Effect of pre-hospital blood products on Acute Traumatic Coagulopathy in a model of severe military trauma

NORDMANN, GILES, RICHARD

Effect of pre-hospital blood products on Acute Traumatic Coagulopathy in a model of severe military trauma

Thesis submitted for the degree of
Doctor of Medicine

School of Medicine, Pharmacy and Health
Durham University

Giles Richard Nordmann
BSc(Hons) MBChB FRCA L/RAMC

December 2018

Defence Science and Technology Laboratory, Porton Down

Abstract

Acute traumatic coagulopathy (ATC) is seen in 30% to 40% of severely injured civilian and military casualties. Early use of blood products attenuates ATC, but the timing for optimal effect is unknown. Emergent clinical practice has started pre-hospital deployment of blood components (combined packed red blood cells [PRBC] and fresh frozen plasma [FFP]), but this is associated with significant logistical burden and some clinical risk. It is therefore imperative to establish whether pre-hospital use of blood products is likely to confer benefit. This study compared the potential impact of pre-hospital resuscitation with blood components versus 0.9% saline in a model of severe injury.

Terminally anaesthetised Large White pigs received controlled soft tissue blunt injury, controlled haemorrhage (35% blood volume) with or without a primary blast injury followed by a 30-min shock phase. The animals were allocated randomly to one of the two injury arms (blast or sham blast). Within each injury arm the animals were allocated randomly to one of two treatment groups as follows: the shock phase was followed by a 60-min pre-hospital evacuation phase; comprising hypotensive resuscitation (target systolic arterial pressure 80 mmHg) using ***either*** 0.9% saline ***or*** blood components (PRBCs:FFP in a 1:1 ratio). Following this phase, an in-hospital phase involving resuscitation to a normotensive target (110 mmHg systolic arterial blood pressure) using PRBCs:FFP was performed in all four groups. A coagulopathy developed in both pre-hospital saline groups (increase in TEG [thromboelastography] R and K times and aPTT [activated partial thromboplastin time]) that persisted for 60 to 90 minutes into the in-hospital phase. The coagulopathy was attenuated in the pre-hospital blood component groups. Pre-hospital blood component resuscitation may therefore attenuate ATC.

Table of Contents

Declaration

This thesis is based on joint research. Each animal involved in this investigation required the concurrent work of at least 8 team members who worked on multiple constituent sections including: animal anaesthesia, animal monitoring, surgical intervention, blood sampling, management of blast, blood gas measurement and coagulation measurement. The author was responsible for sampling and running of all coagulations measurements and the consequent interpretation of all coagulation results.

Statement of Copyright

Acknowledgments

This project is part of a programme of work and research being undertaken at Dstl Porton Down where a large team of scientists at the Biomedical Sciences department works with dedication and focus to improve the medical care of all military casualties; soldiers, sailors and airmen. I am beholden to Dr Emrys Kirkman OBE, a senior scientist and my lead supervisor on this project, whose unwavering support has buoyed my work from inception to completion; his knowledge, expertise and nagging have been invaluable and I will be forever in his debt. I would also like to thank Dr Sarah Watts for her counsel and guidance on veterinary and animal work and as a valuable sounding board and second supervisor. Mr Bob Gwyther was a key member of the team and I am very thankful for his exceptional effort in providing statistical advice and support during the writing of this thesis. Other special recognition should go to the rest of the team who are too many to reference, although special mention goes to Charles Masey and Callie Wilson, both of whom have been invaluable on the subjects of clotting, coagulation measuring equipment and agriculture.

I am incredibly grateful to Professor Peter Mahoney CBE QHS, who was instrumental from the very start of this project and the design of this programme of research. Without his encouragement I would not have begun this stimulating journey I am now on.

Finally I must thank my family and friends for their eternal patience and who will be glad this thesis is complete. Their sage advice of “I suggest you just finish it” has been regularly ringing in my ears.

Dedications

For Ruth, Charlotte and Lily

Publications and Presentations Related to this Thesis

Principle Publication

Watts S, Nordmann G, Brohi K, Midwinter M, Woolley T, Gwyther R, Wilson C, Poon H, Kirkman E.
Evaluation of pre-hospital blood products to attenuate acute coagulopathy of trauma in a model of severe injury and shock in anaesthetised pigs.
Shock (2015); 44(1): 138-48.

Related Publications

Nordmann G, Jones A, Park C, Mahoney P.
The Presence of Acute Traumatic Coagulopathy in Ballistic Casualties in the Pre-Hospital Environment.
European Journal of Trauma and Emergency Surgery (in editorial review).

Woolley T, Thompson P,.., Nordmann G, et al.
Trauma Hemostasis and Oxygen Research Network position paper on the role of hypotensive resuscitation as part of remote damage control resuscitation.
Journal of Trauma and Acute Care Surgery (2018); 84(6) (S1): S3-13.

Nordmann G, Jones A, Park C, Mahoney P.
Acute Traumatic Coagulopathy is evident on severely injured military casualties within 40 minutes of injury.
Anaesthesia (2016); 71(S2): 68 (Abstract).

Parker P, Nordmann G, Doughty H
Taking transfusion forward.
Journal of Royal Army Medical Corps (2015); 161(1): 1 (Editorial).

Tobin J, Nordmann G, Kuncir E.
Pre-hospital Characteristics of Severely Injured Patients Transported in Operation Enduring Freedom / Op Herrick.
Journal of Special Operations Medicine (2015); 15(3): 72-75.

Curry N, Rourke C, Beer S,…, Nordmann G, et al.
CRYOSTAT. Early cryoprecipitate for major haemorrhage in trauma: a randomised controlled feasibility trial.
British Journal of Anaesthesia (2015); 115(1): 76-83.

O'Reilly D, Morrison J, Jansen O, Nordmann G, et al.
Initial UK experience of pre-hospital blood transfusion in combat casualties.
Journal of Trauma and Acute Care Surgery (2014); 77(3): S66-70.

Nordmann G, Woolley T, Doughty H, et al.
Deployed Research.
Journal of Royal Army Medical Corps (2014); 160(2): 92-98.

Lundy J, Oh J, Chung K, Nordmann G et al.
Frequency and relevance of acute peritraumatic pulmonary thrombus diagnosed by computed tomographic imaging in combat casualties.
Journal of Trauma and Acute Care Surgery (2013); 75 (2S2): S215-20

Keene D, Nordmann G, Woolley T.
Rotational Thromboelastometry (ROTEM) guided trauma resuscitation.
Current Opinion in Critical Care (2013); 19(6): 605-12.

Presentations

The Use of Blood Products in the Pre-hospital Environment.
International War and Disaster Medicine Conference, Estonia. Nov 17.

Beneficial effects of 'pre-hospital' vs immediate 'in-hospital' blood products during resuscitation in two models of severe military injury.
Trauma 2015, Gold Coast, Australia. Oct 15. Poster.

Acute Coagulopathy of Trauma is Evident in Severely Injured Military Casualties Within 40 Minutes of Injury.
Trauma 2015, Gold Coast, Australia. Oct 15.

Beneficial Effects of 'pre-hospital' vs immediate 'in-hospital' blood products during resuscitation in a model of severe military injury.
International Symposium on Intensive Care and Emergency Medicine, Brussels. Mar 15. Poster.

Military Damage Control Resuscitation, What We Do and Why.
Anesthetic Society of America. Annual Conference, San Diego. Nov 15.

Military Pre-hospital Resuscitation; MERT - Tactical Evacuation from the Battlefield.
Anesthetic Society of America, Annual Conference, San Diego. Nov 15.

Frontline Research and Blood Usage.
Uniformed Services Society of Anesthesiologists, San Diego. Nov 15.

Damage Control Resuscitation
Emergency Medicine Section Meeting, Royal Society of Medicine, London. Oct 13.

MERT; Bringing Damage Control Resuscitation to the Battlefield
Confédération Interalliée des Officers Médicaux Réserve, Winter Scientific Meeting, NATO HQ, Brussels. Jan 13.

List of Abbreviations

A

AAGBI	Association of Anaesthetists of GB and Ireland
α_2-AP	α_2-Antiplasmin
ABE	Arterial Base Excess
ABG	Arterial Blood Gas
ADP	Adenosine Diphosphate
ACIT	Activation of Coagulation and Inflammation in Trauma
ACT	Activated Clotting Time
ANOVA	Analysis of Variance
APC	Activated Protein C
APTT	Activated Partial Thromboplastin Time
ARDS	Acute Respiratory Distress Syndrome
AT	Antothrombin
ATC	Acute Traumatic Coagulopathy
ATLS®	Advanced Trauma Life Support
AUC	Area Under the Curve

B

BCSH	British Committee for Standards in Haematology
BD	Base Deficit
BE	Base Excess
BP	Blood Pressure
bpm	beats per minute

C

CA 5/10	Clot Amplitude at 5/10 minutes
CFT	Clot Formation Time
CGOs	Clinical Guidelines for Operations
CK	Creatine Kinase
CMV	Cytomegalovirus
CO	Cardiac Output
CO_2	Carbon Dioxide
COX-1	Cyclooxygenase-1
CPDA	Citrate Phosphate Dextrose Adenine
CRASH	Clinical Randomization of an Antifibrinolytic in Significant Haemorrhage
CT	Clotting Time
CvO_2	Mixed Venous Oxygen Content

D

Da	Daltons
DAMP	Danger Associated Molecular Pattern
DCR	Damage Control Resuscitation

DCS	Damage Control Surgery
DIC	Disseminated Intravascular Haemorrhage
DMS	Defence Medical Services
Dstl	Defence Science and Technology Laboratory
DVT	Deep Vein Thrombosis

E

ECG	Electrocardiogram
ED	Emergency Department
ELISA	Enzyme Linked Immunosorbent Assays
EPCR	Endothelial Protein C Receptor
$ETCO_2$	End Tidal CO_2

F

FI-XIII	(Clotting) Factors I to XIII
FIa-XIIIa	Activated Factors I to XIII
FBC	Full Blood Count
FC	Fibrinogen Concentrate
FDA	Food and Drug Administration (US)
FDP	Freeze-Dried Plasma or Fibrinogen Degradation Product
FDPs	Fibrin Degradation Products
FiiRST	Fibrinogen in the Initial Resuscitation of Severe Trauma
FlyP	French Lyophilised PLasma
FFP	Fresh Frozen Plasma
FWB	Fresh Whole Blood

G

GP	Glycoprotein
GSW	Gun Shot Wound

H

Hb	Haemoglobin
Hct	Haemotocrit
HEMS	Helicopter Emergency Medical Service
HES	Hydroxyethyl Starch
HMGB-1	High Mobility Group Box-1 (nuclear protein)
HR	Heart Rate
HTS	Hypertonic Saline
HSD	Hypertonic Saline Dextran

I

ICP	Intracranial Pressure
IED	Improvised Explosive Device
ICU	Intensive Care Unit
IL	Interleukin

INR	International Normalised Ratio
IQR	Inter Quartile Range
IRT	Immediate Response Team
ISS	Injury Severity Score

L

Li/Ly30	Lysis at 30minutes
LP	Lyophilised Plasma
LSD	Least Significant Difference

M

MA	Maximum Amplitude
MAC	Minimum Alveolar Concentration
MAP	Mean Arterial Pressure
MATTERS	Military Application of Tranexamic Acid in Trauma Emergency Resuscitation
MCF	Maximum Clot Firmness
MERT	Medical Emergency Response Team
MERT-E	Enhanced MERT
ML	Maximum Lysis
MTF	Medical Treatment Facility
MT	Massive Transfusion
MTP	Massive Transfusion Protocol
MW	Molecular Weight

N

NCEPOD	National Confidential Enquiry into Patient Outcome and Death
NHS	National Health Service
NHSBT	NHS Blood and Transplant
NICE	National Institute for Health and Care Excellence
NISS	New Injury Severity Score
N_2O	Nitrous Oxide

O

O_2	Oxygen
OCS	Open Canalicular System
OER	Oxygen Extraction Ratio
OR	Operating Room

P

$PaCO_2$	Arterial Partial Pressure of CO_2
PaO_2	Arterial Partial Pressure of O_2
PAR	Protease Activated Receptor
PATCH	Pre-hospital Antifbrinolytics for Traumatic Coagulopathy and Haemorrhage

PCC	Prothrombin Complex Concentrate
PE	Pulmonary Embolus
PAI-1	Plasmin Activator Inhibitor 1
POC	Point of Care
PRBC	Packed Red Blood Cells
PROMMT	Prospective Observational Multicenter Major Trauma Transfusion
PROPPR	Pragmatic Randomized Optimal Platelet and Plasma Ratios
PT	Prothrombin Time

Q

QC	Quality Control

R

RAC	Resuscitation Associated Coagulopathy
RAF	Royal Air Force
RAP	Right Atrial Pressure
RBC	Red Blood Cell
RCT	Randomised Controlled Trial
RETIC	Reversal of Trauma Induced Coagulopathy
RISC	Revised Injury Severity Classification
ROC	Resuscitation Outcome Consortium
ROTEM®	Rotational Thromboelastometry
RTC	Road Traffic Collision

S

SAGM	Saline Adenine Glucose Mannitol
SaO_2	Arterial Oxygen Saturation
SBP	Systolic Blood Pressure
SEM	Standard Error of Mean
SIRS	Systemic Inflammatory Response Syndrome
STAAMP	Study of Tranexamic Acid during Air Medical Prehospital Transport
sVEGFR1	Soluble Vascular Endothelial Growth Factor Receptor 1
SWB	Stored Whole Blood

T

TACO	Transfusion Associated Circulatory Overload
TAFI	Thrombin Activatable Fibrinolysis Inhibitor
TARN	Trauma Audit and Research Network
TBI	Traumatic Brain Injury
TEG®	Thromboelastograph(y)
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
TIC	Trauma Induced Coagulopathy
TIVA	Total Intra-Venous Anaesthesia

TM	Thrombomodulin
TNFα	Tissue Necrosis Factor alpha
t-PA	Tissue Plasminogen Activator
TPAI	Tissue Plasminogen Activator Inhibitor
TRALI	Transfusion-Related Acute Lung Injury
TRISS	Trauma Injury Severity Score
TXA	Tranexamic Acid

U

UK	United Kingdom
USA	United States of America

V

vCJD	Variant Creutzfeldt-Jakob Disease
vWF	vonWillebrand Factor

W

WB	Whole Blood
WBC	White Blood Cells

Chapter 1. Introduction

My stomach lurched into my mouth as we dropped from a great height down toward the ground and hopeful cover. I peered out of the window to see a high riverbank above us as we flew towards the incident, hugging the river valley and skimming low over the river itself. Leachy and I spread the insulation blanket on the ground ready to receive the casualty; monitoring on my right, blood and plasma on my left – already run through the warmer – ready to go. Drug and intubation bags on the seating to my right hand side and crucially within reach. Ventilator and oxygen cylinder on my left.

The other team members worked hard to clear the rear ramp area of bodies and equipment. The previous job had been unpleasant and the mood on board was heavy with depression. They stacked the body bags on the starboard side bench to make room for the next set of what we all hoped would be live casualties. I left them to their thoughts, I knew they would switch on and be ready for anything when the need arose.

A sharp deceleration and nose up tilt; we were there. One second we were looking back out at the diminishing river valley, green fields and even more distant mountains, the next second the ground seemed to tilt beneath us and we stared at desert almost touching distance away. The forward loadie opened up the minigun; high pitched rotations, rapidly followed by the low timbre of hundreds of rounds towards an unseen enemy. It stopped as the brown out surrounded us; sand kicked up by the landing aircraft's downwash hindered any further vision. It stops the pilots from knowing where the ground is and it floods the rear cabin covering the equipment, and us; blinding everyone. It didn't stop the enemy though, as we provided them with a nice fat target. I failed to detect the "crack and thump" of incoming small arms fire, only seeing the sparks as they hit off what I hoped were inessential electrics in the ceiling. I was yet again, thankful for the ballistic protection on the aircraft's sides.

Millsy was off in a shot, in to the murk and cloud of sand to find the casualties. He was back with a team of US Marines carrying two stretchers unbelievably quickly; they did not look keen to stay long. He had done the usual excellent job of microsecond triage and they dumped the worst casualty with Leachy and I. A quick thumbs up meant he was happy to initially manage the other one by himself. As soon as the Marines were off and clear we immediately lurched skywards. The minigun opened up again and from the corner of my eye an RPG flew past the open rear ramp – how close?

The pilots were working hard to avoid small arms fire and RPGs and threw the aircraft from one side to the next, as we did what we could to determine how our patient was. At this stage a rough overview was all that was possible, at least until they managed to achieve some steady flight. Bilateral high lower limb amputee with right arm amputated through the humerus. His left arm was severely damaged with clear open fractures to humerus and forearm; I hoped it was salvageable. There were no obvious thoracic or abdominal injuries and the ground medics had tried and failed to insert a sternal intra-osseus needle, leaving a distinct subcutaneous swelling over the only area I had left as a place for access.

The whites of his eyes stared out of a blackened, filthy face, he couldn't be older than 20. Unresponsive to voice and pain, he was barely maintaining his airway and breathing pattern was intermittent, no more than 3 or 4 a minute. No carotid pulse. Monitoring failed on blood pressure and oxygen saturation but the defibrillator pads picked up a bradycardic trace, with occasional ectopics. He was barely alive; in a low output state due to trauma. Lack of adequate blood to his brain causing reduced consciousness, and now his heart was beginning to fail. He was salvageable, but how?

I looked at Leachy who had done his usual superb job of stopping any external haemorrhage, first job done. In his hand was the end of the fluid line ready to go. Replacement of blood volume was crucial and its priority

was over and above the airway. I remembered we had put some Swan sheath introducers into the extra kit box; they were the main stay of large access in the hospital. It was his only option. I amazed myself as to how easy it was, putting in at least one a day for the last few weeks probably helped, and the vibrations from the aircraft had little impact on finding the vessel and the subsequent Seldinger technique. Not the cleanest central access procedure, but life saving, and just in time as he was becoming increasingly dysrhythmic. The team worked hard and unexpected charity and help emerged from the cockpit, the RAF padre had been shadowing the crew. He amazed us with; "I'll hold that blood for you, I used to be an ITU nurse!" and we had a new crucial team member. 4 units of blood and plasma, intubation, ventilation, (a little) anaesthesia and a 20-minute flight is easily written but the memory of that flight, the pile of bodies laid out at the back of the cabin, and the fight for his life is etched in my memory.

Having handed over an alive, tachycardic patient with a femoral pulse to the trauma team, we pondered exhaustion and sorting out sand covered kit. We talked through the resuscitation and discussed where we could have improved. There is no possible way litres of crystalloid would have had the same effect. Blood products were an essential, integral part of what kept that boy alive, but mounting pressure was on us to stop taking it forward out of the hospital – to reduce wastage and perceived potential risks of giving it to patients outside of the hospital environment. That debate would have to wait for another time though as we got the next call out, "IED, Sangin, 2 casualties. MERT to flight line". (Nordmann 2010).

Worldwide trauma is a major public health problem accounting for nearly 6 million deaths annually (WHO 2010). In England and Wales approximately 16,000 people per annum die following serious injury (ONS 2018). During 9 years of conflict in Iraq and Afghanistan over 2,000 service personnel were injured in hostile action, 65% of which were from explosions (Penn-Barwell 2015). Uncontrolled post-traumatic bleeding is the leading cause of potentially preventable death among injured civilian patients (Cothren 2007)

with exsanguinating haemorrhage causing approximately 50% of those military casualties who die as a result of ballistic injuries (Champion 2003).

One third of all severely injured civilian trauma patients have abnormal clotting (Brohi 2003, Maegele 2007, Frith 2010) and the prevalence of this increases to almost 40% in trauma caused by ballistic and blast injuries (Woolley 2013). The early clotting abnormality found in trauma casualties, termed the Acute Traumatic Coagulopathy (ATC), has been found to be associated with an approximately 50% mortality in addition to larger transfusion requirements, septic complications, organ injury and critical care stay (Brohi 2003, MacLeod 2003, Maegele 2007). Management of ATC in the UK has improved significantly over the last decade with survival from trauma increasing in both UK civilian (Moran 2018) and military patients (Penn-Barwell 2015).

The Damage Control Resuscitation (DCR) techniques used within the hospital setting to manage these casualties have been well described (Holcomb 2007, Jansen 2009). In Afghanistan they were taken forward onto the evacuation aircraft (the Medical Emergency Response Team; MERT) with the addition of pre-hospital physicians in 2006 and then blood components in 2008. These changes were driven by expert opinion rather than evidence and initial resistance in the clinical community was surprising but evident. Data collection was a challenge in that environment and administrative support to studies lacking. Initial reports struggled to find proof of its efficacy and were mostly discursive (Kehoe 2011, Calderbank 2011) or descriptive (Nordmann 2016). The exact impact of the pre-hospital capability was lacking but there were many aspects to the clinical practice that could be attributed to the impact of the MERT – albeit an impact as judged by the receiving physicians' clinical opinion.

In order to isolate and examine the independent effect of pre-hospital blood in a military pre-hospital environment, an animal model needed to be used, so that the other confounding factors could be standardised. An existing animal model of ballistic trauma in use at the Defence Science and

Technology Laboratory (Dstl) Porton Down was adapted to mirror the timelines encountered on the patient pathway in Afghanistan and included blast and ballistic injury.

The body of work in this thesis explores the background of Acute Traumatic Coagulopathy and the use of blood components in the resuscitation of all trauma, but particularly military trauma, casualties. It describes the mechanisms and pathophysiology behind wounding in civilian and military populations, and the resuscitation regimes used in both domains. It goes onto describe coagulation, the equipment used to evaluate coagulation and the coagulopathies observed in trauma patients. The thesis then describes the programme of work undertaken at the Dstl, Porton Down, where an animal model of shock, haemorrhage, ballistic injury and blast injury was used to determine the effect of different pre-hospital resuscitation regimes. For this thesis these resuscitation regimes were pre-hospital saline compared to blood and plasma in a ratio of 1:1. The ultimate aim being to determine if a trauma induced coagulopathy is attenuated by the use of pre-hospital blood component resuscitation.

The work has been approved by the Stakeholders for the Combat Casualty Care research programme and endorsed by the Defence Medical Services Research Strategy Group. The study was conducted in accordance with the Animals (Scientific Procedures) Act 1986 and entailed Ethical Review and permission. The project was forwarded for review to the Home Office Inspectorate and a Project Licence was granted, a legal mandatory step for such investigations.

Chapter 2. Military Resuscitation Strategies

2.1 Counterterrorism Campaign

The response of America and its allies to the terrorist attacks of September 11th, 2001 had far reaching global consequences for security, human rights, international law, international co-operation and military conflict. The principal military impact was the American-led, global, multidimensional counterterrorism campaign that included within it major wars in Afghanistan and Iraq, leading to the longest sustained conflict for the UK and USA, since World War 2.

These prolonged conflicts have led a transformation in trauma care in both the military (Blackbourne 2012, Butler 2012) and civilian medical environments (Howell 2014). For the UK Armed Forces the focus of the military effort was in Helmand Province, Afghanistan and led to casualties that were high in number and severity. There was a significant and intensive medical effort to deal with them. The form of combat changed from direct small arms fire to the predominant use of improvised explosive devices (IEDs) and in response to this, the medical capability in Helmand improved substantially from a small tented medical treatment facility to a purpose built trauma hospital (in Camp Bastion) supported by a 24 hour pre-hospital evacuation team capable of advanced Damage Control Resuscitation at the “front line”.

2.2 Military Trauma Care

Control of haemorrhage particularly in extremities has been shown to have a pivotal role in the management of military casualties, and the ability to speedily correct it has had a significant impact on preventable deaths. Data

from Vietnam showed limb wound haemorrhage accounted for more than half of all potentially preventable deaths (Bellamy 2005). More current reviews of recent conflicts have estimated that 50% of all potentially preventable battlefield deaths are caused by haemorrhage (Champion 2003).

The UK Defence Medical Services answer to this was the development of separate novel haemostatic products during the recent Afghanistan conflict, which included new field dressings, active haemostatic agents and self-applicable tourniquets (Mahoney 2005). Just as important was the revision of the emergency care paradigm to <C>ABC, in recognition that the leading cause of combat casualty death is external haemorrhage, where <C> stands for catastrophic haemorrhage and its management (Hodgetts 2006). The lessons learnt from contingency operations in the 1990s by the USA were utilised for this model. These included a phased methodology of trauma care for the military, identified according to tactical threat (Butler 1996) that commenced with Care Under Fire (ongoing direct threat from small arms fire or imminent blast). Next came Tactical Field Care (threat diminished but treatment ongoing in a resource restricted environment) and Combat Casualty Evacuation Care (when the casualty is in transit from incident to next level of medical care). In recognition of the importance of catastrophic haemorrhage, the <C>ABC algorithm is used in the four stages of UK military trauma care. These four stages represent the increasing experience and clinical capability of the successive teams in addition to the increasing sophistication of their equipment: Care Under Fire, Tactical Field Care, Field Resuscitation (care at the first role of military medical care which includes a primary care doctor and medics) and Advanced Resuscitation (consultant led teams at a medical treatment facility (MTF)).

Military hospital based trauma care has transformed from Advanced Resuscitation into Damage Control Resuscitation (DCR) in recognition of the intricate relationship resuscitation and surgery have, in order to save the casualty. Indeed it is now deemed that Damage Control Surgery and Resuscitation are not separate entities but concurrent activities, with the

resuscitation team's aim to get the casualty to a normal physiology. Damage Control Resuscitation integrates permissive hypotension, homeostatic resuscitation and Damage Control Surgery (Jansen 2009) within it.

2.2.1 Damage Control Resuscitation

Damage control itself is a term borrowed from the Navy where it was used to describe the ongoing repair of a damaged ship at sea, in order to reduce the size of damage. Its use was coined for Damage Control Surgery (DCS) by military and civilian trauma surgeons and describes the few life saving procedures (typically involving an abbreviated laparotomy), that are required in order to save the patient's life (Rotondo 1993). These are commonly not definitive and, like a damaged ship returning to port, the injured patient will need definitive surgery and full anatomical reconstruction at a later time once their physiology is improved. Physiology improvement is the resuscitation aim and DCS is an integral part of the DCR process.

Damage Control Resuscitation has a number of sub-sets within it and it is beyond the scope of this chapter to discuss these all in detail. Anaesthesia provision is a key aspect of any trauma resuscitation, not just in the securing of an airway but the method in which anaesthesia is provided for surgical intervention and post-operative critical care provision. The correct type and dose of anaesthetic and how this is changed throughout the phases and locations of DCR, from Emergency Department (ED) to Intensive Care Unit (ICU) through the Operating Room (OR), depend on a multitude of factors from type of injury, patient physiology to type of surgical procedure required. It is guided by many factors, some of which are discussed further below, but the main aim is facilitating the normalisation of physiology and reversing the ongoing pathophysiological processes (coagulation and otherwise) caused by the patient's injury. The vagaries of trauma anaesthesia would easily occupy a 5-day educational course and regrettably there are very few peer-reviewed papers that discuss it in detail

(Ortega-Gonzalez 2012, Sikorski 2014). Anaesthesia, imaging, DCS, ICU care and many others are all intimately involved and important parts of the DCR process but will not be mentioned further.

Initial descriptive papers on DCR; define the initial theories behind it. Holcomb (2007) described it as a "*proactive early treatment strategy that addresses the lethal triad on admission to a combat hospital*". Hodgetts (2007) defined it as a "*systematic approach to trauma combining the <C>ABC paradigm with a series of clinical techniques from point of wounding to definitive treatment in order to minimize blood loss, maximize tissue oxygenation, and optimize outcome*."

Fluid resuscitation is one of the features of DCR as is blood pressure control and consequently these two aspects are the main goals resuscitators concentrate on; Permissive Hypotension and Haemostatic Resuscitation. Many authors include other aspects to DCR, including (and not exclusively) rewarming, correction of acidosis and restrictive fluid administration (Holcomb 2007, Jansen 2009, Kaafarani 2014). Rewarming is an active and basic part of anaesthesia care, restrictive fluid administration is part of hypotensive resuscitation and correction of acidosis is one of the main aims of DCR as a whole and will only occur with adequate resuscitation (and anaesthetic techniques). Consequently, these won't be discussed separately. Many authors and medical practitioners discuss Massive Transfusion (MT) as an integral aspect of DCR. The basics of MT are closely related to haemostatic resuscitation and will be briefly considered below

2.2.2 Hypotensive Resuscitation

Sometimes with the added pronoun of *restrictive* or *permissive*, Hypotensive Resuscitation is the strategy of restricting fluid administration until haemorrhage is controlled, by definitive surgical control or otherwise. This is completed while accepting a limited period of suboptimal end-organ

perfusion (Jansen 2009). The aim is to keep systolic blood pressure below normal levels in an attempt to avoid disturbance of any clot formed over the injury site, especially within the first hour (Stern 1993). The key time many have used is the first 60 minutes as it is at this time that any undisturbed clot will have achieved greater than approximately 80% of its tensile strength (Shen 1983). The target systolic blood pressure is approximately 80 mmHg as this was deemed to be a pressure that wouldn't "*pop the clot*" (Sondeen 2003, Krausz 2006).

This method had been adopted by many military medical services both in the UK (Greaves 2002, Battlefield Advanced Trauma Life Support, BATLS 2006) and in the US (Holcomb 2003, Krausz 2006). It is also recommended by NICE who endorsed that in the pre-hospital environment aliquots of fluids should be administered to maintain a radial pulse (NICE: Technology Appraisal Guidance 74, 2004). However things have progressed since the early 2000's.

It must be kept in mind that sustained hypotension leads to an oxygen debt that can and will cause organ injury. The level of oxygen debt is commonly measured by base deficit. A worsening base deficit is associated with injury severity, coagulopathy and mortality (Frith 2010). Oxygen debt activates inflammatory pathways leading to a rise in IL-6, IL-8, TNF (Martin 1997) and HMGB-1 (High Mobility Group Box nuclear protein-1) (Cohen 2009); all predictors of systemic inflammatory response (SIRS; Giannoudis 2008), renal failure, acute lung injury (Cohen 2009), infection (Claridge 2000), multiorgan dysfunction (Kobbe 2008) and death (Martin 1997, Cohen 2009). A balance therefore needs to be made between sub-optimal perfusion, minimising blood loss and ensuring clot stability.

A number of animal investigations have examined this issue using ovine (Sakles 1997), porcine (Stern 1993), and rodent (Burris 1999, Smail 1998, Capone 1995) models. These were evaluated by a systematic review of all animal models (Mapstone 2003), which concluded that hypotension reduces blood loss and mortality. Unfortunately the majority of these models employ

an experimental method that reduces the validity of their findings. The injury sustained was a vessel injury, which is more sensitive to re-bleeding and the anaesthetised animal received a vasodilatory anaesthetic method. Despite the limitations of these studies, their results supported that early, aggressive crystalloid resuscitation is detrimental.

Clinical human studies have found it challenging to demonstrate clear benefits for hypotensive resuscitation. Two studies investigated delayed resuscitation and found that an early resuscitation technique had an increased mortality (Bickell 1994, Schreiber 2015). Bickell (1994) found in penetrating torso injuries delaying aggressive fluid resuscitation (a hypotensive approach) improved outcomes, while Schreiber found that blunt (and not penetrating) injuries may benefit from a controlled, initially hypotensive resuscitation regime. Regrettably the timelines in both were short (less than 60 minutes), both used crystalloid resuscitation and the latter did not achieve significantly different blood pressures (105 vs 98.7 mmHg). Dutton (2002) randomised 110 patients to a hypotensive (70 mmHg) or normal (>100 mmHg) blood pressure goal for resuscitation and found no difference in mortality between groups. The actual blood pressures achieved though were 100 mmHg and 114 mmHg respectively, so a pronounced blood pressure difference was not achieved. Morrison (2011) seemed to have the most promising results initially. They published preliminary findings of a randomised control trial of in-hospital trauma patient resuscitation for treatment of haemorrhagic shock, randomising to mean arterial pressures of 50 or 65 mmHg. Their initial findings showed the hypotensive group had a reduced blood loss and a tendency towards lower mortality. Again, although the intent was there, the mean arterial pressure in the two groups was not statistically different and due to futility and insufficient clinical equipoise (as they found no difference in patient survival and the majority of the studies secondary outcomes) the study was terminated (Carrick 2016).

Despite a lack of strong evidence from human studies to support a hypotensive resuscitation approach, many national guidelines (Spahn 2013,

NICE NG 39 2016) have now adopted it for resuscitation of bleeding patients. This general acceptance of the hypotensive approach is presumably due to the positive results from animal studies and the appeal of not worsening haemorrhage. The problem the military face is that its medical evacuation chain is not guaranteed and long pre-hospital times with a delayed access to surgery is commonplace.

Some have argued that 80 mmHg is too low a target. A paper from World War II (Emerson 1945) studied those battle casualties admitted to a field hospital and found that those arriving in severe shock (systolic blood pressure <85 mmHg) had a mortality of 35% compared to 11% in the higher blood pressure group. The authors stated that a systolic blood pressure (SBP) of 80-90 mmHg was an acceptable target. Although somewhat old, the key aspects of this paper are relevant to the military audience still with prolonged timelines and a predominantly young male patient group.

Two further retrospective papers suggest a higher blood pressure is required. The data of 48,000 blunt trauma patients' records from the UK trauma registry was scrutinised (Hasler 2011). The authors found that the odds of dying increased below a SBP of 110 mmHg and doubled below a SBP of 100 mmHg. In the second paper, the US National Trauma Data Bank was analysed to look at 871,000 patients (excluding traumatic brain injury [TBI]) (Eastridge 2007) and the authors found a correlation between admission SBP, base deficit and mortality. For every 10 mmHg drop in SBP below 110 mmHg there was a 4.8% increase in mortality. They concluded that a "*SBP ≤ 110 mmHg is a more clinically relevant definition of hypotension and shock than 90 mmHg*". There appeared to be correlation in the findings from two different continents with similar well-developed trauma systems.

Patient age though was over-looked. It plays a significant part in these civilian studies. Older patients have cardiovascular co-morbidities that make them unable to tolerate lower blood pressures; especially when up to two-thirds of some age groups could have hypertension (Stomatos 1994)

and the elderly trauma patient is twice as likely to die (Lonner 1995) compared to younger patients.

It is likely any study involving older patients will therefore show a higher blood pressure would be required to resuscitate them successfully. A follow up study re-examined the American National Trauma Data Bank (Oyetunji 2011) as the authors suggested the previous papers might be affected by analytical bias. They studied nearly a million records and optimally defined hypotension as being the model with the highest area under the receiver operating characteristic curve. The optimal SBP value for patients aged 18 to 35 years was 85 mmHg, 96 mmHg for patients 36 to 64 years and 117 mmHg for elderly patients. They concluded "*for patients younger than 65 years, the classical definition of hypotension (is) less than 90 mmHg*". This latter paper gives pertinent advice to those treating a younger adult patient group, such as the military casualty.

Military medical care has to deal with the further problems of extended timelines and blast injuries. Experimental studies, in anaesthetised pigs, undertaken by Garner (2010) have investigated hypotensive resuscitation (SBP goal of 80 mmHg) and the impact of blast in a model that involved liver injury in contrast to vessel injury. During the first hour, the degree of shock, as measured by base excess, increased. With further time the degree of shock became devastating. This was accentuated if performed on a background of combined hypovolaemia (in this case, an experimentally controlled blood loss) and blast injury, rapidly leading to increased mortality (Garner 2010). The response to hypotensive resuscitation for the first hour was tolerable, so they devised a new resuscitation concept. This "novel, hybrid resuscitation" concept was an initial 60 minutes of hypotensive resuscitation followed by normotensive resuscitation in attempt to improve tissue perfusion and oxygenation. This new resuscitation concept worked, and was found to improve survival in their porcine military trauma model (that incorporated blast injury and blood loss) and also significantly extended the evacuation times (Doran 2012). Novel hybrid resuscitation was incorporated into UK military pre-hospital guidelines (CGOs 2017) for

the treatment and resuscitation of traumatic haemorrhage in 2012. Variations on this theme have been adopted into many national and international guidelines, all with an initial hypotensive goal as part of trauma resuscitation. Although most don't specify exact timings, there is a recognition that initial hypotension is warranted for a limited period of time. These include European guidelines (Rossaint 2016) recommending a "*SBP of 80-90 mmHg until major bleeding has been stopped*" and NICE guidelines (NICE NG39, 2016) on trauma resuscitation in both hospital and pre-hospital advising that practitioners should "*titrate volume resuscitation to maintain a palpable central pulse (carotid or femoral)*".

In summary, the majority of research evidence and clinical experience suggests that a lower blood pressure should be targeted early in order to reduce bleeding and preserve the initial clot. Nevertheless, this needs to be limited to reduce too great an ischaemic injury to tissues. Duration and level of hypotension is yet to be clearly defined, but it is recognised that this is a risk:benefit decision depending on a number of factors including pre-existing co-morbidities including cardiovascular diseases, patient age and traumatic brain injury. These patients will all be more vulnerable to an ischaemic injury.

Hypotensive resuscitation is a relevant and crucial part of DCR in order to minimise blood loss before definitive haemorrhage control. In civilian practice and the older population a SBP goal of around 100 mmHg is becoming a more accepted value (Woolley 2018), although it has not made it into any recognised guidelines. In military practice and the younger population a SBP of 80-90 mmHg is acceptable and in more austere locations or prolonged evacuation a return to normotension or at least a SBP of 100 mmHg is what I would aim for in practice. Nevertheless strict adherence to blood pressure goals is probably unnecessary, and instead the aim should be for adequate flow and a trend (of cardiovascular and biochemical indicators) in the right direction. Strictly adhering to the 60 minute threshold is probably unwarranted if near patient blood gas testing

was possible, meaning in this younger patient group changing the SBP goal could be guided by base deficit.

2.2.3 Haemostatic Resuscitation

Haemostatic resuscitation is the process of restoring and sustaining normal tissue perfusion to the patient presenting in uncontrolled haemorrhagic shock, with an emphasis on preservation of effective clotting (Dutton 2012). The basis of it has been driven by the military experience of combat casualty care in Iraq and Afghanistan in addition to scientific research carried out in civilian trauma centres.

The foundation of haemostatic resuscitation lies in the recognition of the pathophysiology of haemorrhagic shock and particularly Acute Traumatic Coagulopathy (ATC). Injury causes damage to tissues; disruption of blood vessels and organs causes haemorrhage and a decreased cardiac output. The sympathetic response to this causes vasoconstriction to non-essential tissues (Dutton 2007) which when coupled with bleeding and reduced cardiac output causes significant tissue hypoperfusion and oxygen debt. Hypoperfused cells release hundreds of mediators (Reilly 1993), toxins, inflammatory proteins (Chaudry 2009) and the by-products of anaerobic metabolism including lactate and potassium. Protein C activation as a consequence of hypoperfusion is fundamental to the development of ATC and its fibrinolysis (Brohi 2008). It is now well recognised that severely injured trauma patients will be coagulopathic independent of any iatrogenic cause (Brohi 2003, Hess 2009). Coagulopathy leads to further haemorrhage and ischaemia progression and consumption of essential elements of haemostasis (e.g. fibrinogen in the early stages (Rourke 2012)). The goal of resuscitation is to restore normal oxygen delivery and reversing the pathophysiological causes of ATC; those trauma patients who exsanguinate with surgically repairable injuries do so with an overwhelming coagulopathy (Cosgriff 1997). There are two aspects to this, which are closely inter-linked; coagulation support and restoring tissue perfusion.

2.2.4 *Coagulation Support*

Early support of coagulation is needed and consequently those fluids that do not fulfill this role – crystalloids and colloids – have no role in DCR (see Chapter 6 for further detail). In the hospital environment immediate transfusion of PRBC, plasma (FFP), platelets and fibrinogen must occur. This needs to be planned for and occur before a complete picture of the patient's physiology and injuries are fully understood and assessed.

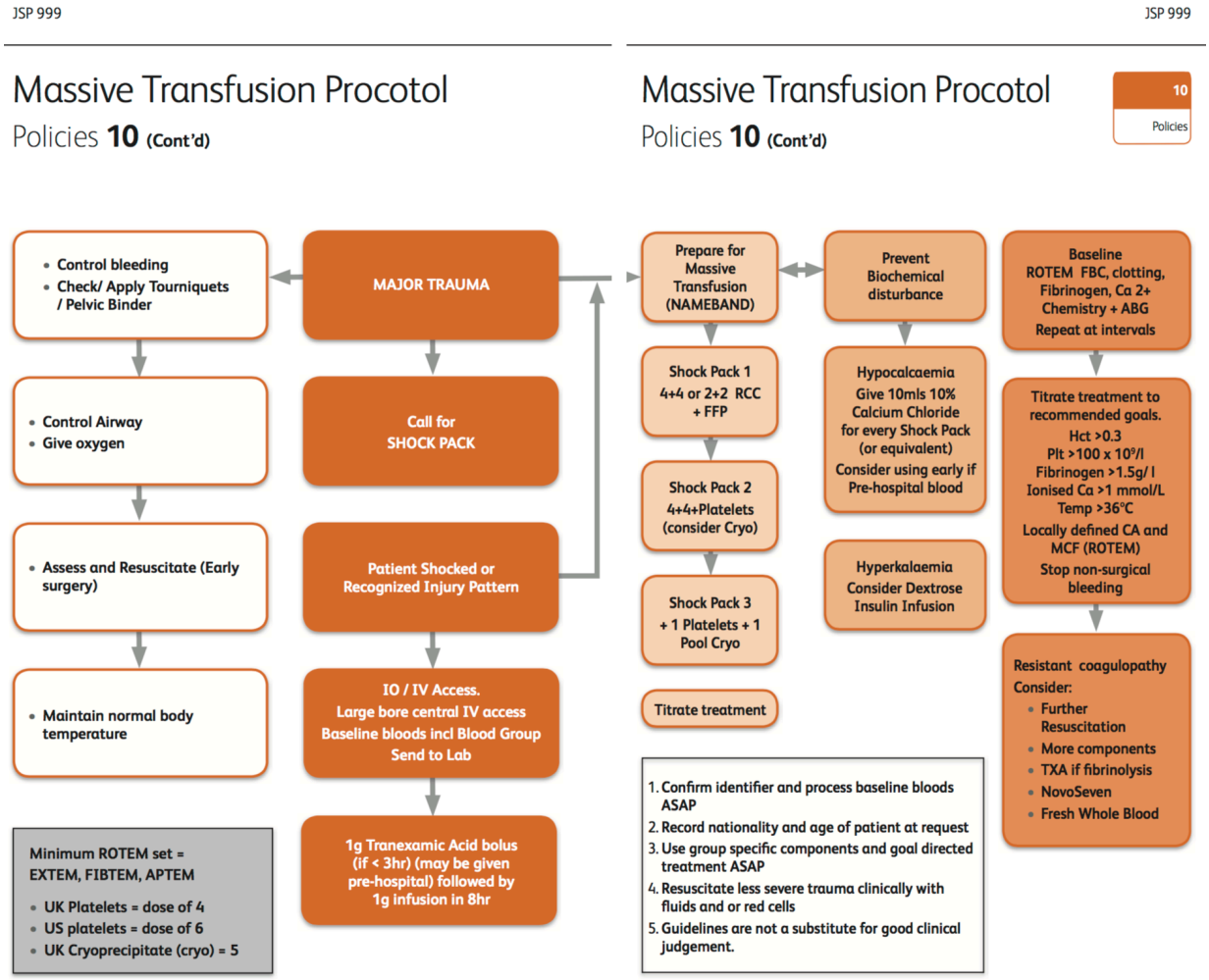


Figure 2-1. Military Massive Transfusion Protocol taken from Clinical Guidelines for Operations, Joint Service Publication (JSP) 999.

Logistics are key and in order to ensure the right blood components are available 24 hours a day the majority of trauma centres have developed massive transfusion protocols (MTPs) (Cotton 2008). These provide a set amount of PRBC, FFP and platelets (and sometimes cryoprecipitate) to the required location in the hospital, with additional help in the form of porters and laboratory staff to enable continued support. The military MTP is typical example (Figure 2-1).

With arterial pressure kept to an acceptable minimum until haemorrhage is controlled, an example of how this element of resuscitation should proceed is illustrated in Figure 2-2.

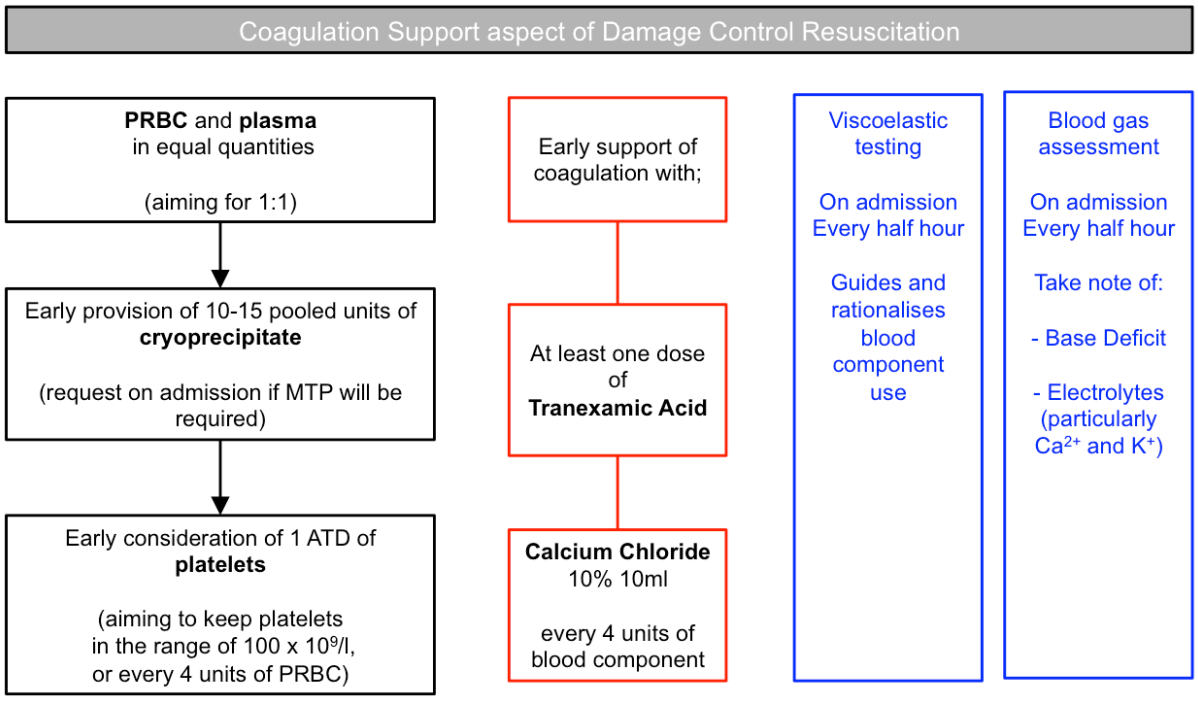


Figure 2-2. The Coagulation Support aspect of Damage Control Resuscitation.

2.2.5 Restoration of Tissue Perfusion

The pathophysiological basis of Acute Traumatic Coagulopathy lies at the level of the endothelium and hypoperfusion of tissues (see Section 4.10 for further details). Restoration of tissue hypoperfusion is the drive to reverse this. There is however little scientific evidence to support this aspect of DCR. It has been briefly described in a few papers (Dawes 2009, Hauser 2010, Giannoudis 2016) and the evidence of its effectiveness in the operating theatres of Afghanistan has been previously published (although not described in full detail) (Morrison 2013a). This latter study was a retrospective assessment of 59 patients admitted to the hospital in Camp Bastion between 2008 and 2010 suffering severe blast injuries, with a combined predicted survival of 50.9%. Intra-operative changes were noted to be; pH 7.19 to 7.45, Base Excess -9.0 to +4.5 mEq/l and Prothrombin Time decreased from 18 to 14 seconds. The method of resuscitation (tissue perfusion restoration) employed by the teams in Afghanistan improved outcome with survival increasing from 50.9% (predicted) to 90% (actual).

