope (Kawasaki et al., 2004) and demonstrated that the CT corresponds to the initiation phase; the CFT reflects the amplification phase where small amounts of thrombin are formed. The thrombin burst that occurs in the propagation phase is reflected by the  $\alpha$ -angle (Rivard et al., 2005; Johansson et al., 2008). This ultimately affects the MCF or overall strength.

#### *ROTEM<sup>®</sup> tests*

This is a whole blood global assessment, but it must be recognised that due to inherent redundancy in the haemostatic process, specific defects may be masked. Consequently a number of tests with inhibitors and activators exist in order to differentiate the components of the process. The activators also initiate the coagulation process which provides more timely results. There are six commercially available tests for ROTEM<sup>®</sup> (Tem Innovations GmbH, Germany) which are listed in table 4.

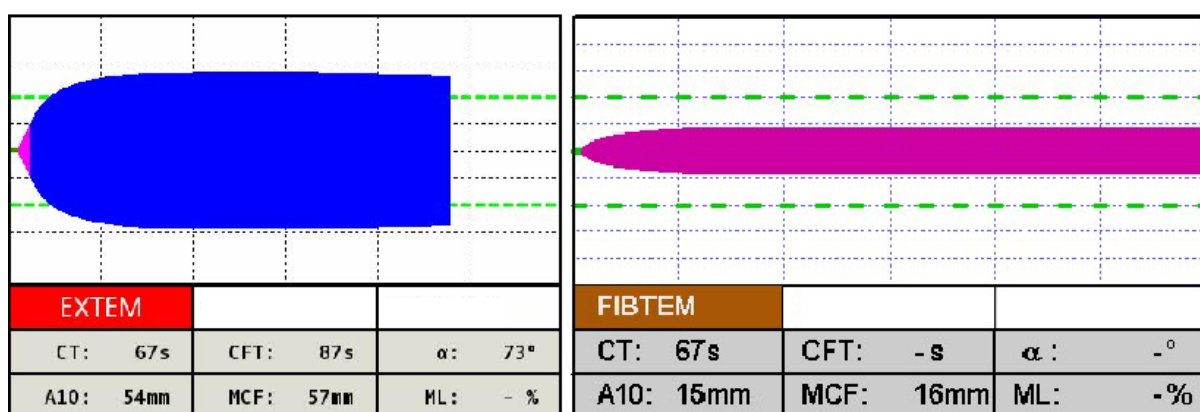
Routinely all tests are carried out at 37°C, although the running temperature of the machine can be altered to mirror the patient's temperature. This would allow a more accurate assessment of the coagulopathic profile of the patient at that moment in time. TEM has been used to determine the alterations of temperature on the coagulation and shown that both CT and CFT are prolonged but not the MCF (Kettner et al., 2003; Rundgren and Engstrom, 2008), suggesting an impairment of the coagulation factors rather than platelets. In the situation of trauma, all steps are taken to prevent hypothermia and if the results show a coagulopathy at 37°C then therapeutic action is required, in conjunction to warming.

Test	Activator/ Inhibitor	Information Provided	Normal Values
<b>EXTEM</b>	Recombinant Tissue Thromboplastin (Tissue Factor)	Assesses factors VII, X, V, II, I, platelets and fibrinolysis	CT: 38-79s CFT: 34-159s MCF: 50-72mm ML: <15% in 60mins
<b>INTEM</b>	Activated by partial thromboplastin phospholipid	Assesses factors XII, XI, IX, VIII, X, V, II, I, platelets and fibrinolysis Sensitive to heparin	CT: 100-240s CFT: 30-110s MCF: 50-72mm ML: <15% in 60mins
<b>FIBTEM</b>	Activated by tissue factor. Platelet function inhibited by Cytochalasin D	Assesses fibrinogen levels and fibrin polymerisation	MCF: 9-25mm
<b>HEPTEM</b>	Activation is same as INTEM with addition of heparinase to remove effect of heparin.	Determines if coagulopathic are due to heparin or not.	Normal values as INTEM
<b>APTEM</b>	Activated as EXTEM. Aprotinin inhibits fibrinolytic processes	Determines if fibrinolysis is true or if additional fibrinogen is required.	Normal values as EXTEM
<b>NATEM</b>	Calcium only added	Allows very sensitive assessment of coagulation activation or inhibition – mainly for in-vitro experimentation	---

**Table 4:** Assessment tests of ROTEM, the activating/inhibiting additive, the information being assessed / provided and the Normal Expected Values. (Ranges validated by Lang et al., 2005).

## ROTEM® graphs

The ROTEM® has four channels allowing four different tests to be run simultaneously. Unless the patient is heparinised, the INTEM result often mirrors the EXTEM trace, with a slightly longer CT/CFT value. By running EXTEM and FIBTEM (normal traces in figure 13), information on the overall clot dynamics can be assessed and as well as fibrinogen levels from the FIBTEM trace (platelets are inhibited). This also allows two patient's samples to be run simultaneously, using two channels for each patient.

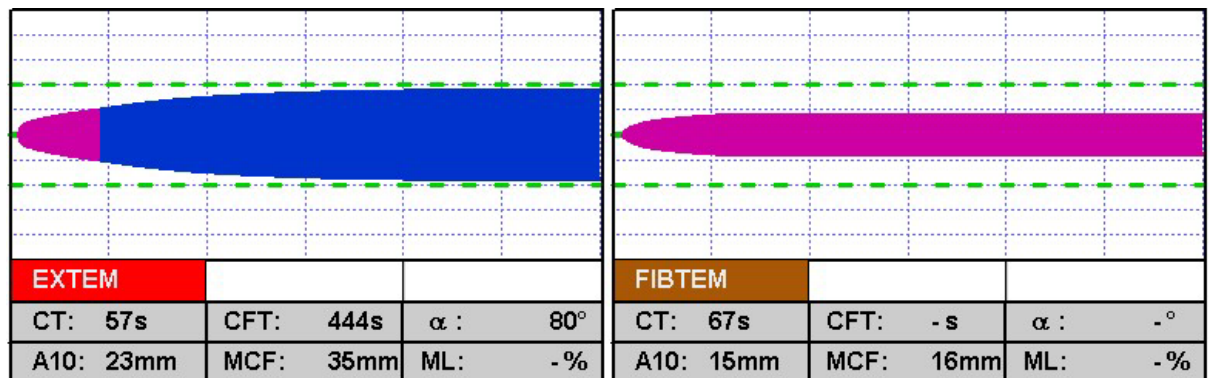


**Figure 13:** Normal EXTEM and FIBTEM traces.

## Abnormal ROTEM® graphs

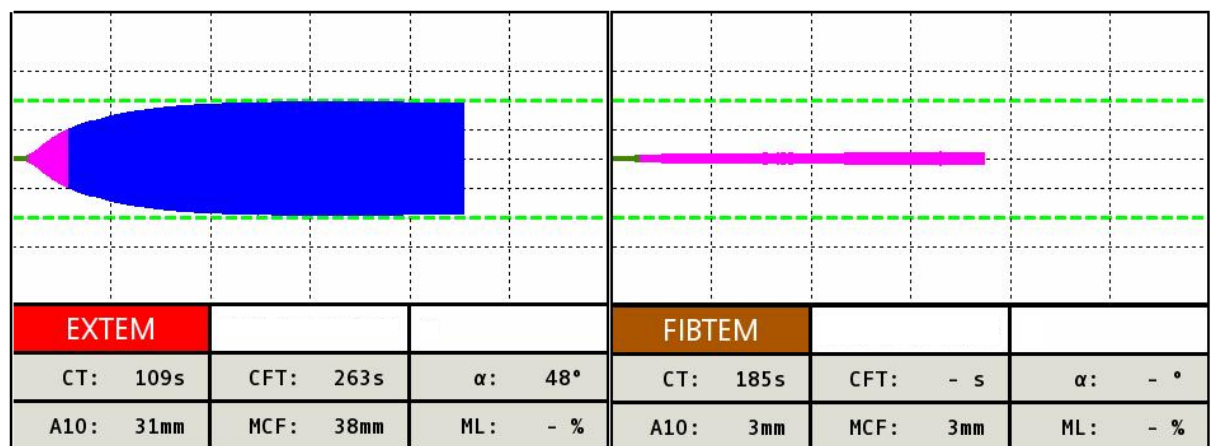
This section illustrates the utility of the graphical representation and clinical interpretation of the output of ROTEM analyses. The pictorial representation of the results makes the test ideal for situations such as a trauma bay in an emergency department, as the traces will develop whilst on-going resuscitation and care of the patient is continuing and any member of staff, regardless of their educational level can easily learn the fundamentals of interpretation. It is then the clinician's role to incorporate the results into the ongoing management of the patient's resuscitation. It should be remembered that TEM methods depend on low shear force and are therefore not suitable to detect defects in early platelet function – for example von Willebrand Factor Deficiency, aspirin or clopidogrel. This should be born in mind if a haemorrhagic patient shows normal results (Kembell-Cook et al., 2005).

Figure 14 shows an example of EXTEM and FIBTEM in a patient with abnormal results. The EXTEM trace has a normal CT, meaning that the initiation phase of clotting is normal but prolonged CFT and decreased  $\alpha$ -angle, and decreased MCF informs the clinician there is a problem with the thrombin burst and subsequent polymerisation of the clot. The FIBTEM is normal, indicating normal fibrinogen levels; thus there is a platelet deficiency / hypofunction in this patient.



**Figure 14:** Platelet deficiency resulting in low MCF in EXTEM but normal fibrinogen levels as shown by normal FIBTEM trace.

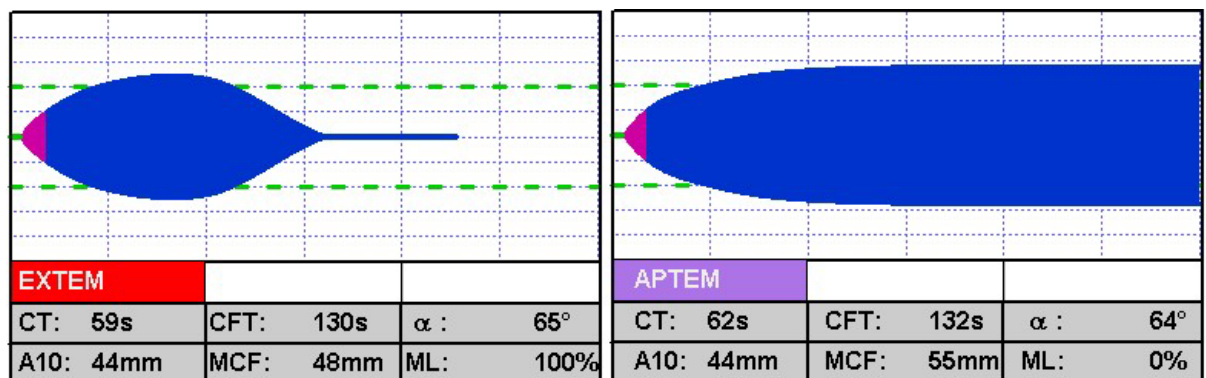
Figure 15 shows an EXTEM trace like the previous with a normal CT, but prolonged CFT, decreased  $\alpha$ -angle, and overall decreased MCF. From the FIBTEM trace, the MCF is significantly reduced indicating a low fibrinogen level.



**Figure 15:** Fibrinogen deficiency, as shown in FIBTEM trace, resulting in a low MCF in the EXTEM trace.

Figure 16 shows an EXTEM trace that has a normal CT but the CFT,  $\alpha$ -angle

and MCF is reduced. In this example the ML is 100% indicating fulminant hyperfibrinolysis. To prove this is the case, an APTEM trace beside it shows a normal trace, as fibrinolysis process has been inhibited by aprotinin. The normal value for fibrinolysis is <15% in 60 minutes, whilst hyperfibrinolysis is a result of >15% in that time. Fulminant hyperfibrinolysis is when the degree of fibrinolysis is >50% in 30 minutes. These appear to be the only definition in the literature to attempt to truly define hyperfibrinolysis in trauma patients (Schochl et al., 2009).



**Figure 16:** EXTEM trace showing hyperfibrinolysis and proven by a normal APTEM when the fibrinolysis process is inhibited.

#### *Citrated vs non-citrated blood and Different Activators.*

The original work on the development of TEM used non-citrated blood (Hartert, 1948) which limits the usability in the clinical setting as there is a requirement for the analysis to be performed with 4 minutes of the blood being drawn to prevent clot formation, but it does avoid of contact activation related to storage sample (Wasowicz et al., 2008). To that end, citrated samples are used in most work on TEM in the clinical setting (Lang et al., 2005; Rugeri et al., 2007; Johansson et al., 2009). There have been concerns that the TEG/TEM results obtained from citrated blood would be different to those from non-citrated samples and that storage time of the citrated sample may influence measurements (Bowbrick et al., 2000; Camenzind et al., 2000; Mancuso et al., 2003; Zambruni et al., 2004). More recent studies have shown that the storage of citrated blood had no discernable effect on the TEG<sup>®</sup> results (Wasowicz et al., 2008), however a hypercoagulable trend has been observed with citrated

samples when compared with non-citrated (Mancuso et al., 2003).

Recent studies conducted by Royal Centre for Defence Medicine/DSTL using ROTEM<sup>®</sup> (E Kirkman, personal communication) has shown that citrated blood samples from normal volunteers does show evidence of enhanced clotting on standing. However, although this change is statistically significant the magnitude is small and clinically insignificant. By contrast, a recent study have shown that citrated samples from coagulopathic patients undergo more substantial (clinically and statistically significant) enhancement of clotting upon standing (Jansen et al, in press) resulting in the risk of underestimating the degree of coagulopathy in these samples.

Further concerns have been raised with both TEG<sup>®</sup> and ROTEM<sup>®</sup> with the divergent activators employed during analysis. TEG<sup>®</sup> traces are predominantly based on monoanalysis using kaolin activation (Johansson et al., 2009), but other papers use celite, tissue factor or combined kaolin and tissue factor (Shore-Lesserson et al., 1999; Avidan et al., 2004; Kashuk et al., 2010). In contrast, ROTEM uses standard activators (TEM-reagents) and Neilson has shown that these negate the discrepancies encountered with TEG (Neilson, 2007). Larsen et al. (2011) raised these discrepancies when considering how many have advocated using TEG/TEM to help guide transfusion and questioned whether the results could be misinterpreted if a range of activators was used. Their study investigated the diagnostic performance and therapeutic consequence of using kaolin-activated whole blood compared with a panel of TEM-reagents to distinguish dilutional coagulopathy, thrombocytopenia, hyperfibrinolysis and heparinisation. The researchers concluded that monoanalysis with kaolin was unable to distinguish coagulopathies caused by dilution from that of thrombocytopenia but these were readily distinguishable using the TEM-reagents. This could mean that transfusion algorithms based on kaolin could lead to unnecessary administration of platelets (Larsen et al., 2011).

*Comparison of TEM results with standard laboratory tests.*

ROTEM<sup>®</sup> has been compared with standard laboratory tests such as PT, APTT,



fibrinogen and platelet count (Savry et al., 2005; Coakley et al., 2006). Savry et al reported that plasma fibrinogen level was strongly correlated with the kinetics of clot formation (CFT) and the maximum clot firmness (MCF) of ROTEM<sup>®</sup> ( $r > 0.7$ , coefficient of Pearson). This result was mirrored by Coakley et al, who reported a significant correlation between FIBTEM MCF and Clauss fibrinogen ( $r = 0.75$ ).

However, the work of both these groups reported that PT and APTT had a weak correlation with ROTEM<sup>®</sup> parameters. It is not possible to make exact comparisons between the PT/APTT and TEM as the laboratory tests are only factor based and provide static end points as compared to the whole blood dynamic test of TEM. Platelet count influences the stability of the clot as measured by EXTEM and INTEM, and in combination with FIBTEM and EXTEM the MCF gives an indirect measurement of platelet contribution to the clot stability (Lang et al., 2009).

Over the past decade, TEM has emerged as a validated and reproducible method for assessing coagulopathy, comparable to standard routine laboratory tests and is beginning to take an important role in improving patient care (Spalding et al., 2007; Ebinger T et al, 2010). This is of particular interest in the role of diagnosing and understanding the pathophysiology of the trauma-induced coagulopathy.

### **3.7 Coagulopathy of Trauma**

Abnormal clotting that occurs after injury is an entity that has been recognised in the literature for over 40 years; however the past decade has seen a resurgence in interest in this subject as it has been recognised in a significant risk of mortality.

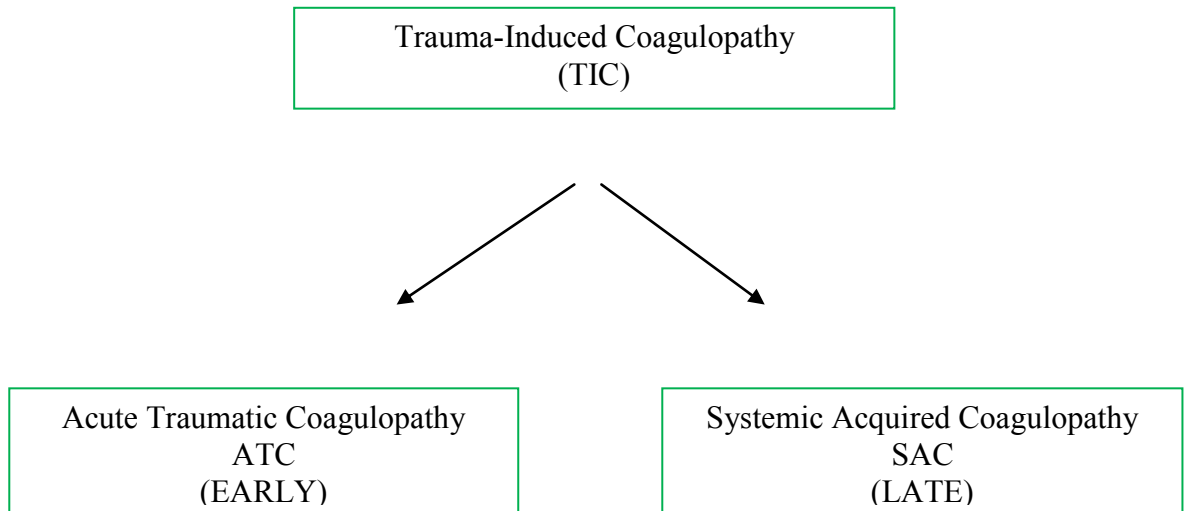
A number of studies looked at the coagulation abnormalities in the Vietnam casualties (Simmons et al., 1969). It was noted that prothrombin time (PT) and activated partial thromboplastin time (APTT) correlated poorly with acute resuscitation efforts and concluded that later complications were reflective of previous shock and massive transfusions. A more detailed analyses of Vietnam results in 1971 recognised that the contribution of thrombocytopenia as the cause of a bleeding diathesis after massive transfusion (Miller et al., 1971).

Over the next two decades it was accepted that coagulation activity was enhanced in the first 24hrs after trauma and then suppressed during the next five days (Kapsch et al., 1984, Risberg B et al., 1986; Gando et al., 1992). The mechanism was thought to be from loss due to bleeding or consumption, dilution from fluid administration or dysfunction of coagulation proteases due to hypothermia and effect of acidaemia (Kashuk et al., 1982, Schreiber 2005, Brohi et al., 2007).

#### **3.7.1 Trauma-Induced Coagulopathy**

One area that has lead to some confusion has been adoption of multiple terms describing different aspects of this phenomenon. It has been described in two phases: early and late. The coagulopathy in the early phase following trauma has been called Acute Coagulopathy of Trauma (ATC) (Brohi et al., 2003); endogenous acute coagulopathy (EAC) (Chesebro et al., 2009) or the Acute Coagulopathy of Trauma-Shock (ACoTS) (Hess et al., 2008). The late phase has been described as Systemic Acquired Coagulopathy (SAC) which is due to the loss or inhibition of the coagulation proteases (Tieu et al., 2007; Ganter and Pittet, 2010).

The term Trauma-Induced Coagulopathy (TIC) is more widely accepted as the over-arching term that encompasses both phases, as illustrated in figure 17.



**Figure 17:** Illustration of how the nomenclature of the coagulopathy associated with trauma is encompassed by the term ‘Trauma-Induced Coagulopathy’.

The clinical incidence and significance of TIC was highlighted by a number of seminal papers describing the early onset of a coagulopathy or ATC and that it is an independent predictor of death (Brohi, 2009). Brohi et al (2003) studied the immediate clotting screen that was taken from 1,088 patients that were brought in by the London Helicopter Emergency Medical Service. The presence of coagulopathy was defined as a PT of greater than 18s, APTT over 60s and a Thrombin Time (TT) over 15s. These values are taken from the definition of coagulopathy stated by the British Committee for Standards in Haematology (2006) and the College of American Pathologists (1994). By these criteria, it was found that 24.4% of patients were coagulopathic at admission and when mortality was looked at as an end point, the patients who were coagulopathic on arrival had a mortality of 46%, which was significantly different from 10.9% for those who had normal clotting parameters ( $p < 0.001$ ) (Brohi et al., 2003). This increased mortality associated with coagulopathy was independent of an increased Injury Severity Score (ISS). There was no correlation with the amount of pre-hospital fluid administered and the development of coagulopathy (although all volumes actually given were low).

A second paper that year also identified a link between early coagulopathy and

mortality. MacLeod et al (2003) took their patients to be coagulopathic with a non-standard definition of PT >14s and APTT >34s. From a population size of 10,790, it was found that 28% had an abnormal PT and 8% had an abnormal APTT. 19.3% of patients with an abnormal PT died, significantly different to the 6.3% who died who had a normal PT ( $P < 0.001$ ). A univariate analysis generated an odds ratio of 3.6 for death with an abnormal PT on arrival to the Emergency Department and 7.81 for deaths with an abnormal PTT. This paper did not look at fluid volumes administered during the resuscitation.

The third seminal paper documenting this acute trauma coagulopathy was an analysis of 8724 patients (Maegle et al., 2007). Using slightly different testing parameters (Quick's test <70% and platelet count <100,000  $\mu\text{l}^{-1}$ ), it was found that 34.2% were coagulopathic on admission to the hospital. Correlation was noted with increased ISS but also with the volume of pre-hospital fluid administered. This is discussed further in section 4.2.

A more recent study (Moore et al., 2009) observed this early coagulopathy in 39% of severely injured patients based on blood that was taken within 15mins of injury. This group corroborated the above findings of having a higher incidence of subsequent multi-organ failure (MOF) and death in that group, highlighting further the importance of understanding the pathogenesis of this ATC.

Within the reported military experience, there are only a few papers published that document the incidence of traumatic coagulopathy in combat casualties (Plotkin et al., 2008). Both were retrospective studies looking at patients brought to the Combat Support Hospitals in Iraq. The incidence of coagulopathy was found to 38% on arrival and again it was noted that this group had a significantly higher rate of mortality. Results of this thesis (Chapter 6) have shown that this incidence does depend on the method of testing as the use of TEG/TEM can indicate a much higher incidence of coagulopathy.

The above studies have looked at hypocoagulopathy; however hypercoagulability after injury is also a major source of morbidity and mortality that occurs later in the timeline of the patient's recovery (Schreiber 2005). It

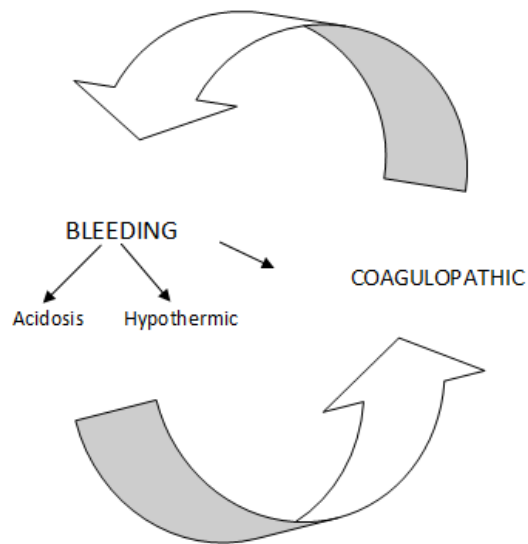
has been shown that 58% of patients who had an ISS >9 had deep vein thrombosis as shown by venography, although there was a high incidence of pelvic/lower limb injury (Geerts et al., 1994). Ganter and Pittet (2010) described that this development of a procoagulant activity was associated with a number of processes including low levels of activated Protein C (APC), diminished fibrinolysis and down regulation of the complement activation. These processes are discussed further in section 3.7.2 below.

The considerable interest in this area the *Educational Initiative on Critical Bleeding in Trauma* (EICBT) which was formed in 2006 with the aim to increase the awareness amongst health professionals that coagulopathy plays an important role during the first hour after traumatic injury (Hoyt 2008). This international panel of experts emphasised the need to understand the physiological mechanisms behind this TIC and to validate the tools by which to recognise these patients.

### **3.7.2 Review of Mechanisms of the Coagulopathy of Trauma**

The “Bloody Vicious Circle” or the “Lethal Triad of Death” are two terms that have been historically linked with the hypocoagulopathy associated with trauma and the subsequent resuscitation. It was suggested that the combination of tissue injury (in part, initiating the clotting cascade) and blood loss lead to acidosis, hypothermia and coagulopathy. Figure 18 depicts this circle that historically thought to be the cause of the TIC.

Although this triad contributes to the later acquired phase of the TIC (SAC), the pathophysiology has been shown to be more complex. More complete and robust measurements combined with the new models of haemostasis (Khierabadi et al., 2007; Rugeri et al., 2007) have provided a global functional characterisation of the causes and effects of traumatic coagulopathy (Hess et al., 2008).



**Figure 18.** “The lethal triad of Death” showing the historical cycle of events that led to coagulopathic bleeding that complicates life-saving surgery and forces the early termination of operations (Schreiber, 2004).

Cosgriff et al (1997) showed that massively transfused trauma patients with a combination of an ISS greater than 25, an acidosis with a pH < 7.10, hypothermia with a temperature < 34°C and a systolic blood pressure < 70 mmHg have a 98% likelihood of developing a life threatening coagulopathy (when all four factors are present). They defined the coagulopathy as a PT and APTT greater than twice normal. Patients with none of these risk factors had a 1% chance of developing such a life threatening coagulopathy (Tieu et al, 2007). Hess published the collaborative work by the EICBT that reviewed 87 publications to conclude that the traumatic coagulopathy is a complex multi factorial process and is contrary to the simplistic, reductionist explanations that have underpinned current clinical understanding of the so-called ‘lethal triad’ (Hess et al., 2008). The following primary mechanisms were identified: tissue trauma, hypoperfusion, inflammation, consumption, haemodilution, acidaemia and hypothermia, with the first three being the key pathophysiology to the early ATC (Ganter and Pettit, 2010). The theories for these mechanisms are described in the following sub-sections.

### Tissue Trauma

A degree of tissue trauma is inevitable in trauma and injury severity has been shown to be closely associated with traumatic coagulopathy (Brohi et al., 2003; MacLeod et al., 2003). As described previously, damage to the endothelial surface initiates coagulation as it results in the exposure of tissue factor, and allows the binding of von Willebrand factor, platelets and FVIIa (Mann, 1999). The endothelial damage increases the release of tissue plasminogen activator (tPA) and this in turn activates the fibrinolytic system (Schneiderman et al., 1991; Kooistra et al., 1994). The increasing levels of thrombin formed by the clotting cascade in turn leads to an increase in the levels of tPA (di Cera 2003). The resulting increase in fibrinolysis is exacerbated by the inhibition of plasminogen activator inhibitor-1 (PAI-1) in the presence of hypoperfusion resulting in hyperfibrinolysis (Brohi et al., 2007). Normally fibrinolysis is to limit clot propagation to the site of vascular injury but with injuries resulting from widespread trauma this localisation appears to be lost (Hess et al., 2008).

Specific organ injuries have been particularly associated with the development of coagulopathy. Severe traumatic brain injury has often been associated with increased bleeding (Zehtabchi et al., 2008) and this has been suggested due to the release of brain-specific thromboplastins which activates the clotting cascade and subsequently causes inappropriate depletion of clotting factors (Stein and Smith, 2004). In one series it was noted that 77% of brain-injured patients who died had a coagulopathy at the time of hospital admission (Hulka et al., 1996). More recent studies have postulated that hyperfibrinolysis is the dominant mechanism for increased bleeding in these patients (Kusimoto et al., 2003; Cohen et al, 2009).

Long bone fractures have also been associated with an increased incidence of coagulopathy. Initially thought to be secondary to a bone marrow-specific pathogenesis (Mellor and Soni, 2001), it is now thought to be driven by tissue injury, hypoperfusion and inflammation (Hess et al., 2008).

### Hypoperfusion

Evidence is accumulating that systemic hypoperfusion (or 'shock') appears to be the prime driver in the pathogenesis of early traumatic coagulopathy (Hess

et al., 2008, Ganter and Pittet, 2010). Brohi et al (2007) showed that there was a dose-dependent association between the degree of hypoperfusion and the extent of the admission coagulopathy (as measured by the prothrombin and partial thromboplastin times). This prospective study looked at 208 trauma patients admitted to a single centre in a 15 month period, and blood was taken on admission for all routine laboratory tests, blood gas analysis and for a range of specialised assays looking at particular components in the thrombomodulin-Protein C pathway. Base deficit (BD) was used as a marker of hypoperfusion, a value greater than 6mEq/L has been associated with an increase in transfusion requirements, acute lung injury, multiple organ failure and mortality. (Siegel et al., 1990, Davis et al., 1996, Eberhard et al., 2000, Durham et al, 2003). As the level of tissue hypoperfusion increased (with increasing BD) both PT and APTT increased. This coagulopathy was absent when there was no evidence of hypoperfusion (Brohi et al., 2007).

It is noted that several authors (including Brohi et al., 2007) have used base deficit as a marker of tissue hypoperfusion. Base deficit (BD) (and lactate) are used clinical indices of shock (inadequacy of tissue oxygenation), which in the case of haemorrhagic shock is the result of hypoperfusion (Wan et al, 2009). In an experimental study BD was shown to correlate significantly with tissue (skeletal muscle) blood flow and oxygenation during haemorrhagic shock and resuscitation (Wan et al., 2009). In this work, although the correlations with BD were significant the  $r^2$  values are not high (0.1274 and 0.1303 respectively for tissue oxygenation and blood flow). This indicates that tissue oxygenation and blood flow only account for a relatively small amount of the variability of BD observed by Wan et al. This is easily accounted for as BD is a whole body measure while in these experiments the blood flow and oxygenation were only measured in skeletal muscle. Nonetheless a number of prominent authors, such as Brohi, do use BD as a marker of tissue hypoperfusion, or rather restricted oxygen delivery.