The basis of restoring tissue perfusion is the reversal of the sympathomimetic response and consequent vaso and veno-constriction. This is implemented with anaesthetic agents to reduce sympathetic outflow and dilate the constricted vascular beds. The right agents for this (inhalational agents and opioids) are not administered immediately as in all likelihood their significant vasodilatory effect in addition to positive pressure ventilation would have a disastrous effect on venous return and be potentially fatal for a severely injured patient. My approach, as a trained military anaesthetic Consultant, is a ketamine (one of the more cardiovascularly stable anaesthetic agents) induction with a small dose of benzodiazepine, followed by a Ketamine/Midazolam infusion. As resuscitation proceeds additional anaesthetic agents (inhalational) and analgesics (opioids) are titrated carefully. It is not uncommon to alternate anaesthetic titration (fentanyl boluses of 50-100μg or inhalational agent

increases of 0.1-0.2 Minimum Alveolar Concentration [MAC]) with blood component boluses (of 100-500ml) judging the effects of both by the resultant SBP and base excess changes. The resultant increasing perfusion to organs and peripheral tissues should reverse coagulation abnormalties, reduce fibrinolysis and inflammatory compound release without exacerbating further haemorrhage. The ultimate aim being to achieve a high flow, low-pressure cardiovascular system and vasodilated patient with stable blood pressure and normalised pH, base deficit, electrolytes and coagulation.

2.3 Military Pre-Hospital Trauma Care

The lack of land evacuation options, increasing injury severity of casualties and troops spread over large distances led the UK to develop its forward medical evacuation in 2006; 16 Air Assault Brigade was spread across the Helmand province with road travel restricted, lengthy and dangerous. The Army clinicians at the hospital in Camp Bastion decided to bolster the RAF Immediate Response Team (IRT) aircraft with a Consultant Emergency Physician or Anaesthetist. The IRT at that time was used to support ground troops with a variety of specialists from explosive technicians to medical personnel. This increase in medical capacity and capability developed quickly into the present Medical Emergency Response Team (MERT) model of a 4 man team led by a Consultant and including Paramedics and an emergency nurse practitioner.

The goal of this manning was to bring forward the capability to initiate DCR earlier, bringing it out of the hospital to the patients at the front line. The medical services were then able to by-pass the role 1 general practitioners and bring damage control resuscitation to the battlefield. By 2006 there was a dedicated medical helicopter (CH-47 Chinook); a large support helicopter able to fly with speed and carry a large numbers of casualties plus the equipment to treat them. By 2008 Packed Red Blood Cells (PRBC) and

Fresh Frozen Plasma (FFP) was carried by the aircraft 24 hours a day, 7 days a week.

The medical techniques and procedures used on the MERT are similar to or exactly the same as that found on any civilian pre-hospital air ambulance within the UK, with a few exceptions. Personal danger is ever present and not unexpected, meaning the location in which medical interventions took place had to be on board the aircraft. Civilian pre-hospital teams complete their interventions at the site of injury and then package the patient for transfer to the next level of care. The risk of small arms and indirect fire meant that the MERT carried out all its interventions whilst in transit to the next level of care. This introduces additional challenges to communication (noisy environment), fine motor skills (excessive high frequency vibrations) and diagnostic skills (it is difficult to feel a radial pulse in the presence of the vibrations found on a CH-47 Chinook and an automated non-invasive blood pressure monitor will struggle to find an accurate blood pressure particularly if it is low). Motion sickness can incapacitate even the best clinician.

A larger team meant a greater number of casualties could be managed, which is useful when the aircraft can take up to 20 or more casualties, compared to the usual one carried on civilian aircraft. Lastly and most importantly was the ability to take blood components into the pre-hospital space.

2.4 Military Pre-Hospital Resuscitation

Treating a trauma patient with active haemorrhage in the pre-hospital environment requires a more nuanced approach. Delivering the least cardiac output necessary to sustain vital organ function is required, whilst attempting to preserve and support haemostasis. The threshold of lethal (or organ specific) ischaemia is heterogeneous across the population; so early resuscitation requires substantial clinical judgement and experience.

Management recommendations are used as guidelines rather than definitive standards of care.

The current UK military resuscitation guidelines for the pre-hospital environment are detailed in Figure 2-3. Its basis lies in the adaptation of current in-hospital DCR regimes and incorporating the crucial aspects of them, with changes being made to overcome the logistical and

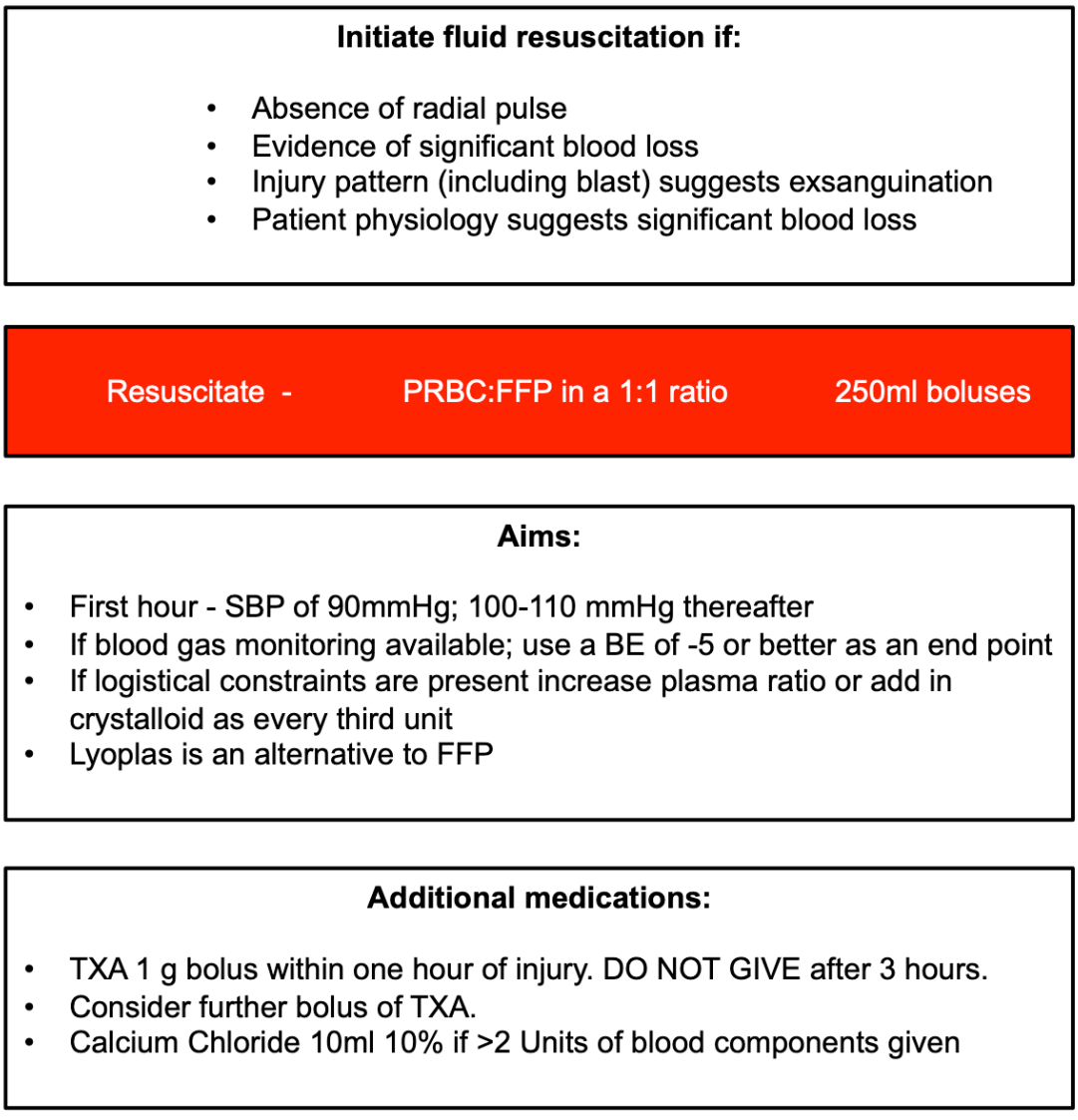


Figure 2-3. Medical Emergency Resuscitation Team Blood Product and Fluid Resuscitation SOP (abridged, modified).

environmental constraints those pre-hospital teams are placed under. Another fundamental dissimilarity needs to be considered; the teams working in that environment are not aiming to resuscitate to incorporate DCS as part of their DCR process. Their aim is to keep the patient alive long enough to reach a location for Damage Control Surgery. Cessation of haemorrhage aside, they need to incorporate the majority of all aspects of Damage Control Resuscitation sufficient enough to reverse or pause the progress of Acute Traumatic Coagulopathy. A vital aspect of this regime is the resuscitation of trauma casualties using blood components in the pre-hospital environment.

2.4.1 Pre-hospital Blood Components

There are national and international guidelines that ensure the quality of blood products. In the UK the "Red Book": the Guidelines for the Blood Transfusion Services, defines guidelines for all materials produced by the UK Blood Transfusion Services for both therapeutic and diagnostic use (Red Book 2013). They reflect the legally binding requirements of the Blood Safety Regulations (UK Statutory Instrument 2005). Its overall aim is to ensure as far as possible the safety of blood transfusion in the UK, for both the donor and the patient.

All blood components for the UK military are provided by the UK Blood Transfusion Services and as UK patients are in receipt of them, the guidelines in the "Red Book" need to be adhered to. Wherever the military are in the World, clinical governance is at the forefront of patient care and patient safety.

Upon delivery of the blood components to the departing port, airhead or airport the onus is on military medical personnel to ensure transportation and storage guidelines are adhered to. Although not particularly difficult, that task becomes more challenging if personnel deploy to more austere locations throughout the globe. Any surgical team will travel with an ability

to both transport and store blood components refrigerated or frozen. Some very small teams will move with a refrigeration capability only and plasma and fibrinogen is taken in lyophilised (powdered) form.

Two main challenges face the medical team ensuring that the guidelines on storage are adhered to. Maintaining temperature within the surgical facility is the easier challenge, but is very much reliant on an ensured power supply. Each team will take with them two generators to ensure they have this at all times. Both fridges and generators struggle to function adequately at temperature extremes, particularly the heat of the desert. Temperature is monitored constantly in accordance with the above regulations.

Ensuring the correct temperature range is adhered to forward of the hospital is more challenging. Working in the back of a military aircraft is a testing environment (Figure 2-4).

Figure 2-4. CH-47 (Chinook) in Medical Emergency Response Team (Enhanced), pre-hospital physician led medical team configuration.

The Golden Hour Box, a sealed container containing a vacuum insulated chamber with an inner, removable thermal isolation compartment (Figure 2-5), is used in this environment and will hold 4 units of PRBC or thawed FFP. It can maintain its temperature range (2-6°C) for 48 hours in external temperatures of up to 45°C. Units are rarely kept in these for more than 24 hours and in normal circumstances they are only given to the pre-hospital team on departure to retrieve a patient.

Figure 2-5. Golden Hour Box (Photo credit: Pelicanbiothermal.com)

The storage temperature is monitored using a temperature indicator tag together with single-use time-temperature indicators (WarmMark® and ColdMark®, ShockWatch, Dallas, Tx). Blood products are only administered if the temperature has remained within range. Components are warmed during administration, using a portable battery operated fluid warming system (enFLow™, GE Healthcare, Waukesha, WI). The hospital is informed during flight if a transfusion occurs so that the blood container may be promptly exchanged for a new one with a full supply of products. This process has the clinical benefit of alerting clinical teams to the likely arrival of a hemodynamically compromised casualty.

The logistic challenges of maintaining a blood supply to austere settings, and the impact of shortage, necessitates a much greater degree of redundancy than in civilian systems. Despite the challenges, wastage of blood products in the UK system has been managed, with 65% of PRBC and 80% of FFP units sent to Afghanistan used, which compares favorably with US experience (Rentas 2012).

Blood directives mandate haemovigilance and the traceability of all blood components. The documentation of pre-hospital transfusion is as per the UK military hospital protocol and consists of a double labelling system. One label remains in the patients' records; the other is returned to the pathology laboratory. The exigencies of administering a transfusion in a cramped aircraft, sometimes under attack, can make this challenging, especially if there are multiple casualties. Nonetheless, the MERT pre-hospital transfusion program achieved 100% traceability. Furthermore, no serious side effects or complications of transfusion attributable to units transfused in the pre-hospital setting have been reported.

Provision of blood products in a pre-hospital environment is a significant logistical challenge. The UK military has been successfully deploying blood products forward since 2008 when the pre-hospital aircraft was co-located with the hospital in Bastion. Despite the lack of robust scientific evidence to justify the challenge of providing pre-hospital blood components, civilian pre-hospital providers within the UK have emulated this practice. The London Helicopter Emergency Medical Service (HEMS) was the first, starting to take PRBC onto the aircraft in 2012 (Weaver 2013). They have proved it is both feasible and associated with overall reduced blood component consumption (Rehn 2017), with other potential benefits (Weaver 2013). Other UK pre-hospital capabilities have followed suit (Lyon 2017). Studies from other countries have shown that pre-hospital PRBC transfusions in trauma are associated with improved outcomes (reduced risk of shock and probability of 24-hour mortality; Brown 2017a), reduced coagulopathy and lower risk of 30-day mortality (in blunt trauma; Brown 2017b).

A plasma only pre-hospital resuscitation investigation (Pre-hospital Air Medical Plasma [PAMPer]; Sperry 2018) illustrated the benefits of plasma for both survival (lower 30-day mortality) and coagulation (decreased PT ratio).

Despite these studies on individual components there is little data on pre-hospital PRBC and plasma (Holcomb 2015a). At Camp Bastion the in-hospital clinicians were in no doubt the effect of having blood products on the aircraft. The overall assessment was that patients were arriving at the hospital in a physiologically much better state than would be expected for their injuries; significant ballistic injuries including blast and multiple proximal amputations. They were being compared to the patients who were admitted by other pre-hospital assets (with no doctor or blood components) or to the collective institutional memory of similar patients admitted earlier in the conflict. There was no scientific proof of benefit though and the cost financially, temporally and logistically of providing forward blood products was not insignificant.

The most notable differences were observed in the most severely injured casualties. It is worth discussing next how these injuries occurred and how they compare to civilian trauma victims.

Chapter 3. Ballistic and Civilian Injuries

3.1 Introduction

In order to further improve military trauma care both the UK and America carried out many Governance led evaluations on their military casualties. These were useful for detailing the type of casualty treated by military facilities in Afghanistan and Iraq. An American review of battlefield deaths between 2001 and 2011 reviewed data on 4,956 fatalities (Eastridge 2012). The majority (87.3%) of deaths occurred in the pre-hospital environment before the casualty reached a surgeon. They estimated that 24.3% of these deaths were preventable and the cause of these deaths was in almost 91% of casualties - haemorrhage.

A UK review of all UK casualties, not just fatalities, between 2003 and 2012 investigated the data for 2,792 UK casualties (Penn-Barwell 2015). 2,227 were due to hostile action, of which 608 (22%) were fatalities. 1,592 (65%) of all casualties from all hostile action were from explosions. Gun shot wounds (GSW) accounted for 684 casualties and represented 31% of all hostile action. The impact of body armour and helmets meant that extremities accounted for 45% of all injuries, with head, face, thorax and abdomen accounting for approximately 10% each.

Data from previous conflicts show that this ratio of GSW to explosive injuries was similar in previous conflicts. The Falklands had approximately 30% GSW injuries, compared to almost 60% of injuries secondary to explosions in the land campaign (Jackson 1983) with Vietnam having similar statistics. The Wound Data and Munitions Effectiveness Team (WDMET) American database on 3 years of the Vietnam War states injuries secondary to explosions (fragments, burns, blast) accounted for 71% of all injuries (WDMET 1970).

An American comprehensive review of combat injuries from past conflicts back to and including the conflict in Vietnam (Champion 2003) found approximately 50% of those who died, did so as a result of exsanguinating haemorrhage, with approximately 80% of these from torso injuries, with 20% of deaths caused by potentially preventable extremity haemorrhage.

For blast injuries the cardiac and apnoeic effects of blast injury may well be a causative reason for death, however at short distances mutilating trauma or the total body destruction from the explosion is more likely. Fragmentation is the most common wounding mechanism (Bellamy 1995, Champion 2003, Penn-Barwell 2015). The incidence of blast lung is significantly higher in the more severely injured casualties of explosions. It has been found in 63% of critically ill ICU blast injured patients (Gutierrez de Caballos 2005) and there has been evidence of blast lung in 47% of blast immediate fatalities (Frykberg 1989). In survivors its incidence is variable from between 75% (Gutierrez de Caballos 2005) to 38% (Katz 1989), which is probably a reflection of the physics of each explosion, and its proximity to casualties.

In Afghanistan and Iraq, blast lung incidence between 2003-2009 in UK casualties was present in about 113 casualties (Smith 2011). Blast accounted for 1678 casualties out of a total 3109 UK casualties. Of those injuries from explosion 233 had a thoracic injury and 113 had blast lung. The mean ISS of those with blast lung was 52.6 – severely injured – which fits with previous descriptions. At the peak of injury incidence in each conflict blast lung accounted for 7.3% of injuries in Iraq and 11% in Afghanistan – twice previous estimates (Ritenour 2008).

In summary the majority of injuries from hostile action were primarily blast and fragmentation trauma resulting from explosive devices, with extremities being the most likely region to be wounded. Fatalities were predominantly secondary to haemorrhage and occurred before the patient reached a medical facility.

3.2 Civilian Terrorist Injuries

Historically, terrorists have predominantly used explosives to achieve their aims on civilian targets. It is not a new phenomenon for the UK; one of the earliest recorded incidents being caused by Irish terrorists who attacked in 1867 (bombing Clerkenwell prison in order to free Fenian prisoners). Throughout the 1970's on the UK mainland, financial and military establishments were targeted with a view to disruption rather than civilian loss of life. This has changed in recent times with the Internet leading to easier access to bomb making instructions and materials.

In July 2005 over 650 civilians were injured, with 56 fatalities (8% of casualties) in three separate bombings on the same day in July in London (Aylwin 2006). Blast and fragmentation injuries predominated with most of the fatalities dying at the scene. This was one of the first of a new breed of terrorist event, large explosions aimed at killing civilians indiscriminately. Various terrorist attacks have occurred since 2005, with even more being foiled by the security and counter terrorism services. Explosive related attacks such as at the Manchester Arena in 2017 have had the greatest impact on indiscriminant loss of life, secondary to blast and fragmentation injuries. That year was both busy for UK terrorist attacks (4 successful, with at least 12 thwarted) but also marked a change in their method of attack within both the UK and Europe. This change of method may well be due to improved security service vigilance. The majority of these have been caused by blunt trauma from vehicles (London Bridge and Westminster in the UK, Barcelona and Nice in 2017; Paris and Berlin in 2016 in Europe) with some penetrating trauma from stabbing (Nice in 2015, Würzberg in 2016 and Westminster in 2017).

Terrorist events have caused nearly 400 deaths and injured nearly 1800 civilians in Europe since 2015. Approximately 60% of casualties have been caused by bombings, with just fewer than 40% caused by blunt trauma (vehicle versus pedestrian). Civilian trauma doctors are familiar with blunt trauma, but not ballistic trauma causing penetrating, fragmentation and blast

injuries. A comprehensive knowledge on how to deal with these injuries ensures their correct management. In the UK military doctors work in NHS hospitals and their knowledge and skills have been invaluable in some of these cases both at a local level (MTN 2014) and Nationally (Bowley 2018).

Management of civilian terrorist blast injuries is the same as military management of ballistic war injuries. Blunt trauma management is normal practice in civilian trauma centres.

3.3 Civilian Trauma Injuries

In the UK blunt trauma predominates. The Trauma Audit and Research Network (TARN) allows investigation into the epidemiology of UK trauma. Subsequent to the National Confidential Enquiry into Patient Outcome and Death report of 2007 (NCEPOD 2007) the NHS formed Regional Trauma Networks and Major Trauma Centres. With the formation of these a greater number of hospitals logged data for TARN. A recent study of this data (Moran 2018) shows over the last decade the causes of UK trauma has been predominantly blunt with less than 5% from penetrating injuries. Road Traffic Collisions (RTC) account for nearly 30% of injuries, with falls accounting for almost 60% of UK trauma. Throughout the UK, management of this trauma is improving despite an increased frequency of older patients with a greater number of co-morbidities; the odds of survival from severe injury has increased by 19% (Moran 2018). When taken into the context of probably forty to fifty thousand trauma cases in the UK per annum, this improvement translates to thousands of lives saved in the UK in the next 5 years (Lockey 2018).

What is the physical impact of these differing mechanisms of injury? Does blast pose a different medical management challenge to the trauma physician?

3.4 Physical Impact of Penetrating Injury

Low velocity penetrating – stab – injuries involving knives have become a high profile incident in the UK (Campbell 2018). Knives are not omnipresent in causing stab injuries which can be due to anything from scissors, to barbecue forks to ice picks. The results are similar; depth of injury can be significantly greater than skin injury length with the impact force concentrated at the tip. The effect is determined by angle and depth of penetration (weapon trajectory both on insertion and removal), the type of tissue involved, location of injury and how compressible the area wounded is (e.g. the abdomen can indent significantly so that the area penetrated can be at a greater depth than the weapon; Butt 2009).

Injuries can compromise the respiratory system (pneumothorax), cause airway complications (disrupt trachea patency) and produce visceral injuries like intestinal perforation. Depending on the location though it is vascular injuries (aortic, carotid) and injuries to highly vascular organs (spleen) that are the most concerning, producing a casualty with minimal external signs of violence but suffering substantial exsanguinating haemorrhage, although the incidence of this extreme is low (Lockyer 2013).

3.5 Physical Impact of Ballistic Injuries

The projectiles in ballistic injuries are most commonly bullets from firearms or fragments of explosive devices, however they can include anything from the surrounding environment of an explosion. The damage to the tissues caused by the projectile depends on a number of factors (Table 3-1).

The kinetic energy of the projectile is key ($E_k = ½ m v^2$; where *m* is the projectile mass and *v* its velocity) and the damage is determined by how much of this is deposited after striking the casualty. Shape and size will determine how it travels through the tissue. A missile crushes the tissue it

strikes creating a permanent wound channel (permanent cavity; Figure 3-1), the size of which is determined by the yaw of the projectile. A bullet fired from a weapon with spiral grooves (rifling) will be stabilised by the spin imparted on it leading to nose first flight and presenting a smaller surface area for the creation of a permanent cavity. In contrast: a bullet arriving at 90° (side on); a soft- or hollow-point bullet that deforms on impact creating a significantly greater surface area; or an irregularly shaped fragment will create larger permanent cavities (Russell 2014).

Projectile Kinetic Energy	The kinetic energy of the projectile determines the amount of energy it can deliver to tissue
Projectile Size and Shape	This determines how the object will behave in contact with tissue and how much kinetic energy it will give up
Tissue Type	The nature of the tissue and particularly how elastic it is, influences the amount of energy it can absorb before damage occurs
Intervening Structures	Between the projectile and the tissue, for example body armour

Table 3-1. Factors influencing damage to tissues in ballistic injuries (Russell 2014)

Once the projectile hits tissue any spin will be insufficient to maintain its nose forward orientation through a denser medium (Hopkinson 1967) and the heavier base will move forward. This rolling motion (yaw) crushes the maximal amount of tissue, slowing the projectile down rapidly as its energy is used to move tissue radially away and the force expended in this roll (yaw) creates the temporary cavity (Figure 3-1). Yaw occurs to a much lesser extent in soft/hollow-point projectiles as on contact with the tissue

they “mushroom”, rapidly making the heaviest part of the projectile at the forward edge. It is also unlikely to occur in irregular fragmentation projectiles, which travel predominantly heavy section first (Hollerman 1990).

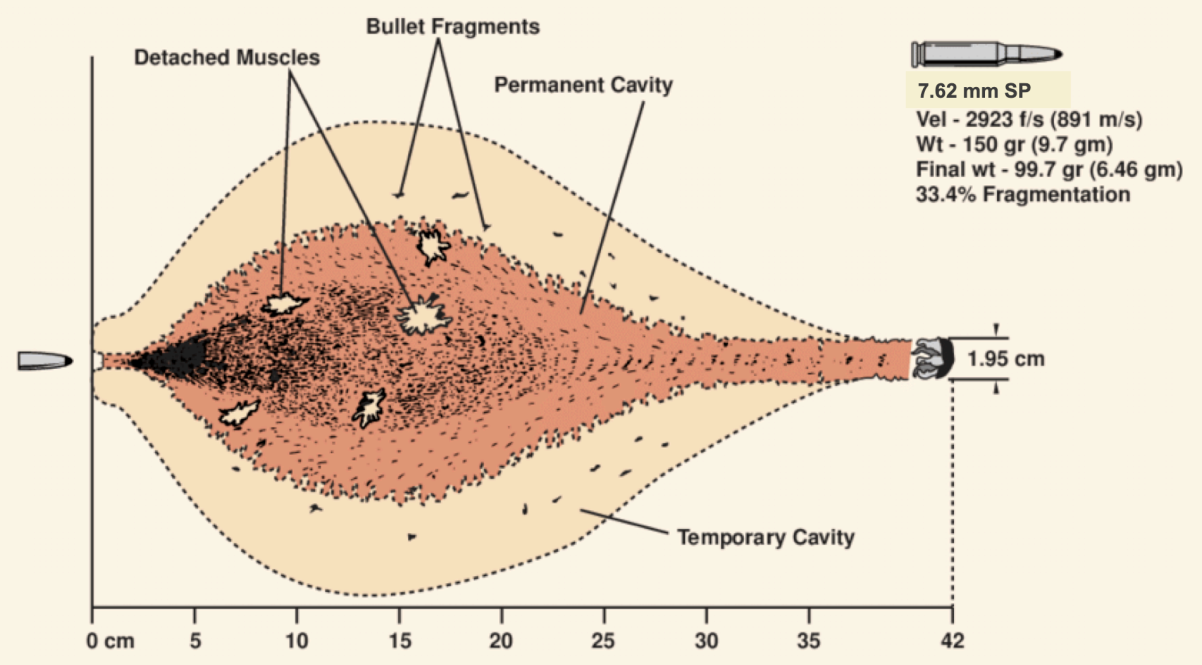


Figure 3-1. Wound profile of NATO 7.62 mm round (Fackler 1996).

The wound produced depends on the density and elasticity of wounded tissue, thickness of body part and the points at which the projectile yaws or when fragmentation occurs (Figure 3-2). A heavier, slower bullet crushes more tissue with little temporary cavity compared to a faster, lighter projectile with less crush and greater cavitation. The heavier, slower projectile will cause more severe wounding in elastic tissue, than the lighter projectile, which causes cavitation (which is well tolerated by elastic tissue, such as lung or muscle). In non elastic tissue (such as liver or brain) the temporary cavity will produce a more severe wound (Fackler 1996, Hollerman 1990, Russell 2014). Although the influencing factors are many as to the degree of injury, the results are consistent – significant tissue

damage and potentially substantial haemorrhage if large vessels are involved.

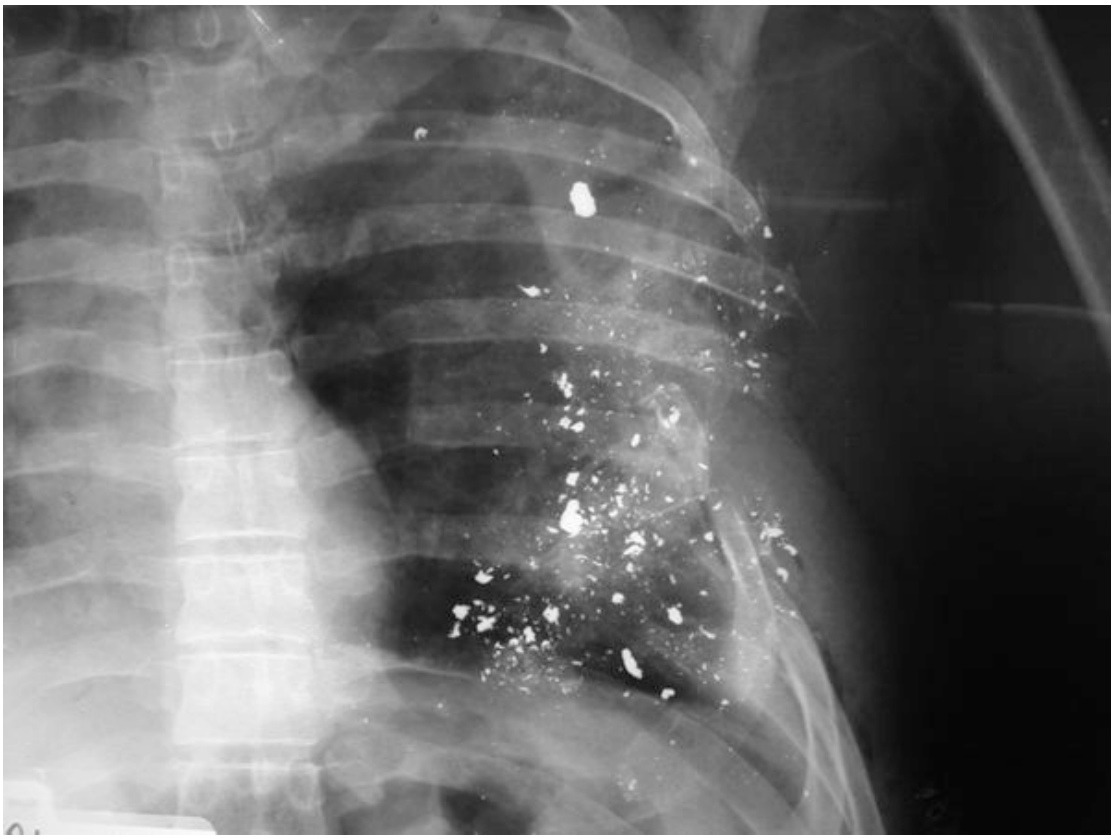


Figure 3-2. Gunshot wound to the thorax from high velocity rifle bullet, illustrating fractures, missile fragmentation and soft tissue disruption. (www.medscape.com Forensic Pathology of Firearm Wounds. Frost et al 2015)

3.6 Physical Impact of Explosions

3.6.1 The Physics of Explosions

An explosion is a violent expansion in which energy is transmitted outwards as a shock wave. High order explosive detonations result in the rapid transformation of the explosive material into a highly pressurised gas, which releases energy at high speeds in a very short space of time (Wolf 2009). This energy “shock wave” travels faster than the speed of sound and it

compresses a rim of air around the ball of explosive products moving out. This energy “shock wave” travels faster than the speed of sound away from the explosion (Figure 3-3).

The magnitude of the peak over pressure rapidly decays with increasing distance from the centre of the explosion. The explosion also pushes and accelerates the air producing a blast wind (dynamic over pressure), which carries with it energised fragments from the explosive container, the explosive itself and near by objects. The pressure differences that occur as part of the blast wave are well described by Friedlander (1946; Figure 3-4).

The outwards movement of the blast wind eventually dissipates leaving a volume of low-pressure gas nearest the point of explosion initiation. There is then a reversal of air movement to correct this under pressure, leading to a pressure oscillation until the norm is restored (Wolf 2009).

The importance of the peak over pressure and blast wind depends on the location of explosion. In an incompressible medium (water) the blast wave is transmitted much further compared to air where its compressibility allows energy to accelerate and heat the air. Water will reduce the movement of the dynamic over pressure and fragments reducing their radius of effect compared to air.

Interaction of the shock wave with solid surfaces will result in reflection, so that a shock wave can reverberate within a structure. In addition the shock wave can be amplified by up to eight times at a reflective surface, so that the reflected wave can interact with the incident wave resulting in peak over pressure summation (Wolf 2009). More severe injuries can therefore occur in confined spaces due to these reflections and summations.

There are four classical categories of blast injury (Mellor 1988, Table 3-2) and these are discussed in the following sections.

Figure 3-3. Photo of explosion and blast wave (arrowed)(from Defence Research and Development Canada, www.canada.ca/en/defence-research-development).

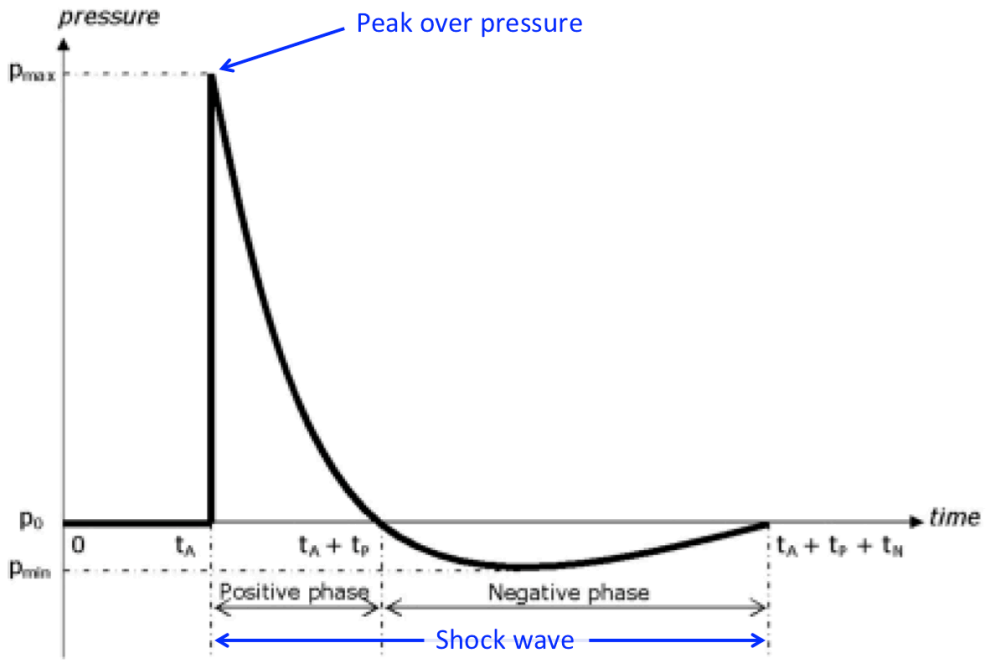


Figure 3-4. Illustration of the blast air pressure changes in an idealised shock wave – a Friedlander curve illustrating the peak overpressure and subsequent negative pressure after an explosion (Mediavilla Varas 2011)

3.6.2 Primary Blast Injury

Primary blast injuries are caused by the interaction of the shock wave with the body. The shock wave couples into the body and travels through the tissue water, principally depositing energy as it moves from areas of high acoustic impedance to low acoustic impedance e.g. gas filled spaces (Guy 1998).

The organs affected are those that are gas filled such as the lungs (causing contusions, haemothorax, pneumothorax and pseudocysts), the gastro-intestinal tract (haemorrhage and perforation) and the middle ear (tympanic membrane rupture and middle ear damage). Eye globe ruptures (DePalma 2005) and traumatic brain injury (with no physical signs of head injury; Ling 2009) have also been described.

Blast Injury Type	Mechanism of Injury
Primary	Shock wave has direct interaction with the body depositing energy throughout but principally at liquid/gas interfaces. The main organs injured involve the lungs, bowel, ear and brain in addition to a physiological response to the blast comprising of apnoea, bradycardia and hypotension.
Secondary	Impact from energised fragments from the explosive device or environmental debris accelerated by the blast wave. The majority of these injuries are penetrating.
Tertiary	Physical displacement of the body or body parts by the blast causing impact with the ground or fixed objects and/or traumatic amputation.
Quaternary	Assorted other injuries as a result of the blast that include flash burns from explosion heat, burns caused by combustion to the environment, crush injuries from fallen buildings, the effects of noxious gases and psychological effects of being involved in a blast.

Table 3-2. Blast injury classification. (Mellor 1988)

3.6.3 Primary Blast Injury - Blast Lung

The shock wave impacts on the body surface resulting in rapid acceleration and movement on the body's surface with resultant transmission of shear and stress waves through the tissue (Horrocks 2001). This tissue stress leads to mechanical failure and resultant injury in primarily gas containing structures, particularly the lungs. The direct effect of the blast wave at the largest air/tissue interface; the lungs, produces the primary blast injury of "blast lung".

Blast lung is a significant cause of mortality both at the scene and in initial survivors (Stein 1999). The shock wave causes rupture of alveolar capillaries with resultant macroscopic haemorrhage and fluid extravasation into lung tissue (Brown 1993). There is an initial respiratory compromise as a result of this intra-pulmonary haemorrhage and oedema (Grobunov 2006). Free radical mediated oxidative stress and the subsequent inflammatory response has also been recognised to have a role in blast lung pathogenesis (Gorbunov 2005).

Microscopically the histological changes observed are initially leucocyte accumulation and haemorrhage (and little evidence of direct endothelial damage) within 12 hours. Subsequent development of inflammation leading to epithelial and endothelial cell damage is observed by 72 hours after injury. These changes lead to impairment in lung mechanics and the physiological process of gas exchange, and it results in a severe pulmonary injury which is defined by haemorrhages, contusions and oedema resulting in both vascular and alveolar injury (Knapp 1990, Stein 1999).

Macroscopically haemothoraces and pneumothoraces are common. Fistulas (both bronchopleural and alveolar vasculo-pulmonary) can present with evidence of subsequent air embolism. Patients present with a range of respiratory symptoms including non-specific breathing difficulties, cough, haemoptysis and chest pain. The respiratory signs evident are tachypnea, hypopnea, cough, haemoptysis and ultimately hypoxia (Kirkman 2010b).

3.6.4 Primary Blast Injury - Cardio-respiratory Response to Blast

The cardiorespiratory response to blast is mediated by the autonomic nervous system. It results in bradycardia and hypotension – with resulting diminished cardiac output – apnoea or rapid shallow breathing (Ohnishi 1997, Guy 1998, Kirkman 2011a). The delay in these responses (2-4 seconds) after blast suggests that these responses are secondary to a reflex (Irwin 1999), rather than a direct effect of blast on the tissues. The reflex is predominantly vagally mediated (Ohnishi 1998, Irwin 1999), although hypotension has additional contributory factors. Vagotomy will abolish the bradycardia and apnoea, but only partly abolish the hypotension (Ohnishi 2001). The reduced peripheral vascular resistance and therefore hypotension and diminished cardiac output can last many hours (Harban 2001) and is probably secondary to a rapid Nitric Oxide production (Zunic 2000).

3.6.5 Secondary Blast Injuries

Secondary injury is caused by the effect of projectiles causing penetrating and fragmentation ballistic injuries (Mellor 1998). Ballistic injuries from primary fragments (that are part of the weapon) and secondary fragments (resulting from the explosion) are the leading cause of death and injury in both military scenarios and civilian terrorist attacks (except in cases of major building collapse; DePalma 2005). The physics and consequent injury from these are similar to projectiles as described in section 3.5 except they are potentially greater in number, their velocity diminishes faster and they are irregular in shape (Fackler 1996).

3.6.6 Tertiary Blast Injuries

Tertiary blast injuries are due to the effects of persons being thrown by the blast wind. Tertiary blast injuries are primarily seen as blunt and sometimes

penetrating trauma, leading to fractures, traumatic amputations and not uncommonly traumatic brain injuries. Large airborne fragments lead to extensive blunt trauma (and crush injuries). Flying smaller fragments cause penetrating and ballistic injuries (Mellor 1998, DePalma 2005).

3.6.7 Quaternary Blast Injuries

Quaternary blast injuries refer to explosion-related injuries, illnesses, and diseases not due to primary, secondary, or tertiary injuries and can include burns, asphyxia and exposure to toxic inhalants (DePalma 2005). They include injuries due to structural collapse, in which few people survive (Arnold 2004). A quinary pattern of a hyper-inflammatory state manifesting as hyperpyrexia, sweating, low central venous pressure and positive fluid balance has more recently been described (Kluger 2007), its mechanism being secondary to unconventional materials used in the manufacture of explosives.

3.7 Physical Impact of Blunt Injury

In the UK blunt trauma from vehicles or falls are the commonest form of injury (Moran 2018). The sequelae to any blunt injury will depend on the amount of energy transferred and the tissues or organs involved. The pathophysiological insult is essentially varying amount of tissue damage, with the individual anatomical areas involved producing differing additional effects.

Blunt abdominal trauma predominantly causes injury to solid organs. If both spleen and liver are involved these casualties are more likely to have lower systolic blood pressure, higher acidosis and lactate and a higher Injury Severity Score (ISS) and transfusion requirements. This is mirrored by a higher mortality and length of hospital stay (Malhotra 2003).

Chest blunt trauma severity and the existence of associated injuries is related to the presence of rib fractures. Haemo/pneumothorax incidence is less than 7% in patients with no rib fractures compared to 81% in those with more than 2 rib fractures. Mortality is understandably greater in this latter group particularly in older patients or in those with associated additional injuries (Liman 2002).

Blunt Traumatic Brain Injuries have consistently accounted for 30-35% of all UK trauma injuries in the last decade (Moran 2018). They are not closely associated with significant haemorrhage or massive tissue injury, however their mortality is ten-fold higher than patients without head injuries (Patel 2005) and for survivors and their families the consequential neuropsychological and social costs are substantial.

Blunt injury is the main civilian injury seen in UK hospitals and the revision of trauma care within the UK has improved post-blunt injury survival (Moran 2018). Individual injuries are associated with specific anatomical sequelae and the result of potentially massive tissue damage.

3.8 Cardiovascular Response to Haemorrhage

3.8.1 Simple Haemorrhage

Haemorrhage without tissue damage in trauma is uncommon; however examining it allows discussion on how other factors found in a trauma patient (pain, tissue damage, pharmacological interactions) affect the simple physiological response to it.

Acute blood loss reduces venous return, decreasing cardiac stroke volume, which in turn leads to a drop in systemic arterial pulse pressure. The cardiovascular system and other homeostatic mechanisms that play a role

in the maintenance of substrate supply to the tissues react to blood loss and the drop in cardiac output. In an attempt to preserve blood flow to certain vital organs such as the brain they reduce flow to other organs such as the kidney. The arterial baroreceptors play a key role (Secher 1985) in this function; they are sensitive to both absolute blood pressure, but also degree of change within a pulse (Angell James 1971).

The initial drop in pulse pressure is sensed by the carotid sinus causing an increase in sympathetic outflow to the heart and vasculature and a reduction in parasympathetic vagal stimulation. There is a rise in chronotropy and an increased peripheral arterial vasoconstriction in order to maintain blood pressure in addition to veno-constriction leading to improved venous return to the heart (Secher 1985, Little 1989).

Sympathetic stimulation of the adrenal glands stimulates the release of catecholamines into the blood, reinforcing the effect of sympathetic stimulation on the heart and vasculature. This increased sympathetic outflow has little influence on both cerebral and coronary circulations, but they both benefit from the vasoconstriction that occurs in other organs (e.g. gastro-intestinal, skeletal muscle and renal). Blood is moved away from peripheral vessels decreasing blood flow to certain inessential organs such as skeletal muscle. The kidneys can receive 25% of the cardiac output, which is far in excess of their oxygen requirements. Renal vasoconstriction moves blood away from the kidneys and their diminished blood flow also causes oliguria leading to retention of salt and water, aiding blood volume maintenance. Reduced organ blood flow results in poor oxygen delivery and a subsequent acidosis that is sensed by the chemoreceptors. The chemoreceptor reflex strengthens the baroreceptor reflex and activates further the sympathetic/adrenergic system.

The increased venous return from veno-constriction results in an autotransfusion that limits cardiac output reduction. The arterial baroreceptors are able to transduce information on pulse pressure resulting

in continued compensation despite no drop in systolic or mean arterial blood pressure. It is postulated that haemorrhage actually leads to an increased sensitivity of the arterial baroreceptor reflex such that a given change in blood pressure evokes a greater alteration in heart rate (Little 1984), which would explain the increased efficacy of this mechanism in haemorrhage. A fairly consistent blood pressure can initially be kept by these mechanisms in a physically fit human being, in the face of on-going haemorrhage.

Changes in Starling forces across the capillaries result in net reabsorption of interstitial fluid into the blood to increase plasma volume at about 1 litre per hour (Carroll 2007, Klabunde 2012). A reduced capillary hydrostatic pressure leads to net reabsorption in the capillary of fluid including electrolytes and some protein, but no cells. It leads to an increased plasma volume and haemodilution of blood resulting in a drop in red cell haematocrit.

The initial acute cardiovascular response to haemorrhage is therefore tachycardia, arterial vasoconstriction and veno-constriction in an attempt to maintain blood pressure and blood flow to vital organs.

3.8.2 Delayed Cardiovascular Response to Haemorrhage

Delayed responses to blood loss help aim to restore plasma volume, proteins and red cells. Volume restoration is secondary to a number of humoral actions; renal renin release leads to increased angiotensin II and aldosterone. Increased ADH (Vasopressin) release occurs due to input from volume receptors. These result in increased thirst mechanisms and increased sodium and water reabsorption from the kidneys. This renal mechanism is important in the long-term recovery from blood loss.

Hepatic protein synthesis restores plasma protein levels to normal levels in a few days. Bone marrow erythropoiesis is stimulated leading to increased

erythropoietin levels. Reticulocyte levels will peak at 10 days with full red cell restoration by 4 to 8 weeks after blood loss.

3.8.3 Experimental Studies

In experimental studies in response to a “pure” haemorrhage with no tissue injury or nociception there is a distinct second staged response to haemorrhage. After significant blood loss in excess of 20% of blood volume a depressor reflex occurs exhibiting as a bradycardia and vasodilatation leading to a fall in blood pressure (Barcroft 1944, Secher 1985; Figure 3-5). This is a vagally mediated depressor response arising from the heart (Evans 1989) and is mediated possibly via cardiac C fibres (Öberg 1972). Studies on animals deficient of C fibres (Öberg 1972) and in animals with vagotomies (Little 1989) have shown a markedly attenuated bardycardic response. The exact mechanism is not clear as it is preserved after cardiac transplantation (total denervation of the heart) suggesting other signaling pathways are involved. It has been postulated this is a basic reflex in order to reduce cardiac workload at a time when coronary blood flow is compromised (Little 1997).

The initial response to simple haemorrhage is therefore biphasic (Figure 3-5); an initial tachycardia and vasoconstriction maintaining mean arterial blood pressure in the face of reduced cardiac output associated with moderate hypovolaemia. As haemorrhage continues beyond 20% of blood volume a vagally mediated depressor reflex results in decompensatory bradycardia and vasodilation, which increases hypotension.

A further third phase has been shown to emerge before irreversible shock occurs. When over 40% of volume has been lost a pre-terminal phase of tachycardia and hypotension occurs (Jacobsen 1990, 1992) and appears to be sympathetically driven, probably driven by cerebral hypoperfusion (Foëx 1999).

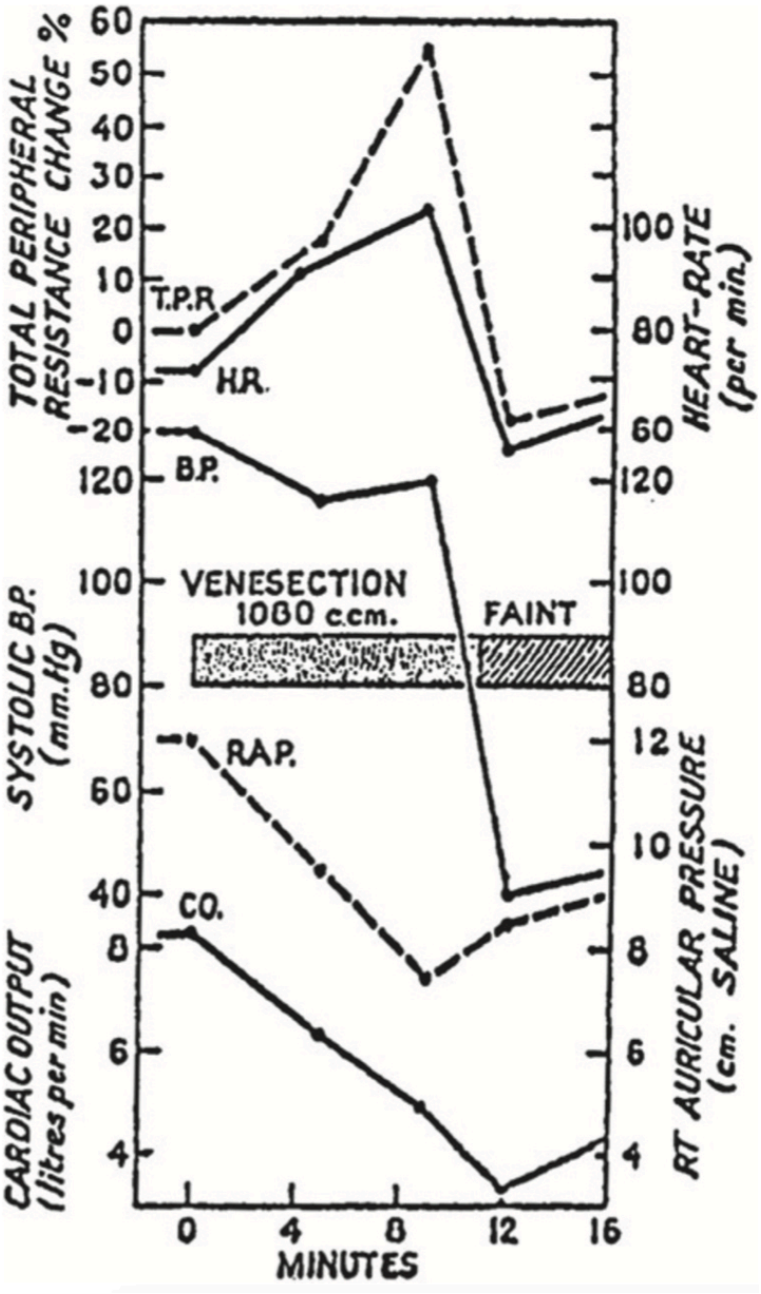


Figure 3-5. Simple haemorrhage in a male volunteer showing biphasic response. Blood removed by venesection until volunteer fainted. Total Peripheral Resistance (TPR), Heart Rate (HR), Systolic Blood Pressure (BP), Right Atrial Pressure (termed Auricular) RAP, Cardiac Output (CO). (Barcroft 1944)

Simple or “pure” haemorrhage is a rare event and it is more commonly associated with pain or musculo-skeletal injury. The cardiovascular

changes that occur in simple haemorrhage are significantly diminished by the presence of coexisting injury (Little 1989).

3.9 Cardiovascular Response to Haemorrhage and Injury

Isolated musculoskeletal injury itself diminishes the vagal outflow producing an increase in heart rate and elevating arterial blood pressure via increased sympathetic outflow (Redfern 1984). The effect of experimental animal injury on the cardiovascular response to hemorrhage has been studied and shown to attenuate or completely prevent the vagally mediated bradycardia reflex in the biphasic response to haemorrhage (Little 1989). This protects the animal against the hypotensive effects of severe haemorrhage meaning a larger loss of blood was required to produce a fall in blood pressure in the injured animal.

This attenuation of the bradycardic reflex is most likely to be due to central inhibition of the medullary central depressor region by afferent nociceptor C fibres from the injured area (Quest 1972).

It appears the preservation of a better blood pressure though is at the cost of peripheral vasoconstriction leading to organ ischaemia and damage exacerbating the resulting injury. Unlike simple haemorrhage where blood flow is redistributed away from skeletal muscle the opposite occurs with haemorrhage and injury. In the latter blood is diverted from the gut towards skeletal muscle (Mackway-Jones 1999). An understandable reflex for survival in fight or flight where skeletal muscles will be used but in the experimental animal model or indeed the patient receiving medical treatment and no longer "*in the fight*" the net result is diminished survival. Oxygen delivery is wasted on inactive muscle at the expense of the metabolically active gut. Ischaemic damage to the intestines may result in both an enhanced endotoxin translocation (Xu 2004) and a resultant increased inflammatory response (Wu 2010).

Therefore although injury reduces the initial bradycardic response to haemorrhage and enables a greater tolerance of blood loss it results in reduced survival in an animal model (Wang 1949). When haemorrhage is superimposed on real rather than simulated tissue injury, the tolerance to blood loss is reduced even further (Rady 1993).

3.10 Cardiovascular Response to Haemorrhage and Blast

The cardio-respiratory effects of primary blast injury producing bradycardia, hypotension and apnoea have already been described (Guy 1989, section 3.6.4). Vagotomy completely abolishes the bradycardia and apnoea and partially abolishes the hypotension (Ohnishi 2001). The majority of blast casualties will have ballistic injuries secondary to fragmentation, with resultant haemorrhage (Cooper 1983). Simultaneous musculo-skeletal (blunt) injury attenuates the depressor response to haemorhage (section 3.9), but this is not so in the case of primary blast injury. Blast injury will augment the bradycardic, hypotensive response to haemorrhage with the bradycardic element occurring after significantly less blood loss. It appears blast augments the depressor response to haemorrhage seen in pure haemorhage, abrogating the baroreceptor reflex; inhibiting the initial compensatory response (tachycardia and vasoconstriction) and accentuates the secondary phase of bradycardia and hypotenson (Sawdon 2002).

This response is further modulated by morphine, which is known to reduce the depressor response to blood loss (Evans 1989, Ohnishi 1997). Intravenous morphine prevented the bradycardia associated with severe haemorrhage and delayed the onset of hypotension (Sawdon 2002). Thus, contrary to injury and haemorhage where the cardiovascular depressor effects of blood loss are attenuated by concurrent injury, in primary blast injury these depressor effects are augmented. The incidence of isolated

haemorrhage and primary blast injuries are very low though, with the majority of casualties having concurrent penetrating ballistic injuries.

3.11 Helmand Casualties

How does the physical impact of these injuries and the responses to them translate into the actual casualties observed in Helmand? Two articles give a good insight into these. One prospective study investigated the coagulation status of casualties injured by Improvised Explosive Devices retrieved from the front line by the MERT (Nordmann 2016). Their demographic data is detailed in Table 3-3. Ten severely injured patients (at least one limb amputation, NISS 47.8±12.63), with systolic blood pressures of 55.0±10.8mmHg had their coagulation measured by rotational thromboelastometry (ROTEM®). They were found to be severely coagulopathic with evidence of hyperfibrinolysis and significantly reduced fibrinogen - the common features of Acute Traumatic Coagulopathy. This coagulopathy was evident within 40 minutes of injury.

A retrospective intra-operative study based in the UK hospital at Camp Bastion studied intra-operative resuscitation methods on 51 casualties (Morrison 2013a). It gives an idea of the status of patients on admission to the hospital (Table 3-3). Both studies describe a severely injured patient group that has suffered blast and fragmentation injuries. They are hypotensive, acidotic and coagulopathic.

For these patients to survive in the pre-hospital environment they need to form a clot to avoid bleeding to death, yet we see evidence in them of a coagulopathy (reduced ability to clot). What is the cause of this coagulopathy? Has the large amount of clot needed to survive consumed the clotting factors, or does the physiological response to injury (combined

Nordmann	Median	IQR
Age (years)	22.4	20.8-25.3
NISS	50	32-58
SBP (mmHg)	65	52.5-70
Coagulation	Hyperfibrinolysis and low fibrinogen	-
Morrison	Median	IQR
Age (years)	25	21-29
ISS	30	23-37
pH	7.19	7.10-7.29
BE (mmol/l)	-9.0	-13.5 to -4.5
PT (secs)	18	15-21

Table 3-3. Military ballistic casualty demographics. Patient demographics from two studies describing patient physiology in the pre-hospital space (Nordmann 2016) and on admission to hospital (Morrison 2013a) having been transferred by the Medical Emergency Response Team (MERT) and admitted to the military trauma hospital in Camp Bastion, Afghanistan. (SEM, Standard Error of the Mean; IQR, Inter Quartile Range; NISS, New Injury Severity Score; ISS, Injury Severity Score; BE, Base Excess; PT, Prothrombin Time).

blast, haemorrhage and ballistic fragmentation) have an influence? Can we do anything to improve or reverse this coagulopathy?

In summary, the general effect of injury is varying degrees of tissue damage and haemorrhage. Blast injuries can cause injuries peculiar to it, but most blast casualties also suffer injuries in common with the rest of the injured population: blood loss and major tissue damage. The physiological consequence to these injuries is well documented with casualties from Helmand illustrating that they are hypotensive, acidotic and coagulopathic. Haemorrhage is known to be the leading cause of death and for these patients to survive they need to form a clot to avoid bleeding to death.

There is evidence here that the coagulation system becomes altered over time, not only secondary to consumption of clotting factors, but also as a result of the physiological derangement caused by these injuries. In the next chapter the coagulation system and how it can be impacted by trauma are discussed in more detail.

Chapter 4. Haemostasis

4.1 Introduction

Haemostasis is the process of preventing blood loss. Upon blood vessel damage this action is mediated through blood vessel constriction and the balance of interaction between platelets, the coagulation system (clot formation) (Figure 4-1) and fibrinolysis (breakdown of clots). The end result is an impermeable plug, or clot, of platelets and fibrin formed at the site of injury. The activation of the platelets and the coagulation system is restricted to the site of injury only, to ensure the plug or thrombus does not spread into the vascular lumen. This final regulatory process of homeostasis is assisted by plasmin-mediated fibrinolysis.

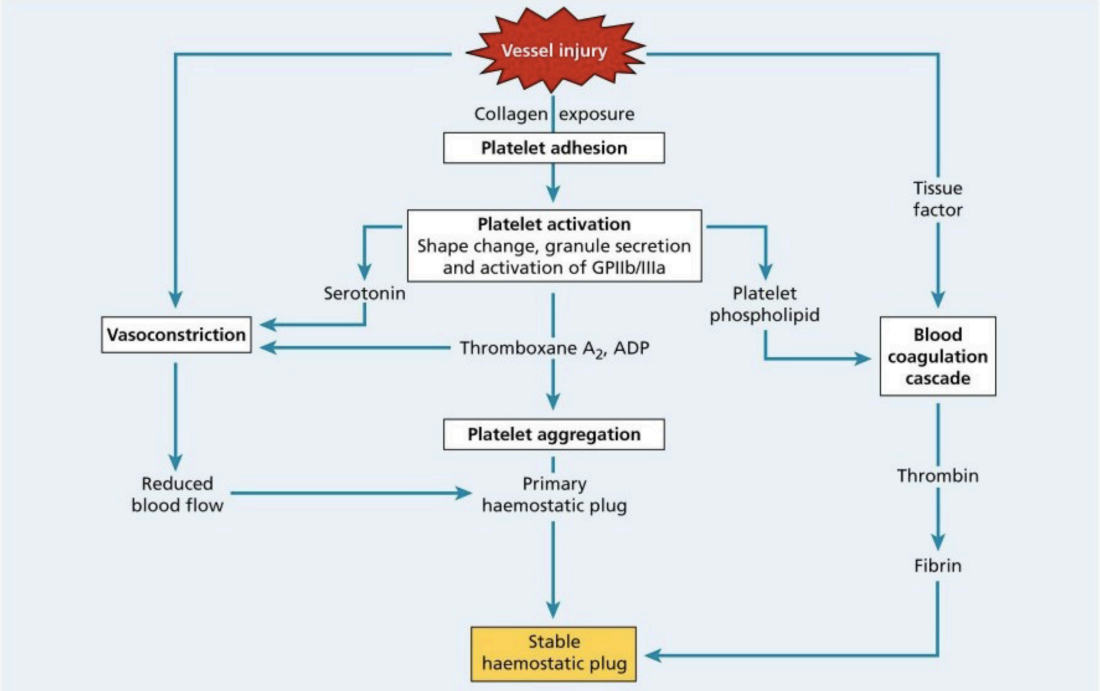


Figure 4-1. Homeostasis overview. (From Hoffbrand 2011 *Essential Haematology* 6th Ed.© 2011 Wiley-Blackwell Publishing)

A fine equilibrium exists between blood fluidity and thrombus formation and this is managed by a tight regulation of platelet activation, coagulation initiation formation, and fibrinolysis – the “haemostatic balance” (Astrup 1958).

4.2 Vasoconstriction

When blood vessels are damaged, circular smooth muscle in the vessel wall contracts. The vascular contraction or spasm reduces the blood flow to the area and increases the chance of a stable blood clot forming (Hoffbrand 2011).

4.3 Platelets

Platelets are discoid structures 2-4μm in size that circulate at the margins of blood vessels (Loscalzo 2003). The platelet ultrastructure is shown in Figure 4-2. The plasma cell invaginates into the cell producing canaliculi, which increase the cell surface area in order to better absorb coagulation proteins. The platelet surface is packed with functional glycoprotein receptors that facilitate platelet activation and interactions with the subendothelial matrix, other platelets and other blood cells (Hoffbrand 2011).

Within their complex structure lies a number of granules within the cytosol (Jurk 2005) that upon activation can fuse with the platelet membrane and release their contents into the surrounding media (Harrison 2005). α-granules are the most prevalent and contain adhesive proteins (vonWillebrand Factor [vWF]), growth factors, clotting factors (FV, FVII, FXI,

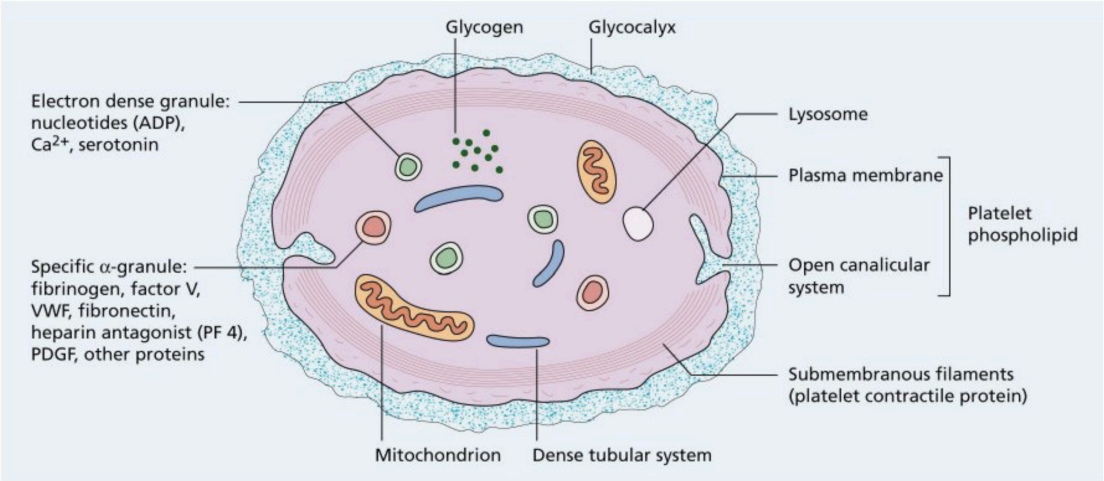


Figure 4-2. Platelet *ultrastructure* (From Hoffbrand 2011 *Essential Haematology* 6th Ed.© 2011 Wiley-Blackwell Publishing)

FXIII) and regulators of coagulation (protein C, plasmin activator inhibitor 1 [PAI-1], tissue factor pathway inhibitor [TFPI]). Electron dense granules contain secondary agonists of platelets which will signal for further platelet activation once released in addition to Calcium; a vital co-enzyme in coagulation and platelet activation (Jurk 2005).

The open canalicular system (OCS) is a network of cell membrane lined channels that pass through the platelet and provide an initial area in which exocytosis can occur and a cell membrane "store" for when platelets divide or expand. A dense tubular system forms a cytoskeleton that is involved in allowing shape change.

4.3.1 Platelet Adhesion

Once the vessel wall is damaged platelets adhere to the exposed collagen in the subendothelial matrix mediated by synergistic reactions occurring between several receptors in the platelet surface (Figure 4-3; Furie 2008). Initially Glycoprotein Ib (GPIb) interacts with vWF binding the platelet to the area of damage. Activation of these various receptors leads to intracellular

signals that cause a conformational change in the platelet GPIIb/IIIa complex. This exposed binding site can then bind to vWF fixing the platelet in location or it can bind to fibrinogen enabling crosslinking.

4.3.2 Platelet Activation

Once bound to the subendothelial matrix the platelet becomes activated (Furie 2008) which initiates a change in platelet morphology: they sphericise and extrude pseudopodia, which enhance interactions between platelets and vessel walls. The cytoskeleton changes cause the classical "fried egg" appearance: long and flat with centralised granules and organelles. The next stage is for further platelet recruitment, which needs the transmission of the activated state of adhered platelets to newly incoming platelets. This occurs through autocrine and paracrine signaling released from intracellular granules, or directly from thrombin produced via the clotting pathways.

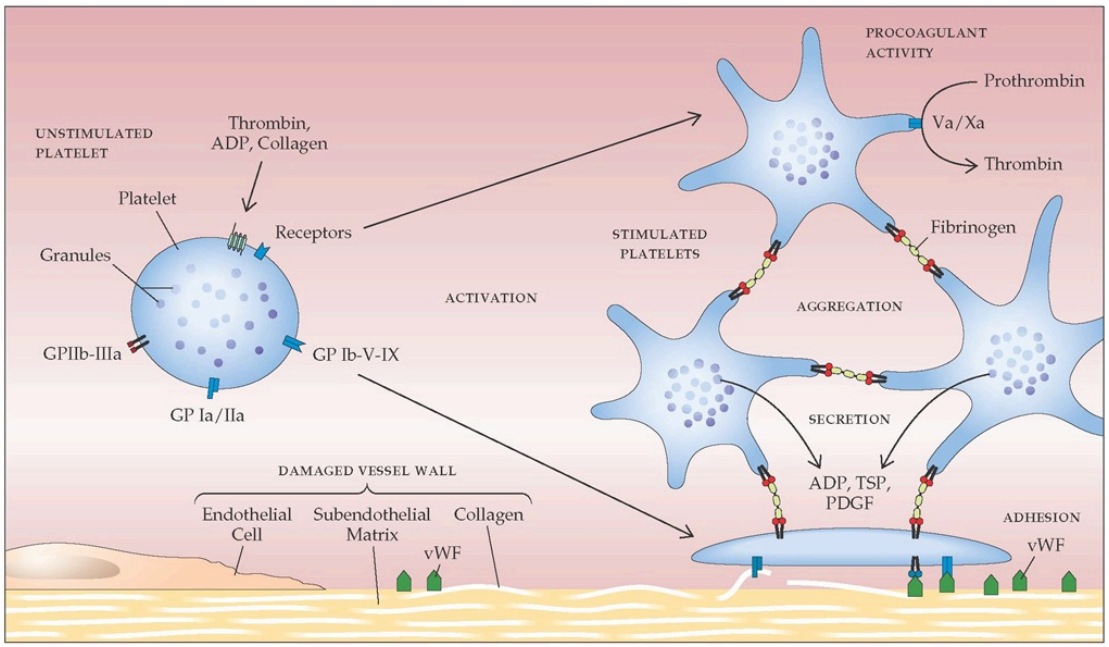


Figure 4-3. Platelet receptors and interactions: adhesion, activation and aggregation (From *http://what-when-how.com/acp-medicine/hemostasis-and-its-regulation-part-1/*)

4.3.3 Platelet Aggregation

Once the initial layer of platelets has covered the subendothelial matrix, adhesion continues in the form of aggregation with two platelets binding to the same fibrinogen molecule (Figure 4-3). These aggregated platelets then form a plug, which (in addition to vasoconstriction) blocks the blood loss from the vessel. Microvesicle extrusion provides a large surface area for large amounts of thrombin to be generated, which in turn leads to clot stabilization by fibrin cross-linking. The final aspect is platelet contraction mediated by the platelet's cytoskeleton (Loscalzo 2003).

4.4 Coagulation Cascade

4.4.1 Tissue Factor

Vessel injury exposes tissue factor (TF) - a class II cytokine held in vascular smooth muscle cells and pericytes - to the plasma and it binds to coagulation factor 7 (FVII, where F and Roman numerals are the recognized nomenclature for coagulation factors). These form a complex on cellular surfaces that triggers the coagulation cascade.

4.4.2 Classic Clotting Cascade

The "classic clotting cascade" was traditionally described as consisting of an "extrinsic pathway" which was initiated by TF interacting with FVII and an "intrinsic pathway" initiated by Thrombin and involving FVIII and FIX (Figure 4-4).

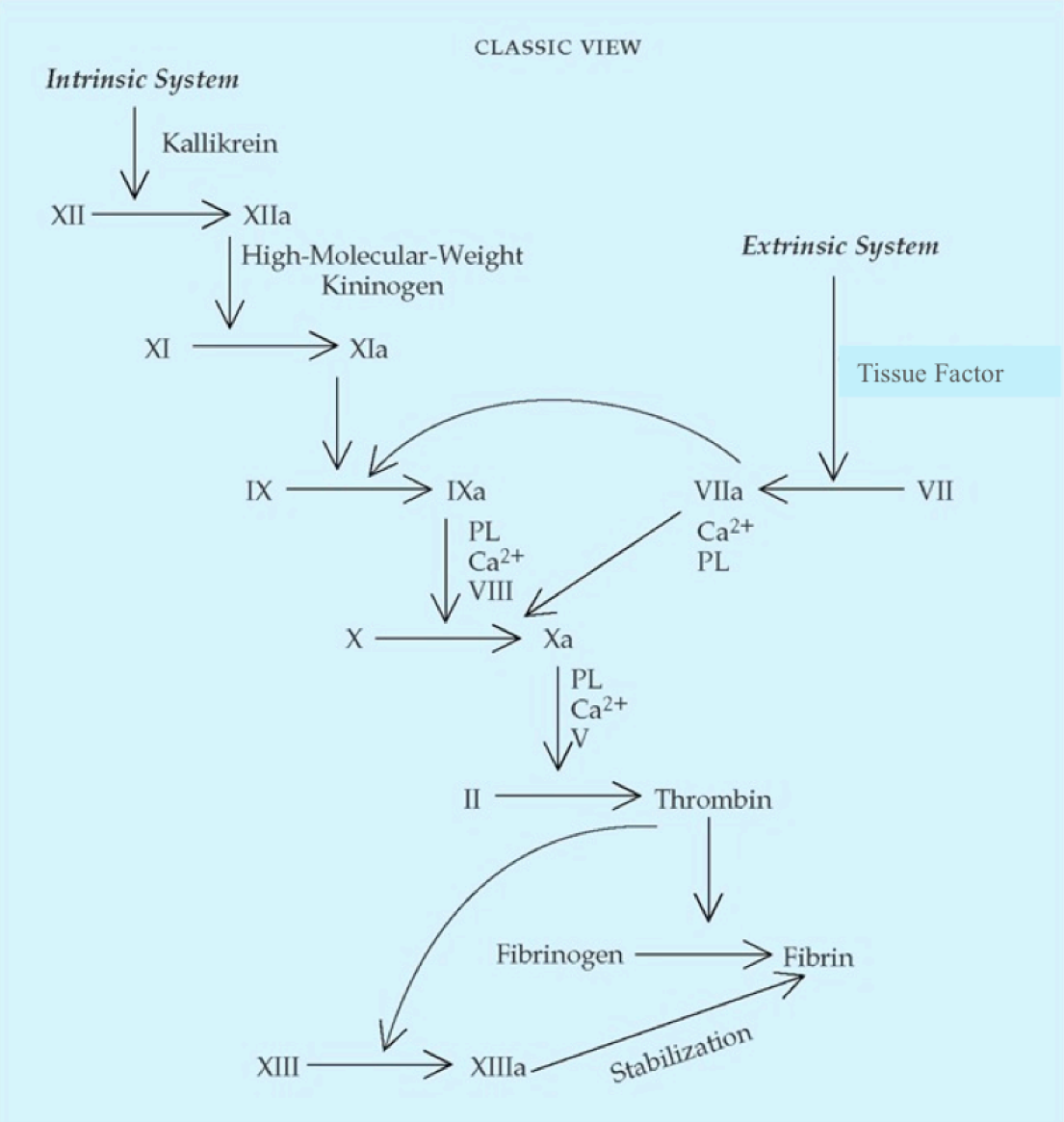


Figure 4-4. The classic view of the clotting cascade, the intrinsic pathway is initiated by the exposure of blood to a negatively charged surface (e.g., thrombin or glass) and the extrinsic pathway is activated by tissue factor or thromboplastin. (From *http://what-when-how.com/acp-medicine/hemostasis-and-its-regulation-part-1/*)

Both of these pathways resulted in activation of their relevant coagulation factors, eventually coming together to form a “common pathway”. This pathway resulted after the activation of coagulation factor 10 (FX), which bound to factor 5 (FV) to form the FXa/FVa complex (where the *a* describes an activated coagulation factor) known as pro-thrombinase (Dahlback 2000). This description has been superseded by a different concept of haemostasis.

4.5 Cell Based Coagulation

The modern concept of coagulation, that has replaced the concept of extrinsic and intrinsic pathways, is the cell-based theory, so called because cell surfaces are the keystone of its progression. Cell-based coagulation consists of highly regulated reactions that take place on cell surfaces (Hoffman 2001, Monroe 2002) in three phases: initiation, amplification and propagation (Figure 4-5). These phases start with the exposure of TF-bearing cells (such as myocytes or adventitial fibroblasts) from the cell wall, resulting in the production of pro-thrombinase complex (FXa/FVa complex). This initial pro-coagulant signal is amplified involving the activation of platelets and finally leads to the propagation of the thrombin generation (Monroe 2006, Mann 2003a, Roberts 2006), known as the "thrombin burst" which ultimately results in fibrin formation.

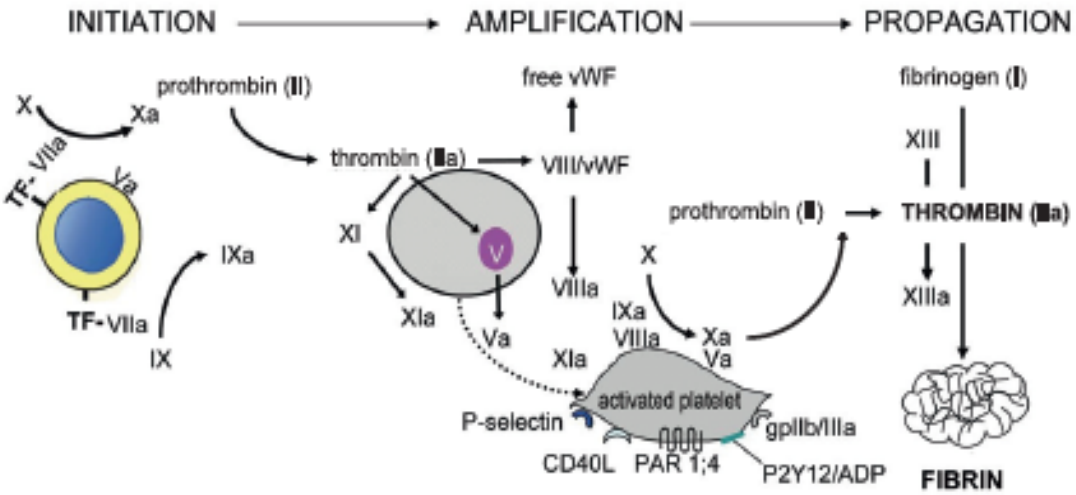


Figure 4-5. Cell based coagulation (From *General mechanisms of coagulation in Thrombosis and Haemostasis © 2013*)

4.5.1 Thrombin

Thrombin is central to the whole process of haemostasis and the regulation of homeostasis. It is has both pro- and anti-coagulant properties. Produced early in the clotting process: it activates platelets initially and then the "thrombin burst" causes fibrinogen conversion to insoluble fibrinogen. It stabilises the clot by activating FXIII to form the fibrin stabilising factor (FXIIIa) and inhibits fibrinolysis through its action in thrombin activated fibrinolysis inhibitor (TAFI). Thrombin is also anti-coagulant as it activates protein C once it is bound to Thrombomodulin. Thrombin is involved in all 3 phases of cell based coagulation: initiation, amplification and propagation.

4.5.2 Initiation

Initiation is localized to TF-bearing cells exposed after vessel endothelial injury. The proteolytic TF/FVIIa complex activates coagulation factors FIX and FX. On TF-expressed cells, FXa binds with FVa to form the pro-thrombinase complex. This complex cleaves prothrombin (FII) to generate a minor quantity of thrombin (FIIa) (Figure 4-6), the enzyme responsible for fibrin formation. The amount of thrombin generated is not enough to produce a significant amount of fibrin, and a subsequent clot, but that is not its function in this part of the clotting pathway; its function here is for the initiation of the next stage – amplification.

4.5.3 Amplification

In the amplification stage, platelets adhere to the injury site and are activated by the low concentration of thrombin (FIIa) initiating the release of more FV and a positive feed back loop is formed; thrombin activates circulating FV, releases FVIII from von Willebrand factor (vWF) and activates it. vWF also has a key role to play in platelet adhesion. The FVa (which binds with FXa) and FVIIIa (which binds with FIXa) in turn bind to

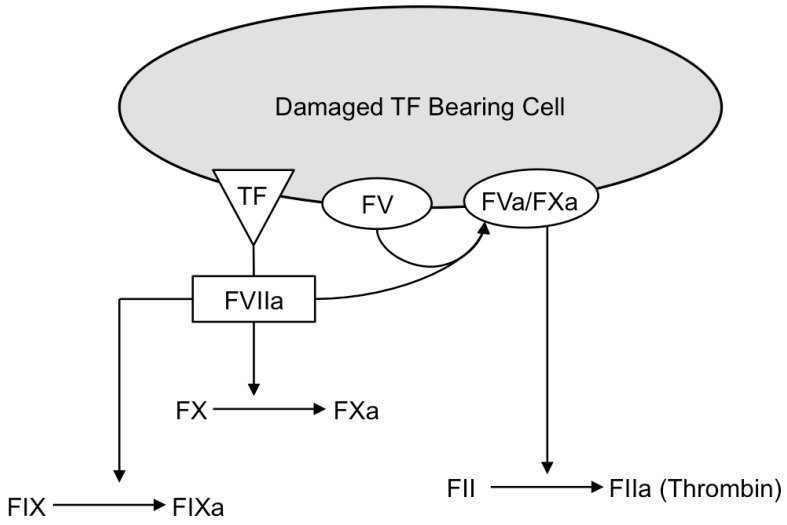


Figure 4-6. Clot Initiation. TF: Tissue Factor, FII: Prothrombin, FII: Thrombin, FV: Proaccelerin, FVII: Proaccelerin, FVa/FXa: Prothrombinase complex, FIX: Christmas factor, FX: Stuart factor

platelet surfaces and act as co-factors for a massive generation of thrombin produced in the propagation phase (Figure 4-7).

4.5.4 Propagation

The propagation phase results from the two complexes – FVIIIa/FIXa "intrinsic tenase" and FVa/FXa "prothrombinase"– assembling on the activated platelet surface and accelerating the production of FXa and in turn thrombin (FIIa). The brisk linking of FXa and FVa results in a "thrombin burst" which in turn converts fibrinogen (FI) to fibrin.

The soluble fibrin monomers polymerise to form fibrin protofibrils. These are stabilized by FXIIIa (also activated by thrombin) to form a solid fibrin mesh network that stabilizes the assembled platelets to form a platelet/fibrin thrombus (Figure 4-5 and 4-7).

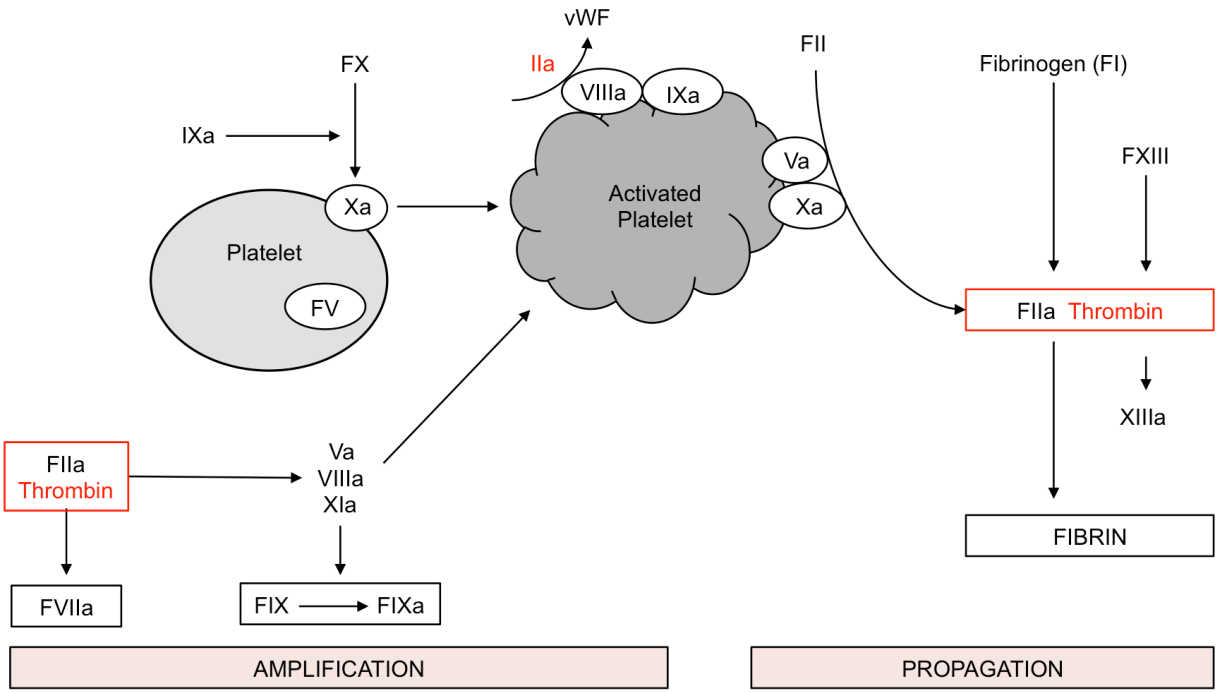


Figure 4-7. Clot Amplification and Propagation. FI: Fibrinogen, FVIII: Antihaemophilic factor, XIII: Fibrin stabilizing factor.

Coagulation is essentially comprised of a number of enzymatic processes, the activation of each factor or complex results in a magnification in the next resultant reaction so that thrombin production is the result of significant amplification; one FXa molecule generates approximately 1000 molecules of thrombin (Mann 2003b).

4.6 Anti-coagulation

The activation of coagulation, its “thrombin burst” and the formation of fibrin need to be regulated or there is the potential for pathological thrombus formation. To control this process and ensure it is confined to the precise location of injury, there are a number of regulatory mechanisms including enzyme inhibition and alteration of cofactor activity. The main inhibitory systems are anti-thrombin, protein C and protein S (and to some extent

TFPI) as the most important regulators of coagulation initiation (prevention of inappropriate clots forming). In addition there is the fibrinolytic system (breakdown of clots) led by plasmin.

4.6.1 Tissue Factor Pathway Inhibitor

Tissue factor pathway inhibitor is a circulating plasma protease inhibitor that is synthesized by the microvascular endothelium. Approximately 20% of TFPI circulating in plasma is associated with lipoproteins; the majority remaining associated with the endothelial surface bound to the glycocalyx (Lupu 1995). TFPI has a very low plasma concentration and inhibits FXa. The TFPI/factor Xa complex becomes an effective inhibitor of tissue factor/factor VIIa (TF/FVIIa complex).

4.6.2 Antithrombin

Antithrombin (AT) is a protease inhibitor and inhibits the majority of the enzymes generated during the activation of coagulation. As it inhibits preferentially free enzymes, rather than those that are part of the intrinsic tenase or prothrombinase complexes, it is probably fundamental to reducing and localizing coagulation to the sites of vascular injury plus protecting the circulation from liberated enzymes (Dahlback 2000, Monroe 2006). AT by itself is inefficient but the heparin-like molecules on vascular endothelium stimulate its activity.

4.6.3 Protein C and S

Thrombomodulin (TM) is a molecule present on vascular endothelium, which binds thrombin. This thrombin/TM complex converts protein C to its activated form (Esmon 2003); activated protein C (APC). Activation to APC via thrombin is a slow process but is accelerated 100 fold when thrombin

binds to TM and another 20 fold when this complex binds to endothelial protein C receptor (EPCR) - a receptor which offers protein C to the thrombin/TM complex. This suggests that this the mechanism for endothelial control of thrombosis (Esmon 2003).

Activated protein C regulates or suppresses coagulation by reducing the activity of co-factors, in particular FVIIIa and FVa (Dahlback 2000)

Protein S, like protein C, is another Vitamin K dependent co-factor that supports the anticoagulant activity of APC. APC and protein S form a membrane bound complex that can split FVIIIa and FVa even when these are part of fully formed pro-coagulant complexes. FVIIIa is normally bound to von Willebrand factor, which prevents it from reacting with the phospholipid membranes. APC can split off FV even if bound to phospholipids and so the anticoagulant FV produced works in collaboration with protein S as a cofactor with APC in the degradation of FVIIIa.

The importance of the protein C system is illustrated by the symptoms associated with protein C or S deficiency in humans (Jalbert 1998), which produces a severe thrombo-embolic disease.

The thrombin/TM complex, although anti-coagulant in action here with its establishment of protein C into inhibiting clotting factor activity, also has pro-coagulant anti-fibrinolytic activity. Thrombin/TM activates thrombin activatable fibrinolysis inhibitor (TAFI), which by its action on fibrin inhibits fibrinolysis (Bouma 2003).

Thrombin therefore has a key part in both coagulation and anticoagulation; at a site of vascular injury it is pro-coagulant in contrast to the intact vascular system where it has anticoagulant activity when it binds to TM and activates protein C.

4.7 Fibrinolysis

The process of clot breakdown is termed fibrinolysis, which exists to limit or prevent excessive thrombus production (Figure 4-8). The process is driven by plasmin, the main action of which is to cleave fibrin resulting in a number of soluble fibrin degradation products (FDPs). These in turn compete with thrombin and consequently reduce further clot formation.

Tissue-plasminogen activator (t-PA) and urokinase convert plasminogen to active plasmin that in turn breaks down fibrin. t-PA is a proteolytic enzyme produced by intact endothelial cells and is a crucial part of restricting coagulation to the site of vessel damage. The half-life of t-PA is short and it is a poor activator of plasminogen, yet in the presence of fibrin (which acts a co-enzyme) its action is increased 100 fold (Rijken 2009), is prolonged and concentrates the production of plasmin to the clot site.

Reduced activity of t-PA can lead to thrombosis or embolic events, whereas increased activity causes hyperfibrinolysis manifesting as excessive bleeding.

4.7.1 Fibrinolysis Inhibition

Plasmin is the main enzyme in fibrinolysis and its activity can be reduced by the presence of α_2-Antiplasmin (a_2-AP) and thrombin activatable fibrinolysis inhibitor (TAFI). The former inhibits plasmin whereas the latter inhibits plasmin function (figure 4-8). Fibrin terminal lysines are exposed upon limited proteolysis of fibrin by plasmin and act as ligands for the lysine-binding sites of plasminogen and tissue-type plasminogen activator (t-PA) that then produce further plasmin. Elimination of these lysines by TAFI abolishes the fibrin cofactor function of t-PA-mediated plasminogen activation, resulting in a decreased rate of plasmin generation and thus down-regulation of fibrinolysis (Bouma 2003).

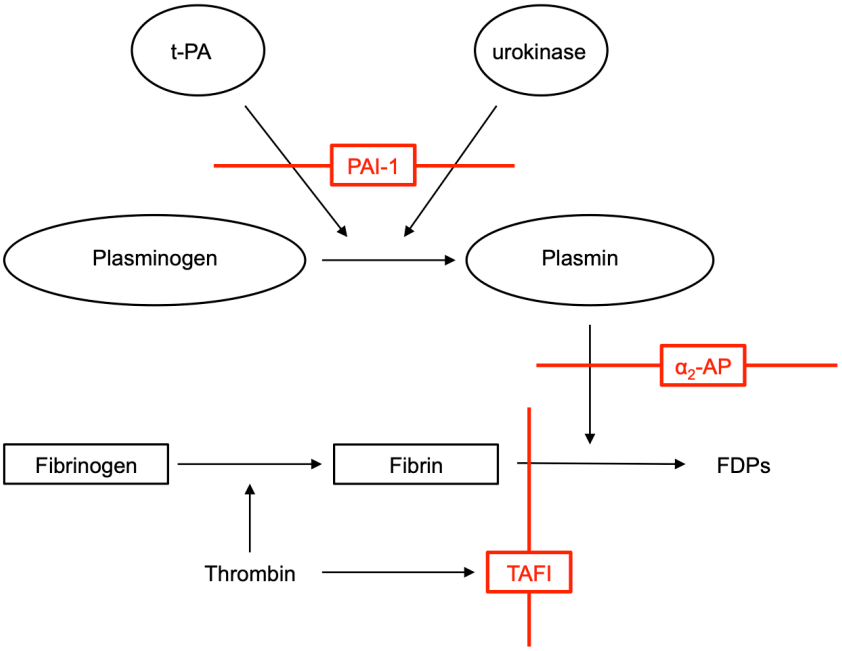


Figure 4-8. Fibrin degradation. Plasminogen is activated by tissue type plasminogen activator (t-PA) or urokinase. These enzymes are regulated by plasminogen activator inhibitor-1 (PAI-1). Plasmin degrades fibrin into soluble fibrin degradation products (FDP) and is regulated by α_2-Antiplasmin (α_2-AP). Thrombin converts fibrinogen into fibrin and also activates thrombin activatable fibrinolysis inhibitor (TAFI) which inhibits fibrinolysis by modifying the fibrin substrate. (Adapted from Bouma 2003).

The conversion of plasminogen to plasmin is controlled by t-PA. t-PA is inhibited by plasminogen activator inhibitors 1 and 2 (PAI-1 and PAI-2). PAI-1 is the most significant and its release is stimulated by various inflammatory and coagulation mediators including thrombin (Cesarman-Maus 2005).

On the contrary excessive APC enhances fibrinolysis by degradation of PAI-1, further evidence of the multiple contrary actions of thrombin, which influences APC generation. This latter aspect of inhibition of fibrinolysis inhibition, or in other words fibrinolysis stimulation as mediated by APC is a critical aspect of the coagulation abnormalities observed in the trauma patient – the Acute Traumatic Coagulopathy

4.8 Coagulopathy

Coagulopathy is used to describe a pathological fault in homeostasis, which leads to either hypercoagulopathy (an increase in thrombosis in either quality or quantity) or hypocoagulopathy (a decrease in thrombus formation through inadequate coagulation or excessive degradation). The focus of this thesis is Acute Traumatic Coagulopathy, which is a hypocoagulopathic state, and as such for the remainder of this text the term “coagulopathy” will be used to describe hypocoagulopathy.

Coagulopathies are either acquired or inherited. Inherited coagulopathies are usually secondary to a deficiency of one aspect of the clotting system resulting in poor clot formation, such as Haemophilia where there is a deficiency in certain clotting factors (paucity of FVIII in Haemophilia A, FIX in B, FXI in C) and in Von Willebrands Disease (missing or defective vWF). Acquired coagulopathies are either iatrogenic or pathological. Iatrogenic are caused by anticoagulant treatment, given for a variety of reasons, but predominantly prophylaxis in conditions where the risk of abnormal thrombus formation is increased. Examples of this are patients with Atrial Fibrillation or those with prosthetic heart valves treated by Warfarin; patients with Acute Coronary Syndrome treated by heparin; or in post-operative prophylaxis against venous thrombo-embolism given Low Molecular Weight Heparins. Examples of pathological acquired coagulopathies are Vitamin K deficiency in liver disease, Disseminated Intravascular Coagulopathy in Obstetric patients or the Trauma Induced Coagulopathies in trauma patients (Kumar 2016).

The causes of Trauma Induced Coagulopathy are multifactorial (Davenport 2011a; Figure 4-9) and were originally described as being the *lethal triad* of dilution, acidosis and hypothermia.

In the last decade the role of hormones, cytokines and the immunological system have all been better understood to have a role in Trauma Induced

Coagulopathy (TIC) and in particular a separate pathological division of that coagulopathy that has been termed Acute Traumatic Coagulopathy.

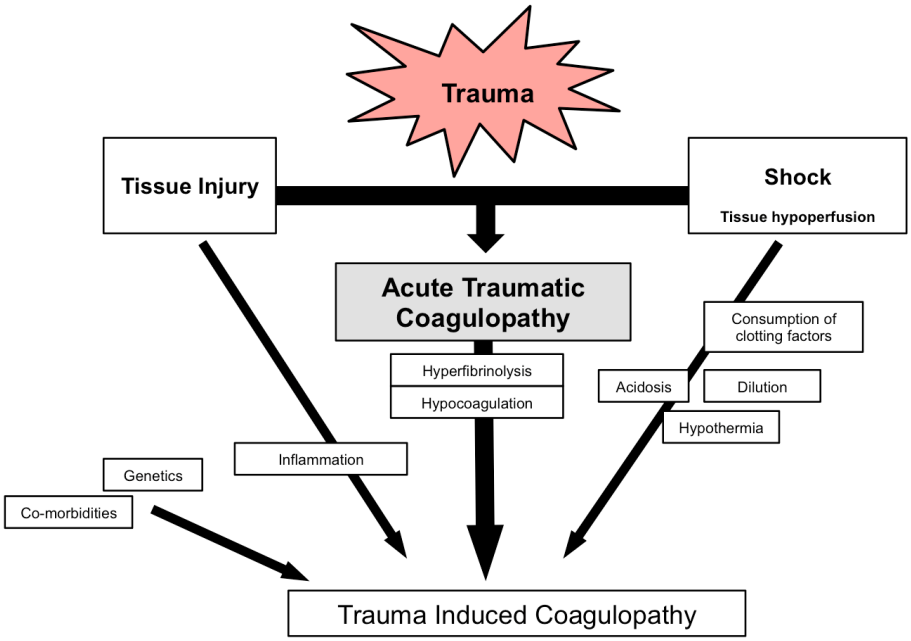


Figure 4-9. Trauma Induced Coagulopathy causes and relationship to ATC. (Adapted from Davenport 2011a).

4.9 Trauma Induced Coagulopathy

Trauma Induced Coagulopathy (TIC) is the term used to describe coagulation abnormalities, particularly hypo-coagulation, associated with and found in a trauma casualty.

Coagulation abnormalities in trauma casualties were identified over half a century ago. In the Vietnam War (Second Indochina War) a direct association was identified between the presence of shock and clotting abnormalities in casualties (Simmons 1969). A couple of decades later a causal relationship was found between a drop in blood pressure (BP) to less than 100mmHg and activation of the clotting system (Risberg 1986) and

that those casualties receiving resuscitation with dilution by crystalloid infusion developed a coagulopathy (Hewson 1985). Until recently crystalloids were the principle initial therapy used in trauma patients as advised for decades by the American College of Surgeons Committee on Trauma and taught on the Advanced Trauma Life Support (ATLS) course (Kortbeek 2008). The advice for its use was continued on this side of the Atlantic by the reasonably recent recommendation in the European Guidelines on the management of bleeding (Rossaint 2016). In this guideline it was only once anaemia developed that the patient was treated with packed red blood cells (PRBC) and other component therapy was not used until much later (Curry 2012) in the resuscitation pathway. This resulted in the dilution of clotting factors establishing a dilutional coagulopathy.

In the first few minutes after injury increased clotting (excessive clot formation) will occur (Riha 2013, Duan 2014). This is an appropriate and reasonable response if it is localised only to injury site, however subsequent significant or excessive clot formation in an attempt to cessate bleeding can lead to a reduction in available clotting factors; a consumptive coagulopathy. Continued bleeding exacerbates this response.

The inadequate resuscitation methods, recommended by ATLS and other bodies, based around the use of crystalloids commonly led to the production of an acidosis. The excessive cold crystalloid infusion, in addition to over exposure of the patient in order to fully examine their injuries led to patients becoming cold. The culmination of all these factors contributed to a worsened coagulopathy. This iatrogenic “resuscitation associated coagulopathy” or *lethal triad* (described later) is generally found later in the patient’s pathway.

A separate pathological coagulation abnormality has been identified not related to the *lethal triad*. This separate pathological process has been found early in the trauma process and temporally seems to be important

initially, with the lethal triad resuscitation based coagulopathy predominating later (Figure 4-10).