The exact reason for this association with hypoperfusion and coagulopathy is still a matter of debate in the literature, but the involvement of protein C appears pivotal (Brohi et al, 2007, 2008; Chesebro et al., 2009.). As the degree of hypoperfusion progresses, i.e. the base deficit decreases, there is an increase in the levels of soluble thrombomodulin (TM) and a decrease in the protein C



levels. As explained in section 3.5.1, TM is expressed by the endothelium and forms a complex with thrombin. Firstly, this reduces the amount of thrombin that is available to cleave fibrinogen to fibrin and secondly, the TM/Thrombin complex activates Protein C. Activated Protein C (APC) inhibits factors Va and VIIa (thus reducing their levels) which in turn leads to a reduction in the amount of thrombin formed. This leads to the “coagulopathy”. In the papers cited above an increase in APC (which was not measured directly) was inferred from a reduction of Protein C.

The APC rise also drives the fibrinolysis by inhibiting PAI-1 (refer to figure 6) and thus to increasing the rate of fibrin degradation and “hyperfibrinolysis” (Brohi et al., 2008).

Chesebro et al (2009) developed a translational mouse model to examine further this mechanistic role of Protein C in the early acute coagulopathy. Mice were subjected to one of four treatment groups – 1) control; 2) Trauma (laparotomy); 3) Haemorrhage; 4) Trauma + Haemorrhage. Both the latter two groups developed significant and severe metabolic acidosis after 60min of haemorrhagic shock, but only in the fourth group of trauma and haemorrhage was the degree of shock associated with an increase in PTT and APC levels (as seen in human trauma patients). This study group also inhibited APC by a selective monoclonal antibody. When this was administered to the trauma and haemorrhage group, there was no resultant rise in the PTT as noted previously, thus preventing the development of the acute traumatic coagulopathy. This adds weight to theory that early acute coagulopathy is mediated by the activation of the protein C pathway (Chesebro et al., 2009).

### Inflammation

Inflammation and coagulation are two main host-defences which are intrinsically linked. Inflammation activates coagulation by a number of mechanisms, including cytokine induction of TF expression, down-regulation of the protein C system, inhibition of fibrinolysis and activation of the complement system. Similarly coagulation modulates inflammatory activity by the components of the

coagulation process which affect the inflammatory cell responses (Petaja, 2011).

The pro-inflammatory cytokines IL-6, IL-1, IL-12 and TNF- $\alpha$  all cause an increase in the overall expression of TF increasing thrombin production. This occurs mainly by increased exposure in vessels after a breakdown in the integrity of the cell-to-cell endothelial junctions and up-regulation of TF synthesis in endothelial cells and monocytes/macrophages. Blockade of IL-6 in animal studies has shown to attenuate the thrombin formation in experimentally induced endotoxaemia (Van der Poll et al., 1994). Inflammation also propagates the formation of microparticles (section 3.3.1) and when they arise from monocytes, they carry significant amounts of TF, increasing their procoagulant state (Osterud and Bjorklid, 2006).

As explained above activated Protein C (APC) appears to be a crucial link in the pathophysiology of ATC, and it also plays a central role in the pathogenesis of sepsis and associated organ dysfunction (Levi and Van der Poll, 2007). Severe inflammation causes a complete down-regulation of the Protein C system by impaired synthesis of the actual protein, a decrease in thrombomodulin activity (resultant of IL-1 and TNF $\alpha$  activity) and an overall down-regulation of the endothelial protein C receptor (EPCR) which would normally stimulate the activity of the APC (Weiler, 2010). The overall effect is that the production of thrombin goes unchecked and can lead to a hypercoagulative state.

Administration of recombinant APC to severely septic patients was thought to decrease mortality (PROWESS trial, Bernard et al., 2001), but newer evidence has questioned that finding (Marti-Carvajal et al., 2011). The physiological sequelae after trauma differs from that of the severely septic patient, however these papers are mentioned here as the vast majority of research studying the interaction of inflammation and coagulopathy have been performed in the setting of severe sepsis. Although a significant number of mechanisms are different between the early response to trauma and sepsis, the detailed insight into the interaction between the inflammatory and coagulation pathways provided by the sepsis studies will undoubtedly contribute to our understanding of alterations in trauma.

Fibrinolysis is an integral component of the inflammation-coagulation balance with TNF $\alpha$  activating it and IL-1 $\beta$  working as an inhibitor. Inflammation causes release of the plasminogen activators (tPA and uPA – section 3.5.2) from endothelial cells which can lead to increase in fibrin degradation. However, its action is tempered by a large, but slower release of PA inhibitor (PAI-1) which blocks fibrinolysis contributing to microvascular thrombosis (Van der Poll et al, 1991; Petaja, 2011).

The coagulation process also modulates the activation of the inflammatory process predominantly by the action of the coagulation proteases and inhibitors on the inflammatory cell responses. Thrombin markedly enhances the level of endotoxin-induced IL-1 in macrophages as well, in conjunction with Factor Xa and fibrin, directly stimulate mononuclear cells and endothelial cells to produce IL-6/IL-8 (Van der Poll et al., 2011). This modulation occurs by binding to protease-activated receptors (PAR). Four types exist (PAR 1-4), all part of the transmembrane G-protein-coupled receptors, and are localised on endothelial cells, mononuclear cells, platelets, fibroblasts and smooth muscle cells (Levi and Van der Poll, 2010). PAR1, 3 and 4 are thrombin receptors and stimulation of these results in an up-regulation of cytokines and growth factors. PAR-2 is activated by factor Xa, TF/VIIa and TF/FVIIa/Xa complexes and activation also produces an increase in production of reactive oxygen species and cell adhesion molecules (Petaja, 2011).

In the context of trauma and its molecular sequelae, inflammatory changes will invariably lead to activation of the coagulation system and *vice versa*, components of the coagulation modulate the inflammatory response. There are many points of 'cross-talk' but importantly with components of the Protein C pathway and fibrinolytic activators/inhibitors. Exactly how the theory that hypoperfusion and activation of the APC pathway driving the early hypocoagulation, however the inflammatory associated with down-regulation of the same pathway could explain the subsequent increase in the risk of thromboembolism that occurs in trauma patients.

### Consumption and Dilution of clotting factors.

Consumption of clotting factors has historically been regarded as a primary cause of traumatic coagulopathy (Schreiber, 2005). This was felt to be that injury initiated the coagulation cascade, as the injury severity directly correlated with the amount of thrombin generated; activation would lead to consumption of factors and hence result in a coagulopathy. This consumptive coagulopathy is the basis for the one of the original theories for ATC, where it was thought the patient was entering Disseminated Intra-vascular Coagulopathy (DIC) (Gando, 2001). Classically DIC has been defined in two stages (Levi, 2005). The first stage involves widespread and unrelated generation of thrombin, inducing platelet activation and aggregation, which along with fibrin is deposited in the microvasculature. Increased PAI-1 prevents fibrinogen breakdown producing an initial hypercoagulable state. The surge in thrombin stimulates the endothelium to release tPA and activates thrombin-activated fibrinolysis inhibitor. It is the action of these anti-thrombotics, in conjunction with the excessive consumption of clotting factors and platelets, that leads to the hypocoagulopathy and fibrinolysis (the second stage of DIC) (Harr et al., 2011). Gando and colleagues have argued over the past two decades that the early coagulopathy is actually the second stage of DIC that results in a substantial consumption of clotting factors, especially fibrinogen (Gando et al, 1992). Hess et al (2008) had concluded that the ATC was distinct from DIC, which prompted Gando (2009) to publish a special editorial expressing his rebuttal to this. Although he agreed that the traumatic coagulopathy was multifactorial, he argued that DIC was the predominant and initiative pathogenesis of coagulopathy at an early stage of trauma resulting in a consumptive coagulopathy and excessive fibrinolysis, and this is what Hess, Brohi et al were calling ATC (Hess et al, 2008; Brohi et al, 2007, 2008).

The evidence against the early ATC actually being a form of DIC is that fibrinogen and platelet counts, which decrease with consumption in DIC, remain within normal limits with patients with ATC (Brohi et al., 2007, 2008). Brohi and colleagues also showed that regardless of the amount of thrombin generated, no change in PT or APTT was noted except in patients who had hypoperfusion.

This continues to add more weight to the argument that ATC is driven by hypoperfusion and is not DIC, however only a clear definition and diagnostic criteria for ATC will help clarify the difference, if one exists.

Dilutional coagulopathy has been postulated as a cause of bleeding in the later phase after trauma, especially after a massive transfusion (Ho et al., 2005). When a patient haemorrhages it naturally leads to a loss of clotting factors and fibrinogen. Over a 2L blood loss leads to significant reduction in levels of prothrombin, FV, FVII and platelets (Hiippala, 1998). The subsequent resuscitation to replace lost volume with clear fluids can add to this dilution. Chapter 4 looks into result of the choice of fluid and how the different compositions affect coagulation.

### Acidosis

Metabolic acidosis in trauma is common and is secondary to tissue hypoxia in states of hypovolaemia and subsequent inadequate tissue perfusion (Siegel et al., 1990). Meng et al (2003) found that a decrease in pH directly influences coagulation by inhibiting the function of the plasma proteases. When the pH was reduced from 7.4 to 7.0 the activity level of FVIIa was reduced by 90%; FVIIa/TF complex by 55% and the rate of prothrombin activation by FXa/FVa complex was reduced by 70%. Using a swine model, Martini et al. (2005) investigated the independent and combined effects of hypothermia and acidosis on thrombin-generation kinetics. An acidosis of pH 7.1 was induced by the infusion of 0.2mol/L hydrochloric acid. Their results showed that an acidosis of pH 7.1 persistently and dramatically inhibited the propagation phase of thrombin generation, indicating severe inhibition of the activation of FV, FVIII, FIX and FX. The same group went on to show these coagulation defects were not immediately corrected by pH correction alone (Martini et al., 2006 and 2007).

In human studies, Engstrom et al. (2006) showed lowering of the pH level significantly impaired coagulation when measured by thromboelastography; resulting in increased clot formation time and reduced alpha angle (an indication of thrombin generation). The clot formation was decreased by 168% at pH 6.8 as compared with pH 7.4 ( $P < 0.00001$ ). Fibrinogen levels are also altered by acidosis, with a correlation with decreasing pH and fibrinogen

degradation (Martini et al., 2007). It is understood that admission base deficit correlates significantly with post-injury organ failure and death (Davis et al., 1996) and base deficit has also been reported to be the best detector of occult hypoperfusion (when vital signs are normal) (Thom et al., 2010). As this hypoperfusion is linked with ATC, it is clear that early correction of this is becoming critical.

### Hypothermia

Clinically significant hypothermia can be defined as a core temperature of  $<35^{\circ}\text{C}$ , and often begins at the time of injury with decreased motor activity and increased heat loss by conduction and evaporation and climatic exposure. This is then exacerbated in hospital by exposure from removal of clothing, anaesthesia and cold fluids (Gregory et al., 1991; Burch et al., 1997).

Hypothermia at admission to hospital has been shown to be associated with a worse outcome (Ferrara et al., 1990), although overall independently it remains a weak predictor of mortality (odds ratio 1.19) (Shafi et al., 2005).

Watts et al. (1998) concluded that the rate of enzyme reactions of plasma coagulation reduces by 10% per  $^{\circ}\text{C}$ . However, more recent papers have concluded there has been little effect shown on coagulation protease function and clinical bleeding at temperatures above  $33^{\circ}\text{C}$  (Meng et al., 2003, Wolberg et al., 2004). Below  $33^{\circ}\text{C}$ , coagulopathy becomes more severe with significant reduction in both enzyme activity and platelet activation. Thrombin generation was reduced by 25% at  $33^{\circ}\text{C}$  and 68% below  $30^{\circ}\text{C}$  when compared with  $37^{\circ}\text{C}$ . Platelet aggregation and adhesion were more sensitive with temperature changes with a decrease of 40% in function by  $33^{\circ}\text{C}$  (Wolberg et al., 2004).

The dynamic changes of fibrinogen metabolism during hypothermia were recently revealed by Martini et al. (2007). Hypothermia was induced in swine to  $32^{\circ}\text{C}$  and sampling was taken to investigate changes in fibrinogen synthesis and degradation. At  $32^{\circ}\text{C}$  there was a decrease in fibrinogen synthesis from the norm but there were no effects on fibrinogen degradation.

Despite these results, it is argued that hypothermia alone is only a weak

independent predictor of mortality and that hypothermia does not contribute to the incidence or degree of ATC (Brohi et al., 2007, 2008).

However, hypothermia and acidosis synergistically impair coagulation. Dirkmann et al. (2008) looked at whole blood that was acidified *in vitro* and then tested by thromboelastography at temperatures from 30 to 39°C. It was concluded that hypothermia produced minor coagulation changes and these changes were greatly enhanced by acidosis. This could be of importance when considering the testing temperature, as thromboelastography performed at 37°C could overestimate the integrity of coagulation during hypothermia in particular in combination with acidosis.

Knowledge of the different mechanisms involved in the pathogenesis of acute traumatic coagulation has a clear significance for the successful management of bleeding trauma patients. The one common thread linking ATC, SAC, acidosis and inflammation is hypoperfusion. Therefore treatment of shock and hypoperfusion in the early stages is vital and then targeting of the progressive systemic acquired coagulopathy with goal-directed resuscitation to replace clotting factors (Kashuk et al., 2008, Stahel et al., 2009). This treatment is discussed in detail in Chapter 4.

## Chapter 4: Resuscitation

### 4.1 Principles of Resuscitation

The objective of resuscitation is to correct or prevent the consequences of shock<sup>1</sup>. In trauma, haemorrhage accounts for 33-56% of pre-hospital deaths in civilian trauma (Kauver et al., 2006) and up to 80% of deaths in the military (Bellamy, 1984; Holcomb et al., 2007). A significant proportion (up to 50%) of this is exsanguinating haemorrhage (Champion et al., 2003). Therefore to have a significant impact on mortality following major trauma, arresting bleeding is of prime importance: firstly by tactical field care (or pre-hospital care); the arrival into a medical facility (the emergency department [ED]); onto definitive surgical intervention if required, and finally into intensive care as required. Assuming the patient survives the initial blood loss secondary consequences develop as a result of the hypovolaemic state. There is a reduction in tissue perfusion and oxygen delivery, leading to metabolic acidosis, coagulopathy and later inflammatory responses. Approximately one-quarter to one-third of all trauma patients will present with a coagulopathy at hospital admission. Resuscitation will be required to not only minimise further blood loss and restore tissue perfusion and oxygenation but also to address this coagulopathic state. A European Multidisciplinary Task Force for Advanced Bleeding Care in Trauma, formed in 2005, recently published their updated guidelines (Rossaint et al., 2010). This outlined 31 recommendations that should be considered in trauma patients with the overall message of rapid control of bleeding, restoration of tissue oxygenation with appropriate volume replacement, prevent hypothermia and management of coagulopathies. This chapter examines the issue of initial volume of fluid resuscitation, the types of fluids available and the developing use of 'Damage Control Resuscitation', with particular emphasis on addressing this Trauma Induced Coagulopathy. The last section will examine the potential role of specific clotting tests (thromboelastography /thromboelastometry - TEG/TEM) in guiding the resuscitation process.

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<sup>1</sup> Shock is defined as a failure of tissue oxygen delivery to meet demand.



## 4.2 Pre-hospital Resuscitation.

The rapid arrest of haemorrhage is saving lives (Hodgetts et al., 2006). In the military this is achieved mainly with the use of tourniquets for exsanguinating limb injuries and a recognition of incompressible bleeding which needs surgery (Brodie et al., 2007; Swan et al., 2009). Fluid resuscitation is often needed to sustain life until the casualty can be evacuated to a surgical facility – the timing, choice of fluid, the volume administered and even the required end-point goal for resuscitation at specific points in the treatment chain is an on-going debate.

Animal resuscitation studies in the mid-20<sup>th</sup> century discovered that prolonged hypotension after controlled haemorrhage resulted in an extracellular fluid deficit. A number of studies recommended correcting the loss of blood by the administration of a three-fold volume of intravascular crystalloid (Stern et al., 2001). Further studies in the 1960's (Shires et al., 1964) showed this three to one resuscitation improved survival. This aggressive resuscitation approach was adopted universally and was widely promulgated in the resuscitation of casualties during the Vietnam War. However excessive fluid volume increases the movement of excess fluid into the 'third space, which resulted in gross pulmonary oedema or the 'Da Nang Lung' in numerous casualties and therefore the benefit was later questioned (Wangensteen and Ludewig, 1969).

The Advanced Trauma Life Support (ATLS<sup>®</sup>) approach to trauma patients was developed in the late 1970's to ensure that clinicians were taught a method of dealing with trauma victims in a timely and appropriate manner. By 2007 over 1 million doctors had trained in the theory of this approach in over 60 000 courses worldwide (American College of Surgeons, 2008). This aggressive and didactic approach was at the centre of the resuscitation of circulation on arrival to the ED and it continues to promote that 3ml of crystalloid fluid should be given to replace each 1ml blood loss – the 3-to-1 rule – with a usual dose of 1-2L of crystalloid being given as a initial loading dose (page 63, ATLS<sup>®</sup> Manual, American College of Surgeons, 2008).

This aggressive initial approach gained support by two key papers in the mid 1980's (Jacobs et al., 1984; Pons et al., 1985), which both described how trauma patients treated with intravenous fluid resuscitation by paramedics were

accredited with improving haemodynamic parameters and overall outcome. However patients in these studies had very short transfer time to hospital and these studies were compromised by the absence of control groups.

However, a number of large studies have questioned this use of pre-hospital fluid, and have suggested it may actually be harmful in certain subsets of trauma patients (Kaweski et al., 1990; Dalton, 1995; Dula et al., 2002; Talving et al., 2005). Revell, Porter and Greaves in a consensus paper in 2002, asserted that IV fluids should not be administered to penetrating trauma patients if a central pulse is present, and that under no circumstances should IV administration delay transport (Revell et al., 2002). The promotion of restricted pre-hospital fluid has been nurtured further by the publication of two recent papers that involve large patient numbers. The first was Canadian prospective, multi-centred controlled trial that compared the intervention of trauma victims before and after a system-wide Advanced Life Support (ALS) program was implemented for paramedics (Stiell et al., 2008 for the Ontario Pre-hospital Advanced Life Support (OPALS) trauma study). Prior to the implementation, the practice had stipulated that IV fluid should not been given in the field. 2867 patients were enrolled and the two groups were well matched regarding demographics, nature of trauma and severity of injury (>90% were blunt trauma). No improvement in morbidity or mortality was demonstrated following the implementation of ALS in all subgroups. Introduction of the ALS protocol did not alter the time to reach hospital as prior to implementation the average time between notification of the accident to arrival at ED was 29.7 mins and 34.2 mins with ALS. However, it was noted that patients with a decreased Glasgow Coma Score (GCS) (<9) did have a worse outcome when given pre-hospital fluid.

The second study is a large retrospective cohort study has been published looking at patients from the US National Trauma Data Bank: records of 776,734 trauma patients studied over a period from 2001-2005 (Haut et al., 2011). They showed that 49.3% were in the pre-hospital IV group, and the multivariable analysis demonstrated that patients receiving IV fluids were significantly more likely to die: odds ratio [OR] 1.11 (95% confidence interval [CI] 1.05-1.17). It was especially marked in patients with penetrating

mechanism OR 1.25; hypotension OR 1.44; severe head injury OR 1.34 and those who need immediate surgery OR 1.35. The OR also increased as the ISS rose; an ISS <9: OR 0.89; ISS≥9: OR 1.14; ISS≥15: OR 1.17; ISS≥25: OR 1.21. They concluded that the routine use of pre-hospital IV fluid should be discouraged in all trauma patients. However, they did not look at the time from the arrival of paramedics at the scene to arrival at the ED and there was no investigation of the actual volumes of pre-hospital fluid administered.

The Eastern Association of the Surgery of Trauma published guidelines suggesting that IV catheters should not be placed in the pre-hospital setting in trauma patients, and advocate that fluids should be withheld in patients with penetrating torso trauma until active bleeding is addressed (Cotton et al., 2009).

In view of the accumulating evidence the revised ATLS® manual still promotes the 3-to-1 rule but has added that aggressive initial fluid management does need a careful and balanced approach with frequent re-evaluation (page 64, ATLS® manual, American College of Surgeons, 2008).

In Chap 3 section 7, attention was drawn to the incidence of the TIC upon arrival at the emergency department and the volume of pre-hospital fluid as a predictor of coagulopathy. In the London study there was minimal pre-hospital (median 500ml) fluid administration and no correlation was found with either the amount or type of intravenous therapy administered (Brohi et al., 2003). However with such volumes of fluid being so small, the true influence of fluid cannot truly be commented on in this paper. The German retrospective analysis, where a range of fluid volumes were given to patients, did find a correlation between coagulopathy and volume of fluid administered, showing the incidence of coagulopathy increased with increasing volume of intravenous fluids administered in the pre-hospital phase (Maegele et al., 2007). The mean volume of fluid given in this phase was 2198ml in patients found to have a coagulopathy versus 1372ml in the non-coagulopathic group ( $p < 0.001$ ). Coagulopathy was observed in 40% of patients who received more than 2000ml, in more than 50% with more than 3000ml and in more than 70% when more than 4000ml was given, but the authors have not correlated these groups with their severity of injury (recorded as the ISS). It could be the greater volume

was administered to those with the highest ISS, and this could explain the higher incidence of coagulopathy. However, their results would advocate a lower volume approach to pre-hospital fluids.

### **4.3 Low Volume Fluid Replacement.**

In keeping with the move away from an aggressive fluid resuscitation, there has been considerable evolution in pre-hospital fluid strategies focusing on limited (low volume) or hypotensive resuscitation based on both laboratory and clinical data. This hypotensive resuscitation is a strategy of deferring or restricting fluid administration until haemorrhage is controlled, while accepting a limited period of suboptimum end-organ perfusion (Jansen et al., 2009). The blood pressure is allowed to remain below normal levels in an attempt to avoid disruption of nascent blood clots, particularly within the first hour after injury (Stern et al., 1993). After this time an undisturbed clot has achieved more than 80% of its ultimate tensile strength (Shen and Lorand, 1983). To prevent this possible disruption of the clot, the systolic blood pressure is maintained at approximately 80mmHg<sup>2</sup> (Sondeen et al., 2003, Krausz 2006). This is the concept that has been adopted by the military medical services of some nations, especially considering the longer time-lines of extraction (Greaves et al., 2002; Holcomb, 2003; Krausz, 2006; Battlefield Advanced Trauma Life Support, BATLS, 2006). Current UK Guidelines as promulgated by the National Institute for Clinical Excellence (NICE) recommend that IV fluids should not be administered in the pre-hospital phase if there is a radial pulse, and if not palpable, aliquot boluses of 250ml of fluid should be given and reassessed each time (NICE: Technology Appraisal Guidance 74, 2004). Widespread adoption of hypotensive resuscitation strategies by national advisory bodies such as NICE who rationalise “that in patients with uncontrolled or potentially uncontrolled bleeding, vigorous fluid therapy may exacerbate bleeding by diluting blood clotting factors, reducing the concentration of circulating blood platelets, *and by*

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<sup>2</sup> In the field this is assessed as the arterial pressure necessary to produce a palpable radial pulse.

*dislodging early clots forming at the site of haemorrhage*” emphasises the acceptance of the concept colloquially known as ‘popping the clot’ during aggressive resuscitation in patients where the haemorrhage has not yet been controlled. This concept is supported by an extensive evidence base, however much of this may to some extent be biased by the adoption of models which are particularly prone to re-bleeding when comparing hypotensive and aggressive resuscitation strategies. Nonetheless, the available clinical evidence does support the use of hypotensive resuscitation strategies when evacuation timelines are short (see below).

The laboratory data that provided the evidence for this limited resuscitation was carried out on large animal models with an uncontrolled haemorrhage element (a model that would be consistent with a penetrating trauma). Bickell subjected a group of pigs to a 5mm aortotomy and those that were not resuscitated stopped bleeding spontaneously within 5 minutes (Bickell et al., 1989). The same group of investigators followed this work with a larger animal study in which 16 juvenile pigs underwent splenectomy. This is required as a pig has the ability to use the spleen as a reservoir and can return up to 25% of blood volume to the circulation as a form of auto-transfusion during haemorrhagic compromise (Hannon et al, 1985). During surgery a wire was placed in the aorta, that when pulled later, would create a 5mm aortotomy. Animals were randomised to have no resuscitation or to receive Ringers Lactate (RL) at a rate of 3x the rate of blood loss, commenced 6 minutes following the aortotomy. This is a considerable volume of fluid when compared to the 3:1 rule proposed by Shire (1964) which was based on volume. At 2 hours, all the animals that had received no fluid resuscitation were alive compared to none of the resuscitated ones ( $p<0.05$ ) (Bickell et al., 1991). The significant increase of intraperitoneal blood recorded at the end of the experiment in the resuscitated group, the authors postulated that the fluid being commenced at 6 min would have disrupted ‘or popped’ the nascent clot.

Further animal model studies that combined a controlled and an uncontrolled haemorrhage component (Kowalenko et al., 1992; Stern et al., 1993) showed this detrimental increase in haemorrhage when aggressive fluid was given. However in these studies the animals underwent a greater degree of

haemorrhage (due to both a controlled bleed via an indwelling vascular catheter and an uncontrolled bleed with a wire aortotomy) and were resuscitated to different systolic pressures. Those who were resuscitated to a lower pressure (40mmHg) had the same survival rate at one hour (87.5%) as those who were not resuscitated at all, and this was significantly higher than the group resuscitated to 80mmHg pressure (37%). The lower pressure group also had a four-fold reduction in intraperitoneal fluid at post mortem. Their protocol dictated that fluid resuscitation was commenced when the pulse pressure reached 5mmHg after the aortotomy, meaning that fluid administration was instigated almost immediately. This model and protocol have a strong bias towards maximising the risk of re-bleed.

Repeating these experiments with end resuscitation pressures of 40, 60 and 80mmHg, the most favourable outcome from a metabolic profile was the modestly resuscitated group of 60mmHg, whereas the lower pressure group had the worst metabolic profile (with lower lactate levels), indicating the response to the poor tissue perfusion (Stern et al., 1993). Resuscitation was commenced almost immediately following the aortotomy when the pulse pressure dropped to 5mmHg, which is unlikely to provide sufficient time for clot formation and this model is likely to be very susceptible to re-bleeding. None of these studies looked at coagulopathy.

Rafie et al., (2004), looked at resuscitation of sheep after haemorrhage to either a hypotensive level of 65mmHg or to a normotensive 90mmHg with either crystalloid or colloid. The animals underwent a controlled bleed only in this study (with no uncontrolled element) which occurred at three time intervals. The first bleed commenced at time zero (T0) at a rate of 25ml/kg for 15minutes. Resuscitation was commenced at T30 and continued to T180. Two smaller bleeds at a rate of 5ml/kg for 5 minutes starting at time T50 and T70. In the hypotensive group, the target pressure of 65mmHg was compromised by both survival and the poor tissue perfusion (indicated by increasing base excess) and it was concluded that the significantly higher cardiac output and oxygen delivery that resulted from the normotensive protocols would offer an overall survival advantage.

A systematic review of animal studies concluded that hypotensive resuscitation strategies reduced the risk of death when compared to normotensive strategies (Mapstone et al., 2003). These models involved vascular injuries that were most likely to re-bleed when arterial blood pressure was elevated (transection or tear in a major artery), thus biasing in favour of a hypotensive resuscitation strategy. Overall the strategy of using a lower than normal blood pressure as a guide to fluid resuscitation appears to consistently reduce the risk of death regardless of the severity of injury.

However, none of the studies had looked at resuscitation past 120 mins and the fundamental limitation of this research is that it involves only animal studies, so it is not truly clear how they relate to human injuries.

With the penalty for a hypotensive approach being tissue hypoperfusion, it is essential that a balance between the possibility of a re-bleed and this hypoperfusion is considered. In a poly-trauma patient, with multiple injuries that could include a head injury, hypotension would need to be avoided to maintain cerebral perfusion and prevent secondary brain injury.

Studies carried out at Porton Down have looked at prolonged hypotension after haemorrhage and blast, and effects of resuscitation (Parry et al., 2005, Garner et al., 2009; Garner et al., 2010, Jacobs, 2010). The methodology of this work is described in greater detail in Chapter 6. Terminally anaesthetised pigs were randomised into four groups. All animals underwent a controlled haemorrhage (with or without exposure to blast) and were resuscitated using 0.9% saline to either a hypotensive level (to maintain a systolic pressure of 80mmHg) or a normotensive level (with a systolic pressure of 110mmHg). The primary outcome was survival and the end point was 8 hours. The results showed that survival was significantly shorter with prolonged hypotensive resuscitation and this was incompatible with survival after blast injury. The prolonged hypotensive state also found to cause a significant continued drop in base excess, rise in blood pH and poor whole-body oxygen delivery, demonstrating that prolongation of a hypotensive state will result in physiological penalties of low tissue perfusion. It was noted that hypotensive resuscitation of up to an

hour did sustain life (Parry et al., 2005; Garner et al., 2010). These results are of significant importance in the consideration of resuscitation in a prolonged pre-hospital extraction.

This model was then developed further to assess the outcome if the systolic blood pressure was restored to normotensive values after one hour and the outcome assessed (Jacobs, 2010). Animals had a controlled bleed from an indwelling vascular catheter and an uncontrolled bleed was added, caused by a grade IV liver injury, to give an injury that is at risk of re-bleeding. All animals were resuscitated to a hypotensive level of 80mmHg for 60 minutes, allowing time for good quality clot formation. One group of animals continued with a prolonged hypotensive strategy and the other was resuscitated to a systolic of 110mmHg (this initial hypotensive strategy followed by a return to normotensive levels has been termed 'Novel Hybrid' [NH] resuscitation). Again the primary end point was survival over an 8 hour period of resuscitation. The Novel Hybrid resuscitation group showed an increased survival compared to hypotensive resuscitation and reversed the physiological deterioration that had resulted from prolonged hypoperfusion. There was no significant increase in the re-bleed from the liver injury with restoration of pressure.

Thus it is essential that resuscitation considers these systemic, time-critical physiological sequelae of hypotensive resuscitation and prevent the adverse consequences of hypoperfusion.

Clinical evidence for hypotensive resuscitation has been shown in patients with penetrating trauma (Bickell et al., 1994). Bickell randomly assigned hypotensive patients who had penetrating torso trauma to receive standard aggressive pre-hospital crystalloid fluid, continued in the ED versus receiving nothing until after the onset of general anaesthesia in the operating room (OR). The primary end point was survival, with approximately 300 in each group. Survival was significantly better in those in whom resuscitation was delayed (70%vs 62%;  $p=0.04$ ), however it should be noted that there was only an average 75-80 minutes between the time of injury to the OR. The study was



also limited by firstly the high exclusion rate (44%) due to injuries being too severe or not severe enough.

A UK crossover randomised study that aimed to look at resuscitation in those who had sustained blunt trauma showed no difference in mortality between the delayed and immediate resuscitation groups (Turner et al., 2000). Despite over 1300 patients being recruited, the results of this study are widely disregarded due to the poor protocol compliance and the low injury severity score of a majority of the patients.