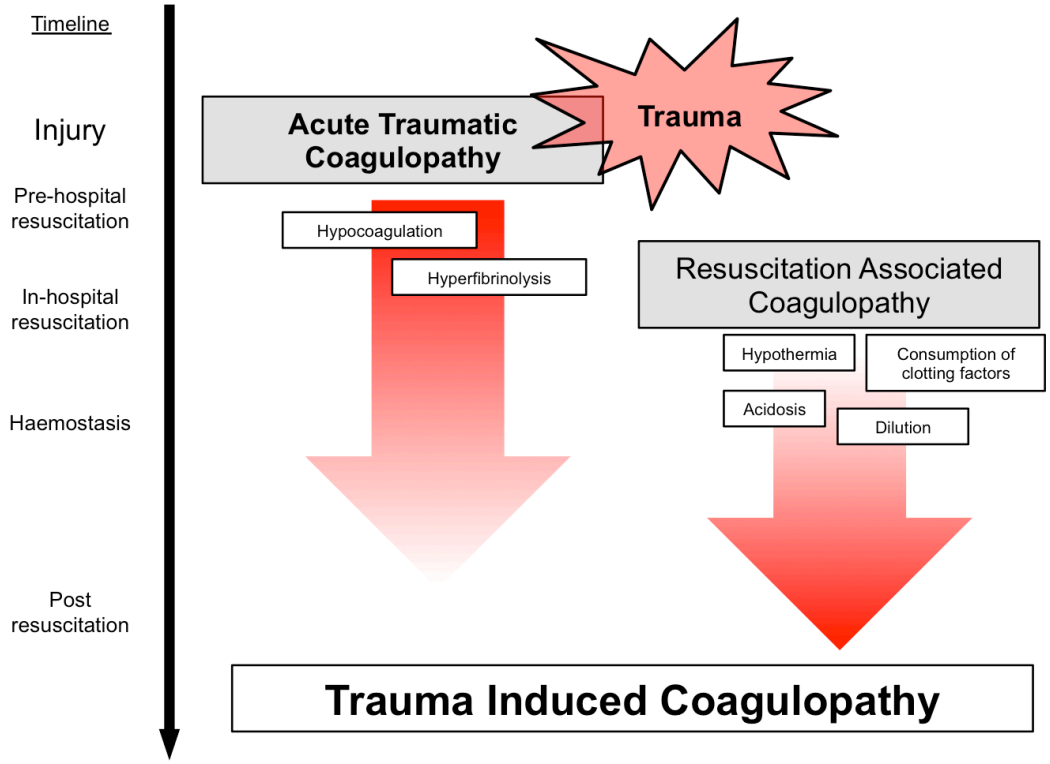


Figure 4-10. Timeline for coagulopathy development in trauma patients. (Adapted from Kushimoto 2017).

Brohi (2003) and his team at the Royal London Hospital - arguably one of the busiest UK trauma centres - studied just over 1000 patients suffering from traumatic injuries. They had a typical UK trauma pattern of 75% suffering blunt trauma and a median injury severity score (ISS) of 20. These patients were predominantly male, had been rapidly brought to the hospital (median 73 minutes) and had received less than a litre of fluid. There was no significant relationship between type and quantity of fluid received and coagulopathy. They had therefore not achieved the

parameters of the “lethal triad” of trauma, yet 24.4% of the patients were coagulopathic on arrival.

Coagulopathy was determined by aPTT or PT of greater than 1.5 times normal and was not affected by age or mechanism of injury (blunt versus penetrating). However the incidence of coagulopathy increased with a rise in ISS; the median incidence of coagulopathy was 24.4% but this rose to 33.1% for those who had an ISS >15 and 61.7% for those with an ISS >45. Key to this paper was the effect on mortality, as coagulopathic patients had a mortality of 46% compared to 10.9% in non-coagulopathic patients. This paper showed evidence of an early coagulopathy that was evident in trauma casualties, related to injury severity and had a direct relationship with mortality. This was a coagulopathy that was not caused by dilution or hypothermia but was a separate additional pathological element to TIC and was termed Acute Traumatic Coagulopathy (ATC).

4.10 Acute Traumatic Coagulopathy

In the last decade a number of trauma centers both in UK and USA have found that some trauma casualties on admission to the hospital are coagulopathic despite little or no haemodilution, no excessive acidosis and minimal reduction in temperature (Brohi 2003, MacLeod 2003, Maegele 2007). These patients are more likely to need a “massive” blood transfusion, develop multi-organ failure and have a four fold chance of dying.

In the USA MacLeod’s team (2003) analysed the data on nearly 8000 trauma casualties from their hospital in Florida over a 5-year period. The population was similar (predominantly male, suffering blunt injury arriving at about 70 minutes post injury). 28% of the patients were coagulopathic and had an increased mortality of 19.3% compared to 6.3% in non-coagulopathic patients. Maegele’s (2007) larger, but retrospective, study of

over 17000 patients from the German trauma registry had a higher incidence of blunt trauma (96%) and found 34.2% were coagulopathic.

These arguably seminal papers were of a civilian population suffering predominantly blunt trauma. The military casualty is different particularly in mechanism of injury with ballistic injuries and high injury severity scores predominating. The proportion of military patients who have ATC remains unclear, although several papers have suggested rates around 40 - 50% (Plotkin 2008, Niles 2009, Doran 2010), which is considerably higher than the civilian population. As the presence of ATC on admission is related to mortality (Brohi 2003, Macleod 2003, Maegele 2007), detecting its presence and finding ways to reduce it or indeed ameliorate the treatment of it are at the forefront of every military physicians and researchers mind.

4.10.1 Pathophysiology of ATC

Continued work into this coagulopathy is improving our knowledge into its pathophysiology. The underlying processes appear to be an unwarranted activation of haemostasis that leads to excessive coagulation and fibrinolysis (Brohi 2007a, Doran 2012). Collagen and Tissue Factor (TF) exposure with tissue damage leads to thrombin generation that is related to the amount of TF exposed (Dunbar 2009). Excessive TF exposure causes a disproportionate activation of haemostasis.

A number of observational studies have illustrated some of the changes that occur in ATC. These have led to a generalized theory of the ongoing processes, the pathophysiology of ATC and in particular the role of activated protein C (Brohi 2007a, Cohen 2012).

The initiator of this coagulopathy is excessive thrombin. High thrombin generation has been illustrated by a number of studies (Dunbar 2009, Chandler 2010, Woolley 2013). These and others (Brohi 2007a, Shaz 2011, Floccard 2012, Rourke 2012) have illustrated low quantity of clotting

factors, reduced protein C levels, low platelet counts, low fibrinogen and low antithrombin – an indication of consumption of vital coagulation reagents. Fibrinogen is one of the first coagulation proteins to fall to critically low levels (Rourke 2012, Curry 2012) and FV is consistently the lowest clotting factor (Bickell 1994, Yuan 2007, Rizoli 2011).

This reduction in fibrinogen is as a result of a number of elements, but of note is the role of thrombin. Thrombin is pro-coagulant in leading to fibrin polymerization and deposition but it also triggers the endothelium to release tissue plasminogen activator (tPA), which in turn leads to plasmin production and therefore fibrin breakdown. This release of tPA is augmented by the presence of hypoperfusion (Brohi 2008, Hayakawa 2011) and many see hypoperfusion as the key initiating mechanism for ATC.

The endothelium is a vital aspect of the control and initiation of clotting. The surface of the endothelium is lined by a group of proteins linked with glycosaminoglycan chains termed the glycocalyx (Reitsma 2007). The glycocalyx or rather its destruction is an important intercessor for ATC development and hypoperfusion is a crucial initiator of glycocalyx destruction.

4.10.2 Endothelial Glycocalyx

The glycocalyx (Figure 4-11) lines the vascular endothelium and one of the glycocalyx proteins is Syndecan-1, a proteoglycan. Its presence in the serum correlates well with glcocalyx destruction (Rehm 2007). A number of factors associated with trauma disrupt the glycocalyx in addition to hypoperfusion, including direct tissue trauma, catecholamine levels and inflammation (Maegele 2014, Johansson 2011).

A number of potential mechanisms for the role of the glycocalyx have been proposed. Heparan sulphate is present in the glycocalyx and is released

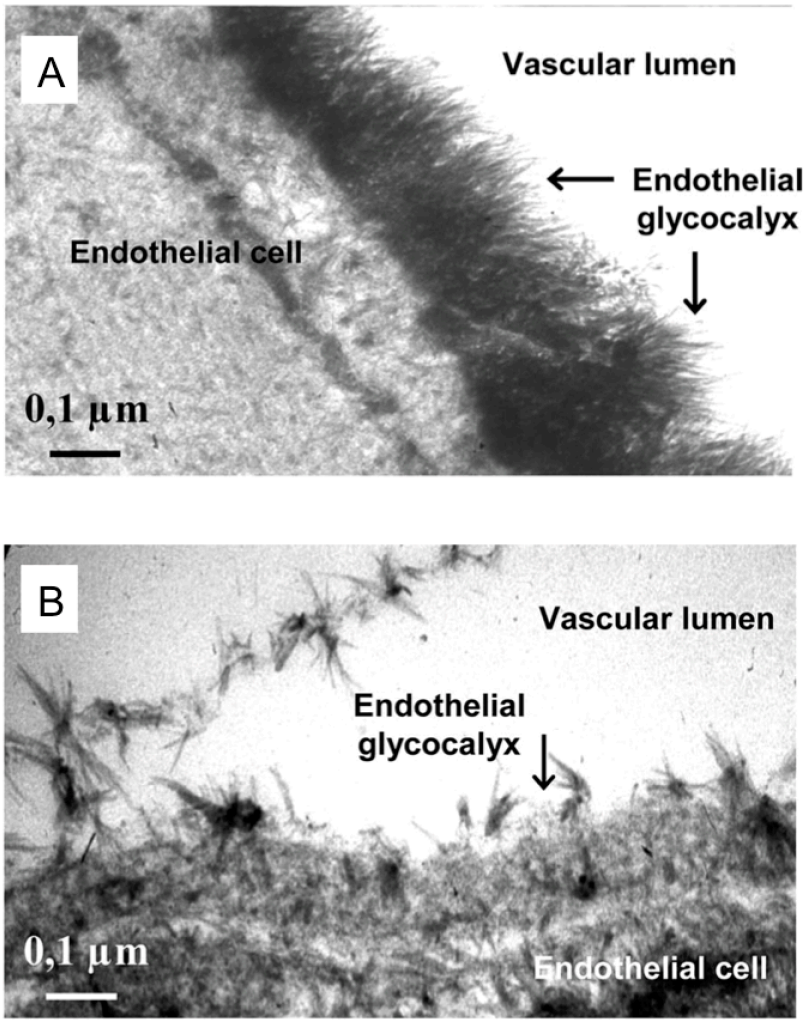


Figure 4-11. Electron microscope views of the glcocalyx. A Intact glycocalyx. B Disrupted glycocalyx. (From Chappell D, Jacob M, Hofmann-Kiefer K, Bruegger D, et al. Hydrocortisone Preserves the Vascular Barrier by Protecting the Endothelial Glycocalyx Anesthesiology 2007; 107:776–84.)

into the circulation upon glycocalyx damage with its heparin-like properties having an anticoagulant effect (Johansson 2010, Liu 2007).

The main concentration of work in this field has been on the link between glycocalyx disruption and the activated protein C (APC) pathway. Johansson (2011) showed that significant endothelial glycocalyx destruction led to increased Thrombomodulin, reduced protein C concentrations,

hyperfibrinolysis and prolonged coagulation times (as measured by conventional laboratory tests - activated partial thromboplastin time [aPTT]).

These results all infer a significant correlation between glycocalyx destruction and Acute Traumatic Coagulopathy development mediated by APC. However when that group looked at the correlation between glycocalyx destruction and directly measured APC levels their findings were conflicting (Ostrowski 2012a) and more in keeping to with the auto-heparinisation mechanism. They found more severely injured patients had a higher level of glycocalyx destruction and a lower level of protein C, but APC levels were no different between the severity groups, while heparinisation (measured by TEG) was greater in the higher injury severity group. It may well be that APC is rapidly broken down within the systemic circulation and doesn't correctly reflect local endothelial and injury site concentrations – but this is merely conjecture. The evidence is suggestive that glycocalyx breakdown has probably both auto-heparinisation and APC activity in its role in Acute Traumatic Coagulopathy.

Fundamentally the glycocalyx has a key role in the pathophysiology of ATC with a number of theories merging into how important the endothelium's role has in this: be it termed the "endotheliopathy of trauma" (Pati 2016, Naumann 2017, 2018) or "shock-induced endotheliopathy" (Johansson 2017). Trauma is not the only cause of endothelial pathology however, with a number of factors being shown to produce evidence of glycocalyx disruption including hypoxia (Annecke 2011), sepsis (Nieuwdorp 2009) and sympatho-adrenal activation (in trauma)(Ostrowski 2017). Elements of all these are seen in hypo-perfused trauma patients and so this is not entirely unexpected. The presence of Syndecan-1 (evidence of glycocalyx destruction) correlates with a number of fundamental consequences to trauma including increased vascular permeability (Rahber 2015) and inflammatory changes (Ushiyama 2016, Schmidt 2011).

In summary the glycocalyx is influential in the pathophysiology of ATC, with studies providing evidence of a number of uniting factors: tissue destruction,

endothelial damage, coagulopathy, inflammation and higher mortality. A key finding is the close relationship between hypoperfusion, glycocalyx destruction and activation of protein C.

4.10.3 Hypoperfusion and the Role of APC

Significant blood loss leads to increased sympathetic drive and natural inotrope secretion in order to preserve perfusion pressure to vital organs, resulting in vasoconstriction peripherally and in certain organs. This vasoconstriction to deviate blood flow towards vital organs leads to a reduced blood flow, or hypoperfusion, elsewhere. Tissue hypoperfusion means inadequate oxygen supply to cells and in the case of ATC it is a hypoxic microcirculation – also termed microcirculatory shock – that is the driver for this coagulopathy (Brohi 2008, Johansson 2011, Gruen 2012).

Protein C has been shown to be a crucial aspect of ATC. Tissue hypoperfusion produces pathological amplification of protein C activation (Brohi 2007b). The activated protein C (APC) then has a dual action of both negative feedback on thrombin production and removal of the inhibition of tPA, thus causing a consequent increase in fibrinolysis (Figure 4-12).

Brohi's team investigated this mechanism at the Royal London trauma centre on 208 major trauma patients (Brohi 2007a). Mean Injury Severity Score (ISS) was 17 and initial blood samples were taken at a median time of 32 minutes from injury. Pre-hospital treatment for resuscitation was as per local guidelines and hence none received vasopressors nor colloid, and crystalloid use was minimal (mean volume 150millilitres). The data was investigated for presence of hypoperfusion (as judged by a base deficit - BD - of 6mEq/l or more) and coagulopathy (using conventional laboratory results of either Prothrombin Time [PT] or activated Partial Thromboplastin Time [aPTT] ratios to normal being increased to 1.5) and compared to injury severity. They found that coagulopathy only occurred if BD >6mEq/l. At

this level of hypoperfusion; thrombomodulin levels increased, protein C levels decreased and plasminogen activator inhibitor one (PAI-1) reduced.

The drop in protein C was assumed by the authors to be secondary to an increase in APC formation, although APC was not measured directly. They hypothesized that the hypoperfusion as indicated by increased BD led to increased thrombin-TM complex production, conversion of protein C to APC and subsequent anticoagulation via FVa and FVIIIa inhibition. In addition the reduced PAI-1 (which inhibits t-PA) leads to a rise in t-PA activity resulting in (excessive) fibrinolysis (Figure 4-12).

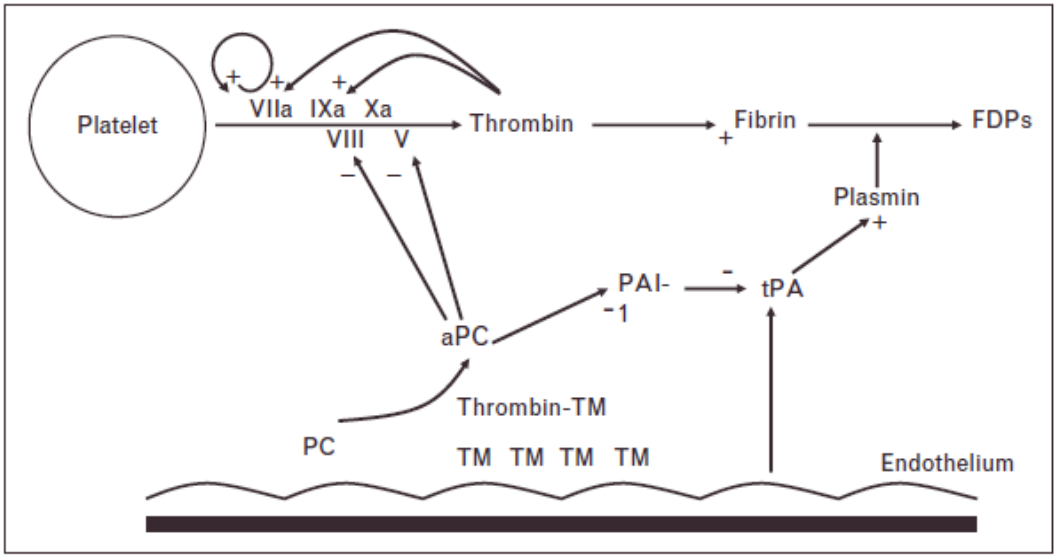


Figure 4-12. Acute Traumatic Coagulopathy caused by activated Protein C. (*From [b]Brohi K, Cohen J, Davenport R. Acute coagulopathy of trauma: mechanism, identification and effect. Curr Opin Crit Care 2007; 13: 680-5.)*

Elements of this theory on ATC pathophysiology is supported by previous and subsequent studies. Animal translational studies (Faller 1999, Doran 2012) and human studies (Brohi 2003, Niles 2008, Frith 2010, 2012, Cohen 2013) have all demonstrated the crucial relationships in this theory. As hypoperfusion or shock worsens, indicated in most studies by worsening of

BD, then coagulation deteriorates. Increased injury severity (ISS) and hypoperfusion synonymously increase the level of coagulopathy.

The presence of a raised APC (and hence ATC) is important for patient outlook. A raised APC in certain patients (with severe injuries and elevated base deficits) is associated with organ injury, raised transfusion requirements, and importantly increased mortality (Brohi 2007a, Cohen 2012). Interestingly if APC is inhibited (in mice), early traumatic coagulopathy is prevented (Chesebro 2009).

The production of APC driven by hypoperfusion appears to be a key part of the complex process that leads to ATC, as it both reduces thrombin production through its ability to inhibit FV and FVIII and also induces fibrinolysis.

4.10.4 Fibrinolysis

The removal of the inhibition of tissue Plasminogen Activator (tPA) by APC leads to increased Plasmin and thus increased fibrinolysis. The presence of severe hyperfibrinolysis (defined as 95% lysis within 30 minutes on ROTEM®) in trauma patients is associated with mortality rates in excess of 70% (Ives 2012, Cotton 2012, Schöchl 2009). The Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage (CRASH-2) trial (CRASH-2 Trial Collaborators 2010) showed a statistical improvement in survival with the use of an antifibrinolytic (Tranexamic Acid) in trauma.

Pharmacological cessation of all fibrinolysis is not the panacea for ACT though, as patients treated with Tranexamic Acid more than 3 hours after injury have an increased mortality (Napolitano 2013), a disturbing fact when all UK trauma centres advocate empiric use of Tranexamic Acid in trauma patients requiring resuscitation (Bozette 2013).

The pathophysiological cause of the hyperfibrinolysis is not clear-cut. The theory on ACT foundation as described above (Brohi 2007a) is supported by other investigators (Ganter 2010, Lier 2011); its presence mediated by an amplified APC pathway instigated by an increased TM release from the hypoxic vascular endothelium. An alternative mechanism is argued by Gando (2011) who believed that it is secondary to Disseminated Intravascular Coagulopathy (DIC) or rather a sub-group of it - DIC with fibrinolytic phenotype - rather than a separate trauma specific pathological process (Gando 2013).

Risberg (1978) described two types of fibrinolysis in early trauma; primary and secondary. Primary systemic fibrinolysis is “shock-induced” from hypoxic endothelium leading to increased t-PA. Secondary fibrinolysis occurs in the microcirculation as a secondary reaction to the deposition of fibrin thrombi due to DIC. It is their belief that this secondary DIC fibrinolysis reaction occurs in trauma and is illustrated by high t-PA levels and low protein C levels (Hayakawa 2011). The problem with this alternative DIC hypothesis is that normal levels of PAI (t-PA inhibitor) are seen in DIC (Gando 2011) compared to reduced levels in the ATC driven hypothesis.

Despite the disagreement on exact process or nomenclature of the fibrinolysis in trauma, its presence is un-argued (Gando 2011, Brohi 2003, Bluth 2011, Levrat 2008, Schöchl 2009) and most importantly its presence significantly increases mortality (Ives 2012, Cotton 2012, Schöchl 2009).

Some investigators have identified three separate “phenotypes” of fibrinolysis in trauma, each having a different effect on mortality (Moore 2014); a normal physiological fibrinolysis which has the lowest mortality (3%), hyperfibrinolysis as identified and described by many with a higher mortality of 44% from typically exsanguination in the first 24 hours, and lastly fibrinolysis “shutdown” described as the inhibition of fibrinolysis. This latter phenomenon has been previously described in elective surgery and was associated with deep venous thrombosis and multiple organ failure

(Chakrabati 1969, Griffiths 1979, Yukizawa 2012, Hayakawa 2012). Moore (2014) showed that fibrinolysis "shutdown" occurred most frequently (64% of patients) and was also associated with a raised mortality (17%). Mortality in this latter group was lower than the high mortality hyperfibrinolysis and occurred later, more characteristically being from multi-organ failure.

Although the presence of hyperfibrinolysis is concerning due to its high mortality, it is potentially diagnosable early through near-patient thromboelastometry and readily treatable with Tranexamic Acid. This latter medication has been readily used in UK practice buoyed by the results of CRASH-2 – 9% reduction in mortality in bleeding patients. The UK Defence Medical Services condone its use, again supported by relevant, although retrospective, data in the military population (Morrison 2012). The concern of a few is the possible harm if Tranexamic Acid is given after 3 hours (CRASH-2 collaborators 2011).

The hypothesis is that hypercoagulopathy occurs after trauma secondary to the activated endothelium responding to the pathological processes of trauma by producing increasing amounts of PAI-1 (inhibited by APC normally in trauma). This now elevated PAI-1 inhibits the t-PA action on plasminogen, resulting in a decrease in fibrinolysis (Selby 2009, Curry 2012). If this is the cause of fibrinolysis shutdown shown in Moore's study (2014), the concern for the clinician is its incidence (64% in his population) and the potential harm that Tranexamic Acid may cause them in a population with an already increased mortality.

Critics of CRASH-2 illustrate the differences between 1st World trauma treatment, the lack of blood received and level of trauma severity in that population compared to what is seen in the US, UK and Australia (Mitra 2014). A randomised controlled trial of Tranexamic Acid use in this population will hopefully guide clinicians better.

Moore's fibrinolytic shutdown group's mortality of multi-organ failure

illustrates the close relationship between the coagulation system and the immune system. The MATTERs (Morrison 2012) paper on military trauma patients receiving a varied amount of Tranexamic Acid and found a difference in mortality after 48 hours compared to CRASH-2 at 24 hours. Many confounding factors may explain this, but one postulation was the effect of Tranexamic Acid on not just fibrinolysis but also the immune system, which is significantly affected in trauma patients and not uncommonly leads to a systemic inflammatory response.

Lysis of fibrin will have an effect on clot stability and strength but some authors have found that fibrinogen has only 31% contribution to clot strength in trauma. The majority of the clot strength being secondary to platelets (69%; Komblith 2014).

4.10.5 Platelet Dysfunction

Platelets and their surfaces play a key role in the amplification and propagation stages of the cell-based model of coagulation (Hoffman 2001). It is not uncommon to find that patients with proven ATC actually have normal platelet levels. In fact low counts have been found to occur in less than 10% of severely injured (ISS>45) patients (Hess 2009). Although trauma patients with a higher platelet level on admission have a higher survival, it has still been recognized that patients with normal platelet counts on admission also had high mortality rates (Brown 2011, Stansbury 2013).

Despite these patients having quantitatively normal platelets the higher 24-hour mortality is associated with abnormal platelet function (Kutcher 2012). This abnormal platelet function in ATC is prevalent, varying from 45% having “platelet hypofunction” (Kutcher 2012) to 86% showing ADP inhibition of platelet function (Wohlauer 2012), with a postulated cause being the extensive disrupted endothelial ADP release producing initial

platelet hyperactivity leading to “exhausted platelet syndrome”. These platelets, although normal in number, are consequently unresponsive despite stimulation.

Using viscoelastic testing (ROTEM®) British military ballistic casualties had an incidence of hypocoagulopathy in 40% (Doran 2010), showing evidence of diminished clot strength secondary to platelet function rather than through deficiency of fibrinogen. These findings not only reflect the important role that platelets play in ATC, but also highlight the fact that normal admission platelet counts do not correlate with adequate function.

Platelet activation is dependent on initiation of the clotting process determined by activity at the endothelium, bringing us full circle to the vital role of the lining of the endothelium – the glycocalyx – and its key position for platelet and protein C activation.

This separate pathophysiological cause for coagulopathy (ATC) is what is immediately seen after trauma. Once medical care and resuscitation commences then the picture becomes more opaque as iatrogenic factors that influence coagulation play a larger part. This resuscitation associated coagulopathy is therefore found later in the patient’s pathway taking a more predominant role and ATC having a lesser impact as time progresses (Figure 4-10). Part of this iatrogenic coagulopathy has historically been described as the *lethal triad* and can be potentially avoided.

The key elements of the *lethal triad* – dilution/consumption, hypothermia and acidosis - were commonly called the lethal triad or bloody vicious cycle (Kashuk 1982, Curry 2012). A term originally used to describe patient pathology subsequent to haemorrhage from abdominal vascular trauma but then readily used consequently in all trauma. The presence of the described triad has been shown to result in worsening haemorrhage and an increased likelihood of death (Kashuk 1982).

4.11 Resuscitation Associated Coagulopathy

4.11.1 Consumption

Initiation, activation and propagation of haemostasis, particularly if excessive or in a high injury severity patient, will cause a subsequent consumption of clotting factors, fibrinogen and platelets - the initial basic intrinsic constituents of coagulation. The presence of early pulmonary thrombus in military ballistic trauma patients (Lundy 2012) demonstrates (abnormal) excessive clot formation distant to the injury site can occur in severely injured patients. This abnormality is for both quantity of reaction, but also location. Excessive clot formation will result in coagulation substrate depletion. Thrombin generation, distant to the wound site, secondary to systemic activation of the coagulation system has also been found in civilian trauma patients (Dunbar 2009). The peak thrombin generation in their patients was at times 300% more than normal patients. This excessive consumption distant to the wound site leads to not only widespread coagulation activation, but also ensuing activation of the fibrinolytic pathways in response to it with subsequent development of a reaction not too dissimilar to disseminated intravascular coagulation (DIC) resulting in excessive clotting factor consumption.

Some authors (Gando 2011) believe that there are high amounts of Tissue Factor (TF) from direct presentation on exposed sub-endothelial TF-bearing cells at the site of injury, and also other systemic intravascular TF-bearing cells such as monocytes and cytokines induced as a result of tissue damage. These authors believed that DIC occurs as a result of a substantial surfeit of thrombin production caused by these high amounts of TF found in the blood immediately after trauma. This predictably leads to extensive coagulation activation and consumption of clotting factors.

Haemostasis does not have a simple on/off switch. Certainly there is one for activation, but the quantity and quality of the resultant reaction is not so clear-cut and a spectrum exists which can be influenced. The presence of

injury, injury severity and type of injury has an influence. High severity, blast injuries (Lundy 2012) cause abnormal clotting pathology in the pulmonary vascular tree and lower limbs – a systemic coagulation response, compared to the less severe, civilian blunt trauma where coagulation is more localized to injury site. There seems to be evidence that these severely injured casualties who have suffered total body injury from the blast wave have a systemic coagulation activation and subsequent consumption of clotting reactants.

Resuscitation methods vary throughout the World. For decades the initial use of an easily transportable fluid has predominated and crystalloids have filled this role. Inappropriate, excessive use of a fluid that contains no clotting products will understandably cause a dilution of clotting reactants and other essential blood components.

4.11.2 Haemodilution

Diluting the blood and therefore red blood cell and haemostasis components contributes to coagulopathy development (Hewson 1985). Part of the body's normal initial physiological response to haemorrhage is an attempt to increase intravascular fluid by movement of extracellular fluid into the vasculature, mounting to a natural dilution of clotting factors (Carey 1973).

Colloid resuscitation in order to increase plasma volume effects clotting in a number of ways. It not only dilutes clotting factors but can also have a direct impact on the fibrinolytic system producing a pro-fibrinolytic state. Dilution of α_2-Antiplasmin reduces its inhibitory effect on plasmin, leading to an increased fibrinolysis. This can be exacerbated by additional dilution of another fibrinolytic inhibitor thrombin activatable fibrinolysis inhibitor (TAFI) (Bolliger 2010). A Cochrane Review on colloids (Perel 2013) looked at 65 trials involving colloids and found there was no justification for their use

compared to crystalloids with no evidence that they reduce the risk of death. Although for most there was no evidence of increased harm – save hydroxyethyl starch which might increase mortality – their expense precludes justification for their use.

Crystalloid resuscitation also causes a diluent effect on factors and like colloids can also potentially produce a pro-fibrinolytic state (Bolliger 2010). As the amount of crystalloid resuscitation increases so does the coagulopathic effect, so that up to 70% of patients will have a coagulopathy and reduced platelet count if they receive 4000ml of fluid (Maegele 2007).

Dilution with crystalloids, colloids and even plasma will also reduce haematocrit and haemoglobin.

4.11.3 Anaemia

Reduced haematocrit due to dilution, through either the normal physiological response to blood loss or as a consequence to iatrogenic means, has an important effect on haemostasis. Margination of cells particularly platelets improve platelet/endothelial cell interaction and adherence. This interaction and adherence is a key component of the normal function of coagulation. In anaemia the reduced haematocrit impairs this effect (Valeri 2007), reducing the interaction between platelets and endothelium and having a negative impact on clotting.

4.11.4 Hypothermia

Defined as the reduced body core temperature of below 35°C, hypothermia has been found historically in nearly 50% of trauma patients on arrival to Emergency Departments particularly if scene times were prolonged (Helm 1995).

Enzyme function is affected by many conditions, one of which is temperature, the extremes of which reduce enzyme function. Haemostasis is reliant on clotting factors, all of which are enzymes in their activated form. It is unsurprising to find therefore that haemostasis is impaired in hypothermic patients. Enzymes do not function at lower temperatures and the activity of proteases, particularly important for activation of clotting factors decreases as temperature drops (Reed, 1990). The activation of factors involved in initiation of coagulation diminish linearly with temperature; FVIIa activity (key in the initiation of coagulation) is only 80% effective at 33°C (Meng 2003).

The effect of low temperatures has an even greater impact on platelets, the activation of which is a key component of the amplification phase of coagulation. Wolberg (2004) found that not only enzyme activity was reduced but platelet function, in particular aggregation and adhesion were diminished to 60% of normal at 33°C. The threshold for these temperature effects on platelets (and enzyme function) appears to be 34°C.

Conventional laboratory tests of coagulation – activated partial thromboplastin time (aPTT) and prothrombin time (PT) – have been shown to be prolonged in hypothermic patients (Rohrer 1992, Staab 1994) but more recently studies on visco-elastic tests (thromboelastography TEG® and rotational thromboelastometry ROTEM®) have yielded more information at different temperatures. Watts (1998) found once patient temperatures dropped below 34°C although PT and aPTT were unchanged, TEG maximum amplitude (MA, which is a measure of clot strength, determined by platelet or fibrinogen function) diminished and this was attributed to platelet function. Dirkmann (2008) found results that supported Watts; using ROTEM, they discovered that platelet function was primarily to blame for haemostasis failure in hypothermia. The maximum clot firmness (MCF, the equivalent to TEG® MA) a reflection of platelet function was reduced. They also found at the lower temperature of 30°C clotting time (CT, a measure of initiation of clot) was prolonged, a reflection of reduced activation clotting factors. Dirkman also found that acidosis acted

synergistically with hypothermia and although there were little effects at normothermia, the combination of both acidosis and hypothermia worsened MCF and CT illustrating reduced platelet function and diminished enzyme efficiency. Martini (2009) looked at the non platelet effects of cold and acidosis, and found they also both effected thrombin generation and fibrinogen metabolism

In all, hypothermia in trauma has been found to be related to injury severity and is an independent risk factor for death, in one study reaching a mortality of 100% when core temperatures were below 32°C (Jurkovich 1987).

4.11.5 Acidosis

Tissue hypoperfusion in trauma causes hypocoagulation by different mechanisms. It is the initiator of the protein C pathway to coagulopathy (ATC). It also causes hypoxia of tissues, the result of which is an acidosis, which has a separate impact on coagulation. Although in Dirkman's in vitro trial (2008), acidosis only had an influence on coagulation in hypothermic conditions, a number of other studies have found that it can significantly affect haemostasis in normothermia. Although these studies generally investigated acidosis in isolation and not in the context of haemorrhage and hypoperfusion, the results remain relevant. Acidosis has been found to inhibit the propagation phase of thrombin generation and accelerates fibrinogen degeneration (Martini 2009). While each clotting factor can be affected differently by acidosis, calcium (a crucial clotting enzyme co-factor) availability is significantly reduced (Martini 2007). Platelet internal structure is altered and their ability to change shape is reduced at pH less than 7.4 (Djaldetti 1979). By pH 7.0 haemostatic platelet functions are completely down regulated (Etulain 2012), with one study finding platelet count reduced by 40% at pH 7.1 potentially due to some impact on aggregation, but in reality the actual cause was unclear (Martini 2007).

In Martini's animal model (2007) the effects of a pH of 7.1 were manifold: PT and aPTT were both prolonged (by approximately 20%); TEG Maximum Amplitude was reduced by 20%; there was 50% inhibition of thrombin generation and fibrinogen degradation was accelerated by 1.8 fold.

The majority of the aspects of this resuscitation-associated coagulopathy can be avoided by using good resuscitation regimes. Acidosis is the exception to this as it will be pathologically produced by the body's reaction to trauma and the consequent hypoperfusion. Usefully, acidosis can be measured with base excess, which is a useful marker of both physiological compromise and response to resuscitation.

Longer term the effect of trauma on the casualty moves away from coagulation and more towards inflammation. There is a correlation between inflammatory changes, sepsis and the endothelium being instrumental in the causation of ATC. An analysis of a subset of patients in the PROPPR (Pragmatic, Randomised Optimal Platelet and Plasma Ratios) trial (Wei 2018) found that evidence of glycocalyx disruption (measured by Syndecan-1) after massive transfusion was associated with developing sepsis. This supports previous work by Johansson's team (Ostrowski 2012b) who investigated a biomarker of endothelial activation (soluble vascular endothelial growth factor receptor 1 – sVEGFR1) in trauma patients, the presence of sVEGFR1 previously having been found to have a strong association with severe sepsis (and mortality) (Shapiro 2010). The presence of sVEGFR1 correlated with glycocalyx degradation (syndecan-1 presence) and the presence of hyperfibrinolysis and APC, but also evidence of inflammation (Interleukin-6).

So the glycocalyx is not only influential in the pathophysiology of ATC, but studies exist that provide evidence of a number of uniting factors; tissue destruction, endothelial damage, coagulopathy, inflammation and higher mortality.

4.11.6 Immune System Activation

The immune system mediated “inflammatory response” is to limit damage or more accurately limit additional damage and promote healing. Traumatic injury is often followed by a systemic inflammatory response (SIRS) (Lenz 2007, Tsukamoto 2010), yet despite the reason for its presence (to limit damage) it is also commonly associated with poor outcomes after trauma (Napolitano 2000).

Cytokines (small proteins involved in cell signalling and key to modulating the balance of the immune system) are raised within 30 minutes of injury and remain elevated for days (Gebhard 2000, Frink 2007). The presence of cytokines in trauma patients is associated with an increased mortality rate, a higher rate of sepsis and elevated incidence of multiple organ failure (Hranjec 2010, Jastrow 2009, Patrick 1999).

It has been found that injured cells release endogenous molecules termed danger-associated molecular pattern (DAMP) molecules, or alarmins, which are the signalling mediators of the “sterile” inflammatory response after trauma (Rider 2017). These molecules (including High Mobility Group Box Protein – HMGB) interact with the innate immune cell receptors to trigger cytokine production (Matzinger 2002).

Inflammation has also been attributed to many other factors involved in trauma patients, including surgery and blood transfusion (Sihler 2010, Tschoeke 2007). These are complicating and arguably iatrogenic factors but it is useful to attempt to separate these different components from each other and trying to elucidate their individual effect on outcome and mortality. The alarmins related inflammatory response found rapidly after time of injury are those pertinent to the immediate ATC. The degree to which they are the cause of multiple organ failure is more difficult to elucidate. By the temporal evidence of SIRS in early trauma patients and its close association with the timing of ATC presentation, alarmins and their relationship to initial cytokine production appear the most relevant to coagulation abnormalities.

There is a close relationship between the inflammatory system and coagulation pathways; inflammation leads to an activation of coagulation, but coagulation also modulates the inflammatory system (Petäjä 2011). It is pro-inflammatory cytokines - identified early in trauma patients - in addition to other mediators that have been identified to contribute to activate the coagulation system and down regulating anticoagulant pathways (Levi 2010).

Tissue Necrosis Factor alpha (TNFα) and Interleukin 1b (IL-1b) induce Tissue Factor (TF) expression on endothelial cells (Levi 2002), a vital component to coagulation initiation. TNFα and IL1b are pro-inflammatory cytokines and in addition to other interleukins (6 and 12) not only increase TF expression, but also increase thrombin production. Thrombin can in turn through a positive loop pathway induce further production of TNFα and IL-6 and IL-1.

Animal studies carried out on a blast/haemorrhage model at Dstl have studied the initiator to this cytokine release – alarmins - in particular HMGB1 and cytokines themselves. They found in a hypotensively resuscitated animal there was an increase in HMGB1 expression, supporting the observations that they are key to initiating the inflammatory response (Kirkman 2011b). In a consequent porcine haemorrhage and blast injury model investigating resuscitation strategies, those animals resuscitated to a normal blood pressure after a set period of hypotensive resuscitation (compared to a complete hypotensive resuscitation) had improved survival, a comparatively better base deficit and lower IL-6 levels, suggesting that the tissue hypoxia (a trigger for ATC) was improved with a higher blood pressure and this led to modulation of the inflammatory response after injury (Doran 2012).

The role of the immune system in trauma is complicated especially when relating its interaction with the coagulation system. The evidence suggests trauma activates both and their consequent reactions can be detrimental.

There appears to be a role for immune-modulation and certainly the alarmins seem to be a potential target for such treatment.

4.12 Coagulation Summary

Coagulation in trauma and the coagulopathy that appears in the trauma patient is complex and as yet not completely understood. Hypoperfusion, causing tissue hypoxia and impacting particularly the vascular endothelium, appears to be the key initiator of the Acute Traumatic Coagulopathy (ATC). ATC has a number of different components one of the most important being fibrinolysis. Hyperfibrinolysis has one of the highest mortality rates.

It is clear that a third of all civilian trauma patients arrive at hospital coagulopathic and this correlates with worse outcomes. These rates of coagulopathy appear to be higher in the military population. Adequate and correct resuscitation of these casualties is clearly important in avoiding the later resuscitation associated coagulopathy. It is also vital to be able to both determine the presence of coagulopathy within these patients early in order to individualise their treatment and identify those at higher risk of death. This is not only a measurement of their coagulation status but also assessment of other aspects of the patient's physiology that may result from injury and are a potential driver for coagulation (and immunology) abnormalities, such as oxygen delivery. The next chapter discusses methods of assessing coagulation and patient physiology in clinical settings.

Chapter 5. Diagnostic Equipment

5.1 Introduction

There is a large variety of medical equipment and instrumentation that can assist the clinician in the management and diagnosis of a patient's condition. In a trauma patient there are minimum standards of treatment and equipment recommended for use by National and International bodies to ensure the patient is cared for appropriately. These range from International guidance from the World Health Organisation (Mock 2004) to UK hospital guidelines from the National Institute for Health Care Excellence (NG40) to the direct guidance to the clinician on matters such as minimum levels of monitoring for an anaesthetised patient from the Association of Anaesthetists of Great Britain and Ireland (AAGBI 2015).

After initial assessment and resuscitation in the Emergency Department, potentially including radiographic investigation, the commonest journey the trauma patient will take is to the Operating Room (OR) for Damage Control Surgery and Damage Control Resuscitation as discussed in more detail in Chapters 2 and 6 of this thesis. The anaesthetist becomes the principal clinician in charge of resuscitating the patient in the OR and relies on certain equipment to guide patient therapy. There are many demanding clinical issues requiring attention, but two lie at the forefront of the resuscitating physicians' mind; adequacy of resuscitation and presence of coagulopathy. Adequacy and response of the patient to resuscitation is estimated by the presence of metabolic acidosis. Inadequate resuscitation will lead to hypo-perfusion of organs and particularly peripheral tissues with resultant anaerobic metabolism leading to acidosis. Arterial blood gases are accepted as the most useful measurement for this. Various assays within this aid treatment, but it is the standard base excess that is judged to be the most useful assay in metabolic acidosis (Park 2008) and used by the majority of anaesthetists as a guide to resuscitation management.

The presence of coagulation abnormality is measured by a number of different assays available within the laboratory and at point of care. The majority of these are discussed in detail in this chapter.

5.2 Acid Base Balance

Negative base excess or base deficit is one of the most readily described parameters in trauma patients with evidence of tissue hypoperfusion or shock. In trauma, tissues and organs have a reduced blood supply, secondary to the cardiovascular and humoral responses to that blood loss. This is principally because of arterial vasoconstriction reducing blood flow and hence oxygen delivery. The hypoperfused cells in the tissue consequently lack adequate oxygen to meet metabolic demands and resort to anaerobic metabolism for less efficient energy production resulting in the conversion of glycogen or glucose to lactate. This production of lactic acid results in a metabolic acidosis. The swiftest compensatory mechanism is through respiratory compensation with hyperventilation excreting carbon dioxide, resulting in a low arterial carbon dioxide in the spontaneously breathing patient. Base excess represents the concentration of titratable acid needed to normalise the pH of one litre of blood to normal physiological values. Decreased base excess represents the presence of unmeasured anions, which in trauma is assumed to be lactate related.

In trauma patients a negative base excess or base deficit is used as a measure of tissue hypoperfusion in particular when related to trauma induced coagulopathy. In trauma patients a base excess of less than -6 mEq/l is accepted as evidence of hypoperfusion (Brohi 2007a, Curry 2012).

The presence of a base deficit in trauma patients is related to increased mortality. Studies as far back as Vietnam (Simmons 1969) found this, as have more recent ones in the civilian population. Frith et al (2010) carried out a retrospective study of over 3000 patients from 5 International trauma

centres and found that as base deficit increased, so did mortality with a correlation between injury severity, shock and the presence of a coagulopathy. Mutschler et al (2013) also performed a retrospective analysis of the German trauma registry and determined that as base deficit worsened, mortality increased. He demonstrated that a base deficit of between -6 and -10 mM had a mortality of 23.9%, whereas a base deficit of less than -10 mM had a mortality of 51.5%.

In addition to mortality, there is an association between base deficit and coagulopathy. In civilian trauma patients a link has been shown to occur between level of base excess and development of Acute Traumatic Coagulopathy (ATC). Brohi (2007) and Frith (2010) showed that as base deficit increased, this correlated with a prolongation of activated partial thromboplastin time (aPTT) and prothrombin time (PT).

Thus, measurement of base excess is important for the assessment of trauma patients as it indicates not only the presence and level of hypoperfusion, but identifies patients that are at risk of developing a coagulopathy (ATC) and are in a higher mortality risk group.

Base excess along with lactate levels, pH and various other key measurements are standard in an arterial blood gas. There are numerous laboratory instruments that do these assays as well as smaller instruments that can be held in theatre and emergency departments. Smaller apparatus also exist and the UK Defence Medical Services use a handheld device called i-*STAT* manufactured by Abbot and can give laboratory quality results significantly quicker than bench top systems (Ismali 2015)

5.3 Assessment of Coagulation

Clotting can be assessed by a number of measurements, either through whole blood assessment or of individual aspects of the haemostatic system.

The resuscitating clinician requires an assay that gives the most information rapidly to aid a rapidly changing physiological response to the initial traumatic injury and response to resuscitation events. Various methods of assessment are discussed below and related to their degree of practicality and use for patient resuscitation.

5.4 Routine Laboratory Testing

Laboratories offer a number of tests for the different aspects of the coagulation pathway. These can be divided into tests of: whole blood, plasma, platelets and fibrinogen. These are discussed in more detail with particular concentration on the relevance to the trauma patient.

5.5 Whole Blood Tests

The formation of a clot is dependent on the interaction of all the factors of haemostasis, in particular the cellular components. A test that considers all these aspects together is arguably a more suitable investigation of the whole system of clotting factors, cells and fibrinolysis and their combined interactions.

5.5.1 Bleeding Time

This is probably one of the oldest tests of haemostasis, (De Caterina 1994) and unusually for modern laboratory test is completed *in vivo.* It requires minimal additional equipment. It has been deemed as an excellent screening test for platelet function, traditionally described as the vascular platelet phase of haemostasis (Raber 1990). It is also dependent on an

intact vasospastic response in small vessels in addition to an adequate number of functionally active platelets.

The test is performed with a standard incision on the forearm 5cm below the antecubital fossa, after a blood pressure cuff has been inflated to 40mmHg. Blood can be absorbed off the skin, but no direct wound pressure is allowed. The time is measured from the moment of incision to the moment bleeding stops. The time varies depending on the type of blade that makes the incision, the direction of the incision and location on arm. Bleeding normally stops within 7 to 9 minutes.

Bleeding time is no longer practiced routinely due to the large number of variables and lack of standardisation and arguably ethical questionability in the face of alternatives.

5.5.2 Activated Clotting Time (ACT)

For this test, fresh whole blood is added to a test tube containing a negatively charged surface (using kaolin, celite or glass) (Horton 2013) and is placed into a warmed rotating device. A proximity switch is activated once a small magnet within the test tube is displaced by sufficient clot formation. It was traditionally used to test the adequacy of heparin, but it can be prolonged by platelet dysfunction, “intrinsic” clotting factor deficiencies, low fibrinogen and hypothermia (Horton 2013).

ACT and Bleeding Time give a global picture of clotting function and to some extent the clot quality, however despite this advantage they are actually not as clinically useful as the alternatives. Any abnormal result does not give specific information on which particular aspect of haemostasis is abnormal.

5.6 Plasma Tests

Two of the commonest investigations for haemostasis requested nationally are the activated partial thromboplastin time (aPTT) and prothrombin time (PT). Originally designed for investigating inherited haemostasis disorders, such as the Haemophilias, they are now used in routine screening of patients with suspected bleeding disorders or peri-operatively. They were designed around measuring the speed of coagulation and historically tested what was traditionally described as the two pathways of coagulation – intrinsic and extrinsic.

5.6.1 Activated Partial Thromboplastin Time (aPTT)

Originally termed the kaolin-cephalin clotting time, as kaolin and cephalin were originally used as activators, this was a test of the “intrinsic” clotting factor pathway. This test uses centrifuged plasma taken from a citrated blood sample. Calcium is added (to reverse the citrate effect) and a number of activators are used to initiate coagulation. These activators include kaolin, but silica, celite and ellagic acid have also been used. The term “partial” represents the lack of tissue factor used. The time to form a clot is measured optically and is normally 30 to 50 seconds (depending on the laboratory set up and what activator is used). This is a measurement of the time for a clot to form (initiation) and gives no information on clot strength or stability. It will be prolonged in deficiencies of the “intrinsic” pathway factors (FVIII, FIX, FXI, FXII) and the final common pathway (FII, FV and FX). It is typically used to measure the effects of heparin. Inaccuracies occur and mild factor deficiencies (of up to 40%) will still produce a normal result (Proctor 1961).

5.6.2 Prothrombin Time (PT)

PT is an assay of the “extrinsic” pathway. It has a derivative called the International Normalised Ratio (INR), which is the ratio of the patient’s prothrombin time to a normal PT result. A normal range is 12-13 seconds and the INR is a ratio of result to normal range (normally 0.8 to 1.2), so that an INR of 1.0 is therefore a normal result, >1.2 is a prolonged time to coagulation and <0.8 is an indication of clotting occurring more rapidly than normal.

The PT test is similar to aPTT, except it uses tissue factor as the activator (normally thromboplastin from brain tissue; Laffan 2017). The PT is the time taken for the clot to form and like aPTT is not a reflection of clot strength or stability. PT will be prolonged in deficiencies of the “extrinsic” system (FVII) and the final common pathway (FII, FV and FX). It is typically used to measure the effects of Warfarin and other factors that impact on Vitamin K production/function. INR is the standard test for Warfarin therapy and standardised internationally after the World Health Organisation regulated the preparation of the thromboplastin activator (Poller 2012).

Although the most common laboratory tests undertaken, these standard haemostatic measurements have two main failings; prolonged time to obtain results from the laboratory and a reliance on a historical and arguably outdated understanding of haemostasis.

The wider acceptance of the cell based model of coagulation and the pivotal role of platelets within this means these plasma based studies are less appropriate for the assessment of haemostasis in a trauma patient. They provide information on the initial aspect of coagulation; initiation up to fibrin formation and do not make any assessment of clot strength or stability.

Although laboratory platelet tests exist, they are predominantly assessments of platelets in isolation.

5.7 Platelet Tests

5.7.1 Platelet Count

Platelets are a key aspect of haemostasis and the formation of an adequate clot. A minimum number of platelets are required to form a normal clot and European and UK Defence guidelines state that in trauma for a bleeding patient platelet count should be kept greater than $1000x10^9$/l (Spahn 2013, JSP 99, 2012). Platelet counts can be done manually using a haemocytometer, but it is more common for laboratories to use an automated analyser. These are normally flow cytometers that count all blood cells (including red cells, leukocytes and platelets) concurrently. The problem with this assay is that it is merely a measurement of quantity not quality, nor function. Abnormal platelet activity and function has been found to occur after trauma in spite of a normal platelet count (Doran 2010, Davenport 2011, Jansen 2013) and it is this measurement that is also needed in the management of a trauma patient.

5.7.2 Platelet Function

Historically the Bleeding Time was used as a test for platelet function (despite being a test of more than this function of homeostasis) and was replaced in the 1960s by light transmission aggregometry. Born used light transmission aggregometry to test the ability of platelets to aggregate in response to external agents *in vitro* (Born 1962). In the 1980s platelet aggregometry in whole blood (Cardinal 1980), the study of activated platelets *ex vivo* using flow cytometry (Michelson 2006) and other measurements of specific platelet compounds and nucleotides were available (Holmsen 1966).

The recent increased use of antiplatelet drugs in patients with their higher risk of bleeding, especially during trauma and surgery has led to the need for platelet function tests use in the peri-operative setting. Newer, simpler

instruments that are point of care (POC) have led to improved diagnostic capabilities available to clinicians.

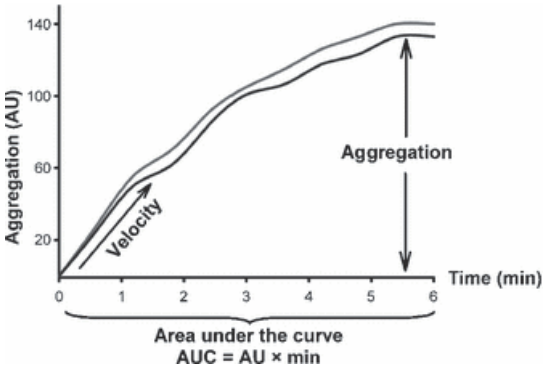
Multiple Electrode Aggregometry (MEA; Multiplate®, Dynabite, Munich) was introduced in 2005. It operates on the basis of whole blood impedance aggregometry, using the fact that platelets become sticky upon activation and adhere and aggregate onto metal sensor wires. The change in resistance of these sensor wires is measured, providing a continuously recorded impedance proportional to the number of platelets adhering to them (Pape 2010). The changes in impedance are plotted against time with the area under the curve produced providing the highest diagnostic power (Figure 5-1A). The main limitation to MEA is the time required to obtain results; the tests takes 20 minutes to perform, however an additional 30 minutes of resting post-sampling of blood is recommended (Jámbor 2009).

TEM International, who produce ROTEM® *delta* (a viscoelastic analyser that is discussed in more detail in section 5.11.2) manufactured an integrated module that can be added to its machines, ROTEM® *platelet.* It also uses whole blood impedance aggregometery and can run concurrently to whole blood viscoelastic testing (Görlinger 2007). It has ready-made agonists and reagents that are ready to use with automated pipetting. It produces a curve and the result is calculated automatically using the Area Under the Curve (AUC) and can give data about platelet function within 10 minutes (Figure 5-1B). It has been shown to detect the effects of anti-platelet drugs (Tóth 2006, Jámbor 2009) in addition to the early effects of trauma and sepsis on platelet function (Adamzik 2012, Kutcher 2012).

Haemoscope Corporation developed TEG® platelet mapping™ initially for monitoring anti-platelet therapy (Bowbrick 2003, Agarwal 2006). Two coagulation assessment curves are produced by the instrument and compared; the first a kaolin-activated test curve reflecting maximal platelet function and the second curve is produced with the addition of FXIIIa (activating clotting factor), reptilase (a snake venom enzyme with similar activity to thrombin) plus one of a number of additives - ADP (adenosine

diphosphate) or AA (arachidonic acid) (both aggregating agent agonists). The activation and aggregation of platelets is quantified by measuring the difference in maximum amplitude (graph width) between unstimulated samples (forming weak fibrin-only clots) and samples with agonists added forming stronger clots containing fibrin and activated/aggregated platelets (Pluthero 2005). These additional additives reduce platelet function and can identify the effect of aspirin or thienopyridine (such as clopidogrel) therapy.

A



B

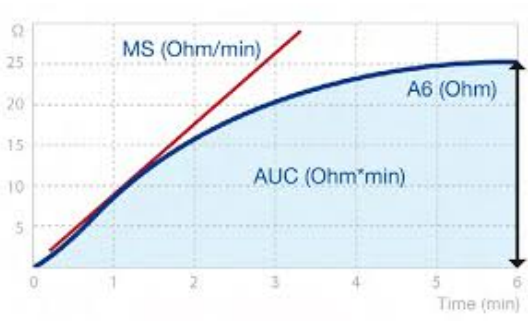


Figure 5-1A. Typical MEA graph of impedance termed as *Aggregation* against time (min). Area under the curve (AUC) produces the indication of platelet function.
Figure 5-1B. Typical ROTEM® *platelet* graph measuring impedance (Ohm) against time (min). AUC is a measure of overall platelet aggregation and Maximum Slope (MS) describes how quickly the platelets aggregate.

Platelets have a significant role in clotting, wound healing and inflammation. In particular their function and activity is being better understood particularly after trauma using the newer viscoelastic additional point of care aggregation tests, chiefly ROTEM® (Ganter 2008, Doran 2010, Solomon 2011). Viscoelastic tests and particularly rotational thromboelastometry can also produce an indicator measurement of platelet function and is discussed below in section 5.11.2. Integral to platelet number and function is their relationship to fibrin. There are a number of laboratory tests that investigate fibrin and fibrinolysis.

5.8 Fibrinogen Tests

5.8.1 Fibrinolysis

The occurrence of fibrin degradation products (FDPs) are used to illustrate the presence and quantity of fibrinolysis, with one specific FDP commonly being measured – the D-dimer. The FDP assay uses antibodies to these products with a test based on agglutination. D-dimer (the most commonly measured FDP) levels are normally less than 500ng/ml. A result of greater than 500 are abnormal but do not indicate a specific disease state, although an increase is an indication of;

- fibrinolysis,
- DIC,
- conditions associated with increased activation of procoagulant and fibrinolytic mechanisms such as recent surgery or presence of thromboembolism
- other conditions such as pregnancy, malignancy and liver disease (Bates 2001, Schouten 2013)

Testing of overall fibrinolysis can also be measured by a euglobulin lysis test that can illustrate a hyperfibrinolytic state, particularly in patients with deficiencies of the controlling enzymes of fibrinolysis – PAI-1 (plasmin activator inhibitor-1) and alpha 2 antiplasmin. There is some evidence that measurement of individual proteins can be useful such as PAI-1 and TAFI (thrombin activatable fibrinolysis inhibitor), but lack of standardisation relegates these methods to research as a whole (Longstaff 2017). Viscoelastic instruments can also detect fibrinolysis to some extent and is discussed in section 5.11.

5.8.2 Fibrinogen Levels

If fibrinogen levels are found to be low on arrival at hospital, this is an independent predictor of both massive transfusion requirement and death in

severely injured trauma patients (Schöchl 2011, Hayakawa 2015, Inaba 2013, Flocard 2012, Rourke 2012). Its timely measurement is imperative to allow correct management of patients' transfusion requirements and is a useful prognostic guide.

There are a number of different assay methods described for measuring fibrinogen levels. Estimations derived from prothrombin time (PT-Fg) are not recommended due to lack of standardisation and numerous variables. Clotable protein assays are very accurate but time consuming and technically difficult. Immunological assays including enzyme-linked immunosorbant assays (ELISAs) have accuracy, but have some variability and lack standardisation.

Clauss fibrinogen assay appears to be the most reliable method for general use in laboratories and remains the gold standard as it measures fibrinogen function directly (Mackie 2003). It requires a plasma sample to be added to a high concentration of thrombin and the clotting time measured and compared to a calibration curve of clotting times from samples of known fibrinogen concentrations and the fibrinogen level is extrapolated from that, giving a result in g/l. The normal range is 1.5 to 4.0 g/l and guidelines suggest in trauma patients their fibrinogen should be kept within this range (Spahn 2013). Unfortunately it can take 30 minutes or more to get the results of these assays.

As the early evaluation of fibrinogen is key (Schöchl 2009, 2011, Theusinger 2015, Kashuk 2010b) for management of trauma patients other more rapid techniques have taken precedence. Viscoelastic methods have become widely used for early fibrinogen level evaluation in trauma patients. Thromboelastometry, especially, has been shown to be of considerable use (Schöhl 2009, 2011, Levrat 2008, Theusinger 2011, 2015). The viscoelastic methods for fibrinogen measurement are discussed in greater depth below.

5.9 Coagulation Tests in Trauma Management

The key problem with management of the bleeding trauma patient and their blood component transfusion requirements is the lack of a rapid and validated coagulation test (Toulon 2009, Segal 2005). The coagulation tests mentioned so far are of limited value for a number of reasons.

Timing is arguably the principal cause for their failure. Laboratory tests in platelet poor plasma require standardised processing, which leads to a delay in the results being available to the clinician for 30 (Toulon 2009) to 45 minutes (Doran 2010). This is an unforgivable delay as the coagulopathy observed in trauma changes rapidly and a historical result is consequently of little use to the physician looking for guidance on transfusion therapy (Brohi 2009, Segal 2005, Curry 2012).

PT and aPTT provide incomplete information on clot initiation (Mann 2003c) and will only detect a reduction in clotting factors of around 40% (Shapiro 1942, Proctor 1961). Both of these tests do not quantify the relative activity of pro-coagulants versus anti-coagulants since neither assay discriminates if a deficient clotting factor is counter balanced by a corresponding anti-coagulant protease (Tripodi 2009).

Platelet count is often initially normal in trauma and does not calculate platelet dysfunction which is common in trauma secondary to the trauma induced coagulopathy and corresponding physiological derangement (Hess 2008).

Fibrinogen levels calculated via Clauss Fibrinogen is a non-functional test and makes no assessment of the cross-linking of polymerised fibrin by FXIII. It can take 30 minutes to get a result.

Finally, there is no laboratory test that examines the relationship between clot production and propagation versus clot lysis or one that determines an assessment of clot strength. Reduced clot strength is a common

pathological consequence of trauma and an inability to measure it will hinder a physicians ability to adequately resuscitate a trauma patient.

In short there are no laboratory tests that can determine the elements of ATC within a useful timeframe in order to aid blood component therapy or help predict the need for massive transfusion (Kashuk 2010a, Dzik 2004). The historical diagnostic indicators for the management of major haemorrhage were based on the need for fresh frozen plasma administration. An INR[1] of greater than 1.5 (Stainsby 2006, Rossaint 2010) was initially used for diagnosis of coagulopathy in trauma. More recent work has reduced this to a level of greater than 1.2 as a more realistic diagnostic level for ATC as a PT ratio of greater than 1.2 in trauma patients was associated with higher transfusion requirements and worse outcomes (Frith 2010).

For the management of a trauma patient therefore the weight is against the majority of diagnostic tests described so far. The reluctance to use them in the trauma patient have been described by a number of authors (Counts 1979, Ciavarella 1987, Yuan 2007) especially when used as an aid to guide transfusion therapy in the face of rapid blood loss. There is recognition by some that they have limitations when screening for, or detecting abnormalities in acute haemorrhage (Davenport 2011b, Johansson 2009a).

5.10 Modern Whole Blood Testing

What is needed for trauma patient bleeding management is a test that is rapid and complete (assessing all aspects of whole blood clot formation, stability and breakdown) using a machine that is mobile and robust enough to be used in the patient's location wherever in the hospital environment they may be. It should also be able to detail the relevant roles fibrinogen

[1] International Normalised Ratio (INR) is a derivative of Prothrombin Time (PT), see section 5.6.2.

and platelets have in clot formation, stability and strength. This need for a global clotting assessment in the trauma patient has initiated a renewed interest in viscoelastic coagulation testing (Johansson 2009a) in trauma patients. It is a method that has been used for considerable time in NHS hospitals for guiding transfusion therapy in elective surgery where blood loss is significant – cardiac surgery (Avidan 2004), hepato-biliary and transplant surgery (Kang 1985) and obstetrics (Rai 2003) – and the move to use it in the emergent bleeding patient has gained increasing popularity.

5.11 Viscoelastic Coagulation Testing

Viscoelastic point of care testing produces information on all phases of coagulation, providing an understanding into the interactions between the plasma and cellular components of whole blood in addition to the activity of the fibrinolytic system (Levy 2010).

The principle method of viscoelastic tests consists of a rotating system moving a pin in a cup. Depending on the manufacturer, either the pin or the cup can be the mobile rotating component. When the system is measuring, a torsion wire usually suspends the pin in the cup. Whole blood is added to the cup and additive reagents to the whole blood initiates clot formation. As the clot forms it entraps the pin and changes movement in the pin. The motion is restricted as the clot strengthens and becomes less restrained as the clot is lysed. The change in movement is interpreted by the machine's software and a graph is produced to reflect the developing clot, which can be related to the clotting process (Figure 5-4).

There are two main manufacturers of devices that measure the viscoelastic properties of blood samples on the market. Both produce equipment that assess the viscoelastic properties of blood and provide a graphical representation of clot formation and fibrinolysis.

5.11.1 TEG® 5000 Thromboelastograph® Haemostasis Analyzer System

The first method; Thromboelastography or TEG® (Haemoscope Corporation, Niles, IL, USA) was originally developed in Germany by Hellmut Hartet who used it for research and illustrating the haemostatic function for whole blood samples (Hartert 1948). The modern version is illustrated in Figure 5-2.

Figure 5-2. TEG® 5000 (from www.haemonetics.com).

TEG was used in the Vietnam War to guide blood transfusions (Hardaway 1988) and in the civilian population Kang pioneered its use to guide blood transfusion in orthotopic liver transplantation (Kang 1985) and increase efficiency of blood component use. The use of TEG in haemostasis assessment and informing blood product use has subsequently been investigated in cardiac surgery (Shore-Lesserson 1999, Spiess 1995, Avidan 2004), obstetrics (Rai 2003) and inherited coagulation abnormalities Sorensen 2005). By the late 1990's, TEG had been used in trauma patients

to guide their resuscitation using blood products, and assess coagulation (Kaufmann 1997). Successive studies on civilian and military trauma patients has confirmed the value of TEG and other viscoelastic testing products in goal directed targeted blood product resuscitation (Johansson 2009a, 2012a, Holcomb 2012, Plotkin 2008, Kashuk 2010a, Tapia 2013).

In addition further studies have confirmed their value in trauma patients requiring blood product resuscitation and guiding the use of adjunctive haemostatic resuscitative agents including fibrinogen concentrate, activated recombinant Factor 7 (rFVIIa), Prothrombin Complex Concentrate (PCC) and the antifibrinolytic tranexamic acid (TXA) (Schöchl 2010, Theusinger 2012, Woolley 2013, Ziegler 2013, Hauser 2012, Branco 2014, Hunt 2015).

The TEG® 5000 uses a whole blood sample that is heated at 37°C in a warmed cup and coagulation is activated using additional compounds (e.g. tissue factor, kaolin, etc.). The cup containing the blood sample is oscillated. The pin placed within the cup is attached to a torsion wire (Figure 5-3).

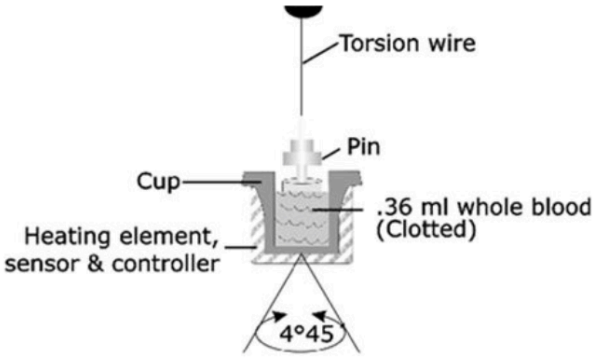


Figure 5-3. TEG pin and cup arrangement (from www.haemonetics.com).

As the oscillating clot forms around the pin, an electro-mechanical transducer measures the movement in the torsion wire attached to it and produces a graphical representation of the movement – a thromboelastograph (Haemonetics 2011). This graphical output illustrates

clot strength on the y-axis measured in millimeters, against time on the x-axis measured in minutes (Figure 5-4).

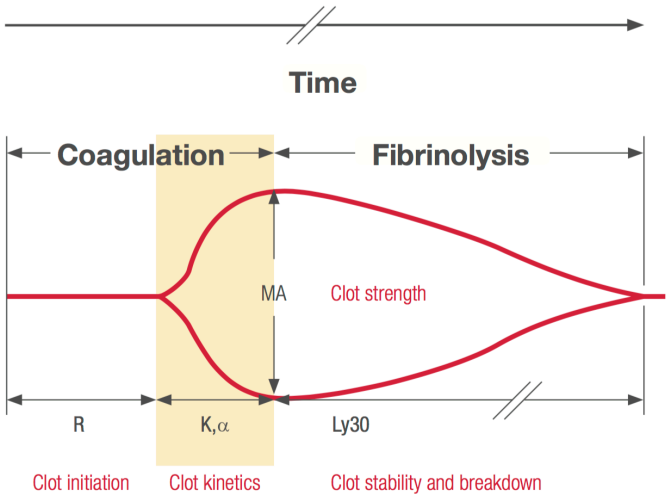


Figure 5-4. Basic TEG graph illustrating key measurement parameters (from www.haemonetics.com).

TEG provides continuous monitoring of the clotting process: initiation, amplification and propagation (kinetics) and fibrinolysis, generating values related to each step (see Table 5-1) and allowing interpretation of the relevant haemostatic process.

Kaolin (and Calcium) are the main initiating compounds used in TEG. Other additives can be used to look at certain aspects of the clotting system. Fibrinogen function can be measured using a glycoprotein IIb/IIIa inhibitor, abciximab. This inhibits platelet function and the assessment of the clot and the contribution of fibrinogen to it can be subsequently assessed by measuring the Maximum Amplitude (MA; Figure 5-4) in comparison to a normal Kaolin sample (Harr 2013).

Platelet function can also be studied using TEG® platelet mapping™, a modified thromboelastography using platelet mapping, which measure the

ability of a platelet agonist (thromboxane A2) to activate arachidonic acid and adenosine diphosphate to stimulate a fibrin-platelet clot independent of thrombin (Bochsen 2007; more details in section 5.7.2).

5.11.2 ROTEM® delta Haemostasis Analyser

The ROTEM® *delta* is the main piece of equipment produced by Tem International and is the current equipment used by the British Defence Medical Services. It uses thromboelastometry to measure haemostasis. (Figure 5-5).

Figure 5-5. ROTEM® *delta* (from www.rotem.de)

Thromboelastometry is based on the principles of thromboelastography but differs from it in a number of ways. In parallel with the latter a pin is placed in the whole blood sample, which itself is placed in a cup. However in thromboelastometry it is the pin that is rotated and rather than a tension wire, a light source is used. The light is directed at a mirror on the pin and reflected onto a detector (Figure 5-6). As the clot forms around the pin, its movement is reduced and this change is detected by the detector and

interpreted by the data processor that converts this into a graphical representation, the thromboelastometric curve (Figure 5-7). The software determines certain parameters from this curve, which are displayed numerically on the screen. (Figure 5-7).

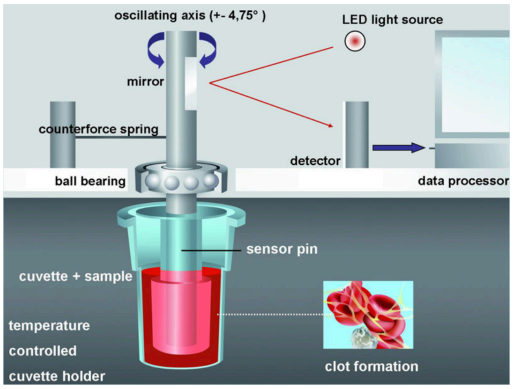


Figure 5-6. Cup and cuvette arrangement in ROTEM (from www.rotem.de)

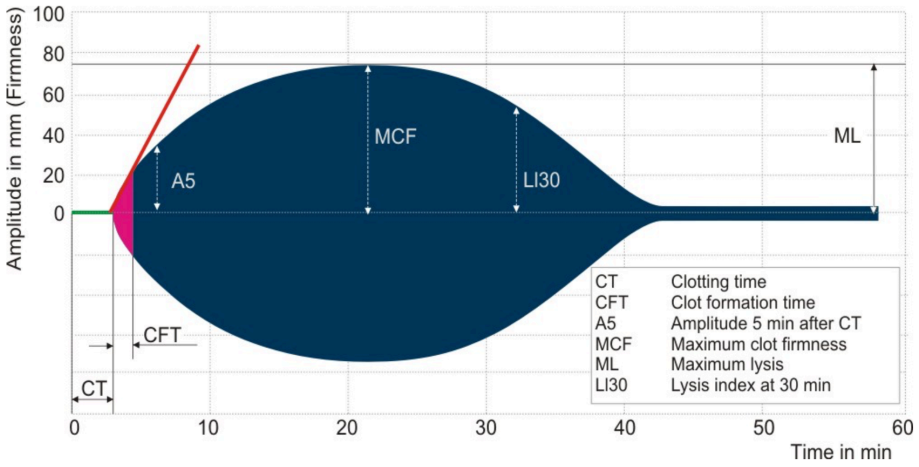


Figure 5-7. Temogram curve displaying measured parameters (from www.rotem.de)

The ROTEM® *delta* has four channels which are temperature adjusted and independent of each other. The plate beneath the channels is warmed to 37°C and includes a warmed trough to hold blood sample tubes. There is a

reagent tray, an integrated personal computer with attached touchscreen and a software assisted automatic pipette. The four channels allow for concurrent specimen testing for additional aspects of the hemostasis pathway using additional assays other than kaolin (Larsen 2011). For the basic test, termed EXTEM, the sample is recalcified with the addition of calcium chloride and tissue factor is added to activate or initiate clotting.

The parameters used by ROTEM® are similar to those used in TEG and can be interpreted to provide information on clot initiation, quality and lysis. The curves and measurements produced by both machines are extremely similar in both normal and abnormal circumstances and consequently in the next sub chapter describing interpretation of the graphs, only temograms will be used.

Both methods demonstrate haemostatic integrity and measure the ability of whole blood samples to form a clot in addition to the details of clot formation or “clot firmness” during the transition of blood from a fluid to a semi-solid state. They have shown good correlation with thrombin generation assays (Rivard 2005, Johansson 2008).

Although the curves and parameters measured by the two instruments are similar it is difficult to directly compare traces from the two devices due to different amounts of sample used, different activators utilised, distinctive cup and pin size and the variability in reference ranges (Nielsen 2007). In one study looking at the use of both in trauma patients the only parameter comparable was clot amplitude (MA in TEG or Maximum Clot Firmness in ROTEM) (Hagemo 2010). While the values of the parameters are not exactly the same and have differing names – their interpretation is similar and is detailed in Table 5-1.

TEG Value	ROTEM Value	Description	Interpretation
R Reaction time (secs)	**CT** Clotting Time (secs)	Time from start of sample measurement to appearance of first detectable clot (amplitude of 2mm)	**Initiation phase** Initial period to thrombin production (dependent on coagulation factor activation intiating the "thrombin burst")
K (secs)	**CFT** Clot Formation Time (secs)	Time for pin to produce a graph of 20mm reflecting a degree of clot firmness	**Amplification phase** Kinetics of clot formation (dependent on formation of fibrin fibres, plus thrombin formation, platelet count and function)
α-angle (degrees)	**α-angle** (degrees)	Angle between centre line and tangent to the curve through 20mm amplitude point (as measured by K)	**Thrombin burst** Maximum velocity of clot formation (representative of thrombin cleaving available fibrinogen into fibrin)
MA Maximum Amplitude (mm)	**MCF** Maximum Clot Firmness (mm)	Maximum clot strength as measured by maximal distance from centre line	**Propogation phase** Reflects maximal clot strength at a fixed time (dependent on a result of maximal fibrin-platelet interaction)
Ly30 Lysis at 30 minutes (%)	**Li30** Lysis Index at 30 minutes (%)	Termination of clot by lysis. In TEG this is the maount of lysis (in % of MA), 30 min after MA achieved. In ROTEM this is % of MCF, 30 mins after CT is achieved	**Fibrinolysis** Measures activity of fibrinolytic system by the dissolution of fibrin-platelet bond between cup and pin

Table 5-1. TEG® and ROTEM® Parameters

As in the TEG where differing compounds added to the activators can examine different aspects of the haemostasis system, so does the ROTEM system. The difference being 4 channels can be run concurrently in ROTEM. The different tests possible are detailed in Table 5-2.

Assay	Additives	Clinical Use
EXTEM	Calcium Chloride Recombinant Tissue Factor Polybrene	Assesses deficiencies of factors involved in “extrinsic” pathway
FIBTEM	Calcium Chloride Recombinant Tissue Factor Polybrene Cytochalasin D	Assesses fibrin polymerisation
APTEM	Calcium Chloride Recombinant Tissue Factor Polybrene Aprotinin	Verifies effect of anti-fibrinolytic drug if given
INTEM	Calcium Chloride Ellagic Acid	Assesses deficiencies of factors involved in “intrinsic” pathway
HEPTEM	Calcium Chloride Ellagic Acid Heptase	Determines Heparin and Protamine effects (in combination with INTEM)

Table 5-2. Commonly used ROTEM *delta* assays

5.11.3 ROTEM Assays

EXTEM

Similar to the laboratory test of prothrombin time, these assay are activated by recalcification (using the star-tem assay containing 0.2mol/l calcium chloride) and the addition of thromboplastin (ex-tem reagents, i.e. recombinant tissue factor and phospholipids). The traditional thought was that the extrinsic pathway initiates coagulation and initial thrombin

generation/clot formation in this test is reliant on FVII, FX, FV, FII and fibrinogen. The addition of polybrene - an inhibitor of heparin - enables the use and interpretation of these tests in patients who are treated by heparin. Clot strength is reliant on fibrinogen formation, platelet number and function and their interaction.

FIBTEM

This modified EXTEM assay contains the additional platelet inhibitor Cytochalasin D, which affects platelet activation and shape, in addition to the expression and activation of GlycoproteinIIb/IIIa (fibrinogen) receptors. Platelet contribution to clot strength (as measured by Maximum Clot Firmness (MCF)) is removed from this assay, meaning the clot formation in this test is based purely on fibrinogen concentration, polymerisation and strength, providing the user with a measurement of fibrinogen function.

The clot strength aspect of this test (MCF) can also be directly compared to the MCF in EXTEM, which is a reflection of both fibrinogen and platelet count/function. The difference in clot strength between EXTEM and FIBTEM therefore allows for estimation of the platelet portion of clot firmness (Olde Engberink 2014).

APTEM

This is the third extrinsic test in the group and includes an antifibrinolytic drug. This was Aprotinin (hence the nomenclature APTEM) but has been replaced by Tranexamic Acid. The inclusion of this drug allows for testing of antifibrinolytic therapy and elucidation of early evidence of fibrinolysis.

INTEM

The “intrinsic” pathway, similar to the activated partial thromboplastin time laboratory test, is tested by this assay. It uses ellagic acid as an activator. The initial thrombin generation and clot formation is deemed to be

dependent on FXII, FXI, FIX, FVIII, FX, FV, FII and fibrinogen. As in EXTEM clot strength is a reflection of both fibrinogen and platelets. It does not contain polybrene; the heparin inhibitor.

HEPTEM

In combination with INTEM this assay – now containing a heparin inhibitor; heparinase – can be used to reveal residual heparinisation or protamine overdose.

Both TEG and ROTEM are capable machines, useful for near patient testing of coagulation, but they both have strengths and weaknesses depending on user need. Clinicians in general favour one over the other, whilst researchers see use in both. Of interest are the new developments in the area with other manufacturers producing competing products. The majority of investigations completed on trauma patients or on animal models, so far, has been done using one of the machines described above.

5.12 Viscoelastic Interpretation

As discussed above TEG traces are very similar to EXTEM traces and the interpretation of the graphs produced for each are similar. ROTEM traces are discussed below, but the analysis of TEG mirrors that of EXTEM traces.

When using thromboelastometric viscoelastic testing to aid resuscitation management of trauma patients INTEM traces offer no additional information to EXTEM and FIBTEM (Doran 2010) – in this study INTEM trace abnormalities were also shown on EXTEM traces and they concluded there is no need for an additional test (INTEM) unless the patient is on Heparin. It is general practice within the Defence Medical Services to run EXTEM and FIBTEM tests together for trauma patients. These two tests will provide information on overall clot dynamics (initiation and amplification)

as well as clot strength and stability (propagation). Fibrinogen levels can be assessed from the FIBTEM trace in addition to an estimate of platelet function. Normal traces are illustrated below in Figure 5-8. The pictorial representation of the results makes the test ideal for patient management in busy Emergency Rooms and Operating Rooms during on going Damage Control Resuscitation and Surgery. Any staff can learn the fundamentals of interpretation and highlight abnormalities to otherwise busy clinicians. The results can be incorporated into ongoing resuscitation management, particularly assisting in decision making on type and quantity of blood component therapy and its speed of administration.

5.12.1 Abnormal Temograms

Viscoelastic testing is a comprehensive test and there a large number of abnormalities it can elucidate. Examples of their interpretation can be gained from the Tem International education website (https://learning.rotem.de/en/interpretation/) and the Haemonetics website (https://teg.haemonetics.com/en/teg-5000-thrombelastograph). Some abnormalities are more commonly observed in Acute Traumatic Coagulopathy or as a consequence of resuscitation. These are discussed below.

5.12.2 Prolonged Clotting Time

Clotting Time is the initial time it takes for the clot to start forming (and the graph amplitude to reach 2mm). It is dependent on the presence of adequate clotting factors. Figure 5-9 illustrates an EXTEM trace with prolonged Clotting Time. The initial green line observed on the EXTEM trace is prolonged and there is a delay until the clot starts to form (appearance of pink thickening trace). The treatment of a patient with this abnormality would be more clotting factors, found commonly in FFP or lyophilised plasma.

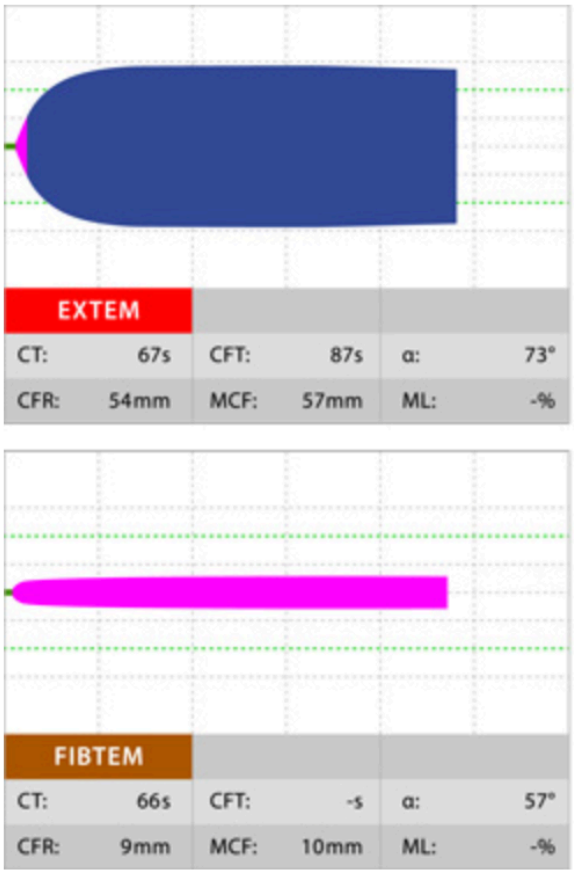


Figure 5-8. Normal Temogram. Normal EXTEM and FIBTEM Traces. EXTEM CT 43-82secs, EXTEM MCF 52-70mm, EXTEM ML <15%, FIBTEM MCF 7-24mm.

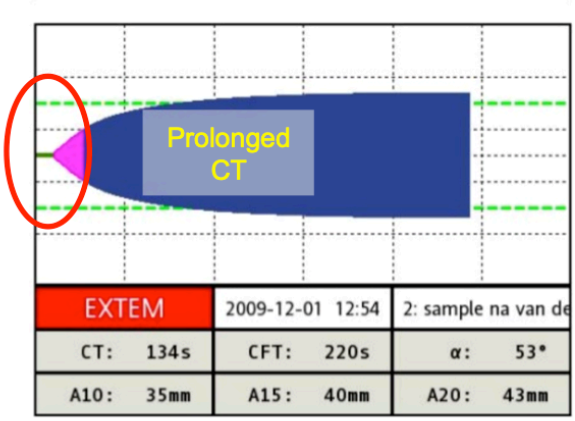


Figure 5-9. Temogram of prolonged CT. The EXTEM CT is prolonged (> 82 secs) which is mirrored by the FIBTEM CT. This is illustrative of clotting factor deficiency.

5.12.3 Maximum Clot Firmness

Maximum Clot Firmness (MCF) is a measurement of clot strength or stability. It is the maximum width the temogram achieves and is a reflection of the interaction between platelets and the fibrin mesh. It is the widest that the blue part of the trace achieves.

An abnormally low EXTEM MCF (<52mm) means the coagulation abnormality is secondary to fibrin(ogen) or platelets; with either being low in quantity or quality/function. A low EXTEM MCF (width) needs to be assessed with the corresponding FIBTEM MCF. The FIBTEM MCF (width) can be normal or low (Figure 5-10).

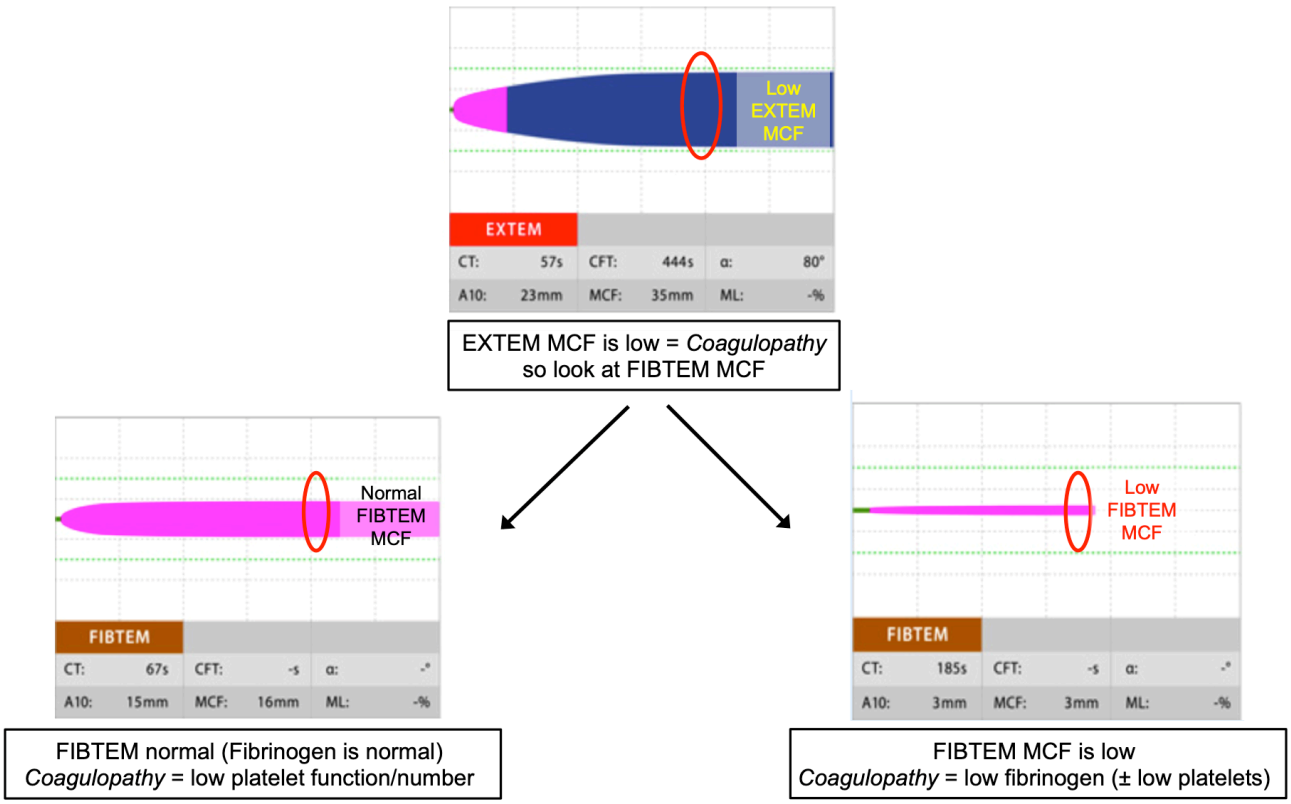


Fig 5.10. Temogram of low EXTEM MCF. If EXTEM MCF is low (<52 mm) and FIBTEM MCF is normal (7-24mm), suggesting a deficiency in platelet number or function or FIBTEM MCF is low (< 7 mm), suggesting a deficiency in fibrinogen.

If the FIBTEM MCF is normal, it indicates that fibrinogen and the fibrin system is normal and the deficiency in coagulation causing the low EXTEM MCF is therefore due to platelet number or function (Figure 5-10).

If the FIBTEM MCF is low (<7mm) then fibrinogen levels are low (Figure 5-10). This may be the cause of coagulation abnormality. However little assessment can be made on platelet number/function. They may also be low (in number or function) but the low fibrinogen levels are hiding their accurate interpretation.

5.12.4 Fibrinolysis

Abnormal clot breakdown is measured by a number of lysis markers, the main one being Maximum Lysis (the maximum amount of clot broken down measured as a percentage of the maximum width obtained at 60 minutes).

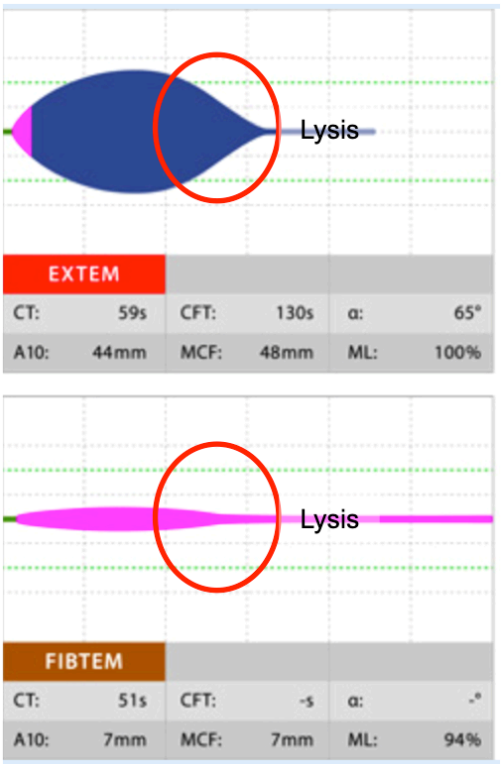


Figure 5-11. Temogram of hyperfibrinolysis. EXTEM (and FIBTEM) ML is >15%.

It is normal to observe some lysis and a value of less than 15% is considered to be normal. More than 15% is hyperfibrinolysis and some authors describe a condition of “fulminant hyperfibrinolysis” as being more than 50% in 30 minutes (Schöchl 2009). Figure 5-11 illustrates an example of hyperfibrinolysis.

5.13 Limitations to Visco-elastic Testing

5.13.1 Initiators

TEG® traces are activated using kaolin, but some investigators have used celite, tissue factor or a combination of kaolin and tissue factor (Shore-Lesserson 1999, Avidan 2004, Kashuk 2010a). ROTEM uses standard activators (recombinant tissue factor) and this has been shown to reduce the discrepancies encountered in TEG (Nielsen 2007). Some investigators have found that analysis with kaolin was unable to distinguish coagulopathies caused by dilution from that of coagulopathies caused by thrombocytopenia, but ROTEM tests could readily distinguish them (Larsen 2011). This potential error caused by TEG analysis could lead to incorrect platelet use.

5.13.2 Clinical

The chief constraint of viscoelastic tests is their insensitivity to the effects of anti-platelet drugs (cyclooxygenase-1 [COX-1] inhibitors like Aspirin and Adenosine Di-Phosphate [ADP] P2Y12 receptor inhibitors like Clopidogrel; Lang 2006, Görlinger 2007). This limitation is caused by the high quantity of thrombin produced in viscoelastic tests which stimulate platelets via protease-activated receptor (PAR) 1 and 4; a thrombin-receptor platelet activating pathway (Kalantzi 2012). As thrombin is a strong platelet activator, the inhibition of the other (arachidonic acid and ADP) pathways

have no effect on viscoelastic tests. Clinicians should be cognisant of this when faced with a haemorrhagic trauma patient with normal coagulation tests (Kembell-Cook 2005).

The UK military in Afghanistan discovered a temporal problem with rotational thromboelastometry. If citrated blood samples are left standing for an hour before being processed EXTEM MCF improves from abnormal to normal (22mm compared to 54mm), showing a spontaneous improvement in coagulation with time. FIBTEM MCF did not change, suggesting it was platelet function that improved in a citrated blood sample over time once blood was removed from the patient (Jansen 2013).

5.13.3 Experimental

One of the key advantages of ROTEM clinically is that the pipetting system is guided by the ROTEM software and performed with an automated pipette drawing and discharging the correct volume of fluid. For an investigator though this is a disadvantage. Having a more utilitarian pipette system such as found in TEG enables certain variables to be better investigating and allows flexibility of testing that is often necessary in research. Different initiators, their volume, and their concentrations can all be more easily investigated with TEG. It is for this reason that despite the considerable weight of support for the use of ROTEM argued in the next sub chapter, it was TEG that was predominantly used for the coagulation tests in this thesis investigation

5.14 Use of ROTEM and TEG in Trauma

ROTEM with its touchscreen, software assisted automatic pipette is very user friendly. The software guides the user though the measurement process with pictures and instructions and a support menu for result

interpretation. Mounted on a wheeled trolley, all these factors reduce inter-user variability (Anderson 2014) and enable it to be used in a number of different environments by different operators.

In comparison to TEG the technical modifications, and in particular the stability of the pin and cup system, of ROTEM make the device less susceptible to vibrations and movement and their interference with the Temogram. It also allows continuous electronic quality control of the pin movement, therefore reagent quality control (QC) is only necessary once a week compared to daily QCs needed for other machines. These factors mean clinicians in a number of different environments generally favour ROTEM. It has been adopted by the UK Defence Medical Services (Doran 2010) and has been used in a number of other austere or outdoor environments (Modesti 2011, Tarmey 2012, Benson 2014). It has been found to be particularly useful in trauma compared to conventional tests (Haas 2015).

Despite the perceived advantages many have observed first hand, the use of viscoelastic tests in the management of a haemorrhagic trauma patient's resuscitation has not been supported by large reviewing organisations such as the Cochrane Library. That group's recent review of the use of viscoelastic tests (Hunt 2015) for diagnosing trauma-induced coagulopathy found little evidence on the accuracy of both TEG and ROTEM and advised they should only be used for research. Since that review more evidence has come to light, although the number of randomised control trials are lacking. Other single centre and larger reviewing bodies argue against the Cochrane authors suggestions and are more supportive of its use in trauma patient management.

Many centres now suggest ROTEM use is a vital aspect of trauma patient resuscitation principally for managing their coagulopathy. Single centre studies have found early Temogram values at 5 (CA 5) and 10 (CA 10)

minutes predict coagulation status in ballistic military trauma casualties (Woolley 2013) and a prospective cohort study from London in over 800 civilian trauma patients found that ROTEM CA 5 was a valid marker for Acute Traumatic Coagulopathy and a predictor of massive transfusion (Davenport 2011b).

A more recent review that included more evidence than the previous Cochrane review (Veigas 2016) found that abnormal temogram amplitudes (MCF, CA 5, CA 10) in EXTEM and FIBTEM were capable of diagnosing Acute Traumatic Coagulopathy; predict the need for massive transfusion and predict mortality. In addition they found the presence of fibrinolysis was also strongly associated with mortality and suggested that "*ROTEM® parameter thresholds to guide blood component transfusion could be clinically useful*."

The French Group on Perioperative Haemostasis went further to declare that for "*the clinical use of these techniques in the setting of emergency and perioperative medicine….viscoelastic tests must be included in algorithms*". (Roullet 2018).

Many US and UK trauma centres now use viscoelastic testing and ROTEM probably has the majority vote from many clinicians due to its robustness, ease of use and more readily available detailed results. It is the main stay of my clinical practice in both civilian NHS and military austere environments.

Over the past few years other companies have entered the market and the manufacturers have developed newer models in the competition to provide smaller, user-friendlier models.

5.15 Viscoelastic Equipment Development

Single use reagents (lyophilised) were developed a few years ago for use with ROTEM *delta* removing the need for multiple pipetting into multiple different containers of liquid initiators/reagents (Rahe-Meyer 2009). This made the ROTEM even more user friendly but unfortunately eliminated the flexibility that can benefit research.

In the last few years both companies have developed a cartridge system, removing the need to pipette completely. Haemeonetics have developed the TEG®6s. This uses a different method for clot strength measurement; the resonance method. An unmetered citrated blood is loaded onto the cartridge. This sample is exposed to a fixed vibration frequency with LED illumination of the blood meniscus. The detector measures meniscus motion and the resonance frequency is identified. The information is converted, by the machine's software, into a conventional TEG readout. The cartridge contains a number of tests within it that will complete 4 tests including the whole coagulation process and fibrinogen. There is an additional platelet function cartridge. Information and publications on its performance capability are beginning to come to light (Gill 2017, Meledeo 2018) with mixed reviews. Gill (2017) found it did not perform well under a considerable vibration challenge. On the contrary Meledeo (2018) put it under a similar test, in addition to others including a direct blow. His group compared it to the TEG5000 and ROTEM *delta* and found it was an improvement over the other systems, stating it was robust and capable enough for austere military deployments.

Tem International has also produced a cartridge system, which is based on their rotational thromboelastometric measurement methodology. A citrated blood sample tube is inserted into the cartridge, which is then tested by the ROTEM *sigma.* Each cartridge contains EXTEM, INTEM, FIBTEM with either APTEM or HEPTEM. A number of trials investigating its use are currently ongoing.

5.16 Equipment Summary

Acid base balance is easily measured by a number of blood gas analysers and small accurate machines exist within the market already. They are readily used to aid resuscitation strategies.

Haemostasis can be measured by a myriad of methods. Time constraints in damage control resuscitation require rapid results and for this reason near patient, point of care testing has greater advantages. Of the few on the market the robustness, completeness of results and ease of use make the ROTEM® *delta* the UK Defence Medical Services' first choice. Clinicians look forward to how the new lighter, more practical machines will fare and await initial investigation results with interest.

Injury mechanism and the appropriate resuscitation regimes have been discussed in earlier chapters. The coagulation abnormalities and how to measure them in these trauma cases have now been discussed in detail. The fluids used in resuscitation and associated adjuncts and how they impact on trauma physiology and coagulation will be discussed in the next chapter.