A third, smaller study (Dutton et al., 2002) randomised patients with either blunt or penetrating trauma to receive either conventional treatment or treated with restricted fluid. They were resuscitated to either a normotensive pressure (>100mmHg) or a hypotensive pressure (70mmHg). No difference was found in mortality in either group. The paper is limited by a number of points; there were only 55 patients in each group, the majority of the patients had a relatively low severity trauma score and the mean blood pressure in the hypotensive group was recorded at 100mmHg.

A Cochrane review in 2003 looked at these three papers as well as two other trials not pertaining to trauma (Kwan et al., 2003). The authors found no evidence from randomised controlled trials (RCTs) for or against early or larger volume of intravenous fluid administration in uncontrolled haemorrhage. There is still no clear consensus on which protocol offers the best outcome.

The direct physiological effect of low volume or hypotensive resuscitation has not been directly investigated but the resultant poor tissue perfusion resulting in both animal and human studies is clear. With the hypothesis that TIC is secondary to tissue hypoperfusion, permissive hypotension could potentially exacerbate the coagulopathy. Therefore the aim of resuscitation should be to restore tissue perfusion, which could in turn attenuate a developing coagulopathy, but the degree of resuscitation given (especially in the pre-hospital phase) must always be tempered with the knowledge of possible consequence of increased hydrostatic pressure.

#### **4.4 Choice of Fluid for Resuscitation**

Recommendation 19 of the updated European Guidelines for the management of bleeding following major trauma proposes that resuscitation should be commenced with crystalloids, with consideration of use of hypertonic solutions. Limited use of colloids could be considered in the haemodynamically unstable patient (Rossaint et al., 2010). It is argued that in the initial stages of a trauma patient resuscitation, the precise fluid given is not crucial, as long as appropriate volume is given (Nolan, 1999). The optimal resuscitation fluid would combine volume expansion and oxygen carrying capacity of blood, with restoration of the normal composition and distribution of the body fluid compartments; as no such fluid exists the options essentially remain: crystalloid, colloid, hypertonic or blood and blood products replacement. With the rapid progress in trauma care, an emerging concept in combat casualty care is haemostatic resuscitation: the rapid and proactive treatment of the coagulopathy associated with major injury (Holcomb et al., 2007; Kirkman et al., 2007). The section will look at the choice of fluid to use and discuss further the concept of this haemostatic resuscitation.

##### **4.4.1 *Crystalloid vs. Colloid Solutions***

Crystalloids are solutions of salt(s) in water that are classified in respect to plasma as hypotonic, isotonic and hypertonic; with osmolarity ranging from 252mOsmol/L in 5% Glucose to 1025mOsmol/L in hypertonic saline (Doran, 2007). Glucose-based solutions rapidly become hypotonic following metabolism of the glucose which leaves free water. This distributes quickly throughout all the compartments providing no expansion of the intravascular space. These fluids are not suitable for trauma resuscitation (Mather et al., 2004). The most common crystalloid fluids used are isotonic – 0.9% Saline and Hartmann's Solution (Ringers Lactate [RL] is the equivalent in the United States [US]). Again these fluids distribute relatively quickly to all interstitial spaces, only to a slower degree than glucose based ones, and this is especially the case in trauma patients when hypoperfusion and ischaemia result in net movement of water out of the intravascular space due to the increase in vascular permeability (section 2.2.1). It has been shown at 30 minutes after

infusion that 16% of the infused crystalloid remains in the intravascular space. This was, however, a study in healthy individuals, rather than in the hypovolaemic, injured patient (Watenpaugh and Gaffney, 1998).

Colloids are homogenous, non-crystalline substances consisting of large molecules or ultramicroscopic particles of one substance dispersed through a second substance – these particles do not settle and cannot be separated out by filtration or centrifugation unlike the components of blood (Salmon and Mythen, 1993). Colloids can be subdivided into two major groups: protein and non-protein (also called plasma derivatives and semi-synthetics).

Naturally occurring colloids include human albumin – the solutions (4.5% or 20%) are derived from human plasma. Controversy arose in 1998 when the authors of a meta-analysis concluded that the use of albumin was associated with an increase in mortality of 6% (Cochrane Injuries Group Albumin Reviewers, 1998). This controversial conclusion was questioned with the publication of the SAFE (Saline vs. Albumin Fluid Evaluation) study in 2004 (Finfer et al., 2004). This looked at the use of albumin and saline for fluid resuscitation in the critically ill in a large multi-centred randomised double-blind trial comparing 4% albumin vs. 0.9% saline for intravascular resuscitation. The study found no difference in mortality, intensive care unit (ICU) stay or hospital days. However, albumin is expensive and there is no evidence to use it as part of the initial resuscitation in a trauma patient.

The non-protein colloids are classified into the modified gelatins, the dextran solutions and the synthetic hydroxyethyl starches; all of which are dissolved in a crystalloid carrier solution, commonly isotonic saline. The gelatins (not available in the US) are derived from hydrolysis of bovine collagen and a half-life of up to 3.5 - 4hrs in the intravascular space (Sadler and Horsey, 1987). The dextrans are groups of branched polysaccharides manufactured by the bacterial action of *Leuconostoc mesenteroides* on a sucrose medium. The products available are Dextran 40 and 70 (molecular weight [MW] of 40,000Da and 70,000Da respectively), only the latter is used in resuscitation (Salmon and Mythen, 1993).

The Hydroxyethyl Starches (HES) are the youngest generation of artificial colloids and have evolved greatly over the past two decades. The structure of HES is a branched amylopectin polymer and is broken down by serum amylase in the blood. The polymer can undergo etherification which results in substitution of hydroxyethyl groups for hydroxide. This occurs at the C2, C3 and C6 position of the glucose molecule and increases the resistance of degradation by the amylases; the higher degree of substitution increases intravascular persistence (Trieb et al., 1996). The starch products are described by their MW followed by their degree of molar substitution – i.e. a solution of hetastarch 450/0.7 has a MW of 450kDa and a molar substitution of 0.7. It is the latter that contributes to the physiological or physiochemical characteristics (Jungheinrich and Neff, 2005); the greater the degree of substitution, the greater the side-effect profile.

As the HES preparations have developed, so have the list of pros and cons. Early preparations, especially of the starches, were associated with an increase in bleeding, renal failure, anaphylaxis and itching, although more recent generations have been found to cause fewer negative effects on coagulation, renal function and demonstrate lower concentration accumulation in plasma (Trieb et al., 1999; Vincent, 2007). HES have been shown to be beneficial in septic patients and acute inflammation by the modulation of inflammatory markers (Schmand et al., 1995; Dieterich, 2007). In an animal model of sepsis, rats were subjected to a caecal perforation and then resuscitated using either HES or a gelatin solution. The HES group had a significant reduction in inflammatory modulators and a lower degree of capillary leakage (Feng et al., 2007). It should, however, be noted that the cardiovascular issues are very different between sepsis and hypovolaemia and the results do not necessarily translate well between the two circumstances, although in a murine haemorrhage model, the use of HES also correlated with a restoration of macrophage integrity and thus a lower IL-6 increase (Schmand et al., 1995).

However, the use of HES has been questioned with regard to renal dysfunction and its effect on coagulation. Another study looking at sepsis, the VISEP trial (the Efficacy of Volume Substitution and Insulin Therapy in Severe Sepsis) was published in 2008 (Brunkhorst et al., 2008). The results of this randomised trial

showed that the use of HES was associated with higher rates of acute renal failure and the requirement for renal replacement therapy than RL.

With regard to coagulation, HES has been shown to interfere with haemostasis by its effect on platelets (Van der Linden and Ickx, 2006). The effect of HES appears to be the same dysfunction as found in Von Willebrand's disease (abnormal platelet aggregation). Trieb and Baron reported that after an infusion of 1000ml of HES a significant decrease in factor VIII:C and vWF was noted, more than would be explained than simple dilution (Treib and Baron, 1998). HES have also been found to modify the platelet membrane resulting in a prolonged bleeding time (Van der Linden and Ickx, 2006). The coagulation profile is preserved by the use of smaller molecular weight, lower substituted starches (Roche et al., 2006).

Animal studies have shown differing results regarding coagulopathy using TEG to assess the haemostasis profile. Via and colleagues assessed the effect of HES on coagulopathy after a period of haemorrhagic shock in anaesthetised pigs (Via et al., 2001). No difference was found with resuscitation with HES when compared to albumin and RL. The use of starch in the resuscitation of swine after a liver injury was compared with resuscitation by RL (Todd et al., 2005). TEG revealed that all animals became hypercoagulable after injury, and this effect was attenuated when starch was used, but did not cause an increase in blood loss.

Considering all of these pros and cons, the choice to use crystalloids or colloids remains an on-going debate in the literature. The Cochrane Collaboration have published two reviews looking specifically at the use of colloids in the critically ill (Bunn et al., 2003) and to assess if colloids are more effective than crystalloid in reducing mortality in people who are critically ill or injured (Perel and Roberts, 2007). The first review looked at the benefits of the available colloids with respect to the following outcomes: incidence of adverse reactions, amount of whole blood transfused and death. Out of the 36 trials reviewed, 20 compared albumin with HES and 11 compared gelatin with HES. The pooled Relative Risk (RR) of these sub-groups was 1.17 (95% CI 0.91 to 1.5) for the former

group and RR = 1.00 (95% CI 0.78 to 1.28) in the latter group. Overall, there was no significant difference in mortality rates and as such there was insufficient evidence to determine if one colloid solution is any more effective or safer above its competitors (Bunn et al, 2003).

In the second review, 65 trials were identified with 56 looking at mortality. The use of crystalloid was compared to albumin, HES, modified gelatin, and dextran. They concluded that, *“there is no evidence from randomised controlled trials (RCTs) that resuscitation with colloids reduces the risk of death, compared to resuscitation with crystalloids, in patients with trauma, burns or following surgery. As colloids are not associated with an improvement in survival, and as they are more expensive than crystalloids, it is hard to see how their continued use in these patients can be justified outside the context of RCTs”*. (Perel and Roberts, 2007).

#### **4.4.2 Use of Hypertonic Solutions.**

Although isotonic crystalloids predominate in the resuscitation of patients, there has been substantial interest and extensive preclinical and clinical experience in evaluating the use of hypertonic solutions of both saline and colloid (Kramer, 2003). Hypertonic solutions are solutions that have a greater solute concentration than the cytosol, meaning that when infused into the intravascular system, it causes water to be drawn out of the cell in the solution by osmosis. This has the effect of increasing the intravascular volume which helps to provide vascular support (Cross et al., 1989). The amount of cellular water mobilised is proportional to the osmotic load and reduces the overall volume needs in perioperative patients (Tollofsrun and Mathru, 1998). Research looking at the effect of endothelial cell swelling during shock and surgical stresses demonstrated that the cells swell (Mazzoni et al., 1989) and that the use of hypertonic resuscitation normalises the cell volume rather than reducing it below normal (Mazzoni et al., 1990). The greatest interest has been with 7.5% saline after the first publication of its use in 1980 by a research team in San Paulo, Brazil (Velasco et al., 1980). They resuscitated dogs, after subjecting them to haemorrhagic shock, with 7.5% saline. They infused 10% of the

volume lost and found that the arterial pressure and cardiac output (CO) rapidly returned to baseline values and led to 100% survival. This was thought to be radical at a time when a 3:1 rule for resuscitation was accepted as the norm.

A US team repeated this work with infusions of 7.5% saline in haemorrhaged conscious sheep (Nakayama et al., 1984). They also resuscitated the animals with a bolus of approximately 10% of the blood volume lost and found that the blood pressure and CO rapidly normalised. This was, however, noted to be a transient response. One concern that has been raised is that initial resuscitation with hypertonic saline (HTS) may cause a sudden rise in arterial blood pressure and dislodge any clot that was forming, and thus actually potentiate the degree of bleeding (Gross et al., 1988). This was not the finding of Kentner and colleagues when they studied the use of HTS in rats following uncontrolled haemorrhage (Kentner et al., 2005).

Hypertonic saline (HTS) has also been shown to demonstrate a number of benefits; mainly that it appears to mitigate the inflammatory response to significant injury. Hypertonicity has been shown to suppress neutrophil activation in-vitro (Junger et al., 1998; Deitch et al., 2003) and modulate T-cell activity (Junger et al., 1997). Another group in San Paulo published findings that demonstrated that use of HTS after an ischaemia-reperfusion injury in rats attenuated the neutrophil infiltration into lungs and thus reduced the early inflammatory response to shock and infection (Fernandes et al., 2009). HTS was also found to be beneficial in reducing the alveolar macrophage activity after haemorrhagic shock and thus postulated that it could be beneficial in reducing the development of Acute Respiratory Distress Syndrome (ARDS) (Powers et al., 2003).

The use of HTS after traumatic brain injury has warranted investigation. It has been demonstrated that HTS may prove more effective in decreasing cerebral oedema when compared with mannitol (Himmelseher, 2007). HTS offers the advantage in facilitating an increase in intravascular volume while reducing intracranial pressure without potentially causing or exacerbating hypotension. The HTS mediated increase in the inner diameter of the capillary vessel by dehydration of endothelial cells may promote increased blood flow to regions of

the brain at risk of secondary injury (Tyagi et al., 2007; Pascual et al., 2008). However, an Australian trial looked at the use of HTS with traumatic brain injury and hypotension. There was no significant increase in survival and no differences in neurological function after 6 months (Cooper et al., 2004).

As with HES, concerns were raised about the possibility of change in clotting. These concerns were from *in vitro* studies looking at the coagulant effects of HTS on PT, PTT and platelet aggregation. A prolongation of these times was noted after 10% or more of the plasma volume was replaced by HTS (Reed et al., 1991; Wilder et al., 2002). However, these findings have not been translated in clinical studies (Tieu et al., 2007).

The transient response, described above by Nakayama, to the initial resuscitation with HTS lead to development of combining the hypertonic saline with a colloid to attempt to retain more of the water in the intravascular space. This was the development of Hypertonic Saline Dextran (HSD).

#### **4.4.3 Hypertonic Saline Dextran (HSD)**

The concept of using small volume, hypertonic resuscitation is of particular interest to the military as they possess a significant logistical benefit in terms of reduced weight burden; 250ml of HSD is reputed to be equivalent to 3L of 0.9% saline with respect to early plasma volume expansion (Dubick et al., 2003). HSD is commercially available as 'RescueFlow<sup>®</sup>' (BioPhausia AB, Stockholm, Sweden) and contains 75g sodium chloride (NaCl) and 60g dextran 70. This 7.5% NaCl represents an osmotic load of 2400 mOsm/L and a MW of 70,000Da, giving it a half-life in the plasma of 12hours. A 250ml aliquot represents a dose of approximately 4mg/kg in an average man (Sapsford, 2003).

The results from HSD have demonstrated the same benefits that have been described with HTS. Improved blood flow to the kidney, pancreas and gastric mucosa was significantly improved when HSD in 10% dextran was used in resuscitation and moderate improvement in flow to the myocardium, brain, skeletal muscle and intestine (Kreimeier et al., 1990). The authors postulated



that this improvement in blood flow improved the overall survival. This improvement in flow was shown to result from a substantial increase in capillary flow that was noted by HSD infusion. Endothelial cells, swollen during the shocked state, were shrunk by 20% when HSD was infused (Mazzoni et al., 1990).

HSD, like HTS, has been shown to favourably modulate the immune response to haemorrhage/resuscitation-induced leukocyte activation (Rotstein, 2000), two recent clinical trials have looked at this in prospective, double-blinded randomised controlled trials of trauma patients with hypovolaemic shock. The first was a Canadian trial (Rizoli et al., 2006) that randomised 27 patients presenting with haemorrhagic shock following blunt trauma to receive either a single 250ml intravenous bolus dose of either HSD or 0.9% saline (placebo). Blood was collected prior to infusion and then at 1, 3, 6 and 24 hours after intervention. The leukocyte count dropped in both treatment arms, but more so with HSD (but not significantly). There was a significant difference with the expression of CD11b, a surface marker involved in neutrophil adhesion and activation, as the progressive increase noted with placebo was prevented when HSD was given. HSD was also found to produce a depletion of CD14+/CD16+ pro-inflammatory monocyte markers. Looking at pro- (TNF $\alpha$  and IL-1b) and the anti-inflammatory cytokines (IL-1ra and IL-10); HSD prevented the increased TNF $\alpha$  expression that was seen with placebo, although there was no significant difference with IL-1b. HSD was found to produce an early and sustained up-regulation of IL-10 and IL-1ra (consistent with previous animal results [Powers et al., 2003]).

The second RCT from Seattle looked at samples at 12, 24, 72 hours and 7 days after injury (Bulger et al., 2007), from 62 patients who received either a bolus of 250ml of HSD or Ringers Lactate (placebo). Again they found a significant reduction in CD11b and TNF $\alpha$  12hrs after injury but these differences were not sustained in the recovery period. Neither study found any difference in clotting parameters. The same group published data looking at the ARDS-free survival at 28 days. Again, there was no difference between those who received HSD or placebo.

Returning to the question of whether HSD would improve survival, Smith et al. continued on the work of Nakayama from 1984 by studying the resuscitation of sheep following controlled haemorrhage with either 7.5% NaCl alone or in combination with 6% dextran 70 (HSD) (Smith et al., 1985). In a pig model with severe but controlled haemorrhage, a HSD infusion resulted in improved and sustained haemodynamic variables when compared to either hypertonic or normal saline (Wade et al., 1989).

This improvement in survival opened the door to clinical studies with HSD in resuscitation. Holcroft looked at resuscitation with either HSD versus Ringer's Lactate in patients who had severe haemorrhagic shock and were transported by helicopter to the emergency department. HSD showed the potential to increase survival with a reduction in the fluid requirement during the first 24 hrs of the study (Holcroft et al., 1987). A number of double-blinded trials in resuscitation followed for hypotensive patients demonstrated that patients who had HSD were found to have a higher blood pressure on arrival to the hospital but overall survival to discharge was similar to those that received isotonic fluids. The subgroup of patients with head injuries and particularly those with a GCS of less than 8 showed a tendency toward improved survival (Vassar et al., 1991, 1993; Mattox et al., 1991; Younes et al., 1997). A meta-analysis in 1997 found that HSD may be superior to either HTS or isotonic fluid with an increase in survival to discharge or 30 days. The result was not significant (Wade et al., 1997). More recent work looked at the efficacy of HSD resuscitation for hypotension following penetrating torso trauma (Wade et al., 2003). It did find a significant improvement in survival.

A larger multi-centred trial that was investigating the use of HSD was instigated by the National Institute of Health with the Resuscitation Outcomes Consortium (ROC) but was halted at the interim analysis (Bulger et al., 2011). It was a double-blinded RCT trial of 3 arms comparing a bolus of 7.5% saline (HTS) versus HSD versus 0.9% saline (NS) as the initial resuscitation fluid given to patients in haemorrhagic shock in the out-of-hospital setting: 62% suffered blunt trauma and 38% with penetrating trauma. The primary end-point was 28-day survival, with secondary points including the ARDS-free survival, multiple organ dysfunction score (MODS) and nosocomial infection. Results at interim stage

showed there was no difference in survival between patients who received hypertonic saline solutions, with or without dextran. HSD: 74.5% (95% CI- 7.5 to 7.8); HTS:73%(95% CI- 8.7-6.0); and NS:74.4%,  $p=0.91$ . The mortality rate before reaching hospital or in the emergency department was higher in the hypertonic saline group, although did not reach statistical significance. There were no differences between groups in organ failure, ARDS-free survival or nosocomial infections. A subgroup analysis of this study looked at the use of hypertonic resuscitation following severe traumatic brain injury (TBI) (Bulger et al., 2010). The main outcome was assessment of neurological status at 6 months. Results showed there was no difference in 6-month neurological outcome among groups with severe TBI (HSD vs NS:53.7% vs 51.5%; HTS vs NS:54.7% vs 51.5%,  $p=0.67$ ).

Of interest, these studies did not show any clinical difference with coagulopathy, although two *in vitro* studies found that dilution of whole blood with increasing concentrations of HSD have decreased clot strength, slight prolongation of PT and decreased platelet aggregation (Hess et al., 1992; Coats and Heron, 2003). All authors' did comment that the alterations were not thought to be clinically relevant.

The ROC group concluded that there was no compelling evidence to promote the use of HSD in the resuscitation of trauma patients, but they did complete their paper by recommending future studies being warranted to define the use of HSD in a military environment. This conclusion should be tempered with the understanding that the trial was terminated early on grounds of futility.

The use of HSD has been investigated in a pre-clinical study for the resuscitation in a complex military injury (Jacobs, 2010). Large white pigs were terminally anaesthetised and subjected to two injury patterns: haemorrhage (with a controlled and uncontrolled element from a liver injury) alone or combined with a blast injury. Animals were initially resuscitated with 0.9% saline (NS) or with HSD to a total amount of 7.1ml/kg (then any subsequent resuscitation with 0.9% saline). Resuscitation was to a hypotensive limit for the first 60mins (80mmHg) and then to a normotensive limit thereafter. Results showed HSD was associated with a significantly reduced survival time in the

blast/haemorrhage group when compared to the group resuscitated with NS. In the absence of blast, HSD did confer some physiological advantage to the animals exposed to haemorrhage alone, with a reduced base deficit throughout the resuscitation period.

Considering the greatest threat to military in current conflicts is from blast, and that **no clear evidence** has been defined for HSD in the pre-hospital setting, it is unlikely that HSD could be considered for routine pre-hospital management of battlefield or civilian trauma casualties.

#### **4.5 Damage Control Resuscitation (DCR).**

Damage control resuscitation (DCR) combines the concept of 'permissive hypotension' or hypotensive resuscitation with the developing haemostatic resuscitation and used in combination with damage control surgery (Kirkman et al., 2007; Holcomb, 2007; Jansen et al., 2009). DCR has been developed in military trauma systems and has been defined in two ways:

1. The proactive early treatment to rapidly reverse an acidosis, prevention of hypothermia and coagulopathy (to arrest the development of the lethal triad (section 3. 7.2) on arrival to a combat hospital (Holcomb et al., 2007)
2. A systematic approach that combines the paradigm of <C>ABC to address catastrophic bleeding, airway, breathing and circulation with a series of clinical techniques from point of wounding to definitive treatment in order to minimise blood loss, maximise tissue oxygenation and optimise outcome (Hodgetts et al., 2007). This extends the principle of DCR forward to the point of wounding.

The foundation and understanding for hypotensive resuscitation has been discussed and is primarily set in the pre-hospital environment; however when a major trauma casualty arrives in hospital, then the focus must shift to allow access to the rapid control of major surgical haemorrhage and then fluid and targeted therapy with the early use of blood and blood products directed towards treatment of intrinsic ATC (Acute Traumatic Coagulopathy) and to prevent dilutional coagulopathy. Damage control surgery at this stage has the

overall aim to stop haemorrhage and prevent contamination and runs simultaneously with the haemostatic resuscitation (neither is an adjunct to the other) (Shapiro et al., 2000). The overall objective is to proactively manage the physiological consequences of injury (Midwinter, 2009).

The 'haemostatic' element of resuscitation replaces the lost blood with packed red cells and blood products early to attempt to restore both perfusion and normal coagulation function. These approaches are only required in the most severely injured, which account for approximately 10% of casualties in a military environment (less than 5% in a civilian setting) (Holcomb et al., 2007), and encompasses those that will require a massive transfusion.

Massive transfusion (MT) is regularly cited as the transfusion of more than a patient's circulating volume in 24 hours or more than 10 units of packed red blood cells PRBC in 24 hours (Malone et al., 2006). However, an acutely bleeding trauma patient and those at risk of life threatening coagulopathy receive 85% of their transfusions in the first 6 hours after injury (Kashuk et al., 2008). Protocols have been developed to help counter the dilution and consumption of factors and this, along with reversal of hypothermia and acidosis is the crux of DCR.

The current definition of a MT in the UK military is outline in table 5 as laid down in the Joint Service Publication (JSP) 950; Medical Policy (2009). However the severity of the injury made on clinical assessment (such as bilateral proximal traumatic amputations or truncal haemorrhage with one proximal traumatic amputation) can be used to implement the policy as this degree of severe injury severity has a high likelihood of developing ACT (Brohi et al., 2003).

## Definition of Massive Transfusion in UK as stated in UK JSP 950

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Definition of Massive Transfusion:

1. The replacement of an equivalent amount of blood to an entire circulating blood volume of the patient within 24 hours; or
2. More than 10 units of red blood cells within 24 hours (whichever comes first).

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Additional Criteria in the Acute Military Operational Setting:

1. The transfusion of over four units of red cells in 1 hour; or
2. The replacement of 50% of the total blood volume in 3 hours; or
3. A rate of blood loss of >150 ml/min

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**Table 5** : UK definition of massive transfusion

This allows the prompt delivery of blood and products to the medical staff to ensure resuscitation is prompt and effective. This section looks at the use of blood, blood products and the use of adjunct therapies given during haemostatic resuscitation.

### **4.5.1 Packed Red Blood Cell (PRBC) Transfusion.**

Haemorrhagic shock necessitates the replacement of red blood cells (RBC), and early red cell transfusion has shown to increase CO and preserve oxygen carrying capacity (Dutton and Carson, 2006). The contribution of RBC in the overall haemostatic process is unclear but reports have stated that they help marginalise platelets within the blood vessel and support thrombin generation (Quaknine-Orlando et al., 1999; Peyrou et al., 1999). A drop in the haematocrit (Hct) results in a increase in bleeding time and this parameter returns to normal upon transfusion (Valerie et al., 2001) and it is postulated that the presence of RBCs accelerates the onset of clot formation (Spoerke et al., 2010).

There is no current international consensus on what haemoglobin (Hb) level should be the trigger for transfusion. The updated European guidelines on the

management of bleeding recommend a target Hb of 7-9 g/dL (recommendation 21, Rossaint et al., 2010). The British Committee for Standards in Haematology (BCSH) (2006) guidelines on management of massive blood loss recommend a target Hb of >8g/dL. The Canadian Critical Trials group found there was less transfusion if the target Hb was 7g/dL but there were no significant benefits in terms of multi-organ failure (MOF) or post-trauma infections (McIntyre et al., 2004). That study was not designed to address the trauma subgroup directly, and so a Hb level of >8g/dL is the accepted norm.

Transfusion will always carry a risk of exposure to blood borne infection, mild allergic reactions and ABO incompatibility. With massive transfusions occurring in the most serious ill patients to underpin DCR, it is not without risk. Blood transfusions have been associated with an increased development of MOF, increased ICU stay, increased incidence of ARDS, higher degree of renal impairment, prolonged length of hospital stay and increased mortality (Moore et al., 1996; Claridge et al., 2002; Malone et al., 2003., Charles et al., 2007; Marik and Corwin, 2008; Chaiwat et al, 2009). Therefore blind over-transfusion may result in increased risk and also be a waste of precious resources.

#### **4.5.2 Fresh Frozen Plasma (FFP).**

Considering DCR is designed to rapidly address ATC with the empiric and simultaneous administration of clotting products with the initial PRBC transfusion (Holcomb et al., 2007) and early administration of thawed FFP at a dose of 10-15ml/kg is recommended (Rossaint et al., 2010). FFP is formed by isolating the plasma from the cellular components of blood within 6 hrs of collection, giving a preparation of near normal levels of clotting factors (Stanworth, 2007). There is little direct evidence for the clinical efficacy of using FFP in trauma patients (Stanworth, 2004), however it is widely accepted that replacement of clotting factors is necessary in a bleeding patient, although historically it was felt that FFP should be administered when a coagulopathy was identified by a PT and APTT were >1.5 times normal (Stainsby et al., 2000). However reliance on these laboratory measure given the length of time these results take to return to the clinician renders them of limited value in a dynamic, rapid resuscitation of a trauma patient (Davenport et al., 2011).

Standard clinical teaching of administration of 1-2 units of FFP after 4-6 units of PRBCs adopted from animal experiments in the early 1980s (Counts et al., 1979; Lucas and Ledgerwood, 1981). This thinking was questioned in 1982 when a FFP:RBC ratio of >1.4 was found to increase the likelihood of coagulopathic bleeding and ratios >1.8 were almost uniformly associated with a lethal outcome (Kashuk et al., 1982; Cosgriff et al., 1997).

The more aggressive ratio of FFP:RBC of 1:1 was introduced by military surgeons in Iraq during the past decade (Hess et al., 2006; Ketchum et al., 2006; Borgman et al., 2007). This recommendation was initially instigated with anecdotal evidence, until Borgman published his small, retrospective study to determine whether the ratio of plasma to RBCs transfused would affect survival (Borgman et al., 2007). As the ratio of plasma to RBC increased, mortality decreased with a statistically significant absolute reduction of 46% (from 65% to 19%) for those who had been resuscitated with FFP:RBC in a 1:1.4 when compared to those who received the conventional 1:8. The authors concluded that this lower ratio improved the survival of patients at risk of haemorrhagic shock, whilst acknowledging that prospective randomised trials were needed to compare empiric plasma to RBC ratios on the effect of outcomes for patients with severe trauma. Further publications have looked at this higher ratio and found broadly the same results (Kashuk et al., 2008; Duchesne et al., 2008; Sperry et al., 2008; Maegele et al., 2008; Holcomb et al., 2008; Gonzalez et al., Gunter et al., 2008; Spinella et al., 2008), but all are retrospective studies. The Denver group (Kashuk et al., 2008) found that the 1:1 FFP:RBC reduced coagulopathy but did not translate into a survival benefit. They concluded that 1:2-1:3 ratios were optimal for survival (Kashuk et al., 2010). Analysis of the German Trauma Registry (Maegele et al., 2008) showed that the mortality rates with ratios of RBC:FFP <0.9; RBC:FBC 1=1; RBC:FBC >1.1 were 3.5%, 10% and 25% respectively. They also showed that the frequency of septic complications and MOF was significantly raised in the 1:1 group.

The main problem with these retrospective studies is the survival bias (as in those patients who die early do not survive long enough to get as much plasma). When the timings of the transfusions was evaluated, it revealed that the patients who were given early, high ratio of FFP:RBC were actually in less



shock and less likely to die from uncontrolled haemorrhage, thus the bias is because those selected are less likely to die in the first place (Magnotti et al., 2011). Snyder reviewed their data to adjust for this bias, the association between higher ratios and improved mortality was not there (Snyder et al., 2009).

Two systematic reviews have looked at the impact of altering FFP:RBC ratio on mortality (Zehtabchi and Nishijima, 2009; Murad et al., 2010), analysing 11 observational studies between them. One paper concluded that there was no significant evidence to show that a high ratio of FFP:RBC was beneficial and reiterated the higher relative risks for sepsis, MOF and ARDS (Zehtabchi and Nishijima, 2009). The second paper (only looking at 4 studies) concluded that there was some evidence to advocate a higher ration was associated with a better outcome (Murad et al., 2010), but both papers commented on the degree of bias and the need for prospective work in this area.