CHAPTER 6. Resuscitation Fluids

6.1 Introduction

The ideal fluid to resuscitate a severely injured, hypotensive trauma patient needs to be safe, easy to dispense and easy to look after. It should expand blood volume, increase oxygen delivery, and improve haemostasis, in addition to not increasing bleeding. No such fluid exists. The alternatives all have their own advantages and disadvantages. This chapter will discuss in detail those fluids and some specific adjuncts that are commonly used in trauma resuscitation practice.

6.2 Non-Blood Component Fluids

For decades the preeminent internationally recognised trauma course, run by the American College of Surgeons, was the Advanced Trauma Life Support ® course. It has historically educated thousands of doctors and allied health practitioners that the initial resuscitation fluid of choice in trauma patients with clinical evidence of blood loss was 2 litres of crystalloid, only very recently reducing this to 1 litre (ACS 2012). Recent European guidelines still advised that in the management of haemorrhage in patients having suffered trauma, it should be crystalloids that are applied initially (Rossaint 2010). These recommendations also stated consideration could be given to hypertonic solutions, although previous recommendations have stated that the type of fluid was not important as long as the appropriate volume was given (Nolan 1999). For those many decades that the ATLS® course taught that crystalloids were the fluid of choice, there has been an ongoing debate as to what the appropriate fluid choice should be (Wiles 2015).

6.2.1 Crystalloids

There are significant advantages to crystalloids: cheap, easily available Worldwide, recognisable by all health professionals, with a long shelf life and logistically the easiest fluid to manage. Paramedics can administer them without a Doctor's prescription - covered by National guidelines (AACE JRCALC 2016) and local Patient Group Directives. The majority of them are easily transportable in sealed bags and very stable at a range of temperature extremes, and regularly warmed to 40°C (Lindoff 2002). Some studies have shown they have a beneficial effect on mortality if given (in certain volumes) in the pre-hospital environment (Hampton 2013). Readily transportable and with a logistical ease of care outside the hospital environment, both civilian and military, crystalloids have been the bedrock of fluid resuscitation in trauma patients for decades.

Although they initially appear to be the panacea for trauma resuscitation fluids recommended by distinguished International organisations, there exist a number of problems with their use as resuscitation fluids in trauma. Isotonic crystalloid fluids such as normal (0.9%) saline and Hartmann's Solution (known as Ringers Lactate in USA) distribute quickly to the interstitial space. In the hypovolaemic trauma patient any resuscitation fluid of choice should replace lost blood and remain in the vascular space, yet crystalloids fail to achieve this. They move relatively freely in the extracellular space and redistribute rapidly into the interstitial space so that at 30 minutes after transfusion only 16% of the volume of infused fluid remains within the intravascular space (Watenpaugh 1998) in a healthy patient. In a hypovolaemic, injured patient this is unlikely to be any better. Many disease states, including sepsis and trauma, cause vascular endothelial glycolcalyx disruption (Kolorava 2014, Pierce 2014). As the glycocalyx is the key structure limiting free fluid across the vascular space (Lee 2010), if it is compromised, there will be increased loss of fluid from the vascular space. This suggests crystalloids may spend even less time in the intravascular space in a trauma casualty, although there are suggestions that in some cases of hypovolaemia this may not be the case (Drobin 1999).

Brisk redistribution is only one of the many shortcomings crystalloid has as a trauma resuscitation fluid. Maegele's study of over 17,000 trauma patients in Germany showed the greater the amount of crystalloid given pre-operatively in trauma casualties the more coagulopathic they were, with an incidence of over 40% if more than two litres of fluid was given, up to an incidence of over 70% if over 4 litres of crystalloid was given (Maegele 2007). Although this is a significant finding to observe a direct correlation between amount of fluid given and level of coagulation abnormality, this study raises a key question: is the correlation between volume of fluid given and coagulopathy a direct one? The coagulopathy was measured by the presence of a platelet count of less than 100,000 μl^{-1} and a prothrombin time test (Quick's value) of less than 70%. These could have been caused by dilution of platelets and clotting factors. Or was there a direct pathophysiological effect on coagulation from some aspect of the content or physical property of the crystalloids?

Severely injured patients are more likely to be coagulopathic through a separate pathophysiological process (Brohi 2008, Frith 2010). Severely injured patients are also more likely to receive more fluid when conventional resuscitation goals are followed in order to maintain physiologically normal cardiovascular observations pre-operatively. Rather than fluid having a direct action on clotting or by dilution of factors and platelets; coagulopathy and fluid volume may be independent reflections of injury severity and not directly interrelated.

The relationship is unlikely to be binary and it is probable that both injury severity and volume of fluid are influencing the coagulation abnormality. Within a set injury severity the greater volume the patient received the worse their coagulopathy was, suggesting that the direct relationship between injury severity and coagulopathy is accentuated by volume of fluid given. What is clear though, direct causation aside, is that there is a relationship between volume of fluid given and worsening coagulopathy.

While there have been studies that have shown pre-hospital crystalloid administration has beneficial results, this is not guaranteed. Hampton and the PROMMTT (Prospective Observational Multicenter Major Trauma Transfusion) Study Group found in just over a thousand patients that pre-hospital crystalloids of 700ml (median) conveyed a survival benefit (decreased in-hospital mortality) (Hampton 2013). However there is confounding evidence that suggest that this is not assured. One particular study showed any greater amount of crystalloid given can increase the odds ratio for mortality by over 2, if one and half litres of crystalloid is given to a trauma patient (Ley 2011). Ley et al found in his retrospective study of over 3000 patients that as the amount of crystalloid given in the Emergency Department increased so did the odds ratio for mortality. It rose to 2.6 for three litres given and his findings were further compounded by age, where those patients suffering from trauma over the age of seventy had an odds ratio for mortality of over 8.

Crystalloids, the ATLS and European resuscitation guidelines first line panacea for trauma resuscitation, have the advantage of being easy to manage and handle, but they do not remain intravascular for long, probably causes a worsening coagulopathy and increases mortality in trauma patients. This suggests they are not the best fluid to use. On balance it appears that they have their uses. In a constrained environment where alternatives are realistically unavailable either by logistical challenge (e.g. temperature constraints or resupply times) or by qualification constraints of medical personnel, where the alternative is no fluid for resuscitation there is a clear and valid argument for their use. If alternatives exist, then the weight of argument is against them in the trauma patient group. Valid alternatives do exist. Colloids are one of them, and in common with crystalloids, are another class of fluid that has a low logistical burden. What potential do they have for trauma resuscitation?

6.2.2 Colloids

Colloids are homogenous, non-crystalline compound fluids that consist of particles that are molecularly large which can't be separated by filtration or centrifuge. They can be protein or non-protein derived and their principal argument for use is the comparatively increased time they remain within the intravascular space (Huskisson 1992, Hillman 1997).

Protein derived colloids include human albumin, which is derived from human plasma. It was shown in one meta-analysis to increase mortality (Cochrane Injuries Group Albumin Reviewers 1998), although another study found otherwise in critically ill patients, but as it is expensive they suggested there was no advantage to its use over crystalloids (Finfer 2004). The SAFE (saline versus albumin fluid evaluation) group found trauma casualties had an increased relative risk of death if they received albumin. A subsequent follow up study (Myburgh 2007) confirmed this higher mortality was attributed to patients with traumatic brain injury.

Non-protein colloids include synthetic hydroxyethyl starches, modified gelatins and dextran solutions. The latter are branched polysaccharides synthesised by the activity of *Leuconostic mesenteroides* on sucrose mediums. Generally the two derived products are Dextran 40 and 70, the nomenclature of which originates from the molecular weight (MW) of the polysaccharides – 40,000 Daltons (Da) and 70,000 Da. It is the larger, Dextran 70, that has been more commonly used in resuscitation (Salmon 1993), especially as Dextran 40 has been associated with renal failure, particularly in the presence of hypovolaemia (Haljamae 1997). Both compounds have been shown to reduce platelet adhesiveness and enhance fibrinolysis and have been associated with significant number of anaphylaxis (Nolan 2001), so that now both are rarely used in the UK.

Gelatins are derived from bovine collagen and have an intravascular space half-life time of up to 4 hours (Sadler 1987). The collagen is modified to increase molecular size and hence improve intravascular retention. The

most widespread are Haemaccel (a Urea-bridged gelatin of 24 500 Da derived from cattle bone) and Gelofusine (a 22 600 Da compound derived from calf skin collagen which has undergone thermal degradation). Most of the gelatins are less than 20 000Da and are excreted rapidly through the kidneys, although intravascular persistence is still up to 4 hours (Sadler 1987). They are the most likely of the colloids to cause anaphylactic reactions (Laxenaire 1994) and some *in vitro* studies have shown that they impair clotting (Mardel 1998, Evans 1998) in contrast to one in vivo study which showed that post-operative thromboelastographic coagulation measurements showed hypercoagulability in patients who received gelatin (Karoutsos 1998).

Hydroxyethyl starches (HES) are branched amylopectin polymers, which can be broken down by serum amylase. Substitution of certain hydroxyethyl groups for hydroxides can increase the time the compound remains within the intravascular space (Treib 1996). The compounds are described by molecular weight (kDa) and degree of substitution (ratio); hetastarch 450/0.7 has a MW of 450kDa and a substitution of 0.7. The latter is related to not only prolonged intravascular time, but also other characteristics including side effect profile (Jungheinreich 2005). They are generally divided into high, medium and low molecular weights (Petroianu 2000). Considerable work has gone into the development and investigation of (HES) over the last few decades; especially after some of the original data on outcome benefits were falsified (Shafer 2011). Most of the subsequent work has been predominated by safety and its use in sepsis.

High molecular weight HES had been shown to reduce Factor VIII (clotting factor 8) and von Willebrand factor and will consequently cause a coagulopathy (Petroianu 2000, Treib 1998) to a greater proportion than would be expected by simple plasma dilution. Molecular weight has been considered a key factor in determining the blood coagulation effects of HES (Treib 1998) with lower molecular weight HES being considered to have less effect on coagulation (de Jongee 2001) but their disadvantage has been their shorter intravascular presence. Recent studies have shown that

there is an additional direct inhibition of platelet function occurs through binding of HES to the platelet surface (Deusch 2003). Contrary to previous beliefs this was found to be with a lower molecular weight HES (200kDa). Madjdpour et al (2005) looked at molecular weight specifically in an animal model. Using both traditional laboratory and thromboelastographic measurement of coagulation they found that all molecular weight HES caused a coagulopathy immediately after infusion and sometimes lasting up to 4 hours afterwards. This was evident in all results including Prothrombin Time (PT), Activated ProThromboplastin Time (aPTT), von Willebrand Factor (vWF) activity, Thromboelastography (TEG®) Maximum Amplitude (MA) width and K time.

The US military community has used HES for many decades and up to one litre of Hextend was recommended for use in the Tactical Combat Casualty Care Guidelines in 2002 (Holcomb 2003), with greater volumes not being recommended secondary to coagulation concerns. Its heat tolerance, price and prolonged duration of action won over counter-arguments for its use in the logistically challenging military pre-hospital environment, yet there was still concern amongst pre-hospital providers as almost 50% still used crystalloids in preference (McSwain 2011). More recent US military guidelines have retained its use in preference to crystalloids (Butler 2014).

Its use in civilian trauma has been reviewed in a number of retrospective studies. The Ryder Trauma Centre in Miami found no difference in mortality for those receiving HES, but did find higher rates of ICU admission, blood product transfusion, sepsis and ARDS (Ogilvie 2010). Shock Trauma in Baltimore (Lissauer 2011) found patients receiving HES had a higher in-hospital mortality, higher blood product utilisation and higher incidence of acute kidney injury compared with saline. Both of these studies have their potential problems as found in many retrospective studies, particularly with selector and survivor bias with some lack of clarity on the mechanism of injury (in the Shock Trauma study 85% of HES patients had blunt trauma). The Ryder group (Allen 2014) looked at the potential difference between mechanism of injury in trauma casualties in receipt of a single bolus of HES

(1410 patients) and found that in blunt trauma HES was an independent risk factor for both acute kidney injury and death, but not in penetrating trauma (although the numbers in the latter group were small). A follow up study by the same authors (Allen 2016) found again there was no difference in a penetrating injury group (816 patients), although all deaths in the first 24 hours were excluded.

To date the Fluids in Resuscitation of Severe Trauma (FIRST) Trial (James 2011) is the main randomized controlled trial examining the effects of HES in trauma. No difference in mortality was observed, but penetrating trauma patients appeared to fare better compared to those injured from blunt trauma who required more blood products if they received HES. There are many critics of this trial and it is injudicious to draw conclusions from it for many reasons; no intent to examine mortality, coagulation or renal failure and the fact that it was manufacturer sponsored. Another prospective study is ongoing and its results are awaited – TETHYS, PragmaTic, prospEctive, randomized, controlled, double-blind mulTicentre, multinational study on the safety and efficacy of a 6% HydroxyEthylStrach (HES) solution versus an electrolyte solution in trauma patients.

The most significant work on HES has been on critically ill and septic patients. Although HES has been found to have a beneficial effect in septic patients and in acute inflammation (Schmand 1995, Dieterich 2007) through immunomodulation, there are concerns over their effect on renal function, coagulation and mortality. After Boldt's retraction of over 90 publications (Wilkes 2013) a number of trials were published – 6 S (Perner 2012), CHEST (Myburgh 2012), CRYST-MAS (Guidet 2012) and VISEP (Brunkhorst 2008) – that linked HES with adverse outcomes, suggesting that the use of HES is harmful in septic or critically ill patients increasing their risk of mortality and renal failure. The US Food and Drug Administration (FDA 2013) has now advised health professionals against their use in these patient groups and the European Medical Agency has called for its removal from the market (Cohen 2018).

The Cochrane Collaboration has reviewed the use of all colloids in patients who had suffered trauma, burns or surgery (Perel 2013). They identified 65 trials and examined 56 that had mortality data where crystalloids were compared to colloids. They found "*no evidence from RCTs that resuscitation with colloids reduces the risk of death compared to crystalloids*", there was "*no improvement in survival*", they were "*more expensive*" and concluded that it was "*hard to see how that their use in these patients can be justified*". These are strong comments that have had widespread repercussions certainly in the NHS where colloids are now a rarity. There is little suggestion in the summary that their use increases risk to the patient and all colloids are bracketed together despite some clear differences between them, making it seem that the accountants are the driver for fluid choice decision-making.

The weight against HES use is certainly significant, but there appears to be evidence that it may be of limited use in particular environments. Despite evidence of its detrimental effect on coagulation, in the penetrating military casualty treated by a health professional with a poor logistical supply, there is support for its limited use if there are no alternatives. In all other situations however it would be hard to justify its use.

Largely this gives any clinician a dilemma. Crystalloids and colloids are both easy to transport to the patient in the civilian pre-hospital environment and the battlefield. There is good argument to suggest crystalloids is preferred to some colloids but is there strong enough argument to avoid their use altogether? Are there any alternatives that don't require temperature-controlled storage?

6.2.3 Hypertonic Saline

Hypertonic solutions have a greater solute concentration than the cell cytosol. When infused they create an osmotic draw that pulls water from intra-cellular to extra-cellular space and into the interstitial and finally intra-

vascular space, thus increasing intra-vascular volume (Cross 1989). This ability to potentially rapidly restore tissue perfusion with a smaller volume has attracted many clinicians involved in resuscitation.

In animal studies of haemorrhagic shock hypertonic fluids have been shown to decrease inflammation (Angle 1998, Deitch 2003), organ injury (Smith 1985) and mortality (Traverso 1987) and so it was hoped the same benefit would be seen in humans.

Patients who have suffered traumatic injuries have been shown to experience complications secondary to immune dysfunction such as multi-organ failure and sepsis (Maier 2000, Rotstein 2003). Hypertonic solutions have been shown to have beneficial immunomodulatory effects on haemorrhage induced leukocyte activity (Junger 1997, Kolson-Petersen 2004), in addition to decreasing neutrophil activation (Angle 1998), stimulating lymphocyte proliferation (Loomis 2001) and anti-inflammatory cytokine production (Gushchin 2002). One human studies has mirrored these experimental studies; in trauma patients admitted to an American trauma centre and treated with either a small bolus (250ml) of hypertonic saline or normal saline (Rizoli 2006). Subjects receiving the hypertonic saline showed reduced neutrophil activation, reduced pro-inflammatory tumour necrosis factor production and increased anti-inflammatory interleukins. These immunomodulatoary effects were shown to outlast the transient cardiovascular effects raising the possibility that hypertonic saline may attenuate multi-organ failure after traumatic injury.

One particular concern for hypertonic saline (HTS) has been its effect on coagulation and the potential to increase bleeding following its administration. Increased bleeding has been observed in various animal trauma and haemorrhage models. They have predominantly been large vessel injury models with uncontrolled haemorrhage and resulted in increased bleeding from injured vessels, haemodynamic decompensation and increased mortality (Gross 1989, Matsuoki 1995, Krausz 1992, Solomonov 2008). There has been criticism of the models used and their

relevance to human trauma; furthermore other studies have disputed these findings in similar animal studies (Elgjo 1996) and those with organ injury (Varicoda 2003). In vitro studies illustrated a prolongation of PT, aPTT and time for platelet aggregation when greater than ten percent of plasma volume is replaced by HTS (Reed 1991, Wilder 2002), meaning concerns on its effect on coagulation will remain.

The fundamental attraction of hypertonic solutions was their ability to expand plasma volume by more than the volume infused (Kramer 1989). Animal haemorrhage models illustrated it improved cardiovascular parameters (arterial pressure and cardiac output) (Kramer 2003, Wade 1997) and led to 100% survival in dogs (Velasco 1980). Similar findings were found in sheep (Nakayama 1984, Smith 1985) but were noted to be transient. Most human studies have been inconclusive; although showing an improvement in mean arterial pressure (MAP) and reducing the volume of other fluids required in subjects' resuscitation, mortality rates are not affected (Younes 1992).

There has been significant interest in the use of HTS use after traumatic brain injury where it was shown to more effectual at reducing cerebral oedema than mannitol (Himmelseher 2007). Human studies have shown it reliably and safely reduces raised intracranial pressure (ICP) in patients with acute traumatic injury (Fisher 1992, Härtl 1997) and in some cases it has lowered ICP in patients refractory to other therapeutic approaches (Kerwin 2009). Despite this some studies have found that the trauma casualty with head injury and low blood pressure there was no significant improvement in neurological outcome or survival at 6 months (Cooper 2004). Pre-hospital studies have shown greater benefit with increased blood pressure and survival in some groups (Vassar 1993).

This continued controversy over hypertonic fluids led the Resuscitation Outcome Consortium (ROC) to conduct two separate multicenter, randomized, blinded trials in America and Canada studied the use of HTS in pre-hospital resuscitation; one on hypovolaemic shock in trauma patients

and one on traumatic brain injury (TBI). The TBI trial was stopped for futility reasons as those patients with TBI, but not in hypovolaemic shock, had no difference in 28-day survival and 6-month neurological outcome (Bulger 2010). Bulger and colleagues also stopped the hypovolaemic trial early over potential safety concerns when in one patient group (no blood transfusions received) HTS resuscitation had a higher 28 day mortality (12.2%) compared to normal saline (4.8%; Bulger 2011). Although the authors noted that overall, in all groups (including those that received blood transfusions), there was no significant difference for the 6 hour (in the hypovolaemic study) and 28 day (both studies) survival and argued that the role of HTS, particularly in the pre-hospital environment, remained extant (Dubick 2013).

Although there is significant evidence of the benefits from HTS from experimental injury and animal models, there is little evidence that this translates to human casualties with haemorrhagic shock. While coagulation and increased bleeding has been a potential problem in animal studies this has not translated across to human studies. The greatest concern is from the more recent studies that have shown increased mortality. Some have argued that this mortality increase could have been secondary to fluid overload in patients with pre-existing co-morbidities (Dubick 2013). A second explanation has suggested that clinical misinterpretation of patient colour and cardiovascular parameters subsequent to receiving HTS have led those patients to be mis-managed (Holcroft 2011). If either of these last explanations is correct and can be overcome, then it suggests that there is a role for HTS in the pre-hospital and military environment.

The transient response to initial resuscitation with HTS led to the development of a combined hypertonic saline with a colloid in order to attempt to retain more fluid within the intravascular space – Hypertonic Saline Dextran (HSD).

6.2.4 Hypertonic Saline Dextran

The benefit of using small volumes of fluid for resuscitation that is equivalent to a much larger volume of conventional fluids is logistically attractive to both the military and civilian pre-hospital practitioner. The intention of adding Dextran, a polysaccharide, to hypertonic saline in the production of hypertonic saline (HSD) was to increase the duration of intra-vascular expansion. 250ml of HSD is comparative to 3 litres of normal saline for plasma volume expansion (Dubick 2003). Animal and human studies have shown similar benefits to HTS in improving organ blood flow (Kreimeier 1990) and modulating the immune response (Rotstein 2000, Rizoli 2006, Bulger 2007). Animal studies have also found that haemodynamics improved for longer compared to HTS (Wade 1989). Results on survival however have been varied with head injured sub-groups having a tendency to improved survival with HSD (Vassar 1993, Mattox 1991, Younes 1997), as did one penetrating torso trauma group (Wade 2003). Wade's group completed a number of meta-analyses finding that HSD had a survival benefit for the treatment of traumatic hypotension (Wade 1997a), in TBI (Wade 1997b), and overall HSD was found to have a 30-day survival benefit compared to conventional crystalloids (Wade 1997c). This data (from predominantly retrospective studies) has not led to the widespread use of HSD though. A Cochrane review in 2004 found there was insufficient data to be able state whether hypertonic crystalloid is better than isotonic fluids for trauma resuscitation (Bunn 2004). One prospective trial was carried out subsequent to that review.

The multi-centre trial investigating HTS for the Resuscitation Outcomes Consortium (ROC) mentioned previously (Bulger 2011) also looked at HSD. Their findings were similar with no difference in mortality except for the group not receiving blood transfusions that had an increased mortality of 10% in the HSD group compared to 4.8% in the normal saline group. Again they mused on potential reasons for this including poor recognition of the actual level of hypovolaemia in patients resuscitated with HSD and fluid

overloading patients with unidentified co-morbidities tipping them into heart failure (Dubick 2013).

Due to the uncertain relationship between HSD (and to some extent HTS) and increased mortality these are not in common use in UK civilian practice. The military (and pre-hospital) situation remains extant though where a limited fluid resuscitation with uncertain resupply chain in a population with predominantly penetrating trauma means that in America it is of use. The data is probably too inconclusive to support general use except in certain military situations.

Early blood product use has predominated a significant proportion of research both human and animal in the last two decades on trauma resuscitation. Different protocols and strategies have attracted a number of different nomenclatures including, and not exclusively, hypotensive, haemostatic and damage control resuscitation. These have been discussed elsewhere in more detail (Chapter 2) but their main constituents include blood and blood components. Large volumes of these can be required in order to maintain oxygen carrying capacity and augment clot formation (Rossaint 2010).

6.3 Blood and Blood Components

In the UK, after donated blood is collected it is divided into a number of different components. The need to maximize the length of storage of the blood donated, and its constituent parts have historically driven this process. Although exceptions occur, the commonest components produced by the NHS Blood and Transplant are Red Cells (also known as Packed Red Blood Cells - PRBC), Fresh Frozen Plasma (FFP), Platelets and Cryoprecipitate (The Red Book, 2013). Whole Blood (also known as Fresh Whole Blood - FWB) has been used uncommonly in UK practice, but has

seen increased use in the USA in both civilian and particularly military environments.

6.3.1 Packed Red Blood Cells

Packed Red Blood Cells (PRBCs) are one of the most commonly used blood components. PRBCs are prepared from whole blood by centrifugation or by apheresis collection. Typically, one unit of PRBC is 280 +/- 60ml volume, of which the Haemoglobin (Hb) content is greater than 40g. Anticoagulation is achieved by the collection into citrate-phosphate-dextrose. Additives are used to prolong the components shelf life of 35 days at 4 +/- 2°C (The Red Book, 2013). In the USA adenine is combined to the collected agents and used for storage as CPDA (citrate, phosphate, dextrose and adenine). In the UK the currently used additive solution is Saline Adenine Glucose and Mannitol (SAGM) which allows for 35 days storage at 1–6°C. Leucodepletion is carried out on the starting whole blood material or on the final PRBC component and there must be less than 1 x 10^6 leucocytes per unit. Leucodepletion was introduced in 1999 in the hope of reducing the risk of vCJD transmission with secondary effects being the reduction in other adverse effects such as non-haemolytic reactions and graft-*vs*-host disease (McLellan 2003).

Haemorrhage causes loss of erythrocytes, the only oxygen carrying cell within the vasculature and vital for its transport to the rest of the body. Replacement is essential. Early transfusion of PRBC increases cardiac output and preserves oxygen carrying capacity (Dutton 2006). There is a lack of generalized agreement on when PRBCs should be given. In critical care the evidence suggests a restrictive transfusion policy is best for that patient group (7-9 g/dl) (Hébert 1999). In trauma patients there are a number of recommendations; The British Committee for Standards in Haematology (BCSH) 2006 guidelines on management of massive blood loss recommend keeping a target of Haemoglobin (Hb) of greater than

8g/dl, but the more recent 2014 guidelines recognise that haematocrit and haemoglobin concentrations may be initially misleading after major blood loss and clinical criteria and observations should predominate, stating that red cells should be given if the Hb is falling (BCSH 2014, Hunt B 2015). The Joint UK Blood Transfusion and Transplantation Services Professional Advisory Committee advice keeping Hb at between 8-10g/dl as part of their "Massive haemorrhage toolkit" for Transfusion management of Massive Haemorrhage in Adults (JPAC 2013). The most recent European guidelines on management of bleeding recommend a target Hb of 7 to 9 g/dl (Rossaint 2016) and the British military's Clinical Guidelines for Operations suggest keeping Haematocrit above 0.3 (CGO, JSP 999, 2012).

Haemostasis is affected by the heamorheological effects of red cells. An increase in the haematocrit-related blood viscosity has a beneficial physical effect on interaction between platelets and endothelium (Turrito 1980). Red cells preferentially move down the centre of blood vessels causing margination of platelets positioning them in a preferential site close to the vessel wall (Flamm 2012). Despite this, over transfusion of PRBCs producing a consequential high haematocrit can have a detrimental impact on haemostasis. Although normally the pressure exerted by the centrally flowing red cells progressively aids platelet adhesion, as haematocrit rises from low (10%) to normal levels (35.8%), at higher haematocrit levels particle crowding impedes the formation of aggregates (Goldsmith 1995).

In anaemic patients initiation of coagulation has been found to be mildly impaired as measured by a prolonged rotational thromboelastography time for clot initiation. Correction of anaemia in these patients by red cell transfusion led to a return of clotting time to normal, but led to a reduction in clot strength (Roeloffzen 2010), possibly secondary to the detrimental effect of high haemotocrit on platelet function.

In addition to these rheological effects, red cells have other direct and indirect actions on haemostasis. Their presence appears to be a key part of haemostasis; structurally abnormal red cells present in in stored blood have

a greater strength of adhesion to the endothelium (Sparrow 2015) and under conditions where red cell damage can occur such as oxidative stress and inflammation (both potentially occurring in the trauma patient) red cells can expose phosphotidylserine, producing an active surface for prothrombin activation (Shi 2006). The fibrin system is a key aspect of haemostasis and particularly the coagulopathy found in trauma. Red cells have been found to have a protective effect against fibrinolysis as they have been discovered to decrease fibrin permeability in a dose dependent manner and increase resistance to lysis (Wohner 2011).

These haemostatic effects are multiple, but predominantly dependent on certain pathological conditions. The most likely effect of red cells is probably rheological and their interaction with platelets, illustrating a minimal level of Hb and haematocrit is needed for platelet function, but too high a haematocrit is detrimental - an observation that fits well with the majority of Hb guidelines.

There are potential risks with red cell transfusion; from mild reactions, allergic reactions, exposure to blood borne infections, ABO incompatibility, haemolytic transfusion reactions and death. Massive transfusions carry additional risks and have been associated with electrolyte abnormalities, renal impairment, increased incidence of ARDS and multi-organ failure, increased ICU stay, hospital stay and increased mortality (Claridge 2002, Malone 2003, Charles 2007, Marik 2008, Chaiwat 2009, Fredericks 2017). It is imperative therefore that the transfusion is guided appropriately and links in with the whole resuscitation process and other components such as fresh frozen plasma (FFP).

6.3.2 Fresh Frozen Plasma

Fresh frozen plasma (FFP) is prepared from a single unit of whole blood or plasma collected by apheresis into a citrate-containing anticoagulant

solution. It needs to be ABO compatible with AB being the universal donor type used. After donation it is frozen within 8 hours and can be stored at -25°C for up to a 36 months. It contains all of the clotting factors, some fibrinogen (up to 900 mg/unit), electrolytes and other plasma proteins including physiological anticoagulants (O'Shaughnessy 2004). Before administration to patients it needs to be thawed. Methods for thawing are water bath systems or microwave oven most commonly. The UK Defence Medical Services use a third method that is a dry tempering system (SAHARA-III). Once thawed the UK Blood Transfusion Services require transfusion within 4 hours, or if that is to be delayed it should be kept at 4°C for up to 24 hours. FVIII activity will decline at 24 hours by up to 28% (Blood Transfusion Task Force 2004). The timeline for use can be extended up to 72 hours, at which point 40% of FVIII activity is lost, although the activities of all other factors (including FII and FV) are normal (approximately 20% loss, Shehata 2001). At 5 days FVIII has lost 60% activity, FV has lost 34% activity and the remainder less than 30% of activity is lost (Blood Transfusion Task Force 2004).

Risks of transfusion are similar to PRBC and include infectious disease transmission (ranging from 1:7.8 million for HIV to 1:153,000 for Hepatitis B; O'Brien 2007), transfusion associated cardiac overload, transfusion-related acute lung injury, acute haemolytic reactions and anaphylaxis (Callum 2005, Popovsky 2006).

FFP is a blood component that has been available since World War 2 (Emerson 1945). It was initially used as a volume expander but this has developed into the management of bleeding and prevention of haemostatic abnormalities in bleeding and coagulopathic patients. The proof of its efficacy in the management of massive haemorrhage in a trauma casualty is disappointingly lacking (Stanworth 2004) and it has been used to treat trauma hemorrhage for many years but with very limited knowledge of its utility and application. Inadequate transfusion is potentially associated with poor outcomes and undoubtedly blind over-transfusion can result in

additional donor exposure with increased rates of sepsis and multi-organ failure (Watson 2009, Norda 2006).

Despite this it has been widely recommended for use in major haemorrhage simultaneously with PRBC (Holcomb 2007) at either specific doses of 10-15ml/kg (Rossaint 2010) or to achieve a lab coagulation level of no more than 1.5 times normal (PT and aPTT) (Stainsby 2006). Other guidelines and recommendations counsel that it should be transfused in a specified ratio to PRBC. These vary according to continent and military or civilian use; USA civilian (Hess 2006), USA military (Borgman 2007), UK military circles (CGOs 2012) and European guidelines (Rossaint 2016). However some key guidelines from noteworthy International bodies do not specify a particular ratio; Europe (NICE 2015, Kanani 2017) and USA (ASA 2006, Iorio 2008).

The National Institute for Health and Care Excellence (NICE) is an executive non-departmental public body of the NHS that provides guidance, advice and information services for health and other professionals. It produces key guidelines on a variety of subjects. It has produced guidelines for the use of FFP in major haemorrhage and advises FFP should be given; if there is *bleeding in excess of 150ml/min or 50% blood loss of total blood volume in under 3 hours or (bleeding) leads to a systolic BP of less than 90 or a HR of more than 110 in adults* (NICE guideline (NG24) 2015).

This reason many guidelines don't state a ratio is that there is on going debate on this issue. However despite the paucity of supporting evidence for its use FFP is required in trauma resuscitation and is beneficial to the trauma casualty (Savage 2014, Snyder 2009, Johansson 2012b).

6.3.3 Packed Blood Red Cell: Fresh Frozen Plasma Ratios

One ongoing debate amongst physicians dealing with trauma casualties concerns the amount of plasma used in resuscitation and its quantity compared to packed blood red cells. I had experience of a crystalloid only based approach in MedSTAR, the Washington DC trauma centre in the late 1990s, but at that time it was rapidly being replaced by a blood component resuscitation policy. As a trainee deploying to Afghanistan in 2008 I was instructed into the 1:1 ratio of FFP to packed cells of all ballistic trauma casualties who had suffered major blood loss, especially if there was on going bleeding. There was no access to point of care coagulation (viscoelastic) testing and this empirical ratio had at that time what was deemed strong supportive data. It was being used elsewhere - by both our fellow American military colleagues in Iraq and Afghanistan and it was in use at the Baltimore Shock Trauma Centre where a number of our physicians had just completed Fellowships.

This empirical ratio used in resuscitation of trauma casualties had support from a number of papers. Modeling had an important initial influence with one computer model of exsanguination (Hirschburg 2003) calculating the possible changes in coagulation subsequent to blood loss. It predicted that the optimal FFP:PRBC ratio of 2:3 would be needed to replenish clotting factors. Another mathematical model (Ho 2005) predicted a similar ratio (1 to 1.5 FFP to 1 unit if PRBC) would be needed to prevent a dilution coagulopathy in patients with ongoing blood loss.

Subsequent to these there were a lot of both military and civilian retrospective studies on early empirical ratio haemostatic resuscitation (Spinella 2008a, Cotton 2008, Gunter 2008, Maegele 2008, Johansson 2009b, Dente 2009, Teixeira 2009, Zink 2009, Snyder 2009, Savage 2014). In summary these papers showed that there was a significant association between higher ratios (of FFP to packed cells) and a lowering of mortality in significantly bleeding trauma casualties. These mortality reductions ranged

from 15 to 62% and were from predominantly civilian trauma centres in the USA and Europe.

Some studies have observed optimal outcomes may be at plasma ratios of greater than 1:1 (Teixeira 2009, Kashuk 2008, Gunter 2008) and some use these as evidence of the benefit of higher ratios. Gunter (retrospective 259 patients) found a reduction in 30-day mortality if patients received a plasma:PRBC ratio of 1:1.5. Teixeira (retrospective 383 patients) found the best relative risk of death was in patients who received a ratio of less than 1:2. Kushuk probably discovered the more convincing evidence towards higher ratios (retrospective 133 patients) finding the median ratio of survivors was 1:2 compared to 1:4 (non-survivors), but stated that 1:2 was the threshold for survival inferring a higher ratio is better. They also discovered that a 1:1 ratio gave patients the best improvement in coagulopathy.

The arguably pivotal study and a critical report for military resuscitators, was from Borgman et al based in Iraq (Borgman 2007) who looked at massive transfusions in 246 military combatants. All of these casualties had received more than 10 units of components in 24 hours. Their FFP to packed cell ratios were divided into three groups; 1:8, 1:2.5 and 1:1.4. They found a 55% absolute reduction in mortality between the highest and lowest ratios. Yet they also identified that the median time to death was much longer (38 hours) in the high ratio group compared to only 2 hours in the low ratio group. A number of those authors investigated civilian trauma in their home trauma centres on return from their military deployment (Holcomb 2008). They found a similar survival advantage in the high ratio group, but again a markedly dissimilar time to death (35 hours compared to 4). Although the first paper was used as a particular support to the resuscitation of military ballistic casualties, the authors disclosed that there was a survivorship bias. The patients, who died early, did so before they were able to get more plasma and hence their ratios were high. In contrast a patient with less shock and less physiologically challenged by their injuries survived long enough to get more FFP and as a result of having lesser

injuries their plasma ratio was higher (Magnotti 2011), suggesting injury severity was the cause of mortality difference, not ratio.

Two authors specifically looked at this survivorship bias (Scalea 2008, Snyder 2009). As in the other papers, both found that high ratios had a better survival. Scalea in a prospective study of 806 patients used stepwise regression analysis on their data and when early deaths were excluded, they found no survival benefit for higher ratios. However their mortality was surprisingly low (only 6%) and various amounts of data is missing that would enable a more detailed review of their findings, including intensive care admission times. When Snyder treated the ratio as a time-dependent variable they also found that the survival advantage of high ratios was lost.

The only other prospective study at that time was the London group (Davenport 2011c) who investigated the haemostatic effect of different ratios during resuscitation. It was a small study on only 50 patients in which the authors stated a ratio of 1:1 had no advantage over a ratio in the range of 1:2 to 3:4 for conveying haemostatic advantage. The results initially seem pertinent, but closer inspection reveals that the median amount of FFP received in the first 12 hours was only 4 units, suggesting that a significant number of massive transfusions did not take place. No actual results for coagulation were presented, only the difference before and after treatment, making it impossible to determine how many were actually coagulopathic. Thromboelastometric graph width, termed Maximum Clot Firmness (MCF), is predominantly a reflection of clot strength determined by platelet quantity and quality in addition to fibrinogen. MCF is typically one of the first abnormalities seen in coagulopathic trauma patients and their results here were not particularly conclusive; the maximum difference in mean value variation between all groups was 8mm, producing changes reported to be in the region of 38%, yet a normal MCF is in the region of 40mm. Initial Clotting Time (CT) is a better reflection of the presence and activity of coagulation factors and as such a better reflection on FFP requirement. The CT significantly improved in both low and mid ratio groups. With these minimal differences in a patient group that did not

receive massive transfusions, it is difficult to agree with their maxim that hemostatic effects are maximal at a 1:2 ratio.

In 2010 I deployed to my second Operational deployment to Afghanistan. At this time our resuscitation strategies were becoming well established. My work was predominantly on the Medical Emergency Response Team; the helicopter based pre-hospital team that collected patients from the front line. Injuries in that year were significant in severity and explosive, ballistic, multiple amputations unfortunately predominated. We had just established the ability to provide blood components to the aircraft with an assured cold chain and on each trip we took 4 units of packed cells and 4 units of FFP. Severely injured casualties was resuscitated with a 1:1 ratio and coagulation was measured on admission using rotational thromboelastometry. A prolonged Clotting Time, indicating lack of clotting factors and by inference inadequate FFP, was a rare result to find with these casualties. Was this a reflection of an already adequate resuscitation reducing the coagulopathy of trauma? Was there scope for less FFP to be given?

These are difficult questions to answer however the use of ROTEM in trauma (as described in the Chapter 5) must be remembered. Certainly the definition and diagnosis of Acute Traumatic Coagulopathy (ATC) has differed temporally and between centres and authors. Historically the lab parameters of PT and aPTT were used, however ROTEM (and TEG) are now well established as having primacy in ATC diagnosis. Throughout the literature describing ATC diagnosis on admission it is not CT that is used, but MCF, A5 or A10 – all graphical measurements of graph height – a reflection of clot strength, not lack of clotting factors. These parameters were shown to be predictive of need for Massive Transfusion prediction (Leemann 2010, Davenport 2011b, Schöchl 2011, Tauber 2011, Brockamp 2012, Maegele 2012, Mutschler 2014) and hence are used as an indicator of the presence of ATC. This suggests our findings of a CT within the normal range are a usual finding, even in the presence of ATC, and not a

reflection of our FFP transfusion policy and its adequacy, both in ratio and quantity.

It would be difficult to determine the effect of our pre-hospital resuscitation without pre-hospital blood results, but the question of the amount or ratio of FFP remains. If a normal CT is found in ATC, then it is natural to question the necessity of needing FFP at all. The evidence for its use has certainly been previously questioned (Stanworth 2007), but the weight of evidence that it is beneficial to the trauma casualty remains (Snyder 2009, Johansson 2012b, Savage 2014). More recent studies on plasma only resuscitation have shown its benefit (Sperry 2018) with a likely effect of plasma on the pathophysiological mechanism behind the cause of ATC (Peng 2013, Pati 2016).

My third deployment to Bastion in 2014 had me based in the military hospital at Camp Bastion. Pre-hospital resuscitation was continuing in a similar manner to two years previously. In the hospital we continued to use an empirical ratio of 1:1, which remained throughout the initial resuscitation phase and into the operating theatre, lasting at times many hours in the operating room. Rotational thromboelastometry was available and used on all patients on admission and into the operating room. It was used for goal directed therapy guiding the use of additional components (cryoprecipitate and platelets) and adjuncts (such as Tranexamic Acid) but it was not used particularly for determining FFP use. These protocols were supported by guidance from the Surgeon General's office, advised by his experts (CGOs, Massive Transfusion Protocol 2012). This continuation of the use of empirical ratio throughout resuscitation despite the ability to use goal directed therapy for all products could attract criticism from some quarters, but our data suggested otherwise. Evidence of haemostasis benefit is lacking, but evidence of the impact of our resuscitation methods on the whole patient pathway is clear. The survival of our patients improved with these changes in management with the 50% survival NISS value being 32 in 2003 rising to 60 in 2012 (Penn-Barwell 2015). This improvement in survival was secondary to a multitude of factors but included "*massive*

transfusions….at approximately a 1:1 ratio with early platelet administration….guided by near-patient thromboelastometry". This aspect of continued resuscitation within the operating theatre at an empirical ratio was shown to improve physiological parameters significantly throughout their period in surgery. Indicators of injury severity and degree of physiological damage before and after surgery illustrate how well their damage control resuscitation improved their circumstance, including clotting status; pH improving from 7.19 to 7.45 and PT reducing from 18secs to 14secs (Morrison 2013a).

Certainly one concern with the potential over use of FFP is the additional donor exposure with the risk of increased sepsis and multi-organ failure (Watson 2009, Norda 2006) and TRALI (Transfusion Related Acute Lung Injury; Inaba 2010a), but these findings have not been found in the military population (Starkey 2013).

A multi-centre study involving 16 trauma centres recruited 452 massively bleeding trauma patients and looked at transfusion rates over the first 6 hours. FFP to packed cell ratios were divided into less than 1:4, more than 1:1 and a group between these ratios. They concluded early high FFP to red cell (and high platelet to red cell) ratios improved survival. Although limitations were evident in the study particularly in the difference in head injuries between the groups, this still provided good evidence that reaching high ratios within the early hours of admission is associated with a reduction in mortality (Zink 2009). A recent observational study associated early plasma administration with improved 30-day survival (del Junco 2013, Holcomb 2013) and a prospective multicentre study involving patients' receiving massive transfusion showed that the high FFP (and platelet) to red cell ratio was associated with a survival benefit even when time dependency was accounted for (Brown 2013).

The PROMMT (Prospective, Observational, Multicenter, Major Trauma Transfusion) study looked at timings and ratios from over a thousand patients admitted to 10 US trauma centres. Patients with lower ratios 1:1

compared to 1:2 or greater) had a decreased 6-hour and 24-hour mortality, but this did not extend beyond this time (Holcomb 2013).

One of the most recent and rational studies was the Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial (Holcomb 2015b, Baraniuk 2014). Nearly 700 randomised trauma patients who were suspected to have or had sustained massive blood loss reported that there was no difference in overall survival between early administration of plasma, (platelets) and packed cells in a 1:1:1 ratio compared to 1:1:2. However the patients in the high ratio group (1:1:1) achieve haemostasis and fewer experienced death due to exsanguination by 24 hours. Importantly there were no differences between the groups in safety and side effects.

Further support to higher plasma ratios is given by some recent papers on plasma only resuscitation. The Prehospital Air Medical Plasma (PAMPer) trial found those patients receiving plasma only resuscitation in the pre-hospital environment, compared to conventional regimes, had a significantly lower mortality (Sperry 2018) with minimal adverse effects. This further supports those investigations that found that plasma had a beneficial effect on the vascular endothelium in minimizing its damage (Peng 2013), inflammation and permeability (Pati 2016) in haemorrhagic shock

There is consequently still no clear evidence that one ratio is better than the other. There is certainly evidence that one is no worse than the other. Military resuscitation physicians lean towards resuscitating with higher FFP ratios. Seminal papers, albeit retrospective, have shown the benefit of this and personal experience has proven its worth supported by the evidence that our trauma management methods as a whole are excellent. Importantly there is little proof that it does harm, both in our population and in a civilian population.

Another component of considerable value and to some extent included in the resuscitation ratio debate are platelets.

6.3.4 Platelets

Platelets are produced in two ways; derived from whole blood donations or from direct apheresis. The standard adult dose of platelets is generally derived from a unit of donated whole blood. A unit is centrifuged and the buffy coat (fraction of an anticoagulated that contains white blood cells and platelets) from four such units are pooled together from four ABO identical donors and then resuspended - after leucocyte depletion - in the plasma of one of the donors (male, to reduce the risk of transfusion-related acute lung injury (TRALI)). This produces a pooled platelet component, typically of 300ml volume, containing a platelet count greater than 240 x 10^9 (JPAC 2013).

In apheresis, blood is removed from the circulation, anti-coagulated and separated into components. The apheresis platelets are leucodepleted and typically have a volume of 180ml with a similar platelet count to pooled platelets.

Both types can be stored up to 5 days at 20 to 24°C with gentle agitation with some Blood Services able to extend this to 7 days if they have automated bacterial screening. Risks of transfusion remain extant with acute non-haemolytic transfusion reactions like TRALI remaining low (Eder 2007) and the potential for transfusion transmissible infections with haemolytic reactions a potential secondary to the level of plasma contained within.

Thrombocytopenia is considered a late event in haemorrhage seen only after a loss of 1.5 blood volumes (Hiipala 1998). It is rarely found on patient admission, with one study finding less than 5% of trauma patients having platelet counts of less than 100 x 10^9/l (MacLeod 2003). Platelet count is typically normal during early, otherwise proven, traumatic coagulopathy (Hess 2009, Brown 2011, Floccard 2012), although patients with a higher injury severity have been found to have a lower platelet count (Hess 2009).

Admission platelet count is considered a good indicator of outcomes, it is inversely related to injury severity and there is an association between platelet count and both morbidity (Schnuriger 2010) and mortality (Van Beek 2007, Hess 2009, Brown 2011). One large study found platelet counts can reduce significantly after hospital admission, which is important when you consider that a low platelet count in trauma has been shown to predict an increased mortality risk (Stansbury 2013).

The antithetical relationship between mortality and platelet count is not just confined to abnormal counts but encompasses the lower extents of the normal range. Although platelet counts can be *normal* in proven coagulopathy of trauma, even this is associated with a high mortality (Stansbury 2013, Brown 2011).

The interpretation of this data suggests that adequate platelet numbers are not a vital aspect of ATC, though there is a relationship between them and both ATC and mortality. This suggests that it is not quantity, but quality that is key.

More recent work has concentrated more on platelet function rather than quantity. Abnormal platelet function has been identified as a part of the acute coagulopathy of trauma (Wohlauer 2012) with a decreased platelet aggregation correlating to mortality (Jacoby 2001, Kutcher 2012). Wohlauer suggested there was a role of early platelet transfusion in trauma resuscitation. His team, using TEG platelet mapping, found that platelet function was abnormal within 30 minutes of injury before significant fluid and blood component administration.

There have been a number of studies investigating the effect of platelet transfusion in trauma patients, although it is a challenge to determine the exact influence of platelets alone from these, as a significant number use predefined ratios with other blood components. One retrospective study of over nearly 700 military trauma patients (Perkins 2009) found a higher ratio of platelet to red cell transfusion (greater than 1:8) had a higher survival

(95%) compared to the lower ratios (less than 1:16 survival of only 36%) at 24 hours and at 30 days (75% survival in higher ratio compared to 43%). In a similar retrospective study at a Level 1 trauma centre in USA (Inaba 2010b), 657 trauma patients were identified who had received a massive transfusion where mortality at 24 hours was found to be lowest in the high ratio group (greater than 1:6) compared to the lowest (lower than 1:18). A review of platelet to red cell ratio in early resuscitation of trauma patients run by Hallet (2013) included 7 observational studies with over 4,000 total patients. Although they could not support specific ratios, they found that in studies looking at patients requiring a massive transfusion a lower mortality was observed with higher ratios. This supports Johansson's earlier review (Johansson 2012b), which also included plasma, but looked specifically at patients receiving massive transfusions (which they identified as more than 10 units in 24 hours). They included 16 studies, with a total of just fewer than 4,000 patients and produced a meta-analysis of these, concluding for platelets the higher the ratio the lower the mortality. One recommendation of these papers was a prospective, randomized controlled trial (RCT) should be carried out. Holcomb (2013) carried out a prospective cohort study to answer this need – the Prospective, Observational, Multicenter, Major Trauma Transfusion (PROMMT) study. Looking at specific timings, they found higher platelet ratios achieved in the first 6 hours conferred a decreased chance of death by a factor of 3, although this did not exist at the 30-day mortality mark.