Two prospective cohort studies have been published: the first looking at the outcome in critically injured trauma patients (Scalea et al., 2008) and the second evaluating the haemostatic effects at different ratios during resuscitation (Davenport et al., 2011). These small studies found that the FFP:RBC ratio did not predict or improve ICU stay or mortality, and a ratio of 1:2 and 3:4 resuscitation provided the greatest benefit to haemostasis.

The evidence currently supports the use of early use of plasma in the resuscitation but in what amount? The other outstanding question is how do we administer this practically and how much should be given? Too much FFP may not be the answer.

#### **4.5.3 Platelets.**

Spontaneous bleeding can occur when the platelet count falls below  $50 \times 10^9/L$  (Stainsby et al., 2000), and below which platelet function diminishes exponentially (Samama et al., 2005). The British Committee for Standards in Haematology (BCSH), 2006, guidelines currently recommend that platelets are given at a threshold of  $>75 \times 10^9/L$  in massive transfusion, and the European Guidelines (Rossaint et al., 2010) recommends the count should be maintained

above  $50 \times 10^9/L$  or above  $100 \times 10^9/L$  in multiple trauma. In severe TBI, thrombocytopenia is a strong negative prognostic factor (Van Beek et al., 2007) and so measures to avoid this should be taken in the subgroup.

Even though there is no direct evidence to support the use of platelets in the trauma patient, with the understanding of the role of the platelets are such an integral part of the clotting process, their addition to the resuscitation is essential. Current evidence suggests that platelet function appears to be of greater importance than platelet number in thrombus formation and integrity (Davenport and Brohi, 2009). The use of early platelets has been advocated in trauma resuscitation, especially within the military sphere (Holcomb, 2007; Borgman et al., 2007; Kirkman et al., 2007). Again, data is retrospective. A ratio of platelets:FBC of 1:5 was shown to have a lower 30-day mortality when compared with those who had received less than this ratio (38% vs 61%,  $p=0.001$ ). However, platelet transfusions are associated with a high incidence with Transfusion-related Acute Lung Injury (TRALI) (Khan et al., 2007).

Platelets are included in the development of proactive massive transfusion protocols (MTP). A recent review of the literature with use of plasma and platelets in MTP showed that there was a 74% reduction of odds in mortality and a 15% improvement in 30-day survival (Johansson and Stensballe, 2010). Again the question of how much and how early platelets should be given remains unanswered.

#### **4.5.4 Fibrinogen / Cryoprecipitate.**

Cryoprecipitate is formed when FFP is thawed, centrifuged and the precipitate is re-suspended in sterile saline and is rich in factors VIII, XIII, vWF and fibrinogen. Four units of FFP contain approximately 1500mg of fibrinogen, equivalent to 1 pooled pack of cryoprecipitate (1400mg) (Kashuk et al., 2010). Fibrinogen can decrease to critical levels at an early stage after trauma, even before administration of PRBC becomes necessary (Hiippala et al., 1995; Martini et al., 2005). Fries et al have examined the effect of fibrinogen in an animal model of traumatic injury and blood loss (Fries et al., 2005 and 2006) demonstrating that the administration of fibrinogen normalises the propagation

phase of clotting, reduced the overall blood loss and increased the survival of the animals compared to those treated with placebo. An in-vitro study compared the effects of fibrinogen, factor XIII and FFP in reversing the dilution-dependent changes in clot formation (Haas et al., 2008). Both fibrinogen and FFP allowed the indices of initiation of clotting to return to baseline levels, but clot firmness was only restored with administration of fibrinogen. These conclusions have increased the attractiveness of early replacement of fibrinogen.

Three observational studies have looked at the administration of fibrinogen concentrate (Haemocomplettan<sup>®</sup> P, CSL Behring GmbH, Germany: licensed in a number of European Countries, not UK). Two of which looked at patients with acquired hypofibrinogenaemia and demonstrated that fibrinogen administration alone stopped bleeding (Danes et al., 2008; Weinkove et al., 2008) and as a therapy for massive haemorrhage, the third paper concluded that fibrinogen improved coagulation tests and the need for transfusion of RBC, FFP and Plts decreased (Fenger-Eriksen et al., 2008).

A recent retrospective review of 252 combat trauma patients who received massive transfusion reported a reduction in mortality in the group with higher concentrations of fibrinogen than those with lower concentrations (Stinger et al., 2008). Current recommendations state that fibrinogen should be replaced if plasma levels are less than 1.5 to 2.0g/L or if there are thromboelastometric signs of deficiency. The current recommendation in the European Guidelines is a dose of 50mg/kg of cryoprecipitate or 3.4g of fibrinogen concentrate should be given initially (Recommendation no 26, Rossaint et al., 2010) when a deficiency is found. There are no scientific trials to support pre-emptive fibrinogen replacement early in the treatment of patients with TIC.

#### **4.5.5 Anti-fibrinolytics**

Hyperfibrinolysis is found in the most severely injured patients and is associated with a increased mortality rate (Kashuk et al., 2010). Hyperfibrinolysis occurs when the fibrinolytic activity is potentially greater than fibrin formation such that the clots integrity is threatened (Hunt, 1996) and as explained in section 3.5.2

the term hyperfibrinolysis is poorly defined within the current literature. Thromboelastography has become the accepted method to both detect and define hyperfibrinolysis which is a clot lysis result of >15% within 60 minutes (Luddington, 2005; Schochl et al., 2009). If it is fulminant (where clot lysis is greater than 50% in 30mins when measured by ROTEM<sup>®</sup>) it is almost always associated with 100% mortality (Schochl et al., 2009). The condition can be treated by the administration of anti-fibrinolytics. The publication of the CRASH-2 trial has brought the use of the anti-fibrinolytic, tranexamic acid (TXA) to the fore (CRASH-2 collaborators, 2010). This was a large, international multi-centre RCT trial of 20 000 patients evaluating the use of the tranexamic acid in major trauma within 8 hours of injury. The primary endpoints were mortality with 4 weeks of injury, vascular occlusive events and transfusion requirements. Results demonstrated that the early administration of TXA to trauma patients reduces the death from haemorrhage with no apparent increase in fatal or non-fatal vascular occlusive events. No significant affect on the transfusion requirement was noted.

This trial is of clinical significance but has been criticised for a number of reasons. The majority of patients were recruited in developing countries where the approach and treatment of trauma victims varies greatly to that of developed countries and also there is no definite evidence that patients were bleeding at the time of randomisation. Also only half the patients (50.4% in the TXA group and 51.3% in the placebo group) received blood transfusions. Was this because there was no blood available to transfuse or because the patients were not actually bleeding? Only 47.9% of the TXA group and 48% in the placebo group underwent a surgical intervention procedure – is this because they again weren't actually bleeding or the blood loss was controlled by external pressures? These questions have raised many criticisms about the study (Cap et al., 2011).

A current military study has looked at the use of TXA in combat-related injuries in Afghanistan and its impact on mortality. Initial results have shown that the use of TXA in conjunction with component-based resuscitation following combat injury is associated with improved survival, especially those requiring massive transfusion. Interestingly the data has shown that there is no significant

difference of mortality <24 hrs but there is a significant difference of decreased mortality of deaths at 30 days (Morrison et al., 2012). This reason for this is not clear.

Current recommendations are that TXA should be administered to bleeding patients, especially if hyperfibrinolysis is detected (Rossaint et al., 2010).

#### **4.5.6. Recombinant activated Factor VII**

Recombinant activated Factor VII (rFVIIa) (Novoseven<sup>®</sup>, Novo Nordisk) is manufactured using recombinant DNA technology. Its use in surgical bleeding and in particular trauma is off licence and is controversial. The first prospective trial (Boffard et al., 2005) in trauma demonstrated a reduction of blood transfusions with administration of rFVIIa in blunt trauma, but no reduction in mortality. There was a significant reduction in the incidence of ARDS. This association with reduction of transfusion in blunt trauma has been well documented (Vincent et al., 2006; Patanwala, 2008) and therefore its use is incorporated into most massive transfusion protocols. The results of the CONTROL trial have been published (Hauser et al., 2010). This was a prospective, randomised, double-blinded multi-centred, placebo-controlled trial that was conducted from 2005 to 2008. The aim was to evaluate efficacy and safety of rFVIIa in patients with active bleeding from trauma and had already received 4 units of blood. This trial was terminated early after the enrolment of 573 patients on the grounds of futility following an interim analysis and because of difficulty in recruitment. Results showed that the addition of rFVIIa reduced blood product use but there was no affect on mortality in either blunt or penetrating trauma when compared with placebo.

The current guideline (Rossaint et al., 2010) is that it should be considered if major bleeding in blunt trauma persists despite standard attempts to control bleeding, although it is not a treatment to used as a 'last ditch' attempt in a patient that is devoid of all clotting factors, active platelets and red blood cells. It should be used before it is too late.

#### **4.5.7 Other Factor Components**

Prothrombin Complex Concentrates (PCC) is composed primarily of Factor IX and is most commonly used for haemorrhagic complications of Haemophilia B (Hoffbrand et al., 2006). It also contains concentrations of factors II, VII and X. Administration of PCC results in an increase in thrombin production in patients who have been on vitamin-K dependent oral anticoagulants (Baglin et al., 2006), thus it is recommended for the emergency reversal of these anticoagulants (Rossaint et al., 2010). Use in an animal model have shown that PCC was more effective than FFP in correcting dilutional coagulopathy and correcting bleeding in a trauma model (Dickneite and Pragst, 2009) but there are currently no specific clinical trials to advocate its use in the management of bleeding in trauma patients who are not on anticoagulation medication (Fries et al., 2009). Patients who have received this must be given thromboprophylaxis during the recovery period.

Although not a factor component, ionised calcium levels must be monitored during treatment of bleeding patients and especially those receiving massive transfusion, as it is a co-factor for many components in the clotting process. The citrate used as the anticoagulant in many blood products chelates calcium and therefore transfusion can exacerbate hypocalcaemia (Hoffbrand et al., 2006). A decrease in cytosolic calcium concentration results in a decrease in all platelet related activity and therefore the level of ionised calcium should be maintained above 0.9mmol/L (Lier et al., 2008).

#### **4.5.8 Fresh Whole Blood**

When discussing resuscitation with particular reference to the military sphere, the use of Fresh Whole Blood (FWB) should be mentioned. It has been used in conflict to resuscitate patients since World War I and current US/UK military doctrine permits FWB to be used in resuscitation when standard blood components are not available (JSP 950; Medical Policy 2009). In a recent report of US usage in recent conflict showed that 3% of patients requiring transfusion were given FWB in a five year period from January 2001 to December 2005 (Spinella et al., 2008). This practice is not widely used in the

civilian trauma in developed countries but should be considered when no other solution is available.

All these treatments and recommendations require a rapid diagnosis and ongoing treatments should be tailored to the patient requiring haemostatic resuscitation, this is where TEG/TEM has been widely documented as an essential component of treatment.

#### **4.6 Use of TEM in the Detection & Management of TIC during Resuscitation**

Rapid, real time functional assessment of coagulation status is imperative in firstly the diagnosis of TIC but also to allow goal-directed therapy for specifically identified coagulation abnormalities. The increasing use of TEG/TEM is becoming accepted as essential in routine practice for treating massive haemorrhage: for both the diagnosis of coagulopathy and with the direction of treatment.

##### **4.6.1 Use of TEG/TEM in detection of TIC**

As discussed in Chapter 3 (section 3.6.2), the use of TEG/TEM has been widely published in the sphere of cardiac and liver surgery, however the use in trauma is only now coming to prominence. Kaufmann and colleagues described the usefulness of TEG in the assessment of trauma patients (Kaufmann et al., 1997) highlighting abnormalities in 75% of trauma patients on admission. Moderately injured patients (average ISS=13) were hypercoagulable whereas more severely injured (ISS= 29) were hypocoagulable by detection of TEG, but all had normal PT/APTT results.

The use of ROTEM<sup>®</sup> demonstrated early changes of in vivo coagulation of trauma patients (Rugeri et al., 2007). Results showed a significant correlation between PT and CA15 (the clot amplitude of 15 minutes) in EXTEM; APTT and

CFT in INTEM; and Fibrinogen level at CA10 in FIBTEM trace. There was a significant correlation between platelet count and CA15 in INTEM.

A number of recent clinical studies, all with small numbers, have looked at the early evaluation of TIC with TEG/TEM (Plotkin et al., 2008; Levrat et al., 2008; Schochl et al., 2009; Carroll et al., 2009; Jaeger et al., 2009; Park et al., 2009; Kashuk et al., 2009). Carroll et al looked at samples taken at the geographical site of injury and on arrival to the ED, and found that hypocoagulopathy detected by TEG<sup>®</sup> correlated with fatality, whereas no such correlation was found with standard tests. This group noted that 2% had hyperfibrinolysis (defined as clot lysis >15% in 60 minutes) compared to 6% in the Levrat study (when samples were taken on arrival to ED); these results were found in the most severely injured group and associated with an increased mortality rate.

The development of Rapid TEG<sup>®</sup>, which uses tissue factor as an activator in testing, led to the Wade et al (2009) proposing TEG<sup>®</sup> could assess coagulation on arrival to the hospital and begin to guide the administration of blood and blood products. However, it was highlighted that no large multi-centred trial that was sufficiently powered has yet been undertaken. This call for a prospective and independently validated study using TEG/TEM was echoed by Johansson et al in their review of the assessment of coagulopathy in trauma (Johansson et al., 2009).

A small retrospective study of combat patients (Plotkin et al., 2009) reported that TEG<sup>®</sup> was a more accurate indicator of blood products than PT, APTT and INR, although they were comparing a single TEG<sup>®</sup> trace taken at anytime within the first 24hrs of admission. Use of ROTEM<sup>®</sup> in a deployed military environment (Doran et al., 2010) found that 64% of patients were coagulopathic on arrival to the ED which was significantly different to the 16% as detected by PT/APTT. The ROTEM<sup>®</sup> value at CA10 (the clot firmness at 10 minutes) was found to correlate with an abnormal MCF, meaning that TEM could provide an indication of a coagulopathy quickly after arrival into the ED. Davenport et al have presented work from the Royal London Hospital (Davenport et al., 2011) that correlates with the size of clot firmness at 5mins (CA5) could predict the need for massive transfusion. Patients with a CA5 ≤ 35 mm had a greater PRBC



transfusion requirement (4 vs 1 units,  $p < 0.001$ ) and were more likely to receive FFP (47% vs 12%,  $p < 0.001$ ). A normal CA5 ( $> 35\text{mm}$ ) had a negative predictor value of 99% for massive transfusion. Woolley et al defined hypocoagulopathy as two or more abnormal values of ROTEM<sup>®</sup> parameters relating to clot initiation, clot dynamics and clot strength. (Woolley et al., 2011).

The other advantages above routine laboratory tests are that ROTEM can be set to the temperature of the patient to provide a true assessment of the patient, whereas all standard tests are at 37°C. Fibrinolysis and platelet dysfunction pose diagnostic gaps which may be missed in standard tests. Considering this, these rapid results should be part of the assessment of any coagulopathy in trauma patients as recommended by the updated European Guidelines (Recommendation 12; Rossaint et al., 2010).

#### **4.6.2 Use of TEG/TEM to guide haemostatic resuscitation**

Haemostatic Resuscitation is a well-established but as outlined above the scientific evidence remains poor (Duchesne, 2011). The need to control blind over-transfusion is necessary to avoid donor exposure, and with the increased risk of sepsis and MOF it is essential that all methods available are used to guide the process.

In cardiac surgery, 2 RCTs have looked at the use of TEG/TEM in guidance of blood and blood product usage. Shore-Lesserson et al. (1999) compared TEG<sup>®</sup>-based and conventional protocols to manage post-operative bleeding. Blood and blood component therapy was significantly less in the TEG<sup>®</sup>-guided group when compared to the conventional group, although there was no difference in blood loss between each group. The second study (Royston and Von Kier, 2001) again demonstrated significantly less blood and blood product usage when a TEG<sup>®</sup>-based algorithm was introduced. The current guidelines on antiplatelet and anticoagulation management in cardiac surgery (Dunning et al., 2008) recommends that *'thromboelastography may be used to guide transfusion in the postoperative period and studies have demonstrated a reduction in blood and blood product usage if used in conjunction with a treatment algorithm'*. They conclude that further large, prospective studies are

needed before TEG<sup>®</sup> could become the standard of care for management for transfusion management.

As with cardiac surgery, the recommendation to use TEG/TEM is becoming an integral part of the resuscitation protocol of trauma patients (Stahel et al, 2009; Kashuk et al., 2010, Doran et al., 2010). This is of particular interest with administration of fibrinogen and platelets. The evidence for the guidance of TEM during resuscitation commences with case reports (Brenni et al, 2010; Doran et al., 2010), each highlighting the requirement of 'personalised' goal-directed therapy for each patient. In Europe, the administration of fibrinogen and prothrombin complex concentrate is more widely accepted as an initial treatment for traumatic coagulopathy than in the UK and the USA (Schochl et al, 2010). Two recent papers have highlighted the use of TEM to help identify those who require fibrinogen concentrate and this in turn lead to a reduction in the requirement for allogeneic blood products (Fries and Martini, 2010; Schochl et al., 2011) and thus guiding the clinician in their use of products during the haemostatic resuscitation.

The recently Cochrane Review assessed the use of TEG/TEM in monitoring haemotherapy in patients with massive transfusion (Afshari et al., 2011). Looking at 9 RCTs with a total of 776 patients, the authors concluded that current data demonstrated that the use of TEM showed no significant benefit on mortality but did show a significant reduction on the amount of bleeding. As with other papers, they concluded that further research was required.

A current review of systematic reviews identified four key questions regarding TIC and assessed the literature for answers (Curry et al., 2011). The questions were:

- a) What are the best methods of predicting and diagnosing TIC?
- b) Which methods of clinical management correct coagulopathy?
- c) Which methods of clinical management correct bleeding?
- d) What are the outcomes of transfusion in trauma?

After reviewing 37 systematic reviews in the literature, this cohort of authors concluded that they could not answer these questions conclusively. A better understanding of the pathophysiology is required, with a clear definition of what TIC actually is which allow future clinical trials to a standard to compare with. Although the use of blood, blood components and factors alter coagulation profiles, the question of '*Will survival improve if coagulation parameters are corrected?*' remains unanswered. Transfusion is needed in bleeding patients, but the lack of prospective RCTs into the ratio of products and their timings is not clear. The next decade of research into this subject may answer these questions in time.

## **Chapter 5: Hypotheses and Aims of this thesis.**

The aim of this chapter is to summarise the salient points of the introductory material and derive the hypotheses that will be tested in the experimental work described in the subsequent chapters.

### **5.1 Randomised controlled trial to compare the effects of Novel Hybrid resuscitation with prolonged hypotensive resuscitation on coagulation and inflammation in two models of complex battlefield injury in terminally anaesthetised pigs.**

#### **5.1.1 *Summary of background material.***

Acute traumatic coagulopathy (ATC) is a multifactorial phenomenon (section 3.7.2) which represents a serious clinical problem in a significant proportion of trauma casualties (section 3.7.1). One of the key mechanisms underpinning this early phase of ATC is tissue hypoperfusion, evidenced by a clinically significant base deficit (BD) in trauma casualties (Brohi et al., 2007). Hypotensive resuscitation is often used in the pre-hospital environment and after hypovolaemic shock is likely to result in significant tissue hypoperfusion and base deficit (section 2.1 and 4.2). Within military research a resuscitation strategy was developed that permitted one hour of hypotensive resuscitation and then after that time the blood pressure was restored to a normotensive level. This strategy was labelled as Novel Hybrid (NH) Resuscitation and has been shown to reduce the base deficit when compared to a prolonged hypotensive resuscitation strategy (Jacobs, 2010). Thus it could be postulated that such a NH resuscitation strategy would result in less coagulopathy when compared to a hypotensive strategy. Conversely, it could also be postulated that the NH resuscitation would require more fluid and therefore could run the risk of causing greater haemodilution which in turn could potentially worsen coagulopathy (section 3.7.2). However, as the actual volume of fluid required in the NH resuscitation was comparatively small to that volume administered in a purely hypotensive strategy (Jacobs, 2010), the effect of tissue hypoperfusion is likely to predominate over haemodilution, leading to the overall hypothesis that

a NH resuscitation strategy will be associated with less coagulopathy than a purely hypotensive resuscitation strategy.

Additionally, it is understood that prolonged hypoperfusion and the associated shock state can augment an inflammatory response (section 2.1.3). It is therefore likely that the NH resuscitation strategy will be associated with a reduced systemic inflammatory state when compared to the hypotensive resuscitation strategy.

### **5.1.2 Hypotheses**

1. NH resuscitation will be associated with improved clotting compared to hypotensive resuscitation.
2. A supplementary hypothesis is that NH resuscitation will be associated with evidence of reduced systemic inflammation compared to prolonged hypotensive resuscitation.

### **5.1.3 Aims**

1. To test hypothesis 1 (above) clotting status, assessed by measuring peak prothrombin time (PT) during resuscitation, will be compared between groups of animals given NH and prolonged hypotensive resuscitation. Two models of injury will be employed; both will involve hypovolaemic shock, one on the background of blast injury and the other without blast injury.
2. To test hypothesis 2 (above) blood levels of IL6 and TNF alpha will be used as indicative markers of systemic inflammation and will be compared between groups given NH and hypotensive resuscitation.

## **5.2 Feasibility of using rotational thromboelastometry to assess coagulation status of combat casualties in a deployed setting.**

This was a feasibility study to determine whether a near patient test of whole-blood clotting (ROTEM) could be utilised in deployed military setting and therefore the main part of this study was not hypothesis driven. In the context of this study, feasibility was determined in two parts:

- a. The ROTEM device would perform to acceptable laboratory standards in the deployed environment (i.e. pass manufacturer specified quality control assessments at prescribed intervals).
- b. Samples could be processed by trained staff to provide credible traces and results imparted to the clinical teams in a clinically meaningful timescale.

It was anticipated that ROTEM would return results to clinicians more rapidly than conventional laboratory testing (PT and APTT). Since TEM performs a more in-depth analysis of clotting (looking at clot initiation, dynamics and strength) compared to conventional testing which looks principally at clot initiation (section 3.6) then it was anticipated that ROTEM might identify more abnormalities in patients. A supplementary analysis was therefore preformed to test the hypothesis that ROTEM would identify greater abnormality than conventional testing in the patients studied. However since this was not performed on a randomised sample of patients this is not intended as a definitive study but merely as an initial pilot examination to inform future study design.

### **5.2.1 Aims**

1. To determine whether ROTEM passed manufacturer prescribed quality assessment tests at prescribed intervals in the military deployed setting.
2. To evaluate whether patient samples could be processed and the results of ROTEM tests returned to clinical teams in a clinically meaningful timescale.
3. On a non-random sample of patients to compare the results of ROTEM and conventional laboratory assessment of clotting.

## **Chapter 6: Randomised controlled trial to compare the effects of Novel Hybrid resuscitation with prolonged hypotensive resuscitation on coagulation and inflammation in two models of complex battlefield injury in terminally anaesthetised pigs.**

### **6.1 Introduction**

Uncontrolled haemorrhage remains the leading cause of battlefield deaths (Champion et al., 2003) and coagulopathy accompanies severe bleeding and blood transfusion (Brohi et al., 2003; Spahn, 2005; Kashuk et al., 2010).

Trauma-induced coagulopathy (TIC) has been shown to present in approximately 30% of casualties in civilian data with limited data showing that TIC has a slightly higher incidence (approximately 39%) in battlefield casualties (Brohi et al., 2007; Maegele et al., 2007; Plotkin et al., 2008). The TIC is associated with increased morbidity and mortality (Brohi et al., 2003; Macleod et al., 2003; Curry et al., 2011) and a clinical imperative is to limit and reverse the development of this coagulopathy by early and aggressive resuscitation on arrival to medical facility (Holcomb et al., 2007).

The recently published European Guidelines for Advanced Bleeding in Trauma Care (Rossaint et al., 2010) recommends a rapid approach to gain control of bleeding, to identify and correct coagulopathies and overall maintain adequate tissue oxygenation. In the pre-hospital phase in urban trauma there is an established trend for hypotensive fluid-resuscitation strategy especially when there is a penetrating mechanism (Haut et al., 2011). This has the aim of avoiding the disruption of the initial clot during the first hour following trauma whilst it gains strength and before surgical control can be achieved (Midwinter and Woolley, 2011).

However, the penalty for a prolonged hypotensive period is tissue hypoperfusion and is particularly pertinent in a military environment when the evacuation times can be considerably longer than those experienced in civilian settings, especially in less mature operations (Bilski et al., 2003). Tissue hypoperfusion and ischaemia per se that can result from this period of permitted

hypotension has been linked to the early TIC by activation of the Protein C pathway producing anticoagulation and increased hyperfibrinolysis (see chap 3.) (Brohi et al., 2008; Cohen et al., 2007).

To test these theories, an injury model would need to be representative of a severe but survivable battlefield injury which would require resuscitation. A relevant injury model should ideally include elements of blast injury (primary) with tissue injury and uncontrolled haemorrhage requiring resuscitation over a protracted timeline (to reflect evacuation delays) would be required.

### **6.1.1 Choice of Animal Model.**

Garner et al (2009) developed a large animal model to investigate the interaction of blast injury and haemorrhage on the outcomes from resuscitation strategies after these combined injuries. A subsequent study (Garner et al 2010) utilised the swine model to investigate the physiological consequences of resuscitation strategies after haemorrhagic shock alone and combined with blast injury. They compared the effects of a normotensive (ATLS<sup>®</sup>-type approach) strategy with a hypotensive approach. The pilot study demonstrated that a combination of blast injury and a controlled haemorrhage (30% blood volume [BV] extracted from an arterial line) followed by hypotensive resuscitation was incompatible with survival during a simulated protracted evacuation (up to 8 hours), although survival was good for the first hour of resuscitation. By contrast survival was high over an 8 hour period with normotensive resuscitation. Hypotensive resuscitation after haemorrhagic shock in the absence of blast injury did lead to good survival over an 8 hour resuscitation period, although there was severe physiological deterioration compared to a control group given normotensive resuscitation. The poor survival and physiological deterioration associated with hypotensive resuscitation was associated with poor tissue oxygen delivery and severe metabolic acidosis, both of which might be predicted to have adverse consequences on clotting. Further studies to investigate putative novel resuscitation strategies needed a model incorporating uncontrolled haemorrhage with the capacity to re-bleed during resuscitation.



Uncontrolled haemorrhage refers to blood loss from a damaged vessel that cannot be arrested by intrinsic mechanisms or external intervention (Kirkman and Watts, 2011). Established animal models of haemorrhage fall into three broad categories:

- high pressure/high volume owing to a lesion in a major artery;
- low pressure/ high volume owing to a lesion in a major vein;
- a mixed model owing to a lesion in both arteries and veins.

In the mixed (arterial/venous) model, the greatest volume of blood lost is initially arterial, then as the haemorrhage progresses and the arterial pressure falls, the relative venous contribution becomes more important. Following Garner's studies, it was considered that this mixed-type of model would be the most appropriate to assess any resuscitation model.

A liver injury can be used to create a mixed arterial/venous uncontrolled haemorrhage. Liver injuries are graded by severity from Grade 1 (least severe) to Grade V (most severe). A grade V liver injury is where there is parenchymal disruption involving >75% of hepatic lobe segments within a single lobe and/or juxtahepatic vascular venous injuries (Moore et al., 1995) that would cause massive venous bleeding. Models incorporating Grade V liver injuries have been used to assess the haemostatic efficacy of commercial clotting agents (Holcomb et al., 1999; Pusateri et al., 2004; Klemcke et al., 2005). However, this degree of injury is generally unsurvivable without rapid surgical intervention. It is therefore too severe to allow assessment of battlefield resuscitation as survival is dependent on early surgical packing of the liver (Martinowitz et al., 2001), which is not possible in the field.

In comparison, a grade IV liver injury consists of parenchymal disruption involving 25-75% of a hepatic lobe or 1-3 of Couinaud's segments within a single lobe (Moore et al., 1995) and has been shown to a more realistic model of potentially survivable (but severe) battlefield injury, which involves significant bleeding that is sensitive to therapy (Jeroukhimov et al., 2002). An additional advantage is that haemorrhage from a solid intra-abdominal organ allows the haemorrhaged blood to remain in the intact peritoneal cavity and thus can be

measured. However, if the uncontrolled haemorrhage was the only source of blood loss, it could lead to increased variability between animals and thereby increase the number of animals needed to assess the efficacy of treatment. If the blood pressure profile during haemorrhage were to be different between injury groups, as is the case with haemorrhage on a background of blast vs. no blast, then an uncontrolled model of haemorrhage when used alone for blood loss can lead to systemic bias. This is because haemorrhage after blast results in a faster fall in arterial blood pressure which would result in a greater uncontrolled blood loss from those animals not subjected to a blast injury (Sawdon et al., 2002). To avoid this bias an initial phase of controlled haemorrhage (30% estimated BV) can be incorporated. After the loss of 30% blood volume arterial blood pressure is at a similar level between animals regardless of prior blast exposure (Garner et al 2010). Any subsequent uncontrolled haemorrhage can be expected to result in similar blood loss between injury (blast vs. no blast) strands, thereby obviating the systematic bias. Therefore, in the current study the liver injury was created after the controlled loss of 30% blood volume to allow further uncontrolled haemorrhage in all groups without the degree of bias.

It is known that juvenile swine can sequester up to 25% of their circulating blood volume in the spleen; and in times of systemic stress they can auto-transfuse a large proportion of this blood which would again introduce another systematic bias between the groups (Hannon et al., 1985) should the sympathetic response mediating the auto-transfusion be different between injury strands. This potential problem was overcome by splenectomy after vasoconstriction and maximisation of the circulating volume by topical application of adrenaline to the spleen (Garner et al., 2009). This removed further variability from the groups and also aligned the model closer to the human response.

### **6.1.2 Choice of Resuscitation Strategy**

The initial study looking at resuscitation following blast and haemorrhage compared normotensive and hypotensive resuscitation strategies and the physiological implications over an 8 hour period (Garner et al., 2010). Having

identified a serious limitation of prolonged hypotensive resuscitation for some battlefield casualties (blast-injured casualties experiencing extended evacuation times) an alternative strategy was designed and investigated (Jacobs 2010). It was recognised that no modern resuscitation strategy would include an immediate normotensive element due to the risk of re-bleeding from an uncompressible source of haemorrhage (Bickell et al., 1994; NICE: Technology Appraisal Guidance 74, 2004). The new strategy therefore comprised an initial hypotensive phase (one hour at a systolic arterial pressure of 80 mmHg, corresponding to a palpable radial pulse) followed by additional fluid to maintain a normotensive target thereafter. To evaluate the effects of this strategy on re-bleeding a model incorporating an element of incompressible haemorrhage was used (Jacobs 2010). The animals were divided into four groups and were subjected to either blast injury or sham. All groups received a total of 35% BV haemorrhage, and sustained a liver injury to provide the incompressible haemorrhage. After a 5min shock period, resuscitation (0.9% saline) began to the hypotensive level for the first hour, then according to one of two protocols to either a normotensive level (a systolic blood pressure of 110mmHg) or a hypotensive level (80mmHg). The former strategy (hypotensive then normotensive) is referred to as 'Novel Hybrid' while the latter is the hypotensive strategy. Fluid was given as per the protocol for 8 hours or until the animal died.