A more recent study was the Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial (Holcomb 2015b, Baraniuk 2014). Their patients in the high ratio group (1:1:1 of PRBC: platelets:plasma) achieved haemostasis and fewer experienced death due to exsanguination by 24 hours with no differences between the groups in safety and side effects. A subsequent study on the impact of platelets in these patients found that those patients who received early platelet transfusion had improved haemostasis, fewer deaths from exsanguination and were associated with a significantly decreased 24-hour and 30-day mortality (Cardenas 2018). The majority of studies appear to directly favour a higher platelet ratio, which

clinicians need to keep in mind early in their casualty's resuscitation as platelets commonly take some UK Blood Services a period of time to provide. In addition, once transfused it is not infrequent to find they take time to start functioning normally.

In fitting with this, there is some commonality in guidelines on timing and quantity of platelets requested. One adult therapeutic dose (one apheresis pack or 4 pooled units) of platelets are recommended to be given in both the UK (BCSH 2014, Hunt B 2015) and in Europe (Rossaint 2016), with the latter stating up to 8 pooled units can be given. Both recommend keeping patient circulating platelet levels above 50 x 10^9/l, however the UK guidelines recognise that many NHS hospitals don't keep a stock of their own platelets and so recommend clinicians should order platelets early in major haemorrhage, when the count has fallen to 100 x 10^9/l. British military guidelines also recommend this higher level in their massive transfusion policy (CGOs 2012).

Platelets work hand in hand with fibrinogen and their interaction ensures the stability and strength of clot formation. Fibrinogen is key in activation and ensuring correct function of platelets and hence effective haemostasis. Its provision should have a key part to play in resuscitation. It is available in a number of forms but most commonly given in the NHS as contained within Cryoprecipitate.

6.3.5 Cryoprecipitate

Cryoprecipitate is the cryoglobulin fraction of plasma that is obtained from fresh frozen plasma (FFP). FFP is thawed to 3°C, spun in a centrifuge at that temperature producing the cryoglobulin at the base, which is then frozen rapidly. It should contain at least 140mg per unit of fibrinogen in the UK (JPAC 2013) and Europe (Council of Europe 2006). It is usually prepared as a pool of multiple donors, typically being from five single units.

The risks and side effects are similar to other blood components including TRALI (Chapman 2009), TACO (Transfusion Associated Circulatory Overload; SHOT 2012) and infectious diseases. The latter is probably higher than FFP, as a common adult dose of cryoprecipitate is from 5 to 10 pooled units of FFP from multiple donors (JPAC 2013). This would suggest that it carries a higher risk of viral transmission per dose (Callum 2009).

Fibrinogen was identified as one of the first haemostasis proteins to fall during substantial haemorrhage in 1995 (Hippala 1995) and has been acknowledged as an important aspect of traumatic coagulopathy (Davenport 2011b). Hippala originally found a low fibrinogen was associated with an increased transfusion requirement and an increased mortality. In 2012 Rourke at al found in a prospective analysis of over 500 patients that coagulopathic patients had significantly lower fibrinogen levels upon arrival and that low fibrinogen (less than 1.5 g/l) on admission was found to be an independent predictor of mortality at 24 hours and 28 days (Rourke 2012). Measurement of fibrinogen (usually Clauss Fibrinogen) is not a normal coagulation measurement when patients arrive at an Emergency Department. It was useful therefore that it was noted that a fall in fibrinogen levels was shown to be associated with a rising base deficit, a low systolic blood pressure and a higher injury severity in Rourke's study. Another project found quantitative results illustrating similar correlations, the chance of low fibrinogen (less than 1.5 g/l) being 73% if Hb was less than 10 g/dl and 63% if Base Excess was less than -6.

It is common for trauma centres in USA and UK to use thromboelastometry to assess initial coagulation in trauma on arrival. Schohl found, in trauma, a FIBTEM MCF of 7mm correlated with a fibrinogen of 1.5 g/l (Schohl 2011) and Rugeri found in civilian trauma patients that were "coagulopathic" had a median fibrinogen level of 0.9 g/l and FIBTEM MCF of 6mm (Rugeri 2007). Others have found similar results (Rourke 2012) suggesting thromboelastometry can estimate fibrinogen levels in a clinically useful time, identifying those patients early (who have a higher mortality) and enabling adequate directed resuscitation.

There is clinical data to suggest the benefits of fibrinogen replacement in increasing survival (Stinger 2008, Curry 2014, Curry 2015) and reversing traumatic coagulopathy (Rourke 2012). Rourke found that those trauma patients receiving a cryoprecipitate transfusion (2 pools, 10Units) maintained their Fibrinogen levels. Those patients who did not receive cryoprecipitate had fibrinogen levels that steadily dropped into the level that correlated with a higher mortality risk (Rourke 2012). A feasibility study looking at early administration of cryoprecipitate in transfusion protocols found that those patients who receiving cryoprecipitate had a mortality of 10% compared to 28.6% for patients resuscitated with a standard cryoprecipitate free transfusion protocol. A couple of military studies had similar findings for their ballistics combat trauma casualties. MATTERS II was a retrospective look at cryoprecipitate in association with Tranexamic Acid (TXA) use, which found those patients receiving cryoprecipitate had a slightly lower mortality compared to those who did not (21.4% compared to 23.6%) and when given with TXA the additive survival benefit meant mortality rates were halved (11.6%; Morrison 2013b). Another study of trauma patients at an American Army combat hospital found that a high ratio of fibrinogen to red cell transfusion was independently associated with improved survival to hospital discharge (Stinger 2008), stating the ratio could be achieved by transfusing 2 (10 Unit total) pooled bags of cryoprecipitate for every 10 Units of red cells. The PROMMT authors found little evidence to support use of cryoprecipitate, however very few of their patients actually received it. They did note though that it took over 2 hours for patients to get their first cryoprecipitate transfusion (Holcomb 2013), similar to the ACIT study where it was noted that patients did not generally receive any cryoprecipitate until after 6 units of red cells (Khan 2014). Another UK study found it took 103 minutes (median) to receive cryoprecipitate (Rourke 2012), however a feasibility trial, CRYOSTAT by the same authors, attempted to drive the inclusion of cryoprecipitate into normal massive transfusion protocols had better success and the majority of patients received cryoprecipitate within 90 minutes of arrival at the hospital (Curry 2015).

The amount and threshold of replacement is not well documented, there are various guidelines with little data to support the findings, however it is interesting to note that one study found that a 0.5g dose of fibrinogen (noting one unit of cryoprecipiate is likely to have 0.14g of fibrinogen and a 5U pool 0.7g) would increase a FIBTEM MCF by 1mm (Gorlinger 2012). Doses recommended are from 5 pooled units (CGOs 2012) to 15-20 units (Rossaint 2016 European guidelines).

There are a number of guidelines on using cryoprecipitate as a fibrinogen replacement, many of which recommend administration once plasma fibrinogen levels drop below 1.0 g/l^{-1} (Levy 2013). Recent evidence as described above suggests a higher level should be targeted (Thomas 2010, Fries 2010). The European guidelines on management of major bleeding in trauma (Rossaint 2016) now recommend a level of 1.5 to 2 g/l or a viscoelastic sign of fibrinogen dysfunction as the threshold for treatment, whereas the UK Defence Medical Services state a threshold of 1.5 g/l (CGOs 2012).

The evidence suggests lower levels of fibrinogen are an indicator of worse outcome (Rourke 2012, Hippala 1995, Schöhl 2010), the time to give cryoprecipiate is usually delayed (Holcomb 2013) but fibrinogen supplementation in the form of cryoprecipitate is beneficial (Stinger 2008, Curry 2014, 2015). Rather than waiting for levels to drop before requesting cryoprecipitate, the argument for earlier pro-active treatment of an adequate dose is growing stronger and personally I would look to request at least a 10 unit pool on patient admission and confirmation of their need for a massive transfusion and then conduct a viscoelastic test to determine if more is needed. Cryoprecipitate is not the only form of fibrinogen supplementation.

6.3.6 Fibrinogen Concentrate

Fibrinogen concentrate (FC) is produced from pooled human plasma and stored as a lyophilised powder at room temperature (Rahe-Meyer 2011). It

can be reconstituted rapidly with sterile water, ready for transfusion (Fenger-Erikson 2009). Viral infection risk is minimal as viral inactivation by exposure to solvent or pasteurisation occurs in the manufacturing process (Pereira 2007). Unlike cryoprecipitate, the concentration of fibrinogen is standardized and there is no requirement for cross matching (Fenger-Erikson 2009). It has been shown to be at least as efficacious, if not better than cryoprecipitate in increasing plasma fibrinogen levels. One study showed 2 g of FC would increase plasma fibrinogen by 0.44g/l, compared to only 0.26g/l after a 10U cryoprecipitate infusion (~1.8-2.2 g of fibrinogen). Some have deemed it better than cryoprecipitate; in one study 7 out of 64 episodes of cryoprecipiate infusion was followed by a drop in fibrinogen (Theodoulou 2012).

Although there are a number of manufacturers, the majority of studies have been one particular formula - Haemocomplettan, known as RiaSTAP. Low fibrinogen levels in trauma are discussed in detail above and the need for supplementation remains extant. Those studies investigating Haemocomplettan as the source of fibrinogen supplementation in cases of severe bleeding have found similar results; its use is associated with an improvement in coagulation parameters (Danes 2008, Fenger-Erikson 2008, Thorarinsdottir 2010), there is a direct relationship with plasma fibrinogen levels and survival (Danes 2008) and its use reduces transfusion requirements (Fenger-Erikson 2008, Thorarinsdottir 2010). One study (Schöchl 2010) specifically looking at goal directed resuscitation in trauma using fibrinogen concentrate found actual mortality was less than expected; 24.4% compared to a predicted mortality of 33.7% (calculating using Trauma Injury Severity Score [TRISS]) and 28.7% (using the Revised Injury Severity Classification [RISC]). Despite these favourable results, a meta-analysis carried out in 2017 on 7 observational, mostly retrospective studies found no effect on mortality with the administration of FC in trauma, noting though that there were no RCTs and their findings were due to a lack of good quality data (Mengoli 2017).

There have been some trials subsequent to this review. The FiiRST trial was a feasibility trial looking at 6g of FC given successfully within an hour of hospital arrival (Nascimento 2016). Fibrinogen levels were higher and remained so for up to 12 hours, but there was no difference observed in mortality. This is not surprising though as neither group received massive transfusions, there was no difference in the amount of blood components each group received and both groups received similar amounts of cryoprecipitate. The RETIC trial (Innerhoffer 2017) investigated the use of FFP versus coagulation factor concentrates. They used fibrinogen concentrate predominantly but the trial was abandoned due to the large numbers from the FFP group needing rescue therapy (additional blood component resuscitation out with the study protocol), but this in itself was an interesting finding. 52% of the FFP group needed rescue versus only 4% receiving FC and the number needing massive transfusion was also greater in the FFP group (30%) versus 12% in the other. Despite this study being stopped early their findings suggest that FC should be looked at in a favourable light.

One more recent trial (Yamamanto 2017) undertook a retrospective look at giving FC pre-emptively in trauma patients rather than waiting for threshold results. Nearly 60% of the patients that had an ISS of greater than 26 had received 10U red cells and were found to have had low fibrinogen. The 48 hour mortality of those with ISS greater than 26 was 8.6% in the pre-emptive FC group compared to 22.9% in the other group. For the more severely injured casualties this difference was greater; those patients with an ISS of greater than 41, the pre-emptive FC group had a mortality of 20% compared to 50% in the other group. Although this is a retrospective study it was one of few to illustrate mortality benefit of giving fibrinogen concentrate early and most definitely leads to the question of whether it could be taken into the pre-hospital environment (both civilian and military).

Another lyophilised product in use by the British and American military and by an increasing number of civilian (particularly pre-hospital) providers is lyophilised plasma.

6.3.7 Freeze Dried Plasma

Freeze dried human plasma (FDP) otherwise known as lyophilised plasma (LP) was first introduced in World War II for use in resuscitation. Plasma was converted into a fine lightweight powder in significant quantities to answer the high demand under difficult logistical conditions allied with the need for large volumes in a combat setting. Disappointingly there were high rates of viral disease transmission secondary to pooling of plasma units and inadequate screening and the concept was abandoned (Inaba 2011). Modern screening methods have significantly reduced the risk of virus transmission have meant the concept of lyophilised plasma has re-emerged as primarily a logistically superior alternative to FFP.

The two main manufacturers are from France producing FLyP (French Lyophilised Plasma) and Germany producing LyoPlas N-w. The latter is used by the British market including the Defence Medical Services. LyoPlas production is typical with FFP being thawed and transferred to a glass bottle in sterile conditions with filtration eliminating any blood cells.

LP has been repeatedly tested for functional comparison to FFP. Investigations after World War II demonstrated its haemostatic function was similar as measured by prothrombin time (Oktavec 1954). *In vitro* assays of dried porcine plasma and FFP showed similar coagulation profiles (FII, FVII and FIX, PT, aPTT and fibrinogen in addition to thromboelastograhy assessments) (Shuja 2008, Spoerke 2009). *In vivo* studies using swine models of polytrauma and haemorrhage demonstrated that FDP clotting factor levels were comparable to FFP with only a 14% drop in coagulation factor activity (Spoerke 2009, Lee 2013).

Similar animal trauma models have illustrated it was equally effective as FFP in reversing coagulopathy and improving physiological markers as well as survival which increased from 15% to 100% (Shuja 2008). In addition to coagulation benefits Spoerke et al (2009) also establish that LP reduced IL-6 levels. Levels of IL 6, 8, TNF are found to be increased in trauma and are

predictors of death (Martin 1997), SIRS (Giannoudis 2008), injury severity (Gebhard 2000) and multiorgan dysfunction (Kobbe 2008), so the authors suggested this secondary effect (to the use of ascorbic acid in its manufacturing process) was a further advantage (Hamilton 2011).

There is very little substantial prospective evidence of the efficacy of LP or FDP in a human population. The military however have been using it for some time, acting on the significant animal data, bringing it into use to meet the logistical demands of treating troops deployed long distances from medical support. At present it is used by Dutch, French, Israeli, German and UK armed forces (personal communications). US forces have just received FDA approval. Most studies have therefore been military based.

A recent prospective study conducted by the French military at the Role 3 Hospital in Kabul, Afghanistan reported on the use of LP in 87 military and civilian casualties (Martinaud 2011). They reported an overall mortality of 10% among those patients who received LP, with 67% of these patients in shock when treatment was initiated. This study noted a significant decrease in Prothrombin Time (PT) after LP administration in the 36 patients with complete data sets. Though this study was small and many patients were lost to follow-up, there were no reported complications attributable to LP administration. The French military have probably the greatest amount of experience and regularly extol the virtues of LP and its significant shelf life (2 years); its ease of time to use (3 minutes to rehydrate) and that at the end of its shelf life it still has similar clotting factor and fibrinogen activity to FFP (Daban 2009, 2010, Bux 2013). A further French study, again from Kabul, looked at 72 transfusion episodes of which 63 received LP (average of 3 units). They found a significant improvement in PT back to normal levels with LP, prior to surgical intervention (Patrick 2011).

This small evidence supports the animal model evidence that LP has a use in patients suffering severe trauma and haemorrhagic shock and backs those growing number of medical providers who have been using it in the civilian pre-hospital environment Internationally. An Israeli retrospective

study (Shlaifer 2017) on 109 casualties over a 3-year period (83% penetrating, 50% multiple severe injuries) receiving FDP, showed that it was both easy and feasible to use in this environment. A French retrospective study has shown trauma patients can receive plasma in a 1:1 ratio with greater rapidity if LP is used instead of FFP (Nguyen 2018). The Norwegians have used LP in their pre-hospital HEMS service and published a 12-month review of its use (Sunde 2015). Only 16 patients received it, having received mixed trauma and being hypotensive (Systolic BP < 90mmHg). They reported no transfusion related complications. These studies show the feasibility of using LP in the pre-hospital environment but more prospective studies are needed to evaluate clinical effectiveness and outcomes following LP transfusion.

There has been only one study published so far; the Traucc trial was a prospective, randomised trial comparing FLyP to FFP in trauma patients in a French hospital. One of their primary outcomes was the incidence of coagulopathy and fibrinogen levels (Garrigue 2017). The investigators found in the 48 patients enrolled that those in receipt of FLyP had higher fibrinogen concentrations and a more rapid improvement in their coagulopathy compared to FFP. They also noted that FLyP patients received plasma more rapidly (15 minutes compared to more than 90) and as a consequence achieved a 1:1 ratio in significantly less time.

Lightweight, easily transportable, simply stored for long periods of time; the superior logistical reasons for using lyophilised plasma are clear. The additional *in vitro* and animal model and limited human *in vivo* evidence that it functions as well as FFP makes lyophilised plasma a viable component to use in a trauma patient. Unfortunately its limited availability and cost reduce any physicians ability to use it. These latter reasons are the principle rationalisation for its use only in austere situations in the British military – in the pre-hospital environment or with Units who provide small surgical teams to areas that are more difficult to reach and support.

6.3.8 Whole Blood

There has been a resurgence in the interest in using Whole Blood (WB), both stored (SWB) and fresh (FWB), for trauma in recent years. It has had a long history of use in the military environment since World War I (Stansbury 2017), and many argue that it is a logistically easier and less wasteful option as it contains all the elements of blood components, but in a smaller volume of anticoagulant. A recent review (Spinella 2016) indicated that its use in hemorrhage, results in outcomes as favourable if not better than component therapy resuscitation. Retrospective military studies on the use of FWB on combat casualties have shown it is independently associated with improved survival in Iraq (Spinella 2009) and Afghanistan (Nessen 2013). The advantage of refrigerated storage for one product compared to multiple storage options for components, including agitation requirements for stand-alone platelets means WB has a significant logistical advantage (Cap 2018). Concerns over infection and grouping mismatch are valid, with 2 infections and one transfusion associated graft versus host fatality in 10,000 FWB transfusions on US personnel (Gilstad 2012). Its use is increasing with at least 5 US and Norwegian trauma centres using WB for trauma resuscitation using group O blood with low titres (less than 50 anti-A and anti-B; Yazer 2018) with a number of larger studies beginning to look at the feasibility and potential advantages of using it (LITES 2018)

UK military experience so far has been sporadic and has centred on emergency donor panel provision in response to patient extremis or when platelet provision was inadequate or non-existent. WB has considerable potential, particularly in the pre-hospital environment and in austere military environments.

Unfortunately within the UK at present NHS Blood and Transplant (NHSBT) does not supply WB as standard. Despite this, requests from the UK military and other agencies have had recent impact, and the London HEMS are at present undertaking a study on its use for pre-hospital trauma resuscitation (LAA News 2018).

6.4 Transfusion Adjuncts

6.4.1 Tranexamic Acid

Hyperfibrinolysis is found in severely injured patients and is associated with increased mortality (Kashuk 2010a). The Clinical Randomisation of an Antifibrinolytic in Significant Haemorrhage 2 (CRASH-2) (CRASH-2 collaborators, 2010, 2011) brought Tranexamic Acid (TXA), an anti-fibrinolytic, into the International sphere. The investigators appraised the use of TXA in trauma within 8 hours of injury in a large, international multi-centre trial of over 20,000 patients. They demonstrated that administration of TXA in trauma reduced death from 16.0% to 14.5% and death from hemorrhage from 5.9% to 4.9%. They found no increase in fatal or non-fatal vascular occlusive events. Importantly for bleeding deaths, early TXA was better, with less than 1 hour being more protective than 1 to 3 hours. Of concern was TXA given after 3 hours was associated with increased risk of death.

Despite these significant and important results there has been criticism from a number of quarters, particularly in America and Australia (Pusateri 2013, Mitra 2014). In CRASH-2, 98% of the patients were recruited from developing countries where trauma patient treatment is considerably different (rudimentary pre-hospital and emergency medicine care, minimal blood supply, limited imaging access and basic operative and postoperative care facilities) to developed countries. There is little evidence that the patients were bleeding at the time of randomization; only half (50.4% in TXA group, 51.3% in placebo group) of the patients received blood transfusions and less than half the patients had a surgical intervention (47.9% in TXA and 48.0% in the placebo group) so many question if some groups of patients recruited to CRASH-2 would be expected to actually benefit from TXA. Particularly when there was no reporting of the total number with hypotension, or haemorrhage. It is reasonably likely that patients with massive haemorrhage may not have made it to some of the hospitals involved in the study. Finally the mortality of recruited patients is

significantly higher than that found in major trauma centres in USA, UK and Australia. Hence criticism is understandable, especially from physicians in developed countries where the trauma population is appreciably different from the groups described above (Cap 2011, Napolitano 2013).

The UK military have used TXA extensively in Afghanistan for ballistic casualties and found in a retrospective study that TXA use was associated with improved survival (OR 7.3; 95% CI 3.02 to 17.32), although unlike CRASH-2 there was no difference in mortality at 24 hours, it was found to be thereafter (Morrison 2012). The reason for this is unclear. Of concern though, was that the patients receiving TXA had a higher rate of pulmonary embolism (PE) and deep vein thrombosis (DVT) (2.7% and 2.4%) compared to those who did not receive TXA (0.3% and 0.2%). It is highly likely that this thrombus incidence is even higher as under reporting of PEs was discovered in the UK Hospital in Afghanistan at that time; actual incidence 9.3% in a retrospective study compared to 3.2% diagnosed at injury, with a positive association between pulmonary thrombus and DVT, amputation and TXA use (Lundy 2013).

A number of studies in developed countries have investigated the impact of TXA use in developed countries. The Ryder Trauma Center reviewed 1217 patients over 4 years and compared those who had received TXA to those who had not (Valle 2014). They found that in patients with the highest injury acuity TXA was associated with increased mortality (27% vs 17%, $p<0.024$). The Texas Health Science Center in Houston investigated 1032 patients over a 4-year period who had evidence of hyperfibrinolysis on thrombolealstography. In-hospital mortality was higher in the TXA group (40% vs 17%, $p<0.0001$; Harvin 2015). Another prospective study from London investigated the implementation of TXA use in their massive transfusion protocol. Of 385 patients, 160 (42%) received TXA within 3 hours. Those receiving TXA were more severely injured and coagulopathic on arrival. TXA was found to have no influence on outcome and in multivariate analysis was protective for mortality in “shock” patients only (Cole 2015). All of these studies support the notion that the CRASH-2 trial

results should not be blindly followed and physicians must question its use in a well developed trauma centre with ready access to blood components and near patient coagulation testing particularly in the more severely injured group, and especially when the theory of distinct fibrinolysis phenotypes is considered (Moore 2015).

Despite these misgivings its use is common in the pre-hospital environment across the World. The USA, French, British and Israeli military and the British, Norwegian and Israeli civilian ambulance services use TXA as part of their resuscitation protocols. The Germans have found evidence that TXA given in the pre-hospital environment improved survival (Wafaisade 2016) although there were some limitations to the study; timings of TXA not documented, no TXA algorithm, and cause of death not documented.

Most guidelines produced recommend the use of TXA at the doses used by the CRASH-2 collaborators; 1g bolus to be given within 3 hours of injury, followed by a 1g infusion over 8 hours. This is recommended by the British (CGOs 2012) and US military guidelines (Joint System Control Resuscitation Clinical Practice Guideline; Damage Control Resuscitation 2011) where a pre-hospital TXA bolus is advocated. Civilian guidelines are similar in Europe (Rossaint 2016) where the first dose is recommended en route to the hospital.

Elsewhere guidelines are a little more cautious; the International Trauma Life Support states "*consideration should be given to administration of TXA….in those patients who show signs of hemorrhagic shock*" (Alson 2014). A document endorsed by the American College of Surgeons Committee on Trauma and American College of Emergency Physicians on pre-hospital use of TXA (Fischer 2016) stated that there was a lack of data and recommended any TXA administration be monitored.

There is some convincing evidence for the use of TXA, however this is not without flaws and more relevant and robust data relevant to developed country civilian and military patients is lacking. Military data suggests it has

potential but the one retrospective study played down significant potential side effects and had insufficient data on timings and doses of TXA given. Many clinicians look forward to the results of two on going prospective pre-hospital studies; STAAMP (Study of Tranexamic Acid during Air Medical Prehospital Transport) (Brown 2015c) in America and PATCH (Pre-hospital Antifbrinolytics for Traumatic Coagulopathy and Haemorrhage) (Mitra 2014) in Australia.

6.4.2 Recombinant Activated Factor Seven

Initial investigations into the use of recombinant activated factor VII (rFVIIa) were promising. An early, randomised controlled trial in trauma patients found reduced PRBC usage and reduced ARDS incidence with no increase in adverse thrombotic events (Boffard 2005). A military retrospective study on ballistic trauma casualties found a reduction in PRBC transfusions (Perkins 2007) and another found reduced 30-day mortality, again with no increase in thrombotic events (Spinella 2008b). However there was growing evidence that it was not an uncomplicated treatment option, with the greatest concern being over thrombo-embolic events. Greater experience and larger numbers of patients exposed to it resulted in a number of studies illustrating these concerns; it is found to be related to deaths from thromboembolic events (MacLaren 2005, O'Connell 2006, Thomas 2007), there is a lack of evidence to support its use in penetrating trauma (Duchesne 2008) and it has been shown to potentially cause an increased incidence of multiple organ failure (Wafaisade 2013). A recent randomized controlled trial was terminated early (secondary to a futility analysis) due to unexpected low mortality (Hauser 2010). Mortality was higher (but not significant) in penetrating trauma for those receiving rFVIIa, but there was no increased incidence of thrombotic events, a factor also found in an earlier study (Boffard 2005). Other studies in the pre-hospital space have found little difference with its treatment (no effect on mortality) although adverse events were not increased by its use (Neeki 2017).

The evidence is confounding and contradictory. The US Food and Drug Administration (FDA) does not approve it for use in trauma and the European guidelines on trauma (Rossaint 2016) recommend that it should only be considered if all other attempts to control bleeding by conventional measures have failed, a similar position to British military guidelines (CGOs 2012). Consequently it is rarely used, as for rFVIIa to work adequately the patient should not be acidotic or hypothermic and should have adequate platelet and clotting factor levels – an unlikely situation if all other conventional haemostatic measures used in the resuscitation so far have failed.

6.5 Resuscitation Fluid Summary

Previous chapters have discussed in detail the expected injuries in civilian and military trauma and the physiological outcome of those injuries. Clotting and how it is affected by trauma has been considered with particular attention to the Acute Traumatic Coagulopathy. The resuscitation methods for these patients has also been examined; methodology (Damage Control Resuscitation) and investigation aids (near patient coagulation assessment). Finally the different fluids, including ratios of blood components, and their adjuncts used in trauma casualty resuscitation has been considered within this chapter.

Whilst there is clear evidence that early use of blood products with a high ratio of plasma (and platelets) has benefits in severely injured casualties, the objective evidence is limited to early in-hospital use. Recent UK military practice advanced the use of blood products to the pre-hospital environment based principally on clinical "*common sense*" and risk balance considerations. Nonetheless early use comes at a logistical cost and some clinical risk. The purpose of the study described in this thesis was to investigate the potential impact of pre-hospital blood components on

attenuating the coagulopathies observed in military ballistic and blast casualties.

Chapter 7. Hypothesis, Aims and Methods

7.1 Introduction

The military in-hospital Damage Control Resuscitation practices in Afghanistan involved early aggressive use of blood products for perceived morbidity and mortality benefit (Holcomb 2007, 2008, Borgman 2007, Penn-Barwell 2015). The UK extended this practice into the pre-hospital environment in 2008 with the UK helicopter based Enhanced Medical Emergency Resuscitation Team (MERT-E) carrying four units of PRBC and thawed FFP.

Concern over the logistical burden of supporting this capability, the potential high blood component wastage and increased risk of transfusion related reactions (secondary to the difficult environment it was being used in) meant that the unproven clinical benefits were questioned.

This study was proposed to provide an objective evaluation of the presence of any additional benefit of pre-hospital *vs* early in-hospital administration of blood products. This would then inform the decision of whether or not to recommend pre-hospital use of blood products, in Afghanistan and in subsequent conflicts.

7.1.1 Animal Models of Haemorrhage and Coagulopathy

Haemorrhage

Animal models of haemorrhage are broadly divided into two; controlled and uncontrolled. Each has its strengths and weaknesses, and each can be more suited than the other for particular research questions. The following

list is not designed to be exhaustive, but simply to provide an overview and relevant examples. Uncontrolled haemorrhage is induced by causing a vascular lesion and allowing blood loss to simply occur. A variety of injuries have been used, including aortotomy and liver injury, depending on the animal species being used and the question being investigated. This type of model has an advantage of being sensitive and appropriate to assess treatments and devices designed to stem the initial haemorrhage. The disadvantage of this model is the resulting widely differing volumes of blood loss between individuals, and therefore a high degree of underlying variability in studies designed to assess treatments once the haemorrhage has occurred (e.g. fluid therapies).

Controlled haemorrhage can be induced by removal of a set volume of blood or by removing blood so as to reach a predetermined blood pressure. This approach has the advantage of minimising the variability of the initial haemorrhagic insult and physiological response (from haemodynamic, autonomic and metabolic perspectives) for studies investigating e.g. resuscitation strategies. A disadvantage, if this is the sole approach taken to the haemorrhage, is that it will not be sensitive to problems of re-bleeding or provide appropriate demands on the haemostatic systems.

Coagulopathy

Several models have used measures such as haemodilution and exchange transfusion to induce a coagulopathy (Sena 2013, Koko 2017), but this is not a coagulopathy akin to human Acute Traumatic Coagulopathy. Few previous large animal models studies have developed an endogenous coagulopathy due to combined injury and shock alone, as humans do.

Brohi's group recently reviewed available animal models used to investigate trauma induced coagulopathy (Frith 2012). They identified 23 models using 5 species. Pigs were the predominant large animals used. The Animal (Scientific Procedures) Act 1986 necessitates that the species to be used is

the lowest possible on the hierarchy of sentience. With the majority of these studies a certain volume of blood is required for measuring the necessary assessments. It is crucial that the volume of blood sampled for animal/investigation assessment has little impact on the investigation itself, necessitating a certain size of animal. This means that the species used is most commonly the pig.

The few studies on pig hemostasis have only been conducted in predominantly physiologically normal animals. Generally they have been found to be hypercoagulable relative to humans, with viscoelastic tests showing a quicker clot formation (shorter Clotting Time [CT] and Clot Formation Time [CFT]; Siller-Matula 2008) and a similar (comparable Maximum Clot Firmness [MCF]; Kessler 2011) or a more stable clot (wider MCF; Siller-Matula 2008). Conventional laboratory tests have not generally shown a difference in results (similar PT and aPTT; Siller-Matula 2008) between the two species. Platelet Count has been noted to lie in the upper end of the human normal range and Fibrinogen levels are at the lower end of the normal range (180 mg/dl; Siller-Matula 2008). The model remains relevant though as it is valid to investigate the change from the normal coagulation of that animal and compare any coagulopathy to normal porcine ranges.

There have been very few models of military injury to investigate the effectiveness of pre-hospital resuscitation, as it needs to include other aspects of military injuries to reproduce the pathophysiological response to ballistic penetrating injuries or blast injuries.

With very few ballistic models, no injury induced coagulopathy models and few pre-hospital resuscitation models existing; the development of a model that included all these was necessary.

7.1.2 Requirements of Model

To assess the impact of pre-hospital blood components in Afghanistan, the model needed to reflect the type of typical ballistic casualty that was being transported by the MERT-E and undergoing Damage Control Resuscitation and Surgery in the military hospital at Camp Bastion. These casualties were severely injured, coagulopathic, acidotic, hypotensive casualties, and had received little medical care prior to that provided by the MERT-E. The requirements of the model would need to include;

a. Severe injury including blast.
b. Significant blood loss.
c. Acidosis (hypotensive shock).
d. Coagulopathy induced by injury similar to ATC.
e. Limited resuscitation prior to expert pre-hospital medical care.
f. A pre-hospital phase of similar duration to Afghanistan.

This would reflect a typical trauma patient that a clinician, either pre-hospital or in-hospital, would be particularly concerned about and pose the crucial question on fitness for definitive or damage control surgery.

7.1.3 Dstl Animal Model

The Defence Science and Technology Laboratory (Dstl) at Porton Down had already completed a significant amount of work on the study of resuscitation in ballistic casualties using animal models. A porcine model of severe, survivable battlefield injury had been developed previously to evaluate pre-hospital resuscitation strategies. It was developed to test the effect of resuscitation on mortality and in particular shock (arterial base excess [ABE] and lactate, rather than coagulation (Garner 2009, 2010, Doran 2012).

The model used terminally anaesthetised Large White pigs that were surgically prepared, including line insertion and splenectomy. Pigs' spleens

are invested with smooth muscle and the organ acts as a reservoir for a significant amount of blood (Hannon 1985). This smooth muscle contracts during the sympathetic response to haemorrhage; squeezing blood into the circulation producing an autotransfusion, making its removal essential in order to more accurately mirror human physiology. The animals were wrapped in a Kevlar blanket before exposure to an explosive charge with no casing in order to reduce variability by preventing secondary and tertiary blast injury. Removing a fixed volume of blood through a preplaced cannula induced shock and this was then converted to an uncontrolled haemorrhage with a Grade IV liver injury (i.e. involving disruption of 25-75% of the liver parenchyma; Moore 1995) that allowed an element of uncontrolled bleeding with the possibility of re-bleeding. The controlled haemorrhage was intended to reduce variability in the amount of blood that would be lost, especially between the blast and sham blast groups.

7.1.4 Animal Model for Shock, Haemorrhage and Acute Coagulopathy of Trauma

Several modifications to this original Dstl model were required for the proposed study of pre-hospital blood components. Ballistic injury, either from bullets or from fragment injuries in an explosion usually leads to widespread tissue damage extending a considerable distance away from the wound-track of the projectile. This tissue damage causes release of mediators which can impact on clotting as the body responds to a large volume of damage to macro and, in particular, micro-vasculature. This aspect was replicated in a controlled, reproducible manner in the current study as blunt injury to muscle using a captive bolt pistol. The modifications in summary were:

a. Injury and haemorrhage were adjusted to induce a coagulopathy at a time point corresponding to hospital admission.

b. The endpoints of the trials were to be based on coagulation

parameters rather than mortality. As such, it was necessary for the animals to survive until the end of the investigation.

c. The liver injury had face validity as a potentially survivable wound capable of re-bleeding, but was removed in order to reduce lethality of the model.

d. Since tissue injury can alter the response to haemorrhage (Rady1993) and is a crucial part of ATC development (Frith 2010), a limb injury (intended to mimic the tissue damage resulting from a fragmentation ballistic injury) was included.

e. Changes in the operational scenario in Afghanistan meant that evacuation times were now much shorter and the timing of interventions in the model needed to reflect this.

This current study was designed to extend this earlier research and investigate the use of pre-hospital blood products in an animal model of blast, tissue injury and haemorrhagic shock. The study was also designed to investigate the effects of injuries from both explosive events and non-blast injuries to reflect the different mechanisms of injury seen in modern warfare.

7.2 Hypothesis

The use of blood components (PRBC:FFP) – compared to the use of saline – in the pre-hospital phase of a simulated patient journey, will attenuate trauma induced coagulopathy and over all shock burden at the decision point for surgery (30 minutes after hospital admission).

7.3 Outcomes

7.3.1 Primary Outcome

Coagulation status as measured by Thromboelastography, 30 minutes into the In-Hospital Phase

7.3.2 Secondary Outcomes

Overall coagulation status at end of In-Hospital Phase; burden of shock as measured by base excess at the end of the early In-hospital phase (characterised by degree and trend of change of base excess) and total volume of fluids used in resuscitation.

7.3.3 Tertiary Outcome

Physiological state (base excess) and degree of coagulopathy 210 min after injury.

7.4 Study Design

The study was conducted on terminally anaesthetised Large White pigs (i.e. anaesthesia is induced at the onset of the study, and the animals are maintained under anaesthesia throughout until they are euthanased humanely with an overdose of anaesthetic at the end). The study was carried out in accordance with the Animals (Scientific Procedures) Act 1986.

This was a prospective, randomised, controlled trial comparing early (simulating pre-hospital) *vs* later (simulating immediate in-hospital) administration of blood components (packed red blood cells and plasma) on the pre-operative coagulopathic and physiological state of battlefield injury

casualties. Two models of severe battlefield injury – blunt tissue damage plus haemorrhagic shock in the presence and absence of blast – were studied in terminally anaesthetised pigs.

Efficacy of treatment was assessed at 30 minutes after hospital admission (having received 60 minutes of pre-hospital and 30 minutes of in-hospital resuscitation). This corresponds to an early in-hospital clinical decision making point on the need to perform abbreviated damage control surgery e.g. limb amputation, or whether the patient is fit enough to withstand a more complete procedure e.g. limb salvage.

The main ideology behind the experimental protocol was to mirror the medical capabilities and the timelines that existed at that time in Afghanistan. This would include:

a. Wounding Phase. Injury and haemorrhage (with or without blast).
b. Shock Phase. This corresponds to the presence of a basic pre-hospital medical capability such as a stand-alone medic who would only give crystalloids if blood pressure was too low (hypotensive resuscitation) – 30 minutes duration.
c. Pre-Hospital Phase. A MERT-E (Medical Emergency Response Team – Enhanced) stage followed where Damage Control Resuscitation could take place with blood components or saline, still aiming for hypotensive resuscitation – 60 minutes duration.
d. Early In-Hospital Phase represented the time it would normally take to resuscitate, obtain a Computer Tomography scan, diagnose and decide on surgical plan for the patient. Blood components were used for resuscitation in all groups. The end of this phase was the Primary Outcome time point – 30 minutes duration.
e. Late In-Hospital Phase with continued blood component resuscitation up to a maximum of 6 units of each blood component. This corresponded to a surgical intervention stage, but no surgery occurred in our model

7.5 Experimental Protocol

The animals were randomly allocated to one of four treatment groups as illustrated in Table 7-1.

Injury. All groups received a blunt tissue injury and haemorrhage. Group 1 and 2 received a Primary Blast Injury, Group 3 and 4 did not (Sham Injury).

Treatment. In the Pre-Hospital Phase the groups' treatment diverged. Group 1 (Blast) and 3 (Sham) received pre-hospital blood components in contrast to saline used in the other two groups. The same clinically relevant blood pressure targets were used throughout. All groups converged at the Early In Hospital Phase where all groups received blood component resuscitation.

The timeline for the experimental protocol is shown in Figure 7-1

Injury \ Treatment	Pre-hospital blood products	Pre-hospital saline
Blast injury, haemorrhage and tissue injury	Group 1	Group 2
Haemorrhage and tissue injury	Group 3	Group 4

Table 7-1. Treatment Group Summary

7.6 Licensing and Animal Husbandry

The entire study was carried out in accordance with the Animals (Scientific Procedures) Act 1986. Prior to commencing the study, the programmed work underwent Local Ethical Review (Dstl Porton Down) and gained a United Kingdom Home Office Project Licence (PPL 30/3004). Immature female Large White pigs were sourced from a commercial supplier, whose animals' are classed as Specific Pathogen Free. The animals were acclimatised at Dstl Porton Down for at least 7 days prior to the study and were attended to twice daily being fed and watered *ad libitum* (16% protein home-milled coarse mix). The animals were weighed three times per week and on the afternoon before a trial. Mean trial-day weights were 52.2 ± 0.4 kg (mean ± SEM). Feeding was discontinued 18 hours before planned induction of anaesthesia with free access to water allowed for all animals.

7.7 Blood bank

Blood was collected by exsanguination from terminally anaesthetised female Large White pigs. Anaesthesia and carotid line insertion were carried out as detailed in section 7.9.1 and 7.9.2. For blood collection anaesthesia maintenance was inhalational using Isoflurane. Standard units of blood were collected from a carotid cannula into a citrate, phosphate and dextrose (CPD) blood collection bag (RCB434CCL, Pall Medical, UK), with each unit collected over 5-7 minutes from the carotid cannula and mixed continually during collection. There were pauses between bag collections resulting in an overall bleeding rate of 22.5mL/min.

The blood was processed according to standard UK blood transfusion protocols ("Red Book", NHSBT 2013) to separate the red blood cells from the plasma, which was leucodepleted, while the packed red cells were stored in saline, adenine, glucose and mannitol solution (SAG-M). The buffy coat and platelets were discarded.

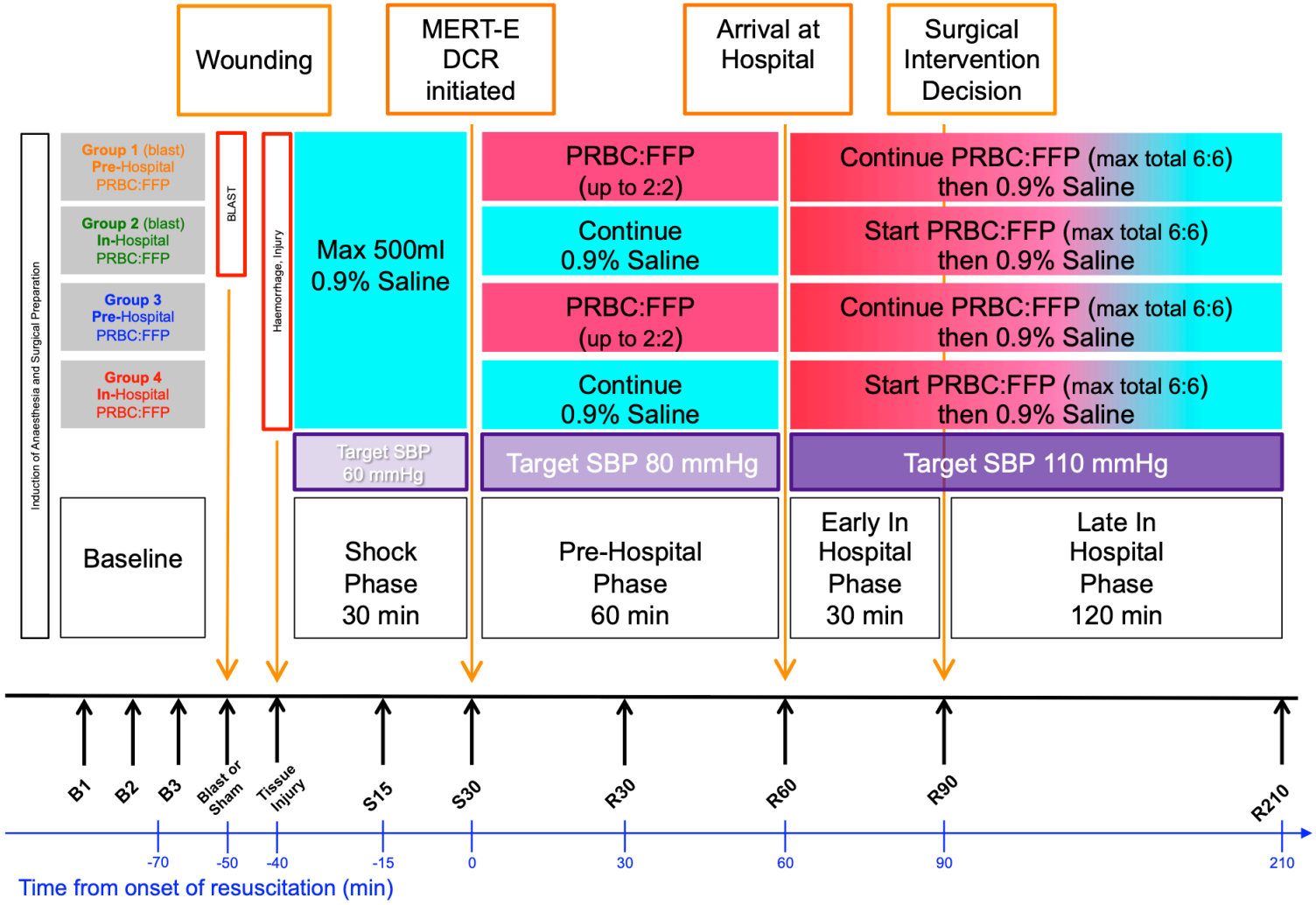

Wounding
MERT-E DCR initiated
Arrival at Hospital
Surgical Intervention Decision
Induction of Anaesthesia and Surgical Preparation
Group 1 (blast) Pre-Hospital PRBC:FFP
Group 2 (blast) In-Hospital PRBC:FFP
Group 3 Pre-Hospital PRBC:FFP
Group 4 In-Hospital PRBC:FFP
BLAST
Haemorrhage, Injury
Max 500ml 0.9% Saline
PRBC:FFP (up to 2:2)
Continue 0.9% Saline
PRBC:FFP (up to 2:2)
Continue 0.9% Saline
Continue PRBC:FFP (max total 6:6) then 0.9% Saline
Start PRBC:FFP (max total 6:6) then 0.9% Saline
Continue PRBC:FFP (max total 6:6) then 0.9% Saline
Start PRBC:FFP (max total 6:6) then 0.9% Saline
Target SBP 60 mmHg
Target SBP 80 mmHg
Target SBP 110 mmHg
Baseline
Shock Phase 30 min
Pre-Hospital Phase 60 min
Early In Hospital Phase 30 min
Late In Hospital Phase 120 min
B1
B2
B3
Blast or Sham
Tissue Injury
S15
S30
R30
R60
R90
R210
-70
-50
-40
-15
0
30
60
90
210
Time from onset of resuscitation (min)

Figure 7-1. Experiment Protocol and timeline. Black bordered boxes show the clinical phases. Orange bordered boxes represent key timelines. Four groups of animals (1 - 4) are displayed and diverge at the simulated pre-hospital phase where casualties may be treated by the MERT-E. In hospital phase is divided into early and late phases separated by the primary outcome (coagulation assessment with TEG) and represents the decision point for surgical intervention. Treatment is identical for all groups during the in-hospital phases. Ratios indicate ratio of PRBC to FFP.
Time points: These are forwarded by the induction of anaesthesia and surgical preparation of the animal (including splenectomy). **B1** to **B3** - baseline measurements after completion of surgical preparation and transportation. **Blast/Sham** - samples taken 5 min after exposure to blast (immediately before injury). **Injury** - samples taken after musculoskeletal injury and controlled haemorrhage. **S15** - samples taken after 15 min of shock. **S30** - samples taken after 30 min of shock and is the start of resuscitation (equivalent to R0). **R30, R60** etc - samples taken 30, 60 etc min after onset of resuscitation. **R90** coincides with the “decision on surgery” clinical event; this time point was used for the primary endpoint of the study. This is 90 minutes after initiation of resuscitation and 120 minutes after injury.

Collected units of blood were processed within 90 min of collection. The resulting units of PRBC were stored at 4°C (LabCold Blood Bank, UK) and used within 14 days of collection. The plasma was fast-frozen (MP1100 Plasma Freezer System, Thermogenesis, US), stored at -30°C (LabCold Plasma Freezer, LabCold, UK) and used within 6 months of collection. The Fresh Frozen Plasma (FFP) was thawed at 37°C in a dry tempering device (Sahara III Maxitherm, Sarstedt, Germany) immediately before use. Prior to use all donor products were forward and reverse matched to recipient blood. In addition, since PRBC and FFP from different donors were used for resuscitation, they were also cross-matched with each other.

7.8 Initial Anaesthesia and Surgical Preparation

7.8.1 Anaesthesia

The study was conducted under terminal anaesthesia, i.e. anaesthesia was induced and maintained throughout the study, followed by humane killing at the end with an overdose of anaesthetic without recovery of consciousness.

Induction and Intubation

Sedation was achieved with 5ml of Midazolam Hydrochloride (approx. 0.1mg/kg) via intramuscular injection. Once sedated, anaesthesia was induced by inhalation of Isoflurane 5%, driven by a 1:1 ratio of Oxygen (O_2) and Nitrous Oxide (NO) via a nose cone. Endotracheal intubation with a size 8.0 silicone oral/nasal tube (Vygon, France) followed induction. Aspiration, chest wall movement and end-tidal Carbon Dioxide ($ETCO_2$) monitoring (Propaq 106 EL, Dräger Medical) confirmed correct tube position. The endotracheal tube was then secured with strong adhesive tape and the mouth loosely packed with gauze.