There was a clear, statistically and clinically significant difference in survival times between resuscitation strategies (Jacobs, 2010). Novel hybrid resuscitation was associated with a significantly increased survival time in blast injured animals. This difference in survival time was not apparent after haemorrhage in the absence of blast injury since survival time was good even in animals given hypotensive resuscitation. Even in the absence of blast injury, novel hybrid resuscitation was associated with significantly improved physiological status. Although arterial blood pressure was elevated without surgical intervention to control the liver injury, there was no apparent re-bleeding in the animals who received novel hybrid resuscitation. Presumably this was because a sufficiently robust clot had formed during the one hour

hypotensive phase to withstand the subsequent elevation in arterial blood pressure (Jacobs, 2010).

## **6.2 Aims**

This study addresses the hypotheses and aims detailed in section 5.1. In summary, the aim of the study in this chapter is to compare the effect of Novel Hybrid (NH) resuscitation against prolonged hypotensive resuscitation on coagulation and systemic inflammation in a model of complex battlefield injury, since it is anticipated that the improved tissue oxygen delivery associated with novel hybrid resuscitation might (in theory) attenuate both acute trauma coagulopathy and the later development of an inflammatory response.

## **6.3 Methods**

This was a randomised controlled trial to compare the effects of Novel Hybrid resuscitation with prolonged hypotensive resuscitation on coagulation and inflammation in two models of complex battlefield injury in terminally anaesthetised pigs, conducted in a laboratory setting. The study was conducted on 24 terminally anaesthetized crossbred, immature, female Large White pigs (41-56 kg). The animals were housed indoors and were fed on a complete wheat-soya based ration at 1.5–1.7 kg per day. They were allowed water *ad libitum*. The study was ethically reviewed and conducted in accordance with the Animal (Scientific Procedures) Act 1986.

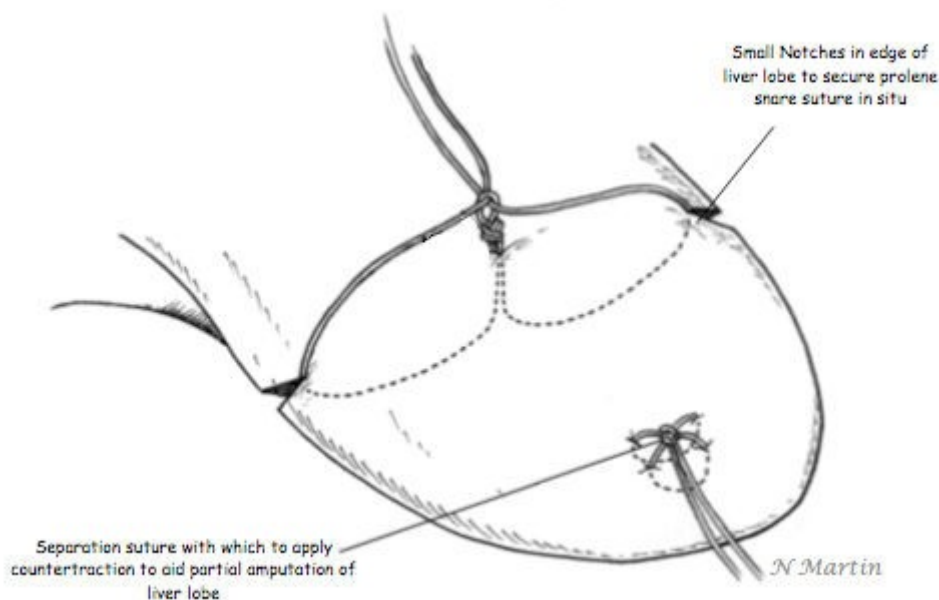
### **6.3.1 Surgical preparation**

The animals were fasted for 18 hours before the surgical procedure, but allowed water *ad libitum*. After pre-medication with intramuscular midazolam hydrochloride (0.1 mg/kg), anaesthesia was induced by mask with isoflurane (5%) in a mixture of oxygen and nitrous oxide (1:1) and the animals intubated. Surgical anaesthesia was subsequently maintained with isoflurane (1–2 %) in a mixture of oxygen and nitrous oxide (1:2) in animals subjected to intermittent positive pressure ventilation using a Manley ventilator. Initial monitoring

consisted of end-tidal CO<sub>2</sub>, pulse oximetry via a tail probe and skin surface electrocardiogram electrodes (Propac 106EL, Protocol Systems Inc., Oregon). With the animal positioned supine surgical preparation took place after skin preparation with povidone-iodine solution (10% w/v, Betadine Aqueous Antiseptic Solution, Seaton Healthcare Group plc, UK).

The left carotid artery, both internal jugular veins, left femoral artery and vein were all cannulated (Portex 8FG, Hythe, UK). Once venous access had been established anaesthesia was maintained with intravenous alphaxolone (Alphaxan, Vetoquinol UK Ltd, UK) and the isoflurane discontinued.

A midline laparotomy was performed, the spleen contracted by topical application of adrenaline (1.0- 1.5 ml of a 1 mg.ml<sup>-1</sup> solution) before removal. A surgical snare was inserted into the left medial lobe of the liver for later induction of a Grade IV liver injury, and the snare exteriorized via the laparotomy. The placement of snare was measured to amputate 2/3 of the middle Riedel's lobe. A schematic illustration is shown in Figure 19 below. The bladder was catheterized by open suprapubic cystostomy. All incisions were closed *en masse*.



**Figure 19:** Schematic illustration of liver snare arrangement designed to create a grade IV liver injury with amputation of the lower 2/3 of the lobe (reprinted by kind permission of the artist, Major N Martin).

Animals were allowed to breathe spontaneously for the remainder of the experiment unless they displayed marked respiratory depression, at which stage Synchronized Intermittent Mandatory Ventilation (SIMV) was instituted in an attempt to maintain adequate oxygenation and prevent severe hypercapnoea. The animals recovered from surgery under anaesthesia for one hour before baseline measurements were made during which time they were transported to the physiological monitoring suite near the explosives arena.

#### *Cardiovascular monitoring*

Arterial blood pressure was recorded via the carotid artery cannula using a strain gauge manometer (Sensoror 840, SensoNor a.s., Norway) and recorded using a computerized data acquisition system (Maclab 8/s, ADInstruments, UK) and associated software (Chart v4.2.3, ADInstruments, UK) for subsequent analysis. Zero pressure for all transducers was set at heart level. Body temperature was maintained at approximately 38°C using external heating/cooling and blankets as appropriate.

#### *Blood gas and related chemistry*

Arterial and venous blood samples were taken 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). Haematocrit was measured by centrifugation, whereby blood samples in capillary tubes were subjected to 13000g for 5 minutes (HaematoSpin 1300, Hawksley, UK) enabling the ratio of packed red blood cells volume to total volume to be read using a Tube reader (Hawksley, UK). Arterial and venous oxygen content was determined by co-oximetry, with oxygen extraction ratio (OER) calculated using standard formulae, equation 4.

$$\mathbf{OER = (CaO_2 - CvO_2) / CaO_2}$$

**Equation 4:** Determination of oxygen extraction ratio (OER) from arterial oxygen content (CaO<sub>2</sub>) and venous oxygen content (CvO<sub>2</sub>) (Little and Edwards, 1993)

### **6.3.2 Experimental Protocol**

The animals were randomly allocated to one of four groups (6 animals in each) at the outset, described below. Two groups were exposed to blast while the remaining groups were not (sham blast). All four groups underwent both controlled (total 35% estimated blood volume) and uncontrolled haemorrhage (from liver injury). The resuscitation strategies employed were either Novel Hybrid (NH, target systolic arterial pressure of 80 mmHg for the first hour and 110 mmHg thereafter) or Hypotensive (Hypot, target systolic arterial pressure of 80 mmHg throughout).

**Group 1:** Blast / Haemorrhage / NH

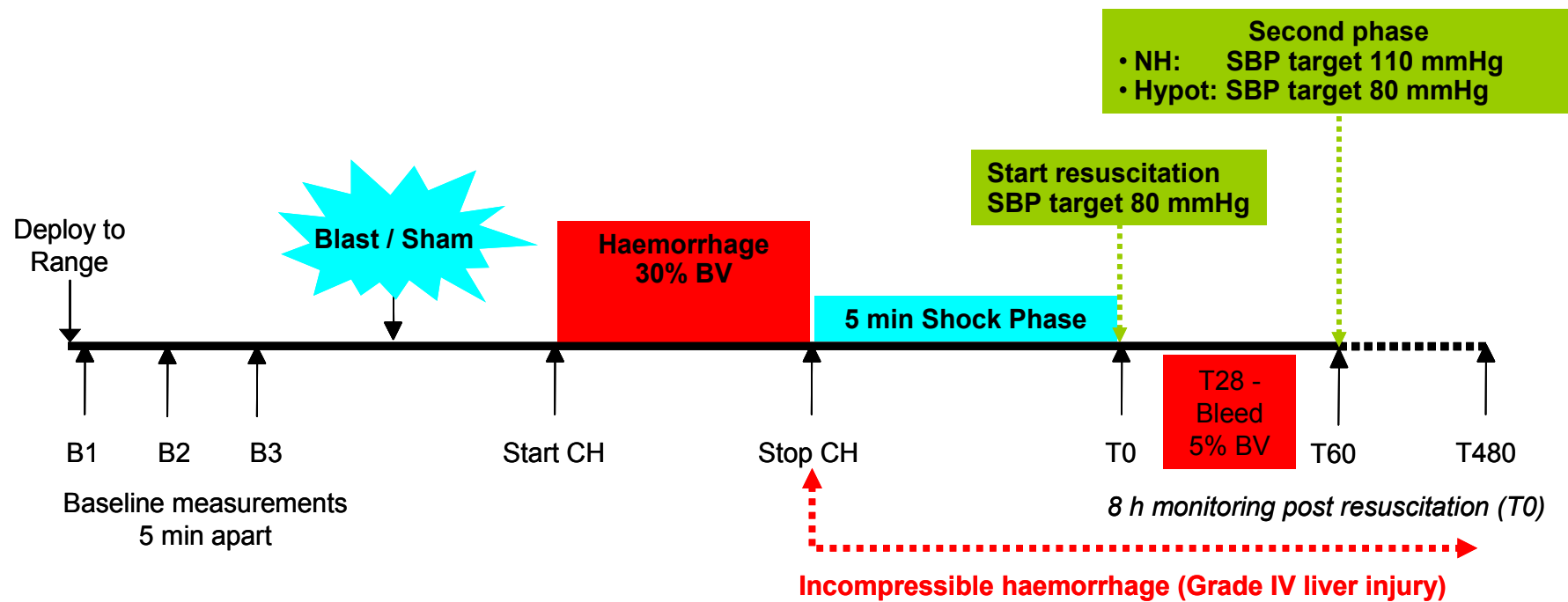
**Group 2:** Blast / Haemorrhage / Hypot

**Group 3:** Haemorrhage / NH

**Group 4:** Haemorrhage / Hypot

The protocol is summarized in Figure 20 on the following page.

One hour after the end of surgery three cardiovascular measurements were made 5 minutes apart and paired arterial and mixed venous blood gas samples taken at the time of the first and third baseline cardiovascular measurement. After the baseline measurement the animals were moved outdoors, wrapped in a Kevlar blanket to protect from secondary and tertiary blast effects and positioned on a trolley 2.15 m from a cylindrical charge of EDC1S explosive (2.2 kg) which was then detonated remotely. Animals subjected to sham blast were treated identically but not exposed to blast. The set-up of the blast arena is shown in the picture illustrated (Figure 21.)



**Figure 20:** Experimental protocol which commenced approximately 1 hour after the end of surgery. CH, controlled haemorrhage; BV, total estimated blood volume; SBP, systolic arterial blood pressure; NH, novel hybrid resuscitation; Hypot, hypotensive resuscitation.





**Figure 21:** Photograph of blast arena. Charge was fastened to central cardboard tube and the animal positioned on trolley to the right wrapped in Kevlar blanket.

Immediately after the blast (or sham blast) the animal was returned to the physiological monitoring suite and twenty minutes later, all animals were subjected to a controlled haemorrhage of 30% of their estimated total blood volume ( $B_0$ ; Equation 5) over 4 minutes via the femoral arterial cannula, using a computer-controlled pump (Masterflex® L/S® model 7550-17, Cole Palmer Instrument Company, Chicago, IL). The rate of bleeding reduced exponentially as the haemorrhage progressed (Equation 6) to mimic the rate of haemorrhage from a major arterial lesion.

$$B_0 = 161.4751(W^{0.2197})$$

**Equation 5:** Equation used to estimate total blood volume.  $B_0$  = total blood volume ( $\text{ml.kg}^{-1}$ ) and  $W$  = body weight (kg). (Bush et al., 1955).

$$V = B_0(1 - e^{-0.04t})$$

**Equation 6:** Rate of blood loss during controlled haemorrhage.  $V$ , total blood loss at time  $t$  in  $\text{ml.kg}^{-1}$ ;  $B_0$ , initial blood volume in  $\text{ml.kg}^{-1}$ ;  $t$ , % time until death;  $B_0$ , initial blood volume (Stern et al., 1993)

Following haemorrhage, the animals underwent a 5-min shock period where no treatment was administered before commencement of fluid resuscitation (time =  $T_0$ ) (Figure 20).

Both resuscitation protocols utilised 0.9% saline given in aliquots at a rate of 3 mL/kg/min to attain and maintain the relevant target systolic arterial pressure. The blood pressure profile in NH was initially hypotensive (for the first hour) and thereafter normotensive as described above. By contrast hypotensive resuscitation employed a hypotensive target blood pressure throughout. All animals were subjected to a simulated re-bleeding episode by controlled removal of 5% total estimated blood volume 28 min into the resuscitation phase.

Resuscitation fluid was administered according to the relevant protocol for 8 hours or until the animal died, if sooner. Cardiovascular and blood gas measurements were made before and after haemorrhage, at  $T_0$  and then at 15 min intervals until 60 min after the onset of resuscitation ( $T_{60}$ ) and thereafter at 30 min intervals for the remainder of the study.

#### *Assessment of coagulation and inflammatory responses*

Arterial blood samples were collected 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  $-80^{\circ}\text{C}$ . Prothrombin time (PT) was determined using the ACL Elite (Instrumentation Laboratories) by turbidometry. Interleukin 6 (IL6) and TNF- $\alpha$  levels were determined by ELISA (R&D Quantikine<sup>®</sup> sandwich ELISA kits, R&D Systems Ltd, UK).

#### *Post mortem*

At the end of the study the animals were humanely killed with an overdose of intravenous sodium pentobarbitone and a post-mortem examination was performed immediately after death. The majority of intra-abdominal fluid, mainly blood from the liver injury, was suctioned into a pre-weight container. Pre-weighed gauze swabs were then used to swab the abdominal cavity dry before repeat weighing of these swabs to accurately calculate the weight and then the volume by equation 7.

$$V = W / 1.036$$

**Equation 7:** Determination of blood volume ( $V$ ) in ml from a measured weight of blood ( $W$ ) in grammes, determined previously for this strain of pigs in our laboratory.

### **6.3.3 Sample Size Calculation and Randomisation**

The primary (overall) purpose of the study comparing NH to hypotensive resuscitation was to compare the effects of these two strategies on survival time in a model of complex battlefield injury in terminally anaesthetised pigs (principally reported by Jacobs 2010). Data to inform the power calculation was derived from an earlier study by Garner et al (2009). I am indebted to Dr R Gwyther (Dstl Statistician) for the survival power calculation.

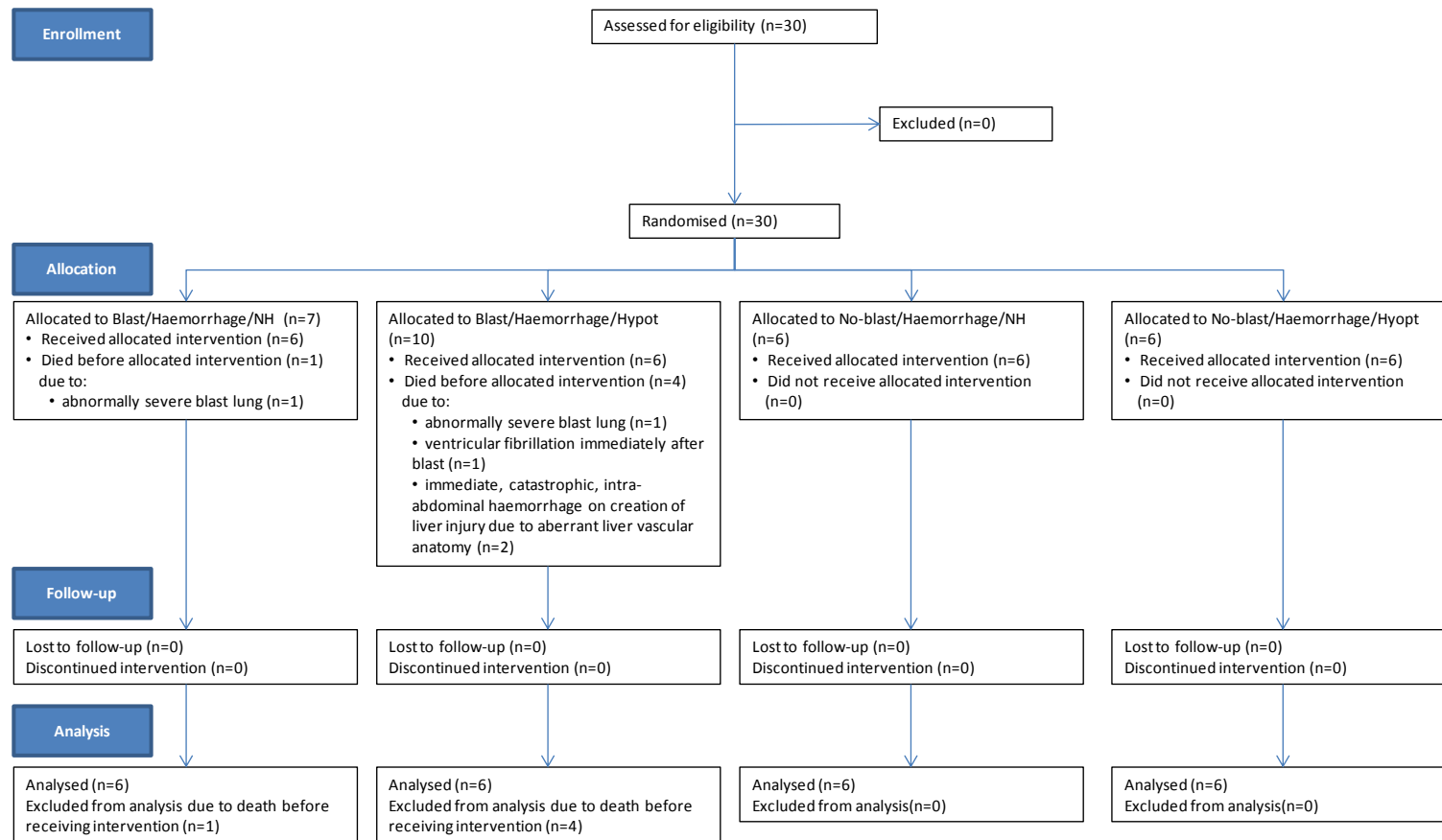
A power calculation, based on an increase from 0.1 to 0.8 in the proportion surviving to 8 hours from the onset of resuscitation indicated that 7 animals would be required in each of the hypotensive and NH groups (Power 0.8, Alpha 0.05, Chi Squared test). An interim analysis by a statistician (independent of the study team) was planned and performed when  $n=6$  had been attained in each group to determine whether a clear (statistical) conclusion had been attained and, if not, how many additional animals would be required to provide an unequivocal conclusion. No meaningful power calculation could be performed with respect to the clotting data since there was no background data available to quantify the degree (or variability) of coagulopathy in this model of complex battlefield injury. However, data from this study can now be used to inform future power calculations.

#### *Randomisation*

Animals were randomised to treatment allocations prior to anaesthesia. The study was not designed to be 'an intention to treat' analysis but rather an investigation of physiological effects of resuscitation. Despite the injury being standardised as far as possible (e.g. reproducible explosive charges, fixed distance between charge and animal, placement of liver snares to anatomical landmarks) a few animals succumbed e.g. due to air emboli during blast (rare,

known complication of blast injury) or catastrophic blood loss during the uncontrolled haemorrhage (verified at post-mortem examination) before instigation of treatment. As such the few animals who did not reach the point of treatment initiation were excluded as they contributed no meaningful data to these physiological responses. No animal data was excluded after the treatment allocation was commenced.

Therefore, although the intended group for each individual animal was pre-determined according to a random numbers table, if a death occurred prior to commencement of treatment then the animal was excluded and the following animal allocated to the treatment paradigm. The flow of animals through the study is illustrated in the CONSORT diagram listed in figure 22.



**Figure 22:** CONSORT 2010 diagram indicating the passage of animals through the study, using the format prescribed by the CONSolidated Standards of Reporting Trials group (quote ref Schulz KF, Altman DG & Moher D (2010). CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ 2010;340:c332)

#### **6.3.4 Statistical analysis**

All data are presented as mean $\pm$ s.e. mean unless indicated otherwise. Data were assessed for normality and transformed if necessary prior to statistical analysis 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 could not be adequately transformed non-parametric analysis was used as indicated. A significance level of  $P\leq 0.05$  (two tailed) was used unless indicated otherwise.

Progressive loss of animals (due to death) from treatment groups can lead to bias in a study, especially when the loss is predominant in particular groups. To avoid this bias time series analysis was restricted to the first 180 min in the blast groups (loss of one animal from one group only up to this time-point) or 320 min in the sham blast groups (loss of two animals from one group). To ensure that graphical data was not misrepresented open symbols are used on all time-series result graphs when the proportion of survivors fall to 66% and no data is plotted when survival is 50% or below. The exception to this principle was the analysis of survival data. Survival times were compared using Kaplan-Meier survival analysis (Peto's log rank test) and the data stratified with respect to blast or sham blast injury and analysed using Minitab (v14). Data from animals still alive after 8 hours were treated as right-censored.

## **6.4 Results**

### **6.4.1 Baseline Measurements**

Baseline, pre-injury, variables were similar between groups (Table 6). Small, statistically significant, differences were seen between groups for arterial base excess, however these are unlikely to be of clinical or physiological significance. Group 1 showed a very high mean IL6 level at baseline, however this was due to a single animal displaying a much higher level than any of the other animals in the study.

Mean arterial blood pressure (MBP), arterial base excess (ABE) and haematocrit were significantly reduced at the end of a 5 min shock phase following haemorrhage and blast (or sham blast) (Table 6). Shock also led to significant increases in HR, OER and small (but statistically significant) increases in PT and IL6 levels. In addition, MBP at the end of the shock phase was found to be significantly lower in the blast injured groups compared to those without blast injury.

### **6.4.2 Effects of resuscitation**

Following intravenous fluid resuscitation commencing at T0, the target systolic blood pressure (SBP) of 80 mmHg was achieved with no differences between groups during the first hour of resuscitation by infusion of 0.9% saline. Beyond the first hour, T60, a higher target SBP of 110 mmHg was obtained in all animals given NH resuscitation whilst those in the hypotensive groups (Hypot) continued at the initial target of 80 mmHg. Thereafter, there was a significant ( $p < 0.001$ ) difference between the two different resuscitation strand groups. There were no significant differences in the SBP profiles achieved between the blast and sham groups within each resuscitation strategy ( $p = 0.793$ ). The respective SBPs were successfully maintained up until the stage at which each individual animal began to decompensate (where it was unresponsive to fluid resuscitation) when blood pressure entered an in-correctable decline leading quickly to death.

Despite a SBP of 80mmHg maintained in all groups, arterial base excess (ABE) fell significantly in all groups during the first hour of hypotensive resuscitation and the oxygen extraction ratio remained high in all groups (Figure 23) (Jacobs 2010). At T60 when the two strata of resuscitation diverged, the negative ABE was reversed in the NH groups whilst it continued to fall in the Hypot groups. Concurrent with these changes OER fell significantly in the NH groups, while it remained high in the Hypot groups ( $P=0.007$ )(Figure 23).

Contrary to expectations, the actual volumes of resuscitation fluid infused were not significantly different between resuscitation strategies ( $p= 0.3641$ , figure 24), when expressed as a function of time. In keeping with this finding, there were also similar haematocrit values between treatment groups (figure 25). The mean haematocrit in all study groups declined during haemorrhage and the shock period prior to commencing fluid resuscitation. An early trend was observed between the animals exposed to blast and the sham-blast animals, with the former demonstrating increased initial resuscitation fluid requirements and correspondingly lower haematocrits (haematocrit at T60; blast/ sham blast  $p=0.0019$ , prospective NH/ Hypo strategy  $p=0.9566$ ). This difference lessened with time, and no significant difference between resuscitation strategies was observed (haematocrit at T180; blast/ sham blast  $p=0.1915$ , NH/ Hypo  $p=0.5437$ ).

In order to help explain the attrition of the animals during the experiment, the number of animals alive in each group at each time point (in the following graphs) is listed in table 7.

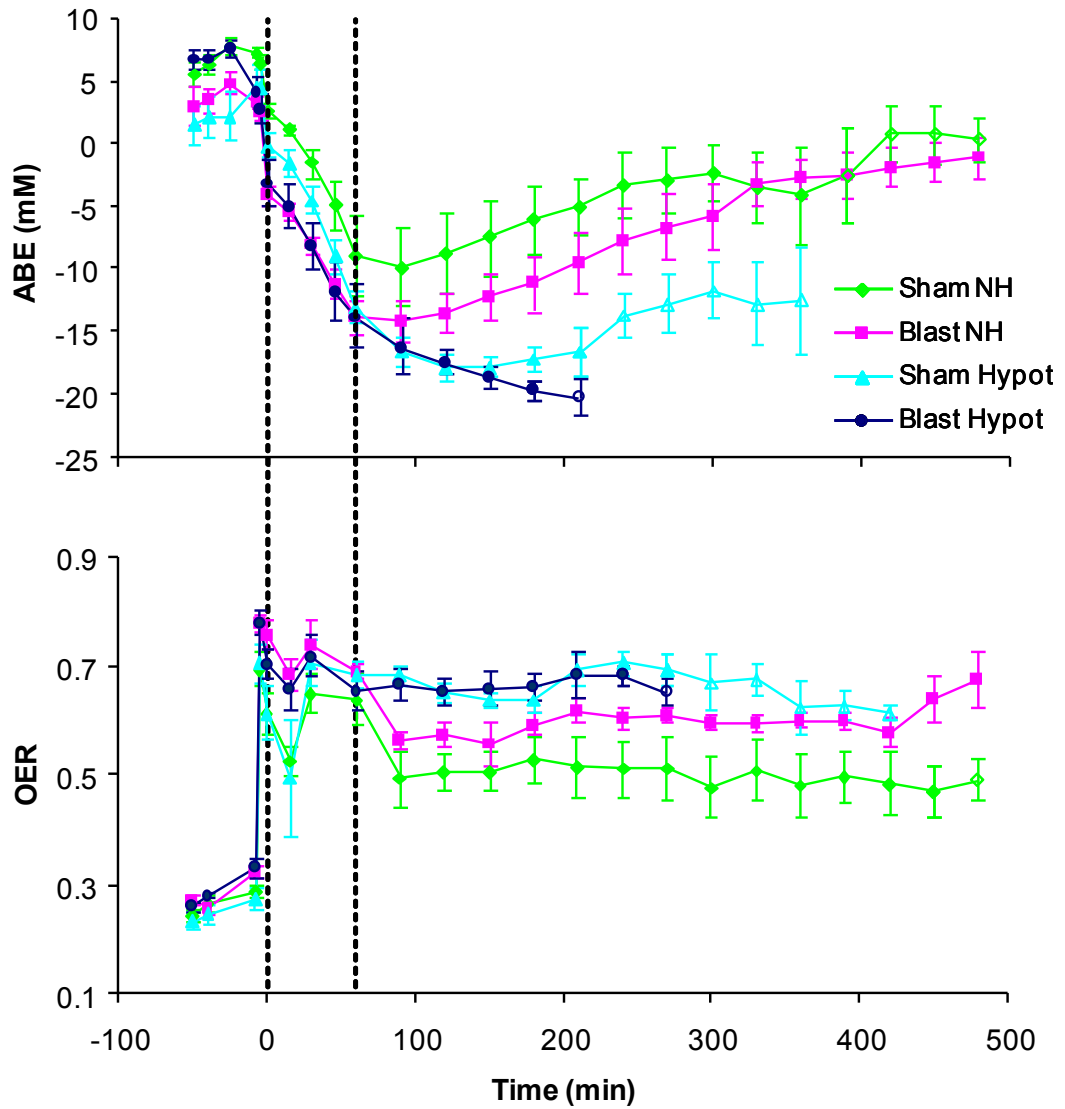


		Blast NH	Blast Hypot	Sham NH	Sham Hypot		
Variable		Group 1	Group 2	Group 3	Group 4	Blast vs Sham Blast	Shock vs Baseline
<b>MBP</b>	Baseline	120.2±7.6	125.5±2.4	124.2±6.3	125.3±6.6		
(mmHg)	Shock	31.8±3.2	37.3±4.7	51.3±4.8	49.0±6.6	P=0.025	P<0.0001
<b>HR</b>	Baseline	158.0±8.3	146.0±12.2	157.2±10.9	165.7±9.2		
(b/min)	Shock	228.9±5.0	209.5±9.7	210.2±14.9	234.2±8.6	P=0.40	P<0.0001
<b>PT</b>	Baseline	1.03±0.02	1.02±0.02	1.00±0.01	1.02±0.01		
Proportion of baseline	Shock	1.10±0.03	1.12±0.03	1.04±0.02	1.09±0.01	P=0.07	P=0.0001
<b>IL6</b>	Baseline	130±50	66±27	71±17	300±120		
(pg/ml)	Shock	252±91	174±45	94±19	410±141	P=0.50	P=0.04
<b>PaO2</b>	Baseline	9.1±0.3	9.9±0.4	8.5±0.4	9.3±0.3		
(kPa)	Shock	9.1±0.5	9.8±0.9	10.3±0.7	12.0±0.5	P=0.27	P=0.0002
<b>ABE</b>	Baseline	2.9±1.6	6.6±0.8	5.4±0.9	1.4±1.6		
(mM)	Shock	-4.2±0.8	-3.2±1.8	2.5±0.6	-0.2±1.0	P=0.07	P<0.0001
<b>OER</b>	Baseline	0.27±0.01	0.26±0.01	0.24±0.01	0.23±0.02		
(Ratio)	Shock	0.63±0.13	0.70±0.03	0.61±0.04	0.61±0.05	P=0.18	P<0.0001
<b>Hct</b>	Baseline	39.3±0.6	37.0±0.7	37.8±0.9	37.2±1.5		
(%)	Shock	29.6±1.3	29.5±1.8	31.0±1.6	29.0±1.6	P=0.91	P<0.0001
<b>Temp</b>	Baseline	38.7±0.3	38.6±0.2	38.5±0.2	38.5±0.2		
(°C)	Shock	38.5±0.3	38.4±0.4	39.2±0.2	39.2±0.1	P=0.10	P=0.02

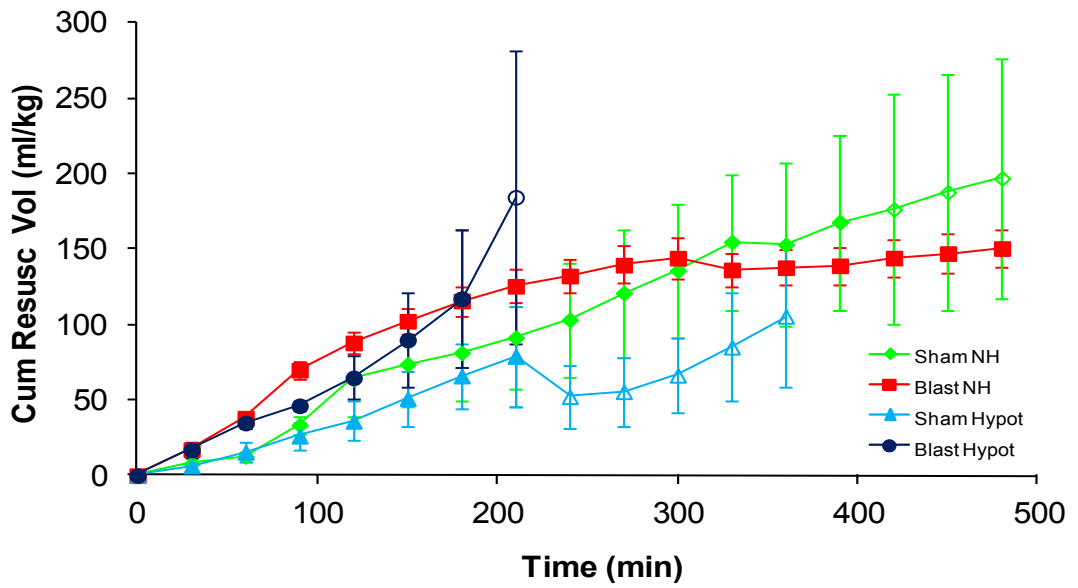
**Table 6:** Baseline and values seen after 5 min of shock following blast or sham blast plus 30% blood volume controlled haemorrhage and Grade IV liver injury. All groups were combined and analysis performed as a two way analysis of variance with time (baseline/shock) and injury (baseline/sham) as the main factors. MBP, mean arterial blood pressure; HR, heart rate; PT, prothrombin time expressed as a percentage of that seen at the start of surgery; IL6, arterial Interleukin 6; PaO2, arterial partial pressure of oxygen; ABE, arterial base excess; OER, systemic oxygen extraction ratio; Hct, arterial haematocrit; Temp, body temperature.

Time from onset of resuscitation (min)	Sham NH	Blast NH	Sham Hypot	Blast Hypot
0	6	6	6	6
15	6	6	6	6
30	6	6	6	6
45	6	6	6	6
60	6	6	6	6
90	6	6	6	6
120	6	6	6	5
150	6	6	6	5
180	6	6	6	5
210	6	6	5	3
240	6	6	4	1
270	6	6	4	1
300	6	6	4	1
330	6	5	4	1
360	5	5	4	1
390	5	5	3	1
420	4	5	3	1
450	4	5	3	1
480	4	5	3	1

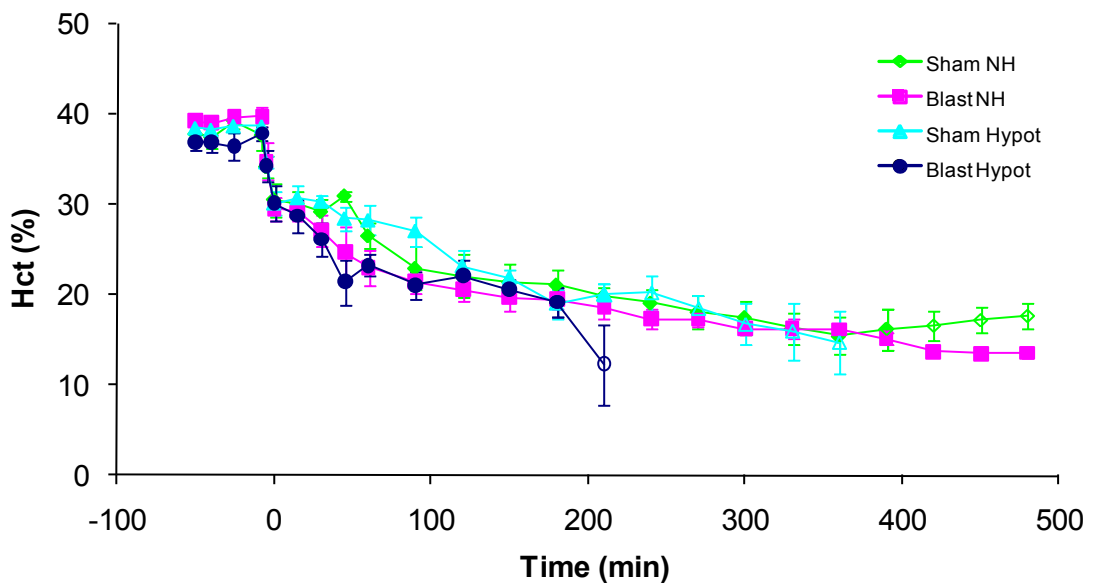
**Table 7:** Number of surviving animals in each group at time-points corresponding to the data-points in figures 23, 24 and 25.



**Figure 23:** Arterial base excess (ABE) and oxygen extraction ratio (OER) in four groups of animals subjected to either sham blast (Sham) or blast (Blast), hemorrhagic shock and resuscitation. The first dotted line represents the onset of resuscitation (hypotensive in all groups). The second dotted line indicates the onset of normotensive resuscitation in NH groups and continued hypotensive resuscitation in the Hypot groups. Open symbols indicate 66% of animals surviving. No data plotted when survivors fall to 50% or below of the original. Mean values  $\pm$  SEM.



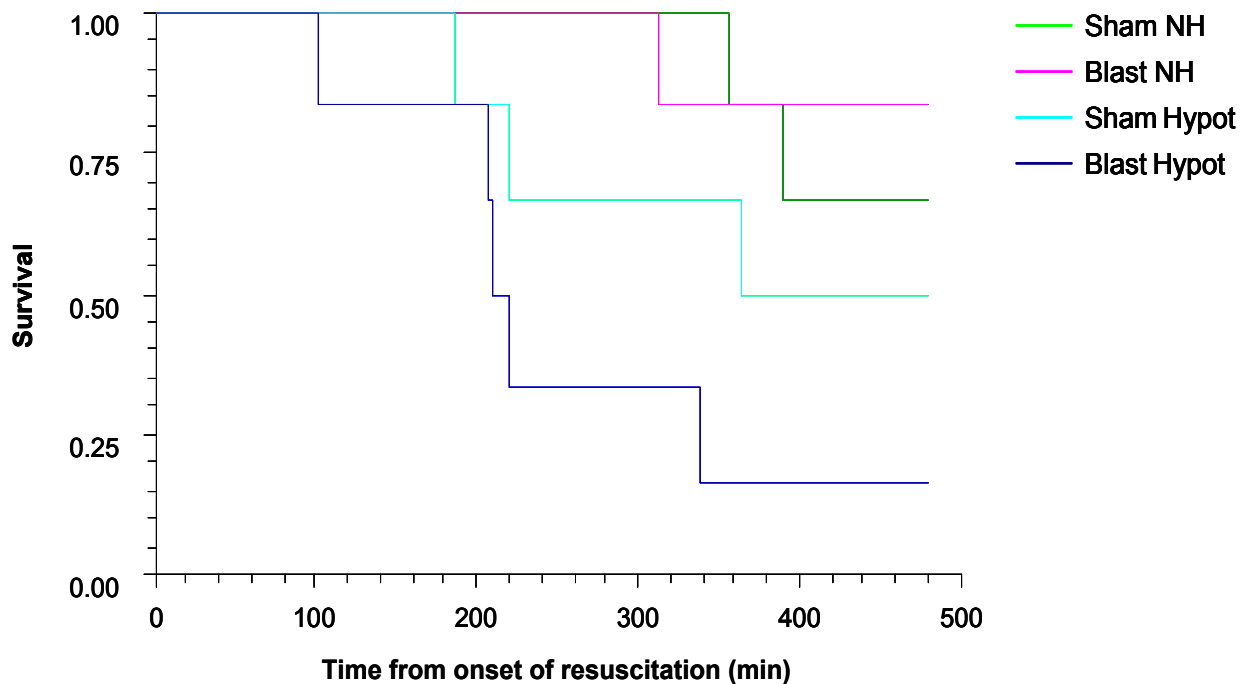
**Figure 24:** Cumulative Volumes of Resuscitation fluids give over time in the four groups of animals. Mean values  $\pm$  SEM. Refer to legend of figure 23.



**Figure 25:** Haematocrit (HCT) in four groups of animals. Mean values  $\pm$  SEM. Refer to legend of Figure 23.

### 6.4.3 Survival

Mean survival time was significantly different between groups ( $P=0.024$ , Peto log rank), with Group 2, the hypotensive group with blast injury (258 [151-366] min, mean [95%CI]) being significantly shorter than Groups 1, 3 and 4 (452, 448[393-502] and 369[247-490] min respectively) (Jacobs 2010). This is clearly shown in Figure 26.

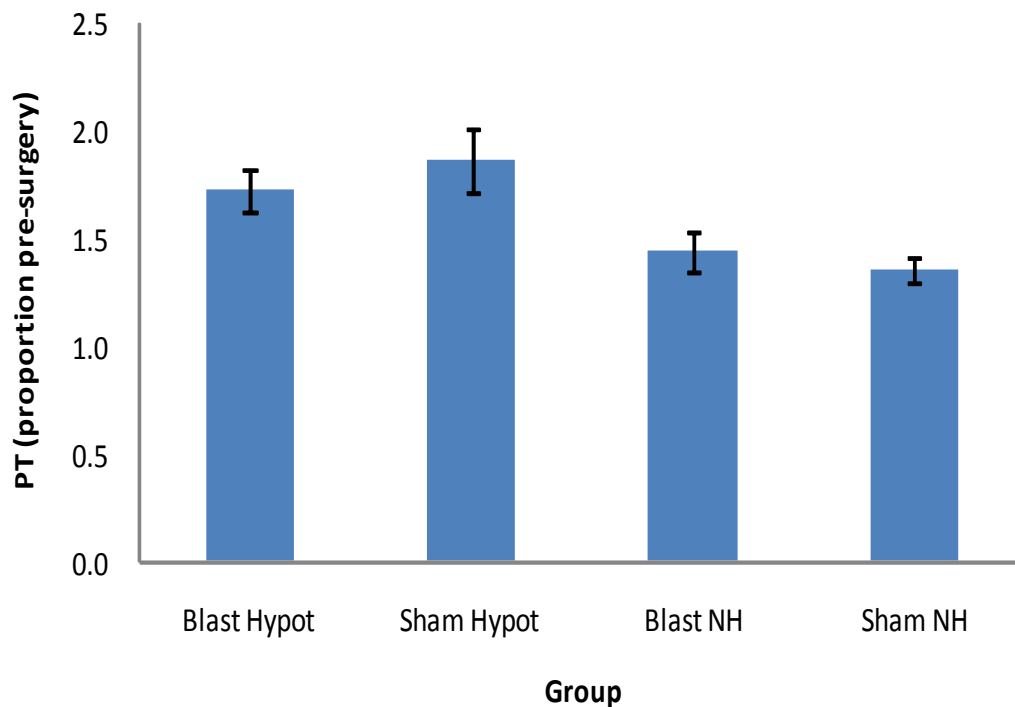


**Figure 26:** Kaplan-Meier survival graph for the 4 study groups.

### 6.4.4 Effects on clotting and inflammation

By the end of the study, NH resuscitation was associated with significantly lower PT compared to hypotensive resuscitation ( $1.44\pm 0.09$  and  $1.36\pm 0.06$  for groups 1&3 compared to  $1.73\pm 0.10$  and  $1.87\pm 0.15$  in groups 2&4;  $P=0.001$ ). This was measured as a proportion as maximum when compared to pre-surgical baseline level. The peak PT levels attained are shown in Figure 27.

By contrast blast injury (vs. sham blast) had no significant effect ( $P=0.56$ ) on the peak levels of PT, even when the analysis was limited to the NH resuscitation groups ( $P=0.24$ ) where survival times were comparable.

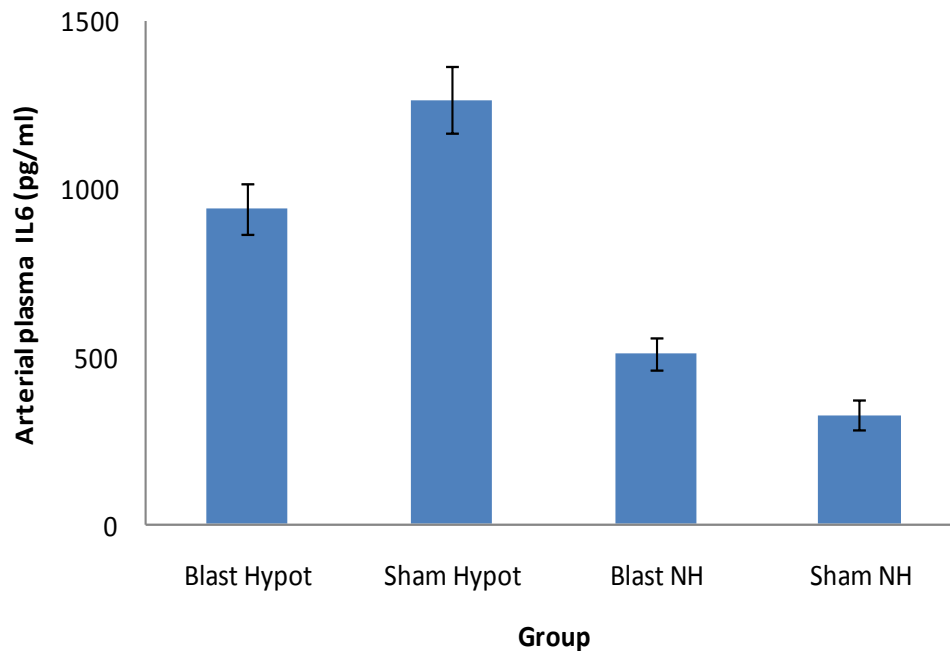


**Figure 27.** Maximum level of Prothrombin Time (PT) in the four animal groups. Mean Values $\pm$ SEM

When data for all of the four groups were pooled there was a significant correlation between clotting impairment, PT and the degree of physiological derangement measured as arterial base excess, ABE, ( $P=0.0004$ , Kendall's rank correlation).

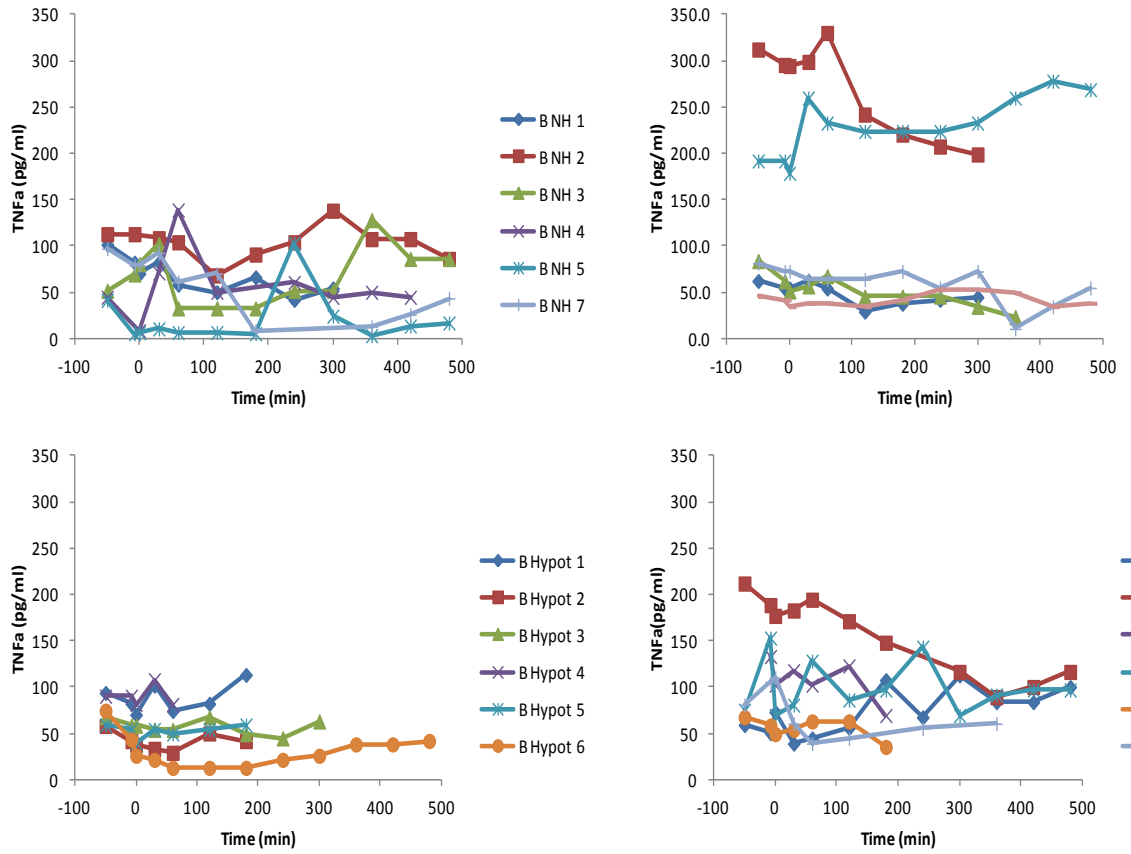
The inflammatory response was assessed by measuring IL6 and TNF- $\alpha$  levels. Peak levels of IL6 were significantly higher in the Hypot groups compared to NH (Figure 28,  $P=0.001$ ), but overall there was no significant effects of blast ( $P=0.34$ ) on this inflammatory marker. However, survival time was significantly shorter in the blast-injured animals given Hypot ( $P=0.017$ ). Due to the expected time course of IL6 release, the levels seen in the short-lived Blast/Hypot group may be reduced. When the effects of blast was examined only in those given

NH resuscitation (where survival times were similar between NH and Hypot,  $P=0.45$ ), IL6 was found to be significantly higher in animals given blast injury ( $P=0.009$ ).



**Figure 28:** Maximum level of arterial Interleukin 6 (IL-6) levels in the four animal groups. Mean values  $\pm$ SEM.

There was no significant change in TNF- $\alpha$  levels over time in any of the four groups. Due to the wide variation in baseline (pre-injury) values between individuals, data from individual animals are shown in Figure 28. Examination of initial white cell counts in the three animals showing highest baseline TNF $\alpha$  levels did not reveal any evidence of pre-existing infection in these animals i.e. the white cell count for these animals was within the normal range.

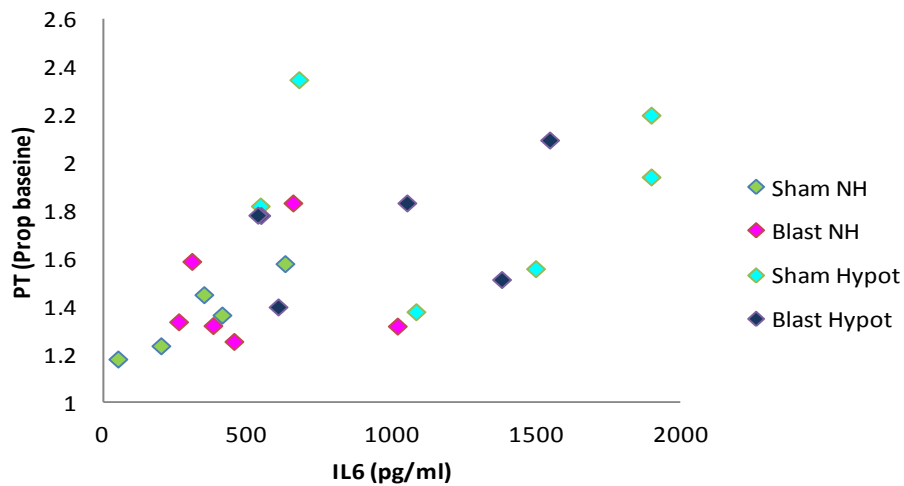


**Figure 29:** Relationship between TNF- $\alpha$  levels in all animals recorded over time.



### 6.4.5 Relationship between coagulopathy and IL6 response to injury, shock and resuscitation

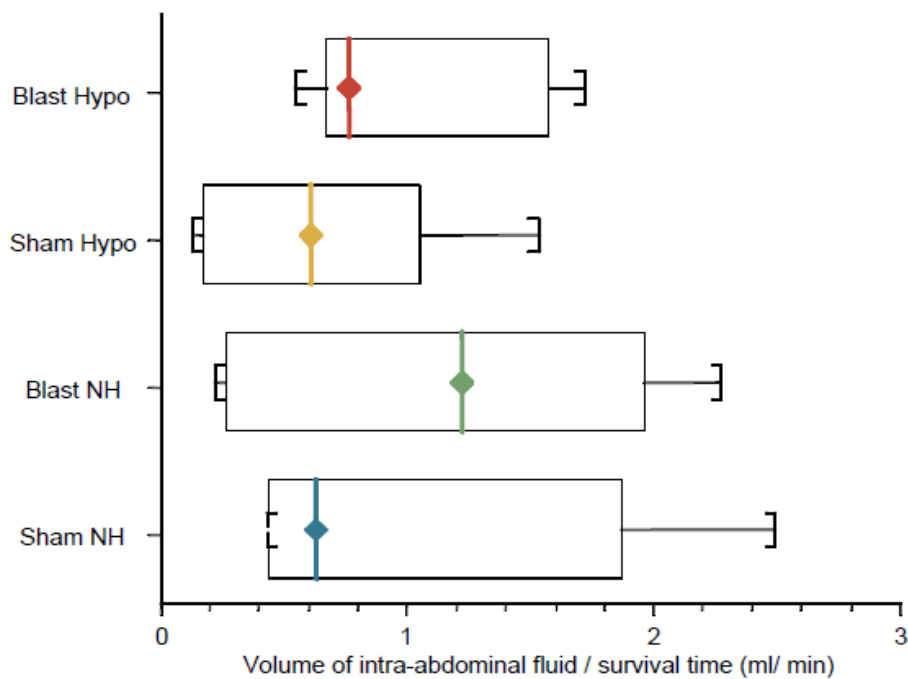
Taking the data from all four groups once more, it was found that there was a significant correlation between the maximum PT and the highest IL6 level seen in each animal after the first hour of resuscitation (when all animals had been subjected to one hour of hypotensive resuscitation) ( $P= 0.0017$ , Kendall's rank correlation). This is shown in Figure 30.



**Figure 30:** Correlation between maximum Prothrombin Time (PT) and the highest Interleukin 6 (IL6) level after the first hour of resuscitation.

#### 6.4.6 Post Mortem results

At post-mortem successful grade IV liver injuries, with amputation of a portion of the Riedel's lobe of the liver, was found in all animals. No evidence of increased intra-abdominal blood loss (as evidence of re-bleeding) was associated with the animals that received NH resuscitation, with no significant difference in the volumes of intra-abdominal fluid ( $P = 0.33$ , 2 way ANOVA). See Figure 30. The volumes were expressed as a function of survival time to enable fair comparisons in groups. (Jacobs, 2010)



**Figure 31:** Volume of intra-abdominal fluid assessed immediately post-mortem, normalised for survival time among the four animal groups. Data presented as Minimum-[lower quartile-medial-upper quartile]- Maximum.

## 6.5 Discussion

The principal findings of the current study are that novel hybrid (NH) resuscitation reduces the clotting impairment and systemic inflammation seen with prolonged hypotensive resuscitation (Figures 25 & 27). These beneficial effects are seen following resuscitation after haemorrhagic shock in the presence and absence of concomitant blast injury.

The beneficial effects of NH on clotting and inflammation are not an artefact of the longer survival time in this group (groups 1&3) and are seen despite, not because of, the reduced survival time in the Blast Hypotensive group (group 2). In the case of PT, it is likely that sufficient time had elapsed for full expression of an elevated PT in most of the animals of the blast group given hypotensive resuscitation since a recent study demonstrated peak levels of PT approximately 60 min post-haemorrhage/resuscitation (Shuja et al., 2008). Furthermore, the enhanced PT seen in the hypotensive groups is not an artefact of the volume of fluid administered; overall slightly smaller volumes of fluid were given to the hypotensive resuscitation groups and the overall degree of haemodilution was similar between all groups as the haematocrit levels were not significantly different between groups (Figure 25). Presumably the time-course of resuscitation was such that the additional fluid given to the NH groups had sufficient time to exit the circulation by a combination of renal excretion and extravasation into the interstitial space.

The likely mechanism whereby NH is associated with improved clotting may relate to increased tissue perfusion and reduced metabolic acidosis, compared to hypotensive resuscitation. However hypotensive resuscitation is the current accepted strategy for pre-hospital resuscitation of civilian trauma casualties suffering hypovolaemic shock (NICE: Technology Appraisal Guidance 74, 2004). However, hypotensive resuscitation is not without a physiological penalty since it results in reduced tissue perfusion and the development of metabolic acidosis). This can become overwhelming in the presence of concomitant lung injury due to blast (Garner et al., 2010).

A statistically and clinically significant metabolic acidosis developed in all groups following haemorrhage, regardless of prior exposure to blast, during the

hypotensive resuscitation phase; shown by the fall in arterial base excess (figure 23). After 60mins (T60), when the resuscitation protocols diverged, the physiological decline was arrested and reversed during the normotensive phase of NH. The animals that continued with hypotensive resuscitation remained severely acidotic. The acidosis progressed until the animals succumbed if there had been a blast exposure in their injury pattern.

The compromise in oxygen delivery was reflected in the oxygen extraction ratios (OER). During the hypotensive phase, when tissue perfusion is low, OER was increased to the theoretical maximum of approximately 80% in an attempt to meet demand. This persisted in the hypotensive group whereas animals given NH were able to reduce OER from the ceiling once perfusion was improved in the normotensive phase (see figure 23).

Much has been written regarding the acidosis induced by large volumes of 0.9% NaCl, referred to as hyperchloraemic acidosis (Vincent et al., 2007). When the literature is unified in concluding that acidosis is associated with adverse physiological effects, there is often a failure to differentiate between the effects attributable to the cause of acidosis and the acidosis itself (Handy & Soni, 2008). In this context, there may be an important difference between the acute effects of saline administered under the unstable conditions found in severe haemorrhagic shock and large amounts of this crystalloid given in a ward setting. In the context of our study, marginally more saline was given to animals subjected to the NH resuscitation strategy, however the degree of acidosis was substantially less in this group. This raises the possibility that an acute hypovolaemic shock restoration of tissue perfusion (and oxygen delivery) may outweigh any acidotic effects of the saline itself. The final word is best summarised by Handy and Soni (2008), "*there is little evidence that in the 50 year of normal saline usage that there has been significant morbidity from the use of this fluid*"

During this study the animals breathed air throughout. An elevation in PaO<sub>2</sub> was noted at the end of the shock period in the sham blast, but not in the blast-exposed animals (see table 6). This effect can be explained by the hyperventilatory response to severe hypovolaemia (Little & Kirkman, 1997). The

underpinning mechanism is a stimulation of peripheral (arterial) chemoreceptors due to stagnant hypoxia as a consequence of both hypotension and a sympathetically-driven vasoconstriction in the chemoreceptors (Acker & O'Reegan, 1981). The resulting increase in ventilation results in an elevation in arterial oxygen tension. It is likely that the elevation in oxygen tension is only seen in sham-blast groups since the effects of blast are known to rapidly initiate an increase in pulmonary vascular permeability and extravasation of fluid which will increase the pulmonary diffusion barrier, in addition to potentially causing an arterio-venous shunt, all of which will have a greater effect on pulmonary oxygen transport when compared with carbon dioxide transport. Consequently there was an increase in PaO<sub>2</sub> after haemorrhage in the sham-blast group, despite no increase in FiO<sub>2</sub> at any time during the study.

This study was conducted in female pigs. It is well documented that there are sex-based differences in outcome after trauma, where females generally have a better outcome (George et al., 2003; Sperry et al., 2008). The mechanisms underpinning this are only partly understood but a number of studies suggest that oestrogen may a key role. Neutrophil activation is blunted in models of trauma and haemorrhagic shock in female when compared to male rats (Dietch et al., 2006; Ananthakrishanan et al., 2005). However, the female advantage is lost after oophorectomy but restored with the administration of oestrogen receptor agonists (Doucet et al., 2010). Female mice have been shown to have a greater tolerance to ischaemia-reperfusion injury when compared to males (Hu et al., 2009). A recent surgical review (Bullard et al., 2010) summarised the key elements of the evidence underpinning sex-related differences in outcome after trauma concluding that “in survival after trauma, men would benefit from being more like women”. However females do not have it all their own way: a recent study indicated that although females appear to exhibit less early trauma coagulopathy but the outcome was significantly worse in females that do develop the condition (Engels et al., 2011). Although there are important sex-related differences in the response to trauma, there are more likely to be quantitative rather than qualitative differences in the context of the variables studied in this thesis. By limiting this study to one sex, the results are not invalidated as far as males are concerned but it is likely to have reduced

variability (compared to mix sex groups) and hence reduced the number the number of animals required to attain a statistically robust conclusion.

The aetiology of acute trauma hypo-coagulopathy is multi-factorial and in part is linked to tissue hypoperfusion and consequences such as acidosis as well as hypothermia, consumption of factors and (where large volumes of resuscitation fluids are used) dilution (Hess et al., 2008; Tieu et al, 2007). More recent views emphasise the importance of tissue hypoperfusion and ischaemia *per se* which can lead to an early hypo-coagulopathy before these other factors are present by changing the balance of clotting pathways from fibrinogenesis to fibrinolysis (Brohi et al., 2008). In support of this view, PT was found to be elevated in a cohort of major trauma patients only when there was concurrent evidence of tissue hypoperfusion (increased base deficit) (Brohi et al., 2007). In our study, the elevation of PT also correlated with increased base deficit (reduced ABE). It is clear from the oxygen transport measurements made in the current study that tissue oxygen delivery is enhanced during the normotensive phase of NH since the reduction in base deficit is paralleled by a fall in oxygen extraction ratio.

It is suggested that tissue hypoperfusion leads to endothelial expression of thrombomodulin and increased circulating thrombomodulin levels. The thrombomodulin, in turn, complexes with thrombin, diverting it to an anticoagulant function via activated Protein C (Brohi et al., 2007). Without measurements of circulating thrombomodulin and Protein C in this study, it is not possible to take the comparison further, but it is interesting to speculate that the association of the improvement in the clotting status seen in animals given novel hybrid (NH) resuscitation may be due to an improvement in tissue perfusion.

By contrast to our findings, a recent report by White et al. (2010) showed that PT (and a number of other clotting parameters) were unaffected by oxygen debt in anaesthetised pigs following a similar volume of haemorrhage plus musculo-skeletal injury. However, in this latter study measurements were made at pre-defined levels of oxygen debt and it is of note that the degree of base deficit was small (approximately -2 mM) even at the most severe level of oxygen debt. A possible explanation for the difference between our two studies is that the

development of the coagulopathy is also time-dependent and that in our study more time had elapsed to allow greater influence of an oxygen debt on both metabolic state (base deficit) and clotting status.

NH resuscitation was associated with a reduced inflammatory IL6 response (figure 28) compared to hypotensive resuscitation. The time-course for the IL6 (inflammatory) response is such that even higher levels of IL6 would be anticipated in the hypotensive groups had they survived longer (Dong et al., 2006). The aetiology of the acute systemic inflammatory response associated with trauma is complex and includes the effects of both shock and resuscitation (Watters et al., 2006). It has been argued that crystalloid solutions used for resuscitation can augment the inflammatory response (Molina et al., 1997). However in our study it appears that the effects of prolonged shock are a more potent stimulus since the hypotensive group displayed higher levels of IL6 than the group given NH resuscitation. The inflammatory effects of tissue hypoperfusion appeared to be greater than the effects of blast *per se*, emphasising the potency of shock as a mediator of systemic inflammation.

We did not find any effect of injury, shock or resuscitation on TNF- $\alpha$ , and consequently no difference in the TNF- $\alpha$  level between the four groups (figure 29) despite seeing clear changes in another inflammatory marker (IL6). This may appear superficially surprising since a number of studies have shown that the inflammatory response that follows haemorrhagic shock and resuscitation is characterised by elevations in a range of cytokines, including IL6 and TNF- $\alpha$  (Cai et al., 2009; Relja et al., 2009). However, although a trauma/haemorrhagic shock – induced TNF- $\alpha$  response is well described in the rat it is less so in porcine models. Studies conducted in pigs that show elevations in TNF- $\alpha$  after traumatic injury and haemorrhagic shock generally involve very profound shock e.g. a sustained mean arterial pressure of 20-25 mmHg (Dong et al., 2010), while a more modest haemorrhage is associated with little or no alteration in TNF- $\alpha$  (Dong et al., 2006). The TNF- $\alpha$  response in porcine models of haemorrhagic shock is often much less than the IL-6 response to the same insult, e.g. a two-fold increase in TNF- $\alpha$  compared to a greater than 360 fold increase in IL6 (Englehart et al., 2008). However, where models of

haemorrhagic shock are combined with an element of sepsis, TNF- $\alpha$  does show a marked increase, confirming that the pig is capable of producing a TNF- $\alpha$  response to other forms of shock (Kubiak et al, 2010). Therefore, in contrast to other species such as rat, pigs do not mount a large TNF- $\alpha$  response to haemorrhagic shock unless the model is very severe or combined with sepsis. In this respect the porcine model appears to be a better representation of the response to trauma in humans than the rat models are since clinical data suggests that man, like pig, does not mount a large TNF- $\alpha$  response to trauma, in contrast to the situation in septic shock (Martin et al., 1997).

The correlations of clotting impairment and degree of physiological impairment and enhanced inflammatory state showed a significant association (Figure 30). The complex interactions between the inflammatory and coagulation 'systems' have been discussed in chapter 3.7.2. It is of note in the present study that the development of coagulopathy after trauma/haemorrhage and hypotensive resuscitation was mirrored by an inflammatory response (elevation in IL-6). A separate analysis of samples from the same series by a colleague showed that HMGB-1 is also increased, especially after hypotensive resuscitation (Jacobs, 2010). Collectively these mirror an observational finding from a clinical study that the development of acute trauma coagulopathy is paralleled by elevations in HMGB-1 (Cohen et al 2009), a known mediator of sterile inflammation and driver of secondary inflammatory cytokines such as IL-6 (Fink et al 2007). It is also known that inflammatory agents such as HMGB-1 can bind to thrombomodulin, although it is currently not known how this might affect clotting (Cohen et al 2009). It is therefore impossible to determine from any of these studies whether there is a causative link between the inflammatory system and acute trauma coagulopathy or whether the link simply reflects common drivers based e.g. on tissue hypoperfusion.

Superficially it might be expected that an enhanced inflammatory response might oppose the acute reduction in clotting associated with trauma. However, the situation in vivo is likely to be complex, with a number of competing influences occurring at the level of the endothelium. Some authors have argued that the inflammatory system in the context of trauma may drive a very complex response comprising of both anticoagulation at the site of injury (leading to



ATC) and a systemic enhancement of coagulation giving rise to disseminated intravascular coagulation (DIC) (Gando et al 2011) i.e. the worst of both worlds. In this circumstance it is argued that a high level of inflammatory cytokines and severe tissue injury activates the tissue-factor-dependent coagulation pathway followed by massive thrombin generation and its activation. Low levels of protein C and antithrombin induce insufficient coagulation control and the inhibition of the anticoagulation pathway (Gando et al 2011). A complex interaction between the cellular and fluid (plasma) elements of the coagulation system has been proposed to reconcile the simultaneous presence of both increased and depressed clotting states (Johansson et al 2010); the situation in circulating whole blood is a consequence of the degree of the tissue injury and is critically related to the degree of endothelial damage, with a progressively more procoagulant endothelium inducing a gradient of increasing anticoagulation towards the plasma phase (Johansson et al 2011). However, a very recent clinical study has found no evidence of early DIC at a time when ATC is apparent in the presence of concomitant elevations in pro-inflammatory cytokines such as Il-6 (Johansson et al 2011). This suggests that the overt pro-coagulant state is a later response in injured patient. The detail of the interaction between the inflammatory and coagulation system(s) remains to be elucidated; to some degree this may be facilitated by investigations into the interactions between the two systems in situations such as sepsis (where it has been studied extensively) although this may also be the source of confusion since there are very important differences between 'sterile' inflammation during the early phase of the response to trauma and the inflammation seen during sepsis.

There a number of potential and actual limitations in any study which relies on a model of a clinical condition. The study was conducted on anaesthetised pigs rather than conscious humans and both the species of animal and the choice of anaesthetic could have significant implications for the conclusion of the study. With the latter, the anaesthetic used (intravenous alphaxalone) has previously been documented to preserve the central nervous pathways mediating the

relevant cardiovascular response to injury, hence it is unlikely to have caused a qualitative alteration in the responses compared to conscious man.

With regard to the species differences, there are differences in the coagulation systems of humans and pigs. However, provided these differences are known and acknowledged then reliable conclusions can be derived regarding the elements of the system that are similar between the species of interest.

Although a porcine model would not be relevant in all aspects of the mechanisms of trauma coagulopathy, the current model does seem to replicate acute trauma coagulopathy which responded to improved tissue blood flow in manner predictable from current human studies. Lastly, this model has relatively little tissue injury (other than the effects of blast in one injury strand). Since the degree of tissue injury can affect coagulation, a future model should incorporate an additional tissue injury (musculo-skeletal) when studying ATC.

## **6.6 Conclusion**

We have demonstrated that the novel hybrid (NH) resuscitation strategy attenuates the development of acute trauma coagulopathy and systemic inflammation in a model of complex injury. This emphasises the importance of the difficult clinical balance involved in choosing a resuscitation strategy for individual patients where the needs of tissue perfusion must be offset against the risk of re-bleeding during resuscitation.

## **Chapter 7: Feasibility of using rotational thromboelastometry to assess coagulation status of combat casualties in a deployed setting.**

### **7.1 Introduction**

The incidence of acute trauma coagulopathy seen on admission to the Emergency Department has been shown to be between 25% to 34% in civilian patients and between 31% to 38% in military patients (Brohi et al., 2003; Macleod et al., 2003; Maegele et al., 2007; Plotkin et al., 2008). This is associated with a 4-fold increase in mortality (Brohi et al, 2003). Concurrently, a higher proportion of military patients (8% to 10%) undergo massive transfusion (MT) when compared to civilian (3% to 5%)(Holcomb 2007; Como et al., 2004). The mortality rate for those receiving massive transfusion ranges from 20% to 50% (Borgman et al., 2007).

As a means of addressing this coagulopathy, haemostatic resuscitation has been developed to rapidly and proactively treat the coagulopathy with blood and blood products (Kirkman et al., 2007). However, current guidelines on the management of massive blood loss rely heavily on the availability of standard laboratory results to guide therapy (Rossaint et al., 2010). The British National Blood Transfusion Service and the American College of Pathologists' guidelines define the presence of coagulopathy as a prothrombin time (PT) over 18 seconds and/or an activated partial thromboplastin time over 60 seconds (College of American Pathologists, 1994). These tests are designed to measure only the clotting in plasma and do not consider the action of the factor and cellular components together and their interaction. The average time for laboratory test results to be available is 45 minutes, during which time the clinical picture can rapidly change allowing coagulopathies to develop and remain undetected. The delay does not allow for timely tracking of the effectiveness or otherwise of therapeutic intervention.

Point-of-care monitoring using thromboelastography (TEG) / thromboelastometry (TEM) is a test that is well established within cardiac and liver elective surgery. A NHS health technology report in 2008 looked the

clinical effectiveness of TEG/TEM concluding that the technique reduced the need for inappropriate transfusions and decreased blood product requirement. The technique has been shown to reduce the number of deaths, complications, infections in cardiac and liver transplant surgery and overall resulted in an increase in survival (Craig et al., 2008).

TEG/TEM provides a timely and convenient method that measures a number of aspects of the coagulation profile, including initial clotting, platelet interaction and fibrinolysis in a sample of whole blood. ROTEM<sup>®</sup> (Pentapharm GmbH, Munich, Germany; figure 1) is a self-contained, touch-screen machine that is analogous with thromboelastography (TEG<sup>®</sup>) (Haemoscope Corporation, Niles, IL, USA) to produce the clot profile. Table 3 (Chapter 3) lists the main features of the two machines. ROTEM<sup>®</sup> has come to the forefront in Europe over the past decade. To clarify the nomenclature used in this thesis, TEG/TEM will describe the technique of measurement of coagulation (whether it was carried out by either ROTEM<sup>®</sup> or TEG<sup>®</sup>). The specific device (ROTEM or TEG) will be named as appropriate.

## **7.2 Aim**

This study addresses the hypotheses and aims detailed in section 5.2. In summary, the aim of this field study was to assess the feasibility of using ROTEM<sup>®</sup> in a deployed setting and to determine if results from the ROTEM<sup>®</sup> could be used to assess the coagulation in the military trauma patient with the future potential to guide resuscitation therapies in MT patients. It was not the intention of this study to use this technique to select patients who might require activation of the MTP, but to observe the changes apparent on admission and relate them to their clinical condition, the results of the standard laboratory assessments of coagulation and the subsequent use of blood and blood products.

### **7.3 Method**

The study was designed as a prospective observational field trial of the feasibility of using TEM in the deployed military environment following preliminary experience in the UK with the equipment. The study method was assessed by the Ministry of Defence Research Ethical Committee (MODREC). Formal consent was not deemed necessary from patients as the blood taken for testing was the residue of that taken for routine clinical assessment and was therefore deemed to be waste material. The committee's opinion was this constituted a technology assessment study of an approved 'CE' marked clinical apparatus being utilised in a novel setting and as the results were available to clinicians managing the cases (if they wished), there was no requirement for separate ethical consent. The ROTEM<sup>®</sup> results were anonymised for analysis. Permission was granted for the use of the two example cases listed in this chapter.

#### **7.3.1 Patient Selection**

The study was performed over 5 weeks in 2009 in the military hospital in Camp Bastion in Helmand Province in Afghanistan. Patients included were those that were admitted into the emergency department (ED) and whose injury mechanism and clinical concern resulted in the trauma team activation, including those requiring massive transfusion. This group of patients, considering the severity of their injuries, were felt to be at risk of developing the acute traumatic coagulopathy. Patients were excluded if they had received blood transfusion and medical care prior to arrival at Camp Bastion and whose condition was not a result of trauma. The patients in whom the Massive Transfusion Protocol (MTP) was activated were defined by the criteria laid down in the Joint Service Publication (JSP) 950; Medical Policy (2009). This is described in detail in Table 5 (Chapter 4).

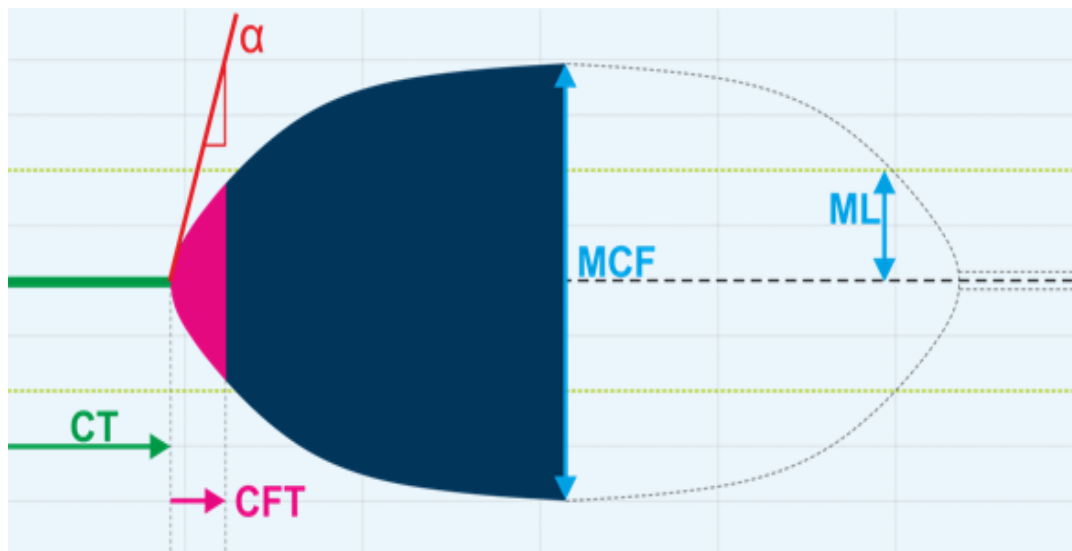
A blood sample (4ml citrated fresh whole blood) was drawn from the patient at the same time as admission bloods, which were tested for routine laboratory tests including standard coagulation tests of prothrombin time (PT), activated partial thromboplastin time (APTT) and platelet count. The normal reference

values for the hospital's laboratory analyser (IL 300, Instrumentation Laboratories UK Ltd, Warrington, UK) were used to identify those as having normal coagulation or abnormal coagulation. A normal value for PT was  $\leq 18$ s and APTT was  $\leq 38$ s. Arterial blood gases were taken on arrival and initial body temperature was recorded with routine observations. The total transfusion of blood and blood products given within the first 24hrs was recorded. Injury Severity Score was calculated using the documented injuries that had been determined at the end of 24hr admission using the guidelines by Baker et al (1974).

### **7.3.2 Thromboelastometry measurement**

TEM was measured using ROTEM<sup>®</sup>. This was chosen as the technique over TEG<sup>®</sup> for a number of reasons including: robustness in extreme environments and continuous movement; the user-friendly technology with the touch screen and automated pipette; the availability of four channels with a coloured coded pictorial result that was easily interrupted by all staff. Several differential ROTEM<sup>®</sup> test assays exist that contain different activators and inhibitors; these tests are outlined in table 4 (Chapter 3); which explains which activator is used, which aspect of clotting process is being tested and the normal expected values. Three tests were performed on each blood sample in this study; EXTEM, INTEM and FIBTEM. HEPTTEM was not used as this population of patients were not heparinised and APTEM was only used in extreme circumstances to prove that hyperfibrinolysis was occurring.

The information from each trace provides the following parameters (an example trace is illustrated pictorially in figure 32):



**Figure 32:** ROTEM<sup>®</sup> trace. ((Produced by kind permission of Tem Innovations GmbH, Germany). CT-Clotting Time; CFT-Clot Formation Time; MCF-Maximum Clot Firmness; ML- Maximum Lysis.

Refer to Section 3.6.2 for a full explanation of the terminology and interpretation of the components of the trace. However, to summarise interpretation, an elongated CT time indicates disturbed activation of coagulation, where a factor deficiency is considered. Decreased MCF in the EXTEM test indicates a problem with the overall clot strength. Concurrent evaluation of the FIBTEM test allows a differential assessment of whether the problem is due to lack of fibrinogen or reduced platelet function /numbers or both. This is because in the FIBTEM test the platelets are disabled by an added inhibitor and hence the FIBTEM MCF is independent of the platelets. A reduced FIBTEM MCF therefore suggests a problem with fibrinogen levels whereas a normal FIBTEM MCF suggests that fibrinogen levels are adequate and the problem lies elsewhere e.g. with the platelets. Further explanation of ROTEM<sup>®</sup> analysis is outlined in Chapter 3.6.2. The cut-off values to define normal/abnormal clotting for each parameter was taken from the manufacturer's reference ranges as documented in table 4 (chapter 3).

All samples were tested at 37°C within 20 minutes of being drawn. After the patient was enrolled into the MTP, a least one (up to 10) further traces were carried out to monitor the changes in the coagulation status during the on-going resuscitation. Although ROTEM<sup>®</sup> is an established clinical test used in the clinical setting in the UK; at the time of this study, the ROTEM<sup>®</sup> had not been

incorporated into the MTP algorithm, so it was not used to direct therapy but the results were made available to the treating clinicians to use as they felt clinically appropriate.

### **7.3.3 Statistical analysis**

Comparison of ROTEM<sup>®</sup> results with standard laboratory coagulation tests (PT, APTT) was by the non-parametric test of paired proportions – McNemar’s Test. Nonparametric data is expressed as median (with interquartile range) and two-group analysis was performed with Mann-Whitney U test. Parametric data were expressed as mean  $\pm$  SEM and were compared using a Student’s t-test. A *p* value of 0.05 was chosen to represent statistical significance.

## **7.4 Results**

In the study period 31 patients had TEM performed, of which 20 were subsequently enrolled into the MTP. One of these 20 patients had received blood products at a forward unit, therefore were excluded from the study. The other 11 patients comprised of 4 in-patients and 7 admissions to the ED. One of the ED admissions was a subarachnoid haemorrhage; this patient and the in-patient cases were excluded from the study, leaving a small group of 6 new admissions who did not receive a massive transfusion. Therefore the overall patient group consisted of 19 MTP and 6 non-MTP patients.

All the patients were men, and the median age was 21 years (interquartile range 18-35 years). In 60% (15 of 25) of cases, injuries occurred after exposure to a blast incident, 36% (9 of 25) resulted from gunshot wounds, and one case was involved in a road traffic crash. The admission physiology of each group is listed in table 8. At the end of the study period, 16 patients (84.2%) had survived from the MTP group; all non-MTP patients survived.

The total amount of blood products the MTP group received was 164 units packed red blood cells (PRBC), 116 units fresh frozen plasma (FFP), 15 units of platelets (Plts) and 13 units of cryoprecipitate (cryo). This is a mean of 8.6



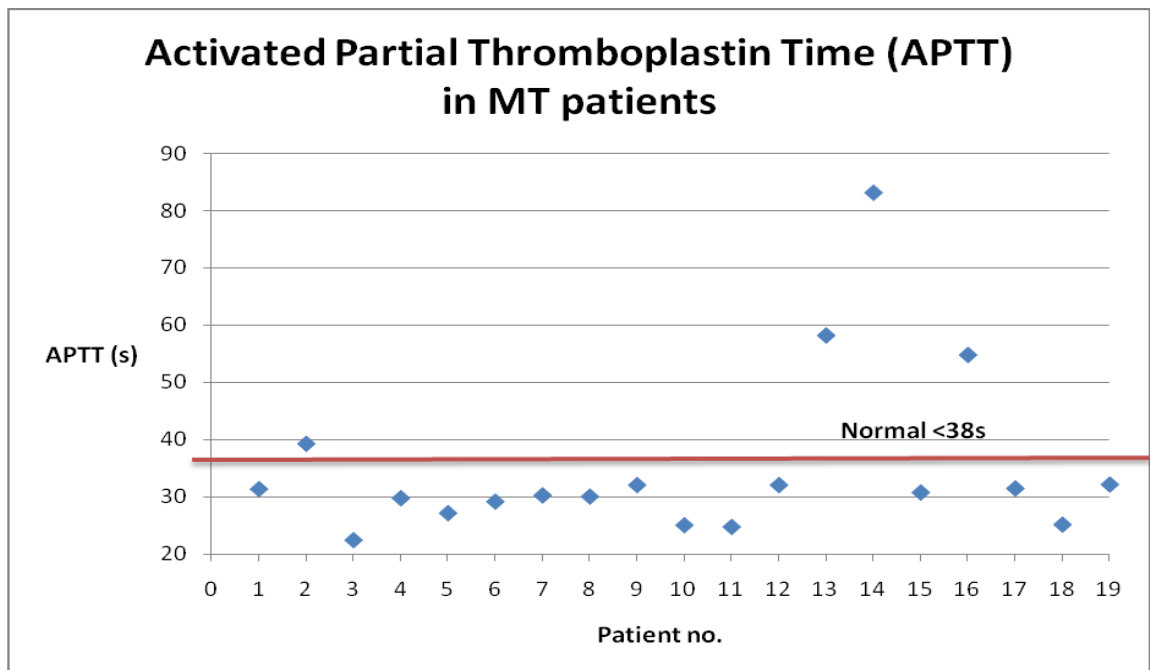
PRBC/6 FFP/1Pit/1cryo per patient. The range of units given per person was 4-16 PRBC; 2-12 FFP; 0-5 Plts; 0-2 cryo. The ratio of PRBC to FFP is 1.4:1. The non-MTP group of 6 patients received a total of 5.5 units PRBC and 3 units of FFP, with no requirement for platelets or cryoprecipitate transfusions.

Parameter	MTP Group (n = 19)	Non-MTP Group (n = 6)	p-value
ISS (IQR)	35 (25–50)	20 (19–20)	p <0.001 <sup>1</sup>
Systolic blood pressure (mmHg)	115 ± 7	142 ± 9	p<0.05 <sup>2</sup>
Pulse rate (beats per min)	122 ± 5	100 ± 10	p<0.03 <sup>2</sup>
Temperature (°C)	34.8 ± 0.3	36.0 ± 0.4	p<0.07 <sup>2</sup>
pH	7.27 (7.19–7.32)	7.36 (7.28–7.40)	p = 0.08 <sup>1</sup>
Base excess (mM)	-5 (-9 – -3)	-2 (-5 – +1)	p = 0.12 <sup>1</sup>

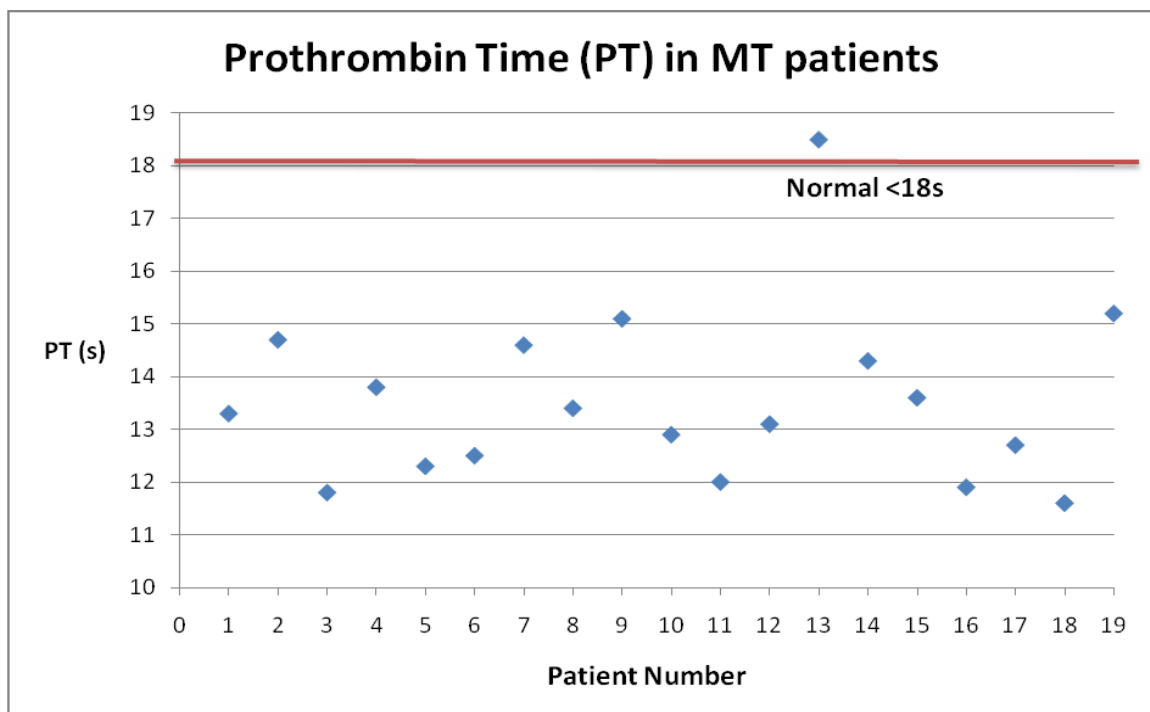
**Table 8:** Admission physiology of two groups of patients: MTP and non-MTP. Median values (with inter-quartile range) are listed; p-values were calculated by Mann-Whitney U (two-tailed) test<sup>1</sup> or unpaired t-test<sup>2</sup>.

Using the normal reference values for the laboratory analyser, (upper limit of 18s for PT and 38s for APTT), 16% (4/25) of the entire group had abnormal results. All 4 patients with abnormal clotting received MTP. Therefore in the MTP group, 21.1% (4/19) had abnormal results. All of these four coagulopathic patients had an abnormal APTT, one of which also had an abnormal PT. The results are shown in figures 33 and 34 showing the distribution of these values. All the patients in the non-MT group had normal PT/APTT results. The average

recorded time from when the blood sample was taken from the patient to when the laboratory results were available to the clinician was 45 minutes.



**Figure 33:** Admission values of APTT for patients who went on to have massive transfusion. Only four values were above the upper limit of normal.



**Figure 34:** Admission values for PT for patients who went onto have massive transfusion. Only one value was above the upper limit of normal.

Analysis of the admission ROTEM<sup>®</sup> traces from all patients (of both the MTP and non-MTP groups), it was found that 64% (16 out of 25) had abnormal results. When these figures were examined statistically using a non-parametric test of paired proportions (McNemar's test), ROTEM<sup>®</sup> returned significantly more abnormalities than the standard, conventional laboratory tests ( $p=0,0005$ ). When the ROTEM<sup>®</sup> results of the two groups, MTP and non-MTP, were examined separately there was little difference in abnormalities detected between them. The MTP group had 63.2% (95% CI; 38.4-83.7%) (12/19) outside the set reference ranges. In the non-MTP group, 67.7% (95% CI; 22.3%- 95.7% (4/6) had an abnormal trace, with all cases having a low MCF in the FIBTEM trace. The normal reference range for MCF in EXTEM is 50-72mm, but a MCF value of <45mm is viewed as a critical level for increased risk of bleeding (Lang et al., 2005). Taking this value and re-examining the results from the two groups, 31.6% (6/19) of the MTP patients fell below this value whereas all non-MTP patients had MCF >45mm.

Table 9 shows the initial EXTEM traces of all 25 patients providing the CT, CFT, A10, MCF and ML values. Patient no. 1-19 are the MTP group and patient no. 20-25 are the non-MTP group. Patient no 16 had a grossly abnormal CT where no clotting was observed after 30 minutes. This patient had critical head injuries along with extremity injuries, and although full resuscitation was commenced initially, this was deemed futile after a CT scan showed unsurvivable head injuries. He died shortly after this information was obtained.

In the MTP group, the CFT was abnormal in 21.1% (4/19) patients with 3 cases going on to develop an abnormal MCF.

Looking at the MCF value in both groups, a total of 9 patients had an abnormal result in their admission trace; 7 of which had an abnormal value at A10. No patient with an abnormal A10 had a normal MCF above 50mm (the lower range of normal reference range).

Patient 22, who did not receive the MTP had abnormal results across all parameters recorded. This patient had been brought in from a RTA having suffered blunt injuries. The exact time from injury to arrival at ED was not

known, but the patient required only treatment of re-warming and crystalloid fluid. No transfusion was required.

INITIAL EXTEM TRACE					
Patient No.	CT	CFT	A10	MCF	ML
	38–79 (s)	34–159 (s)	43–75 (mm)	50–72 (mm)	<15 (%)
1	6	148	49	56	0
2	73	120	40	40	15
3	60	97	49	55	3
4	46	356	30	50	0
5	45	204	34	35	1
6	51	86	51	55	7
7	56	143	41	45	3
8	52	80	54	62	9
9	73	95	51	55	0
10	49	66	54	60	4
11	56	169	50	51	8
12	55	73	56	56	4
13	64	108	44	46	1
14	72	131	40	42	98
15	68	96	49	51	10
16	1897	---	---	---	---
17	45	83	51	54	14
18	72	43	39	43	66
19	73	83	55	55	5
20	51	85	55	59	8
21	55	168	51	58	2
22	116	187	42	47	3
23	50	75	56	60	9
24	74	102	58	62	6
25	37	114	46	46	9

**Table 9:** ROTEM results of initial EXTEM traces for all patients. Patients' no. 1-19 were in the MTP group and patients 20- 25 were the non-MTP group. Highlighted results are abnormal. A dashed line means no result was obtained.

## 7.5 Example Cases

An advantage of TEM is that it can be used to both tailor the direction of the clinical management and to display response to therapy of patients. This is best illustrated by individual clinical cases, two of which are listed below.

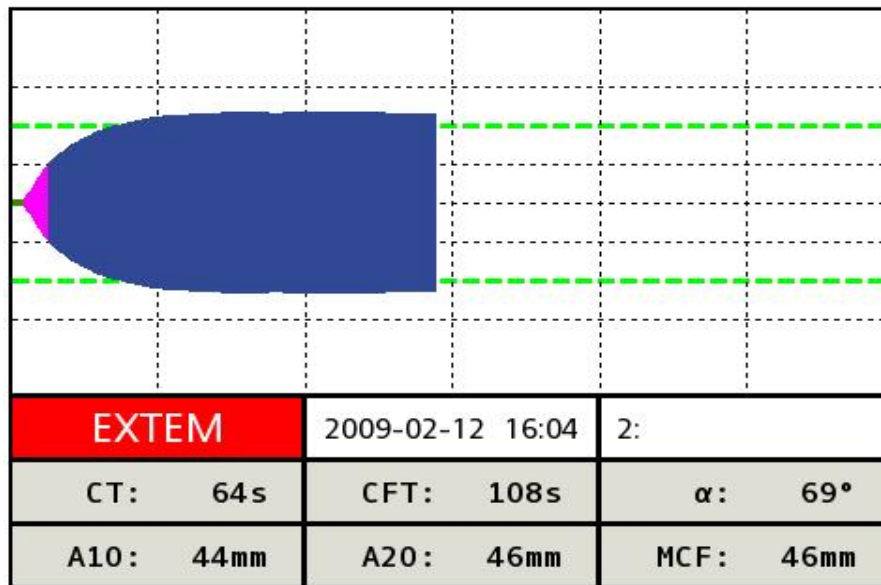
### 6.5.1 Case 1

A 30-kg male was admitted to the ED 2 hours after he had been involved in an explosion. He had sustained significant soft-tissue loss to his right upper leg with an associated compound fracture of his femur. Admission physiological measurements were a pulse of 140 beats/min, systolic blood pressure of 70 mmHg, temperature of 32 °C, pH of 7.01 and a base excess of -18mM. He was anaemic with an Hb of 5.6 g/dL, a abnormal platelet count of  $36 \times 10^9/L$  and a PT of 18.5s, and an APTT of 58.2s. Over the next 18 hours, this patient was transfused 16 units PRBC, 10 units FFP, 5 units Platelets, and 1unit cryoprecipitate. The four traces shown in Figures 35 to 38 are EXTEM traces over this period of time. As ROTEM<sup>®</sup> results were not incorporated into the MT algorithm at this time, the results were not used to direct therapy but the traces were available to the treating clinicians to use as they felt appropriate.

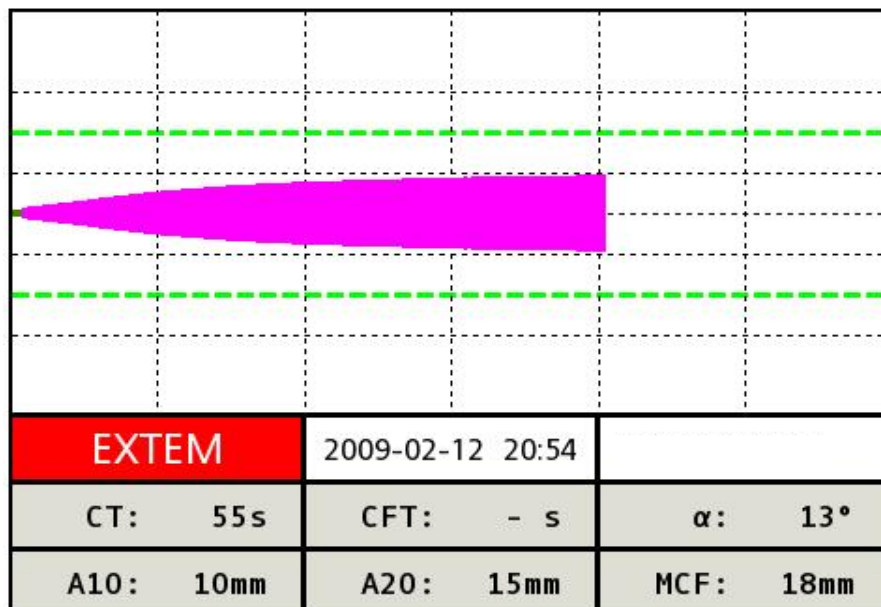
Over the first two hours the patient underwent initial damage control surgery with ongoing resuscitation before being transferred to the intensive care unit (ICU). He received 10units PRBC, 7units FFP, 2 units Platelets and 1unit cryoprecipitate in that time during which his physiology had essentially returned to normal. Despite this his coagulopathy had worsened (refer to Figure 36 and 37). His full blood count showed a Hb of 8.6 g/dL and a platelet count of  $55 \times 10^9 /L$  at that time.

By 0700 the following morning, 15 hours after admission and despite his on-going resuscitation, he remained coagulopathic with a low platelet count. This was reflected by his ROTEM<sup>®</sup> trace at that time, shown in figure 37. A unit of freshly apherised platelets was given and the response in coagulation state was noted in figure 37. This improvement mirrored the subjective assessment of his wounds and his clinical ability to clot, meaning he returned to operating room (OR) for further debridement of his wounds and external fixation of his femur.

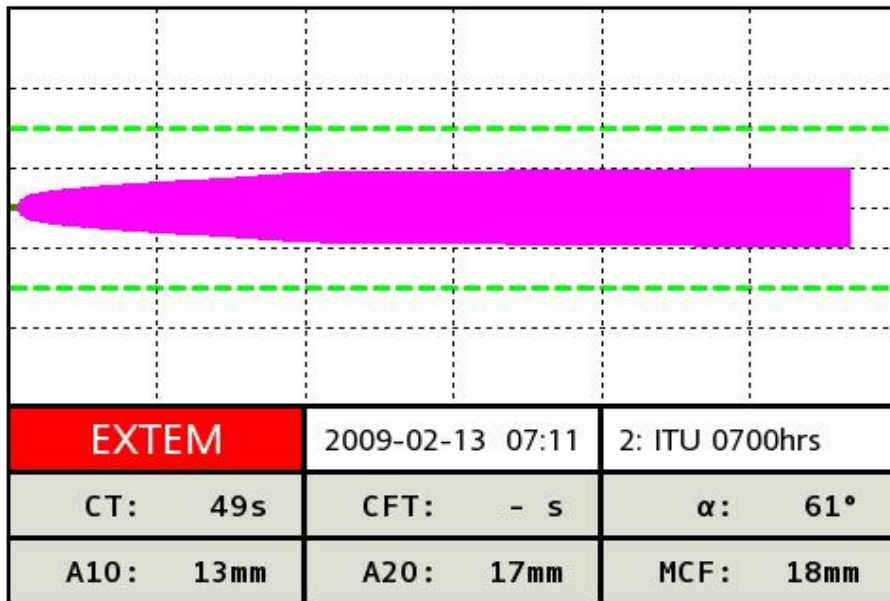
His fibrinogen levels were within normal range after his first unit of transfused cryoprecipitate.



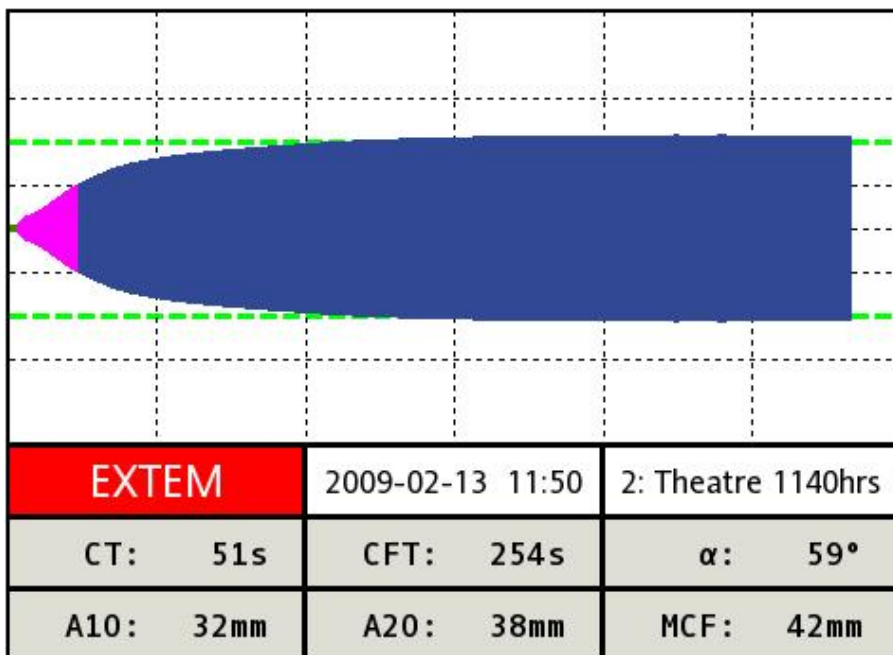
**Figure 35:** Admission EXTEM trace showing normal CT but decreased MCF



**Figure 36:** After initial surgery and admission to ITU after initial haemostatic resuscitation and surgery. This abnormal trace mirrored clinical coagulopathy.



**Figure 37:** 15 hr after admission, after further surgery and resuscitation, patients remained coagulopathic.



**Figure 38:** After apherised platelets were given, significant improvement was noticed in trace and clinically.

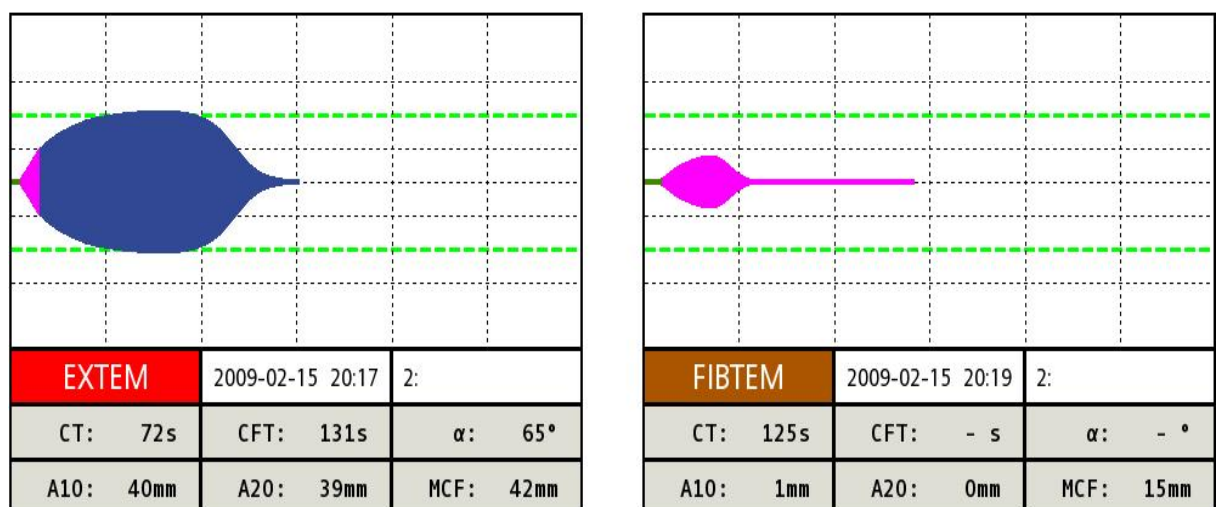
This exemplar case demonstrates the how the ROTEM<sup>®</sup> traces could provide timely and convenient results that mirror the coagulation status and how it could

be used to both potentially guide therapy in MT and to observe response to that treatment.

### 7.5.2 Case 2

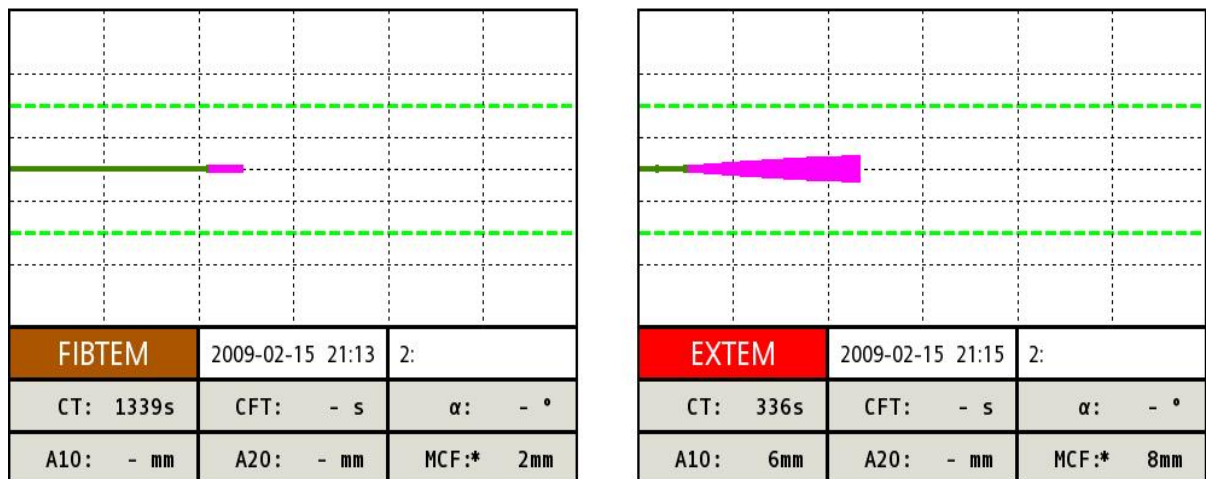
A 65-year-old male admitted after sustaining a GSW to his right flank. He had been brought to one of the International Security Assistance Forces (ISAF) forward operating bases (FOB), so previous history was not provided. Admission physiology was documented as a pulse of 94 beats/min, a systolic pressure of 110 mmHg, tympanic temperature of 34.9°, a pH of 7.01 and base excess of -17mM. Blood results were Hb= 8.4g/dL, Plts= 182 x10<sup>9</sup>/L, PT= 14.3s and APTT= 83.1s. He was taken directly to the OR on arrival to the hospital facility.

His initial EXTEM and FIBTEM traces (Figure 39) showed that there was complete lysis of the clot within 20 min, indicating hyperfibrinolysis. He underwent damage control surgery with aggressive haemostatic resuscitation. His physiology continued to deteriorate despite this and after one hour his ROTEM® trace (Figure 39) showed a completely hypocoagulable state. When taken in context with his injury severity and physiological status, it was considered by the managing clinicians that further resuscitation would be futile. ROTEM added crucial information at a critical time in the decision-making process.



**Figure 39:** Admission ROTEM® trace of case 2 showing hyperfibrinolysis.





**Figure 40:** ROTEM<sup>®</sup> trace after 1 hour of resuscitation and surgery showing a profound hypocoagulable state.

## 7.6 Discussion

Acute coagulopathy of trauma is an increasingly recognised problem, but the prevalence depends on the established definitions that are based on standard laboratory tests. There are two disadvantages with these tests, the first being the time that they take from the laboratory. In the setting of a deployed hospital, the laboratory is situated next to the ED and is dedicated to the testing of new admissions during the reception of trauma patients. Samples were taken to the laboratory for PT/APTT and OR for ROTEM<sup>®</sup> analysis. The average time from when the samples were drawn to PT/APTT results being available was 45 minutes. Jaeger et al (2009) found that their turnaround time for laboratory results was 61 minutes; and Singer et al (2008), with the introduction of a dedicated stat laboratory for the ED patients, reduced the time for results to 30 minutes. However, with the introduction of the haemostatic resuscitation, the evolving situation of rapid trauma damage control resuscitation would make those results less relevant to the real-time clinical situation. The ROTEM<sup>®</sup> tests in this study were initiated within approximately 8 minutes of the patient's arrival by the lead investigator (the fastest being 3 minutes and exceptionally, one test being commenced 20 minutes after arrival). The results could then be observed over the next 20 minutes as they evolve on the screen. Although a full trace can

take up to 60 mins to complete, abnormal traces e.g. a prolonged CT or a decreased  $\alpha$ -angle can be easily seen after a much shorter interval, giving an early indication of a coagulopathic state. This is an advantage of having a near-patient test. Other bedside tests are available commercially, such as ISTAT<sup>®</sup> PT/INR (Abbot Laboratories, East Winsor, NJ, USA), that will measure INR within minutes; however, they do not provide the overall dynamic profile indicating the rate and strength of the clot formation as provided by the ROTEM<sup>®</sup> or TEG<sup>®</sup>.

This study has suggested that the value at A10 could act as an early indicator of an abnormal MCF, as shown by the results in this small study that 80% (7/9) patients who had an abnormal A10, had a final abnormal MCF. This has since been indicated in a follow-up study at Camp Bastion (Woolley et al, 2012). This is of clinical importance as an early result that indicates the level of coagulopathy may allow the haemostatic resuscitation to be directed. In a prospective cohort study at the Royal London Hospital, England has shown that an EXTEM CA5 (the amplitude of the EXTEM trace at 5mins after CT was attained) correlates with the PT ratio. A patient with a CA5 result of  $\leq 35$ mm had a greater transfusion requirements (4 vs. 1 unit,  $p < 0.001$ ) and were more likely to receive FFP (47% vs 12%,  $p < 0.001$ ). A normal CA5 ( $> 35$ mm) had a negative predictive value of 99% for massive transfusion (10+ units) (Davenport et al., 2011). In a separate study of 300 trauma patients, a significant correlation was found between CA15 (EXTEM trace) and the PT (Rugeri et al., 2007). These results suggest that as the ROTEM<sup>®</sup> trace develops, an early warning of coagulopathy may be evident to the clinician in the first 15 minutes of the test starting, rather having to wait for the development of the completed trace.

The second disadvantage of the standard laboratory tests is the actual detection of coagulopathy. This study has shown that the standard laboratory tests of PT and APTT detected only 21.1% results outside their normal range in the severely injured patients. This detection rate is significantly lower than the 64% abnormal results detected by ROTEM<sup>®</sup> when all traces were considered. In the absence of an independent 'gold standard' assessment of coagulopathy it is impossible to be sure whether the conventional testing is returning a number

of false negative values or whether the ROTEM<sup>®</sup> gives a number of false positive values. Clearly further studies are needed to evaluate this, but in the meantime, of the two potential errors, the 'safe' clinical approach is to have a heightened sensitivity to an impending problem rather than having a false security from a negative test. A clear benefit of ROTEM<sup>®</sup> is a provision of an early picture as the test develops.

However, rather than absolute value of any single parameter, the main benefit to the clinician is the trend of any changes and ability monitor further treatment during haemostatic resuscitation.

It is recognised that the same proportion of abnormalities is detected by ROTEM<sup>®</sup> in both MT and non-MT groups in the present study. However, this study was not designed to compare MTP and non-MTP groups and the small sample sizes involved (especially the non-MTP group) has resulted in large 95% CI around the means. A larger, prospective study is clearly required to evaluate if initial ROTEM<sup>®</sup> tests can predict subsequently need MTP, as has been addressed in the civilian population (Davenport et al., 2011).

It is currently not suggested that ROTEM<sup>®</sup> should play a role in the initial activation of a MTP for any patient, however, this technology may allow treatment to be individualised and not given as "one size fits all." The ongoing monitoring of individual patient's traces may also allow the MTP to be "turned off," as was shown in the UK effectiveness report noting that the number of products used was reduced (Craig et al., 2008). Interestingly, the ratio of PRBC to FFP delivered in this study group during the MTP was close to that recommended by Borgman et al based on a retrospective analysis of his data (Borgman et al., 2007).

This study is the first to demonstrate the feasibility of the use of ROTEM<sup>®</sup> in the deployed military setting to assess coagulation status at the time of admission. Prior to this study it was not clear that a ROTEM<sup>®</sup> analyser would function reliably in the ROLE 3 hospital in the conditions currently encountered. The study reported in this thesis formed a basis of a report to both UK and US

Medical Services which formed part of the evidence base for the clinical deployment of the technique (Midwinter et al., 2010).

The ROTEM<sup>®</sup> was placed in OR which was found to be the ideal location. It was directly beside the ED resuscitation bays, and the screen was easily visible for the medical staff during surgery when the majority of blood and blood products were transfused. The touch-screen and automated process meant that all staff members, including operating department practitioners, nurses, and medical staff, were able to learn to run the samples. The pictorial results were visible from a distance and allowed easy interpretation, as clearly shown by the traces in the two exemplar cases described above. Without seeing any actual figures, the staff could easily note when a trace was significantly abnormal. This and the fact that laboratory staff are fully occupied in issuing blood products during an MT make the near-patient assessment of coagulation status in the OR an optimal solution. Figure 40 shows how the machine can easily be seen by all staff in the OR.



**Figure 41.** The ROTEM<sup>®</sup> machine is visible to all staff in the operating room.

The ROTEM<sup>®</sup> machine can be adjusted to conduct the test at the patient's body temperature. However, a decision was made to standardise the test (in accordance with manufacturer's instructions) at a 'normal' body temperature of 37°C. The reason for this was twofold; since all patients have active re-warming, it was felt important to evaluate what the patient's clotting status would be once the temperature had been normalised, since it is imperative to determine if there is an intrinsic coagulopathy independent of the body temperature that would require treatment with blood products. Secondly, this standardisation provided the ability to evaluate two patients simultaneously on one ROTEM<sup>®</sup> device.

The only published paper examining the use of thromboelastography (TEG<sup>®</sup>) in the deployed military setting was a retrospective study looking at single TEG<sup>®</sup> traces that were taken within the first 24 hours (median time 4.5 hr) after admission (Plotkin et al., 2008). In this study, as illustrated by the exemplar cases, it was found that the greatest benefit of using either ROTEM<sup>®</sup> or TEG<sup>®</sup> would be from serial testing to monitor the changes during the resuscitation process and not as an isolated result.

There are restrictions with the technique. It does take some training and education for interpretation, and rogue results can be obtained. It is essential that citrated samples are taken to ensure the correct mix of blood to anticoagulant; failure to do so will give a false impression of coagulopathy. Use of vacutainers mitigates against this problem. The ROTEM<sup>®</sup> does not detect the effects of anticoagulants such as aspirin, clopidogrel and low-molecular-weight heparin and does not detect von Willebrand disease. Failure to detect these effects is not a problem in the population in this study but would be a consideration if used in a civilian trauma centre/ED.

The example case studies are an unusual addition in such reports but are included as it is felt the individualisation of management is the greatest advantage of ROTEM<sup>®</sup> in this setting. This demonstration is particularly pertinent with the current increasing use of blood and blood products in the military sphere. Wade et al (2009) pointed out that a number of published studies (Kaufmann et al, 2007; Johansson et al., 2005) advocate the use of

TEM/TEG in trauma patients to determine the coagulation status but that currently they lack the power to be incorporated into massive transfusion protocols. This study, while still of limited sample size, adds further weight to the argument that battlefield casualties might be managed in a targeted, goal-directed manner and that the use of ROTEM<sup>®</sup> could help with this management, thus ensuring that we are not “empirically and ‘blindly’” administering blood and blood products. Continuing clinical evaluation of TEM/TEG data in a deployed setting will allow the place of the ROTEM<sup>®</sup> in future algorithms to be better defined.

All patients who had an abnormal EXTEM trace also had an abnormal INTEM trace. Unless the patient is heparinised, INTEM does not offer any further additional information. Thus, future cases can be run on two channels, allowing two patients to be run simultaneously.

This study has a number of limitations. This was only a feasibility study without any randomisation of the patients. Although the results were available to all medical staff, there was requirement for them to be used. The principle author was deployed as a member of the surgical team and although other members of the medical staff became proficient at using ROTEM over the study period, but it did mean that not all trauma patients admitted to the hospital during the period of the study were tested. Focus was therefore placed only on those most severely injured.

Future work should be continued with a dedicated researcher ensuring samples taken are correlated with physiological status and resuscitation time points. Continuing use and data collection at Camp Bastion, Helmand Province, Afghanistan, will allow the role of ROTEM<sup>®</sup> in the management of battlefield casualties to be further refined with the intention of including ROTEM<sup>®</sup> parameters in future iterations of the MTP.

## **7.7 Conclusion**

This observational field study shows that it is both practical and feasible to use ROTEM<sup>®</sup> in the deployed military setting. With the immediate availability to the

clinician and pictorial representation of the results, this near-patient test allows for easy real-time evaluation of the patient's coagulation status. The ROTEM<sup>®</sup> detects a greater proportion of coagulation abnormalities upon admission than the standard laboratory tests of PT and APTT. Future studies may refine the role of TEM in MTP algorithms and guide management of individual combat casualties.

## Chapter 8. Conclusions and Future Directions

The principal finding of the experimental part of this thesis is that a resuscitation strategy which targets tissue oxygen delivery (novel hybrid resuscitation) attenuates early trauma-induced coagulopathy and inflammation. Novel hybrid (NH) resuscitation has now been incorporated into the military Battlefield Advanced Life Support (BATLS) strategy, which currently recommends that hypotensive resuscitation the first hour of treatment (Battlefield Advanced Trauma Life Support, BATLS, 2006) except in the case of severe head injuries. If the evacuation (especially of blast injured casualties) is prolonged then consideration should be given to elevating blood pressure to normotensive levels after the first hour with the use of crystalloid in the field or blood and blood products in the transfer. Prior to evacuation, the clinical decision is to balance of risk between either re-bleeding due to the restoration of the blood pressure to a normotensive level or further physiological deterioration due to tissue hypoperfusion and shock due to prolonged hypotension. Based on the findings of this thesis, and the prevailing theories accounting for the acute traumatic coagulopathy (ATC), it is anticipated that casualties given NH resuscitation may experience less coagulopathy than those exposed to a prolonged period (of greater than one hour) of hypotension where the tissue hypoperfusion could exacerbate the ATC. If the treatment of ATC requires reversal of the hypoperfusion by improving tissue oxygenation, blood and blood products used during the resuscitation could obviate this problem but product use might potentially worsen hyperfibrinolysis, in the presence of ischaemia, by increasing the available thrombin level which is available to conjugate with thrombomodulin and drive the production of activated protein C (APC) (Midwinter and Woolley, 2010).

Alternative methods of improving tissue oxygenation can be considered which target the arterial oxygen content aspect of oxygen delivery. The use of oxygen for resuscitation after combined blast and haemorrhage has been shown to improve survival. In this work, the targeted use of oxygen arrested but did not reverse the shock induced by hypotensive resuscitation, which reflects the



limitations of blood flow (J Granville-Chapman et al, 2010). Follow-on studies are being conducted to investigate the effectiveness of enhancing oxygen carrying capacity using haemoglobin-based oxygen carriers (HBOCs), which will have the advantage of increasing oxygen carrying capacity as well as content and should therefore be superior to simply giving supplementary oxygen. It will be interesting to examine the effects of HBOCs on the development of coagulopathy. If early trauma coagulopathy is due to tissue ischaemia then the use of HBOCs may reduce the coagulopathy, which would clearly be of clinical importance as ATC is significantly linked to higher mortality rates (Brohi et al., 2003; Macleod et al., 2003). The results of such work will also give further insight into the mechanism of acute trauma coagulopathy by helping to determine whether the coagulopathy is due to ischaemia *per se* or possibly due to a change in vascular shear which is also part of trauma-shock.

It is interesting to speculate whether the beneficial effect of NH resuscitation on clotting is because of the improvement in oxygen delivery or due to a change in vascular shear associated with improved blood flow; both of which occur during the normotensive phase. There is evidence that both ischaemia and shear can modulate thrombomodulin expression in vascular endothelial cells (Jun et al., 2009; Ishibazawa et al., 2011). An early study into the investigation of shear and thrombomodulin (TM) expression (Malek et al., 1994) suggested that while increases in shear elevated the TM expression, a reduction in shear had little effect. Unfortunately most studies have examined the effects of shear on TM expression over longer time-courses than those relevant for ATC. However, the effects of shear have not been investigated *in vivo* as part of the over-all 'mixing-pot' associated with trauma shock, which may affect the dynamics of the response. Shear could therefore be relevant for ATC and the beneficial effects of NH resuscitation.

The current animal model, when used in conjunction with methods which may improve tissue oxygen delivery by increasing arterial oxygen content, rather than flow, may provide insight into the mechanism underpinning ATC. However, the first step towards this is to further characterise the current model by evaluating whether the coagulopathy seen in the model used in this thesis is

characterised by alterations in protein C (and activated protein C) as described clinically by Brohi et al (2007).

Care would be needed in any follow-on studies using HBOCs to evaluate and if possible control changes in vascular shear. Definitive studies are likely to require *in vitro* experiments where it would be easier to maintain constant shear while changing oxygen delivery by modulating oxygen content of the perfusate. Ultimately both *in vitro* and *in vivo* studies would be required, the former to allow detailed investigation of mechanisms and the latter to ensure relevance to a clinical condition.

The interaction between coagulation and inflammatory systems is an area of rapidly increasing interest. Such is the degree of overlap that a number of authors now view clotting and inflammation as different facets of the same system. The study presented in this thesis showed an association between an attenuated coagulopathy and reduced inflammation. It is impossible to determine whether there is any causative link or whether both phenomena are linked by a common factor such as improved tissue oxygenation and perfusion. An interesting speculative comment was made by Cohen et al (2009) in relation to the interaction between HMGB1 (an inflammatory cytokine, elevated by hypotensive resuscitation in the present study, Jacobs, 2010) and thrombomodulin, namely that HMGB1 might attenuate the maladaptive activation of protein C (and hence the coagulopathy) observed after severe trauma. The basis of this speculation is that thrombomodulin can bind HMGB1 (although the effects of this binding on protein C is unknown)(Cohen et al., 2009). The role of endogenous HMGB1 in ATC could be tested in our animal model; if the hypothesis is correct then the administration of an HMGB1 antibody (already shown to possess anti-inflammatory effects [Shimazaki J et al., 2012]) may attenuate the early trauma-induced inflammation but would worsen the coagulopathy by 'neutralizing' endogenous HMGB1. Such an investigation would be essential before any clinical trials were conducted to assess the efficacy of a HMGB1 antibody in trauma casualties.

Effective targeting of treatment for coagulopathy in casualties requires a timely measurement of coagulation to detect, target and monitor treatment. The principal aim of the clinical study reported in this thesis was to evaluate the feasibility of using ROTEM<sup>®</sup> in a deployed military facility receiving seriously injured battlefield casualties. ROTEM<sup>®</sup> was found to be sufficiently robust to perform well in the environment and provided useful clinical data for those clinicians familiar with the technique. Currently there are no universally accepted definitions of ATC based on thromboelastometry which have been correlated with a bleeding disorder in individual patients. ROTEM<sup>®</sup> did identify a significantly greater number of patients with an abnormality than conventional (PT and APTT) testing. However, it is not yet clear which of the testing systems most accurately reflect a clinical problem in this population of patients. One way to resolve this would be to correlate the findings of both tests against an independent, objective, clinical measure of bleeding. Unfortunately it is likely to be very challenging to find quantifiable independent measures since a number of commonly used indices, e.g. transfusion volumes, are likely to be driven at least in part by measures of clotting. Further directions in clinical research will undoubtedly include assessments of platelet function which, when taken in conjunction with ROTEM, will provide valuable insight into the emerging pathophysiology of acute trauma coagulopathy and provide valuable clinical assessments in the near future.

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