Maintenance of Anaesthesia in Operating Theatre

Once intubated, the animals were moved to the operating theatre, where anaesthesia continued with Isoflurane (1-2%) and a mixture of O_2 and N_2O (ratio 1:2) (Blease Frontline Plus 690). The animals were then ventilated (Blease Frontline Manley MP3 Anaesthetic Ventilator) for the duration of the surgical procedure. Monitoring comprised of a Propaq 106 EL (Dräger Medical) monitor for continuous 3-lead ECG, End Tidal CO_2 and rectal temperature measurements. Once central venous access was secured at surgery, inhalational anaesthesia was replaced by an intravenous (IV) infusion of Alfaxalone (Alfaxan®, Jurox Pty Ltd) – at a starting rate of approximately 1ml/kg/hr using a syringe driver (IVAC Medical Systems™ P2000) and titrated to maintain surgical anaesthesia. Total Intra-Venous Anaesthesia (TIVA) continued for the remainder of the experiment. At the end of surgery mechanical ventilation was discontinued and the animals breathed air spontaneously.

Temperature Control

Warming pads, a silver-foil blanket, a wool blanket and a portable electric heater were all used to maintain the animal's normal rectal temperature of 38.5°C. If the temperature rose above 39°C, the animal was fanned and cooling pads applied. Resuscitation fluids were heated by being transfused through a fluid warming device (Belmont® Rapid Infuser RI-2, Belmont Instrument Corporation). Core body temperature was measured by a rectal temperature probe, connected to the Propaq 106 EL monitor (Dräger Medical).

7.8.2 Surgical Preparation

After intubation, surgical fields were shaved and prepared with Pivodine Iodine (Betadine®, Purdue Pharma) antiseptic. The animal was placed

supine, covered with a large sterile surgical drape, cut to expose the neck, abdomen and left groin.

Vascular Access in the Neck

Both carotid sheaths were exposed via a midline neck incision. The left internal carotid artery and internal jugular vein were cannulated with Portex 8 French lines (Hythe, UK), flushed and sutured in position (Figure 7-2). A pulmonary artery catheter (744MF75 Swann-Ganz, Edwards Life Sciences Ltd, Newbury, UK) was floated through an introducer sheath (Desivalve Catheter Introducer, Vygon, Cirencester UK) in the right internal jugular vein. Pulmonary arterial placement was confirmed by pressure wave monitoring (Propaq 106 EL, Dräger Medical AG &Co. Lübeck, Germany). The neck incision was closed with a silk suture.

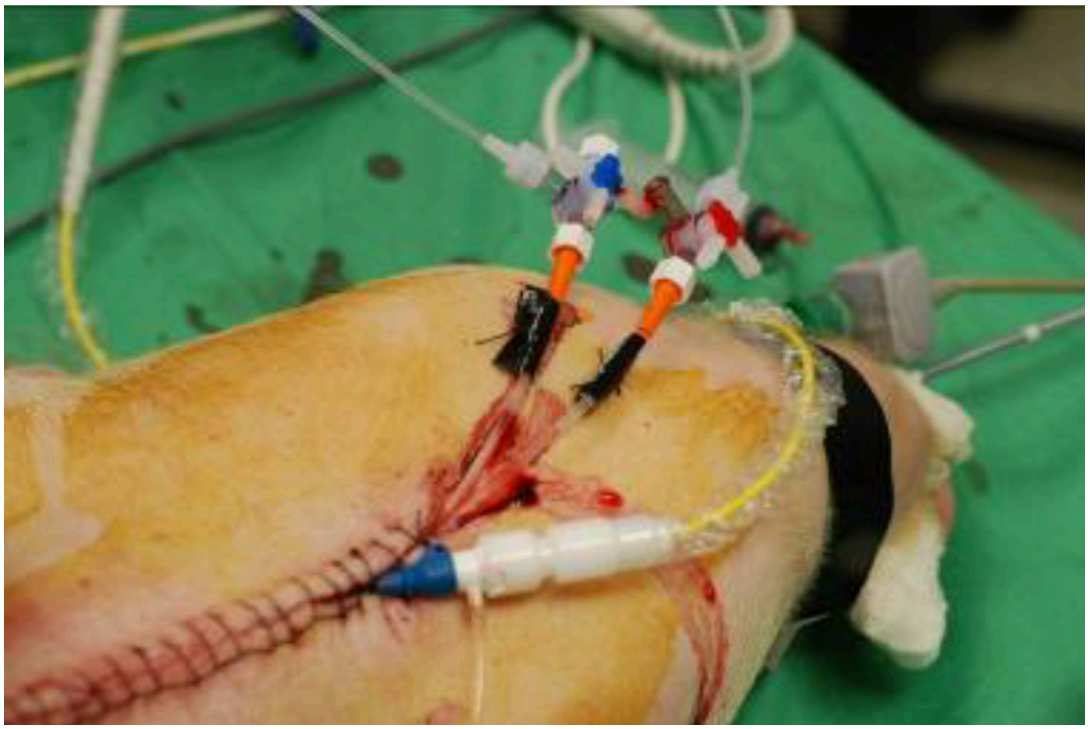


Figure 7-2. Neck and Head Access. Displaying from left to right; sutures, Swann sheath, jugular vein and carotid artery access. Further to the right of that the endo-tracheal tube (with mainstream capnography) and Oesophageal probe are visible.

Splenectomy

As previously discussed, in order to replicate more closely the human response to haemorrhage, the porcine spleen must be removed to disable the auto-transfusion capability this organ encompasses. 1ml 1:1000 Epinephrine was dripped onto the surface and massaged onto the spleen. This contracted the smooth muscle, emptying the organ of most of its stored blood. The splenic pedicle was then double ligated and divided. The spleen was removed and weighed to estimate the retained blood volume.

Open Suprapubic Cystostomy

Two 2/0 Polyglactin 910 (Vicryl™, Ethicon, UK) purse-string sutures were placed on the anterior wall of the bladder. A cystostomy was made with diathermy in the centre of the purse-string and a 14G Foley Catheter placed into the bladder (Bard Ltd, Crawley, UK). The balloon was filled with 10ml sterile water and the sutures drawn up and tied. The bladder was emptied.

Abdominal Closure

The abdominal incision was closed en-mass with continuous size ‘0’ Nylon double-stranded sutures (PDS™, Ethicon, UK) and the Foley catheter were exteriorised without snagging. The wound was dressed with thick black adhesive tape.

Femoral Vessel Cannulation

Approached through a left-sided groin incision the femoral vessels were distally ligated and cannulated with trimmed 8Fr Dog catheters (Arnolds®, Smiths Medical International Ltd. Hythe, UK). Three-way taps (Vyclic-Color, Vygon, Ecouen, France) were placed and the lines secured. The incision was closed with a silk blanket stitch.

Once these procedures were complete a large van was used to transport the instrumented animal for the 15-minute journey from the operating theatre to the blast range located in close proximity to the Laboratory. Anaesthesia was maintained and each animal was accompanied and monitored throughout.

7.9 Animal Assessment Methods

Numerous parameters were measured either continuously (e.g. $ETCO_2$ and ECG) or at certain intervals (e.g. urine output and blood samples). Table 7-2 illustrates the blood sampling protocol.

7.9.1 Cardiovascular Measurements

The animal's arterial blood pressure was recorded via the carotid artery cannula. Pulmonary arterial and central venous pressures were recorded via the flow-directed balloon tipped flotation catheter, which was also used to determine cardiac output as a 6 minute rolling average (Vigilance™ Volumetrics CEDV, Edwards Lifesciences™,USA). Physiological pressure measurements were made using strain gauge manometers (SensoNor 840, SensoNor a.s., Norway): zero pressure for all transducers was set at heart level. Core body temperature was measured by rectal probe (Propaq 106 EL) and maintained at approximately 38°C using external heating/cooling and blankets as described previously. All cardiovascular variables were recorded using a computerised data acquisition system (Maclab 8/s, ADInstruments, UK) and associated software (Chart v4.2.3, ADInstruments, UK) for subsequent analysis.

Time	Coagulation PT aPTT Fibrinogen	TEG	Blood Gas	FBC
Line In 1 (neck)				
Line In 1 (leg)				
Line In 2				
Post-Surgery				
Move to range				
Baseline 1				
Baseline 2				
Baseline 3				
Blast (Sham)				
Pre-Injury				
Injury and haemorrhage				
Post-Injury				
Start shock phase				
S1 (15 mins of shock, post-injury)				
S2 (30 mins of shock, post-injury)				
Start resuscitation				
Start of MERT pre-hospital evacuation phase				
R15 (15 minutes of resuscitation)				
R30				
R45				
R60				
Start of in-hospital resuscitation phase				
R75				
R90 Primary Outcome Point				
R120				
R150				
R180				
R210				

Table 7-2. Measurement parameter timeline. Coloured box equates to time of sample. Full Blood Count (FBC); Thromboelastography tested with TEG ® (see section 7.10.4).

7.9.2 Blood Gas Analysis

Blood gas analysis was performed alongside cardiovascular measurements, but an extra set was taken immediately after blast exposure. Arterial and mixed venous blood samples were taken anaerobically into heparinised syringes from the carotid and pulmonary artery catheters respectively for blood gas, base excess and lactate analysis (Gem Premier 3000 Blood Gas Analyser, Instrumentation Laboratories, Warrington, UK).

7.9.3 Coagulation and Haematology

Full blood counts were conducted using an ADVIA 120 (Bayer, Barmen, Germany). Visco-elastic assessment was completed using thromboelastography (TEG). A TEG® 5000 Hemostasis Analyser (Haemonetics Ltd., UK) was performed on fresh, un-citrated whole blood measuring R time (clot initiation), K time (clot dynamics) and Maximum Amplitude (MA; clot strength). Arterial blood was taken from the femoral cannula and analysed immediately using dilute Innovin® (1:50887 dilution Dade® Innovin®, Dade Behring; marketed by System UK Ltd, UK) as the initiator (Sørensen 2010). All TEG® analyses were performed in triplicate at 37°C.

Prothrombin and Activated Partial Thromboplastin Times (PT and aPTT) and Fibrinogen were measured from arterial blood samples. These were taken into citrated vacutainers (9NC 0.105M Vacutainer 367691, Beckton Dickinson, UK) and centrifuged at 1500 x g for 10 min. The plasma was separated and stored at -80°C for determination of PT and aPTT by turbidometry and fibrinogen concentration (Clauss method) using an ACL Elite analyser (Instrumentation Laboratories, Warrington, UK).

7.9.4 Pre-injury Baseline Measurements

Once at the blast range the animal was taken initially to the laboratory monitoring suite (Figure 7-3) where baseline measurements were taken (see Table 7-2 for details). These measurement were made a minimum of one hour after completion of surgery. The animal was then moved out to the blast arena (Figure 7-4).

Figure 7-3. Laboratory monitoring suite in close proximity to the Blast Arena. This was the location for the remainder of the investigation. From left to right: Monitoring (just out of shot), ventilator and anaesthetic machine, TEG machines, blood gas machines.

7.10 Wounding Phase

7.10.1 Blast Arena

The anaesthetised animal was mounted supine onto the blast rig, with the right thorax nearest to the charge, and wrapped in a Kevlar® blanket to protect from any fragment injury. Intravenous anaesthesia was maintained using a protected battery powered syringe driver. A completely independent second syringe driver (which could be triggered remotely), and anaesthetic cannula, provided a redundant backup system. The animals were monitored continuously throughout this phase and ECG and

respirometry were relayed to a laptop computer in a bunker. Surgical anaesthesia was maintained throughout.

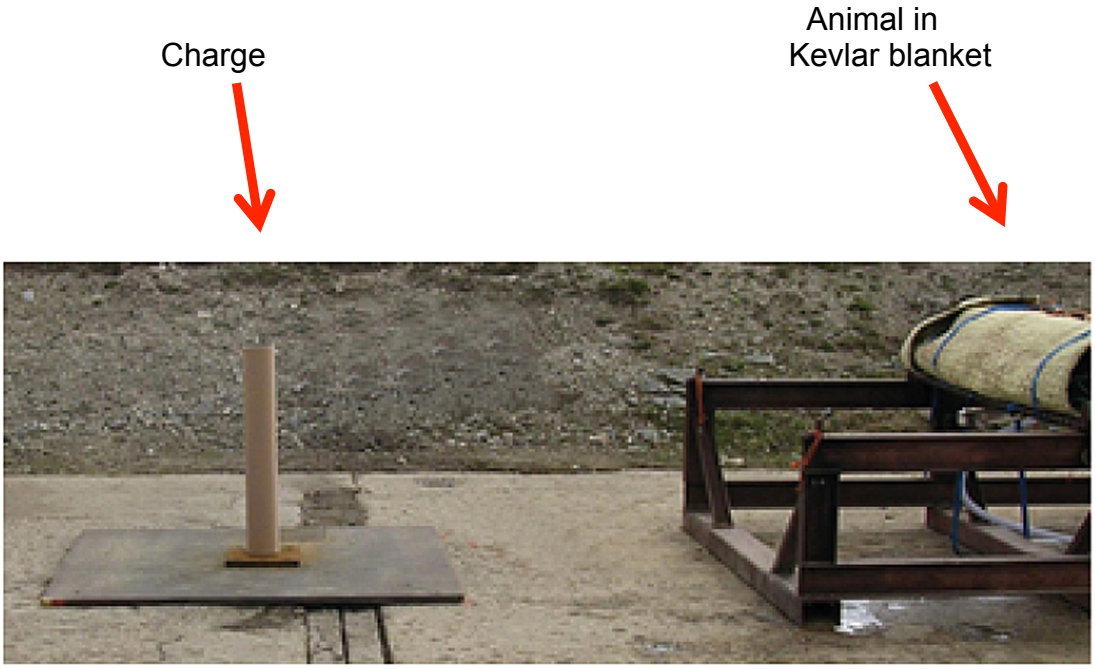


Figure 7-4. Photograph of blast arena. The explosive charge was secured to the cardboard tube. The animal is placed on a trolley wrapped in a Kevlar blanket.

7.10.2 Blast Exposure

A stand-off of 2.15m was measured from the centre of the charge to the body wall at the level of the 8th rib. This protocol was determined based on previous work performed by the Trauma and Biophysics Team at Dstl Porton Down, who have a broad experience in exposing large animal models to blast injury (Cooper 1996). The same batch of 2.2kg uncased cylindrical high explosive *EDC1S* charges was used for all experiments in the series.

After detonation of the charge and a safety check of the area, the surgical team quickly returned to the animal, always arriving within 120 seconds from charge detonation. Airway patency, breathing and anaesthetic

delivery were confirmed. Apnoeic animals were hand-ventilated until spontaneous respiration returned or were ventilated mechanically if they did not regain satisfactory spontaneous ventilation. Concurrently, a blood sample was taken from the femoral arterial line for blood gas analysis. The animal was recovered to the Laboratory (Figure 7-3) for the rest of the experiment. Animals subjected to the sham blast were treated identically but not exposed to blast.

7.10.3 Post-blast

Immediately after the explosion the animal was taken to the Laboratory. The IV infusion of Alfaxan® was continued and was titrated to the depth of anaesthesia. The animal was only ventilated (Evita 2, Dräger Medical) if its Arterial Blood Gas (ABG) showed a PaO_2 < 7 KPa, or a $PaCO_2$ > 6 KPa. Mechanical ventilation was discontinued if the animal showed signs of spontaneous breathing, (to determine if the animal had regained the capacity for adequate ventilation and gas exchange).

7.10.4 Tissue Blunt Injury

Ten minutes after blast (or sham blast), all animals were subjected to a controlled soft tissue injury using a blunt captive bolt pistol (CASH Special Knocker, Accles & Shelvoke, Sutton Coldfield, UK) delivering 4 standard impacts (using 2 Grain .25 cartridges) to the muscle of the right hindquarter. This resulted in widespread deep contusion in the underlying muscle but no fracture to bone.

Serum myoglobin was measured to assess the impact of tissue injury in each animal, (AQT90 FLEX, Radiometer). Creatine kinase (CK) is considered as a more clinically sensitive marker for muscle injury because myoglobin has a short half-life (2-3 hours), but in the context of this thesis myoglobin short half-life is a strength rather than a weakness. CK can take

12 hours to show a rise, peaking at 1-3 days after injury (Huerta-Alardin 2004), making it impractical for use in this investigation. Myoglobin is released into plasma as a result of muscle damage, making a rise in serum myoglobin (normal 3-80 µg/l) a good indicator of muscle damage. It rapidly returns to normal 1 to 6 hours after muscle injury due to rapid clearance of myoglobin by both renal excretion and metabolism to bilirubin (Poels 1993).

A summary of measurements is displayed in Figure 7-5 for myoglobin levels at R60 (time of hospital "admission", after 60 minutes of resuscitation). Baseline myoglobin levels were <42 µg/l in all animals. At R60, all animals had a significantly raised myoglobin level illustrating significant muscle damage. There was no difference between treatment groups in either strand: sham blast strand (P = 0.876), blast strand (P = 0.302; independent t-test based on log-transformed data in each case). The mean for each group was: Grp 1 1076 µg/l, Grp 2 797 µg/l, Grp 3 747 µg/l, Grp 4 814 µg/l.

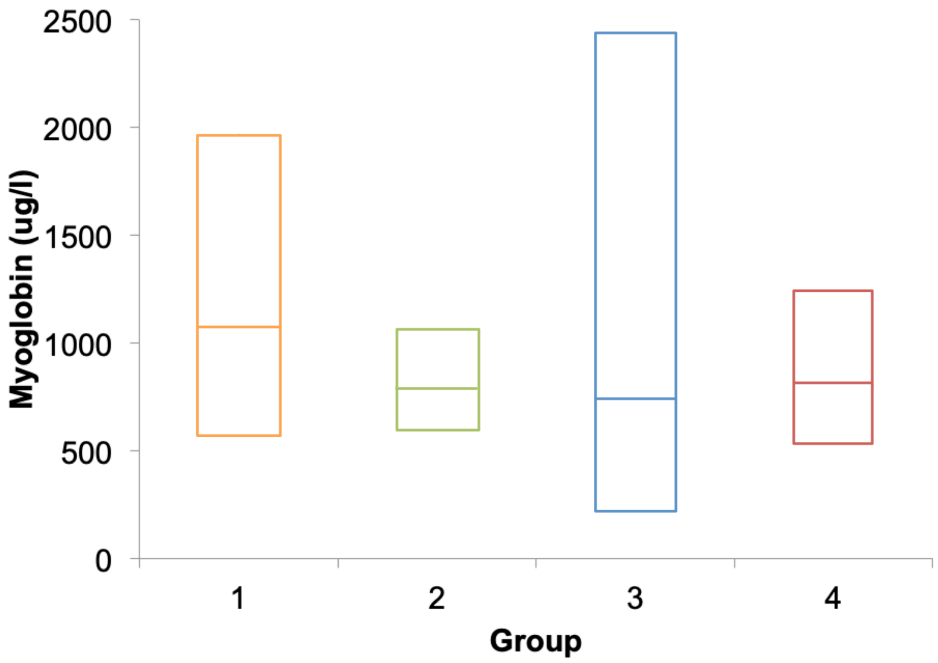


Figure 7-5. Serum myoglobin at R60 for all groups. Group 1 and 2; blast strand. Group 3 and 4; sham blast strand. Values are mean and upper and lower 95% confidence limits. Data were log transformed for analysis, and the resultant summary statistics detransformed for presentation.

7.10.5 Controlled Haemorrhage

Five minutes after the tissue injury a haemorrhage of 35% of the estimated total blood volume was performed over 9 min 40 sec 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 to mimic the rate of haemorrhage from a major arterial lesion. For blood volume and bleeding rate equations see Stern 1993 and Garner 2010.

7.11 Shock Phase

Following haemorrhage, the animals underwent a 30 minute shock period during which a capped volume of 0.9% saline (500 mL maximum) was administered as necessary to maintain a target systolic arterial blood pressure (SBP) of 60 mmHg, reflecting an aspect of current clinical practice by Combat Medical Technicians. Resuscitation infusions were warmed to 37°C and administered at a rate of 200 mL/min (Belmont Rapid Infuser, Belmont Instrument Corporation, US).

7.12 Pre-Hospital Phase

The next phase of the protocol represented a 60 min pre-hospital evacuation phase. Warmed resuscitation fluid was administered according to the relevant protocol, with a final target SBP of 80 mmHg in each group. Group 1 and 3 were given aliquots of 0.9% saline (representing standard of care) to attain and maintain the target SBP, while Group 2 and 4 received Packed Red Blood Cells (PRBC) and Fresh Frozen Plasma (1:1 ratio, PRBC and FFP given simultaneously). The volume of blood products in the pre-hospital phase was capped at 4 units (4x300 mL approximately) per

animal (2 units PRBC and 2 units FFP). Once the maximum amount of blood product had been used resuscitation continued to the same pressure target using 0.9% saline for the reminder of the pre-hospital evacuation phase. All infusion volumes were measured accurately.

7.13 Early In-Hospital Phase.

The in-hospital phase represented more aggressive resuscitation to a normotensive target (SBP 110 mmHg) using PRBC:FFP (1:1) in all groups. Supplementary oxygen (minimum FiO2 0.3) was given and titrated to attain an arterial oxygen saturation (SaO2) of 98%. At the end of this phase (after 30 minutes) the necessary blood test measurements were taken as this time point corresponded to the Primary Outcome Time Point (R90).

7.14 Late In-Hospital Phase

This phase was a continuation of the Early In-Hospital Phase. Due to the limitations of the blood bank the total amount of PRBC:FFP used in any one animal was capped at 6 units of each product (including the blood components used during the pre-hospital phase in Groups 2 and 4). Once the total permissible amount of PRBC:FFP had been given resuscitation continued to the same pressure target using 0.9% saline in all groups. In practice few animals received saline in the in-hospital phase.

7.15 Study End

At the end of the study, after 210 minutes of resuscitation (R210), the animals were killed humanely with an overdose of anaesthetic; Phenobarbitone (Euthatal, Merial Animal Health Ltd), administered intravenously.

7.15.1 Post-Mortem Examination

An immediate post mortem examination was performed after animal death, confirmed by cessation of circulation and breathing. The thoracic cavity was opened and the lungs and heart removed. The lungs were examined for blast injury (contusion; Figure 7-7). Froth or clots in the trachea were noted. The heart was inspected for contusions.

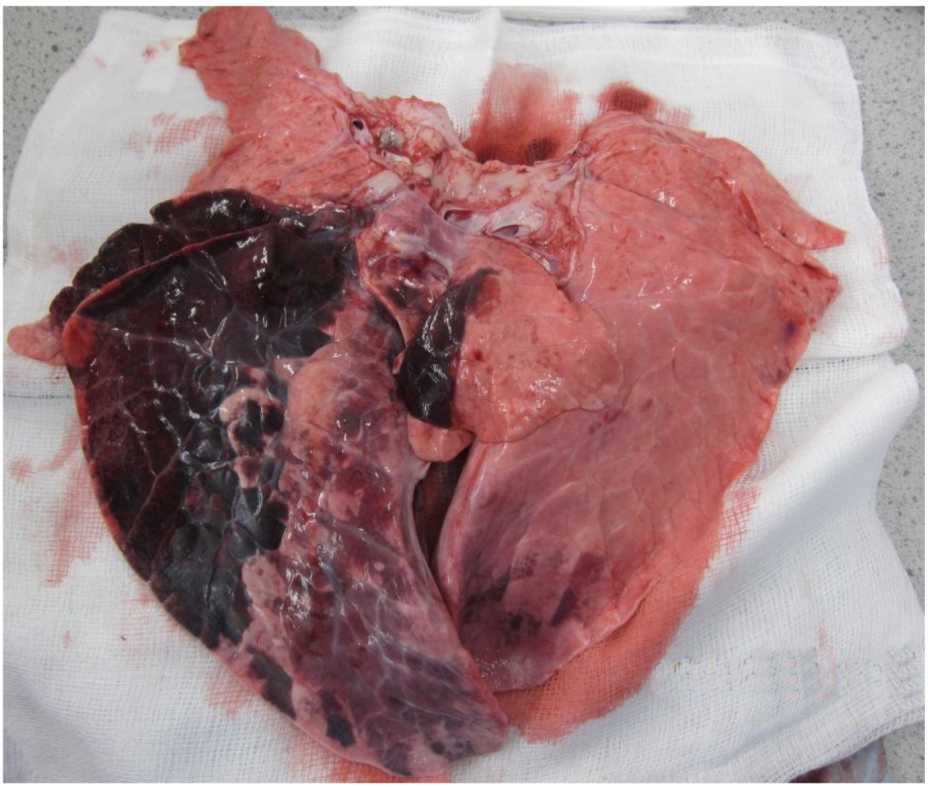


Fig 7-7. Post-mortem blast injury to right lung (side facing blast), with no injury to left.

7.16 Statistics

Statistical analysis was carried out using a NCSS V11 statistical analysis package, and was conducted on data collected at pre-determined intervals, pre-treatment and post-treatment.

A two-way Mixed Model repeated measures ANOVA using Treatment and Time as Main Factors was conducted on data recorded from critical measurement variables. Identified baseline values for each treatment condition was entered into the model as a covariate term such that treatment effects could be observed as a function of the mean covariate baseline estimate. For the period from initial baseline to the end of the shock phase the first baseline (B1, Figure 7.1) was used. Post-treatment analysis utilised the last time point (end of shock phase, S30 Figure 7.1), immediately before the commencement of resuscitation as the covariate baseline.

The distribution of variables was examined graphically and tested for Normality using the Anderson-Darling test procedure before being entered into the analysis model. Data shown to be non-normal were transformed where appropriate. Significant Treatment effects at critical time points indicated by the analysis model were tested using pre-planned contrast of Treatment means as follows:-

- Group 1 v Group 2 in the Blast Condition (injury strand)

- Group 3 v Group 4 in the Sham Blast Condition (injury strand)

These contrasts were achieved using by a Fisher LSD t-test procedure at an alpha level of rejection of $p \leq 0.05$ on the condition that the overall F value for the Main Treatment Effect of the ANOVA was $p < 0.1$.

Measurement variables shown to be non-normal and resistant to transform were analysed at critical time points using a non-parametric one-way

Kruskall Wallis ANOVA followed by a Dunn's post hoc comparison of contrast. Treatment medians were significant at an alpha level of rejection of $p \leq 0.05$

Single time point comparisons between two treatment groups were made with an independent t-test (parametric data) or Mann Whitney U test (non parametric data). Statistical significance was taken as $p \leq 0.05$.

Chapter 8. Results

8.1 Introduction

This chapter is divided into two separate sub-chapters in order to describe the results of blast and sham blast injuries independently. Each sub-chapter is divided into 4 separate time periods corresponding to those illustrated in Figure 8-1:

- *Baseline*
- *Injury Period* (consisting of Wounding and Shock Phases) ending at S30.
- *Primary Treatment Period* (consisting of the 60 minutes of Pre-Hospital Phase and the 30 minutes of Early In-Hospital Phase) ending at R90 (primary outcome point).
- *Final Treatment Period* (consisting of the Late In-Hospital Phase) ending at R210.

8.2 Baseline Results – Sham Blast Groups

All animals that survived to S30 (end of Injury Period) survived to the end of the investigation. Baseline data is shown in Table 8-1. There were no significant differences between groups in the majority of the parameters. Group 4 had a slightly prolonged TEG R time compared to Group 3 with the groups being within 15% of each other. Mean arterial pressure (MAP) was within 10% of each other with no significant difference between the groups in systolic blood pressure (SBP). Consequently blood pressure and mixed venous oxygen content (CvO_2) differences were not physiologically or clinically significant.

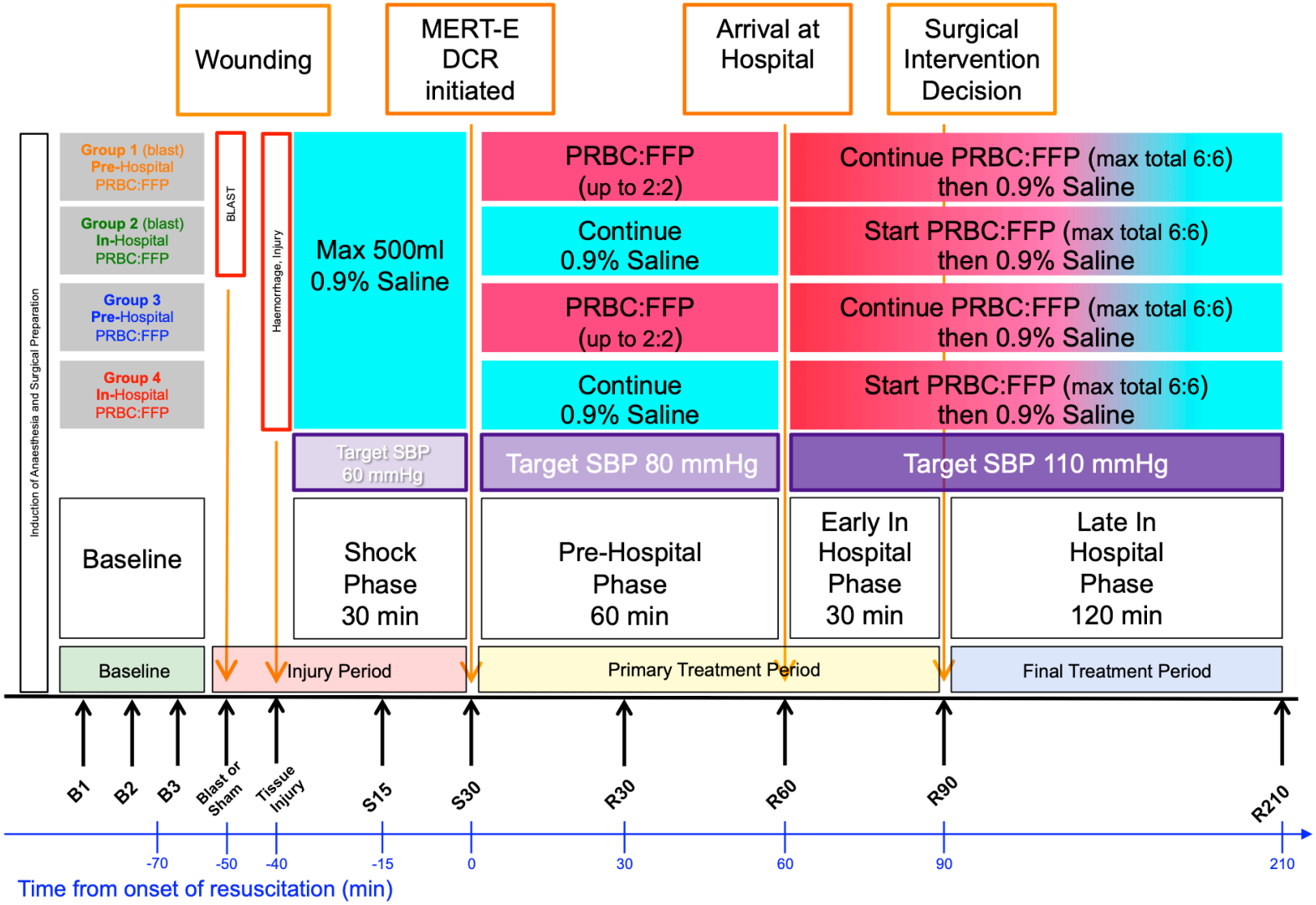


Figure 8-1. Experiment protocol illustrating timeline and division of time periods: Baseline, Injury, Primary Treatment and Final Treatment Periods.

			Baseline		Statistical *P*	S30/R0 (End of Injury Period)		Statistical *P*	Effect of injury and haemorrhagic shock Statistical *P* between baseline and S30/R0		
			Pre-Hosp PRBC:FFP	In-Hosp PRBC:FFP	Between groups at Baseline	Pre-Hosp PRBC:FFP	In-Hosp PRBC:FFP	Between groups at S30/R0	Changes over time	Differences between groups	Differences in pattern over time between groups
			Group 3	Group 4		Group 3	Group 4				
Demographics	No of animals		9	9	1.000						
	Body temp °C		38.3±0.1	38.3±0.1	0.056 *						
	Body weight Kg		52.0±1.2	52.3±1.1	0.862						
Coagulation	TEG	R (sec)	12.1±0.5	14.2±0.7	0.016 *	14.6±2.9	18.6±2.7	0.289	0.034	0.842	0.93
		K (sec)	5.2±0.2	5.7±0.2	0.102	4.7±0.6	5.8±1.5	0.093 *	0.028	0.360	0.451
		MA (mm)	66.1±1.5	66.9±1.7	1.000 *	67.2±1.9	67.6±2.8	0.818	0.118	0.185	0.805
	PT (min)		12.6±0.2	12.8±0.1	0.453	12.7±0.2	12.8±0.2	0.706	< 0.001	0.030	0.400
	aPTT (min)		12.5±0.3	13.8±0.6	0.085	13.5±1.0	13.6±0.7	0.386 *	0.152	0.532	0.254
	Fibrinogen (g/dl)		2.3±0.1	2.6±0.1	0.124	2.0±0.1	2.1±0.1	0.387	< 0.001	0.204	0.184
Cardiovascular	Systolic BP(mmHg)		154±7	168±3	0.078	69±4	74±4	0.378	< 0.001	0.139	0.700
	MAP (mmHg)		131±4	145±2	0.017	47±4	41±3	0.416	< 0.001	0.101	0.622
	Heart Rate (bpm)		149±13	152±9	0.848	239±7	253±6	0.145	< 0.001	0.516	0.665
	Cardiac Output (l/min)		7.98±0.43	7.56±0.24	0.416	2.69±0.21	2.42±0.22	0.479 *	< 0.001	0.253	0.272
Physiology	PaO2 (kPa)		9.4±0.2	9.1±0.4	0.373	11.7±0.4	12.8±0.2	0.036	< 0.001	0.105	0.144
	PaCO2 (kPa)		6.5±0.2	6.5±0.1	0.839	5.1±0.3	4.5±0.2	0.107	< 0.001	0.170	0.187
	Arterial pH		7.43±0.01	7.45±0.01	0.211	7.37±0.02	7.40±0.01	0.359	< 0.001	0.755	0.990
	ABE (mM)		7.2±0.4	8.5±0.7	0.139	-2.18±1.3	-3.3±0.8	0.269 *	< 0.001	0.070	0.214
	Lactate (mM)		1.0±0.2	1.0±0.2	0.722 *	6.4±0.8	7.3±0.4	0.356	< 0.001	0.312	0.668
	CvO2 (ml/dl)		11.9±0.2	10.6±0.3	0.009 *	3.7±0.4	3.5±0.3	0.569	< 0.001	0.921	0.224
	OER		0.28±0.02	0.29±0.01	0.817 *	0.78±0.04	0.76±0.02	0.949 *	< 0.001	0.674	0.999
Haematology	Hb (g/dl)		13.2±0.1	13.0±0.1	0.085	11.4±0.2	11.4±0.2	0.926	< 0.001	0.130	0.348
	Hct		0.40±0.01	0.39±0.004	0.098	0.35±0.01	0.34±0.01	0.750	< 0.001	0.194	0.230
	Platelet count (10/l)		387±43	373±21	0.777	354±31	346±32	0.857	< 0.001	0.061	0.616
Biochemistry	K (mM/l)		3.8±0.04	3.8±0.04	0.315	5.1±0.4	5.2±0.2	0.826	< 0.001	0.777	0.936
	Ca (mM/l)		1.3±0.01	1.3±0.01	0.424 *	1.2±0.01	1.2±0.02	0.446	< 0.001	0.050	0.038

Table 8-1. Initial animal data: Baseline and S30/R0 for SHAM blast injury groups. * Comparison performed using Mann-Whitney U test. Values are mean ±SEM.

8.3 Injury Period – Sham Blast Groups

The effect of blunt injury and haemorrhagic shock – Injury Period – ending at S30, is illustrated in Table 8-1.

8.3.1 Coagulation – Injury Period (Sham Blast Groups)

Injury and haemorrhagic shock led to significant temporal changes in R (*P* = 0.034) and K times (*P* = 0.028) with a fall in both immediately after injury. This was followed by a rise by the end of the 30 minutes shock phase. There was no significant difference between groups (see Table 8-1 for *P* values and Figure 8-2). PT and aPTT did not show an early fall immediately after injury but did rise during the shock phase. These changes over time and between groups were significant for PT, but not aPTT. However, by the end of the shock period it is important to note that there were no significant differences between groups in either PT or aPTT. By contrast, MA showed no significant change over time or between groups (see Figure 8-3 and Table 8-1). There was a small significant drop in fibrinogen observed in both groups, with no significant difference between groups (Table 8-1).

Both groups therefore displayed a period of increased coagulation, assessed by TEG, immediately after tissue injury. This increased clotting was not reflected in conventional laboratory measurements (PT, aPTT) and appeared to resolve spontaneously prior to any resuscitation attempts. Notwithstanding the temporary reductions in K and R time, the results illustrate that both groups' coagulation was similar at the end of the injury phase.

8.3.2 Physiology – Injury Period (Sham Blast Groups)

Blunt injury and haemorrhage produced a significant challenge to the cardiovascular system in both groups. There was a marked and statistically

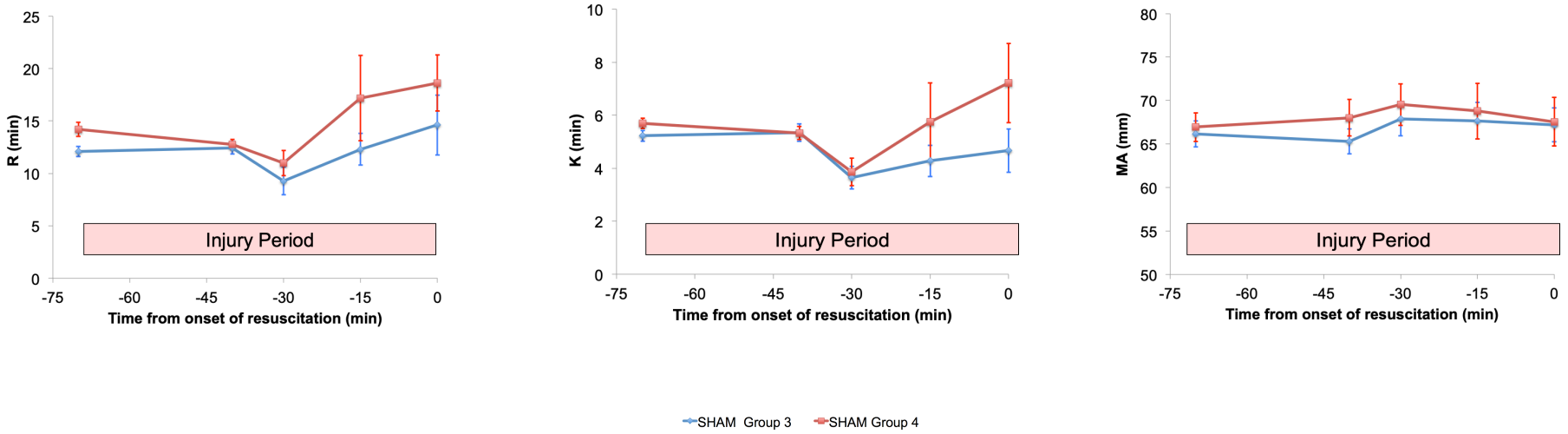


Figure 8-2. Effects of blunt injury and haemorrhage on TEG R time (clot initiation), K time (clot dynamics) and MA (clot strength) in the SHAM blast injury groups, prior to resuscitation. Time 0 corresponds to S30. Group 3 - planned pre-hospital blood component resuscitation. Group 4 - planned pre-hospital saline. Mean values ± SEM.

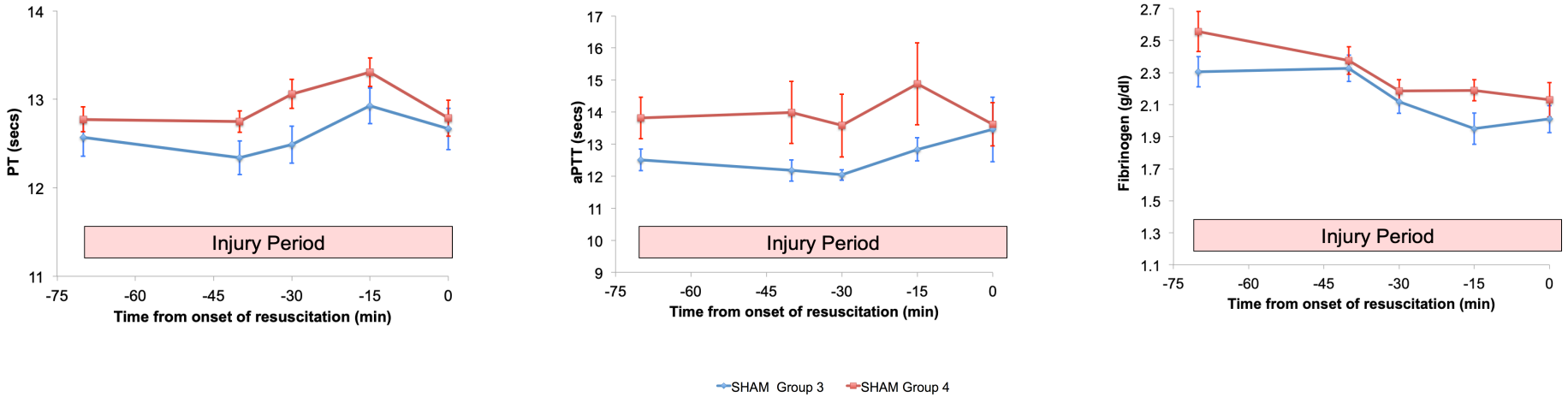


Figure 8-3. Effects of blunt injury and haemorrhage on PT and aPTT in the SHAM blast injury groups, prior to resuscitation. Time 0 corresponds to S30. Group 3 - planned pre-hospital blood component resuscitation. Group 4 - planned pre-hospital saline. Mean values ± SEM

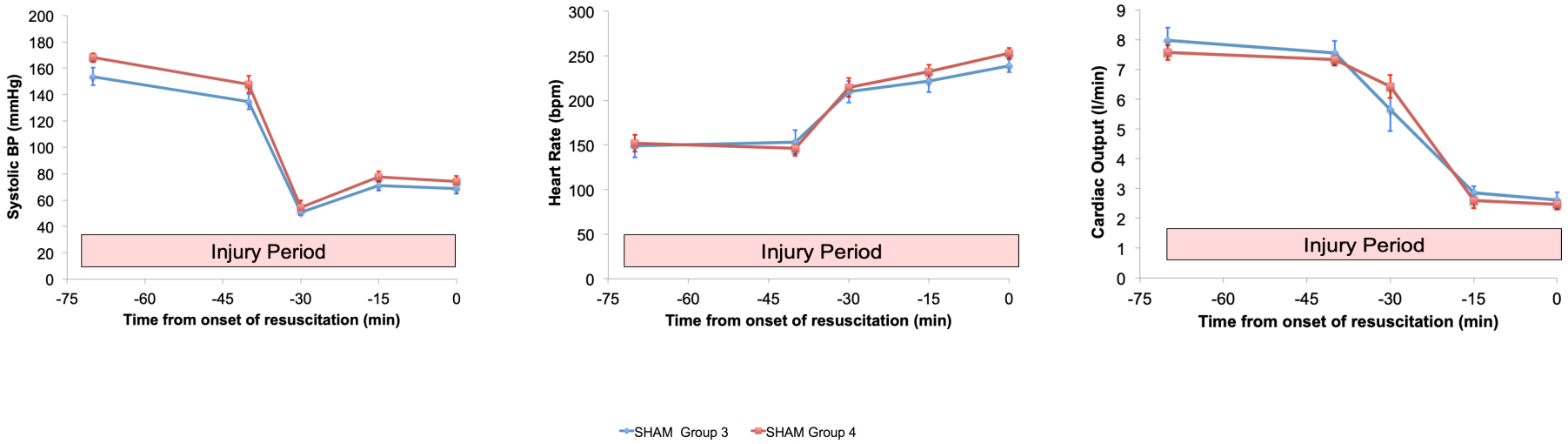


Figure 8-4. Effects of tissue injury and haemorrhagic shock on Systolic Blood Pressure, Heart Rate and Cardiac Output in the SHAM blast injury groups prior to resuscitation. Group 3 - planned pre-hospital blood component resuscitation. Group 4 - planned pre-hospital saline. Mean values ± SEM.

significant drop in systolic blood pressure (SBP) and cardiac output (CO) and a significant increase in heart rate ([HR]; $P < 0.001$; for all parameters changing over time; Table 8-1). There was no significant difference in the cardiovascular response to haemorrhage between groups (see Table 8-1 for P values and Figure 8-4).

The degree of shock was evaluated by measuring changes in arterial base excess and lactate. Both groups showed a marked clinical and statistically significant fall in arterial base excess and a rise in lactate (difference over time $P < 0.001$) with no difference between groups ($P > 0.268$ for all parameters; see Table 8-1 and Figure 8-5).

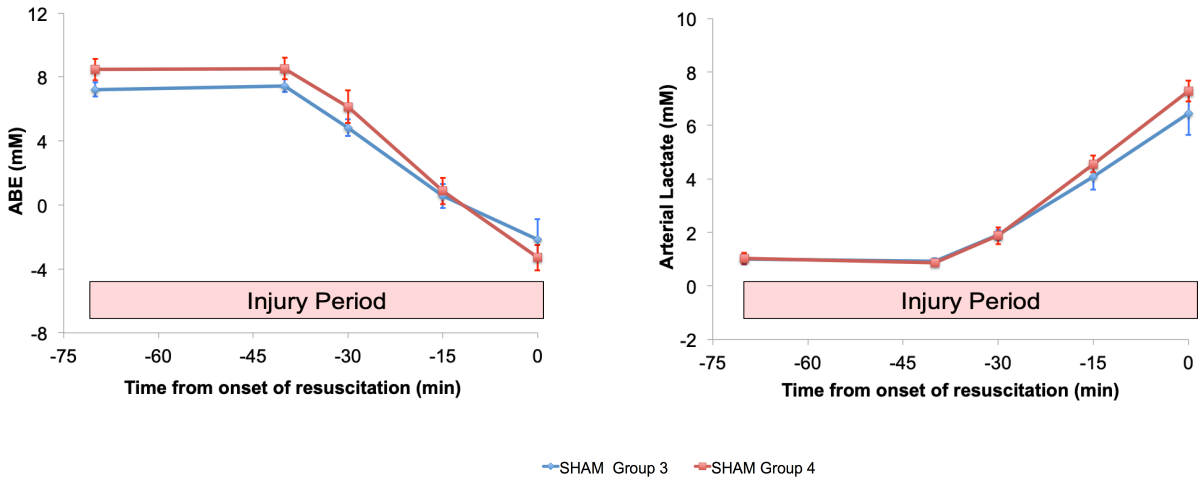


Figure 8-5. Effects of tissue injury and haemorrhagic shock on arterial base excess (ABE) and arterial lactate in the SHAM blast injury groups prior to resuscitation. Group 3 - planned pre-hospital blood component resuscitation. Group 4 - planned pre-hospital saline. Mean values ± SEM.

All animals exhibited an increase in respiratory effort (respiratory rate and tidal volume, noted by simple observation) in response to injury, which resulted in a significant increase in arterial oxygen partial pressure (PaO_2) and a significant decrease in arterial carbon dioxide partial pressure ($PaCO_2$; $P < 0.001$ for change over time of both parameters). There was no

difference between groups (P = 0.105), bar the single time point difference in PaO_2 at S30/R0, which is probably of little physiological incidence (11.7 compared to 12.8 kPa; see Table 8-1).

Mixed venous oxygen content (CvO_2) dropped significantly and the oxygen extraction ratio (OER) increased markedly for both groups (difference over time $P < 0.001$) with no difference between groups at S30 ($P > 0.569$ for both parameters; see Table 8-1 and Figure 8-6).

By the end of the Injury Period, the injury and haemorrhage had caused equal physiological insult to the animals in both groups. Both treatment groups displayed a cardiovascular response to haemorrhage (rise in HR and drop in BP) and the level of shock was reflected equally by a developing acidosis and a marked rise in the peripheral extraction of oxygen.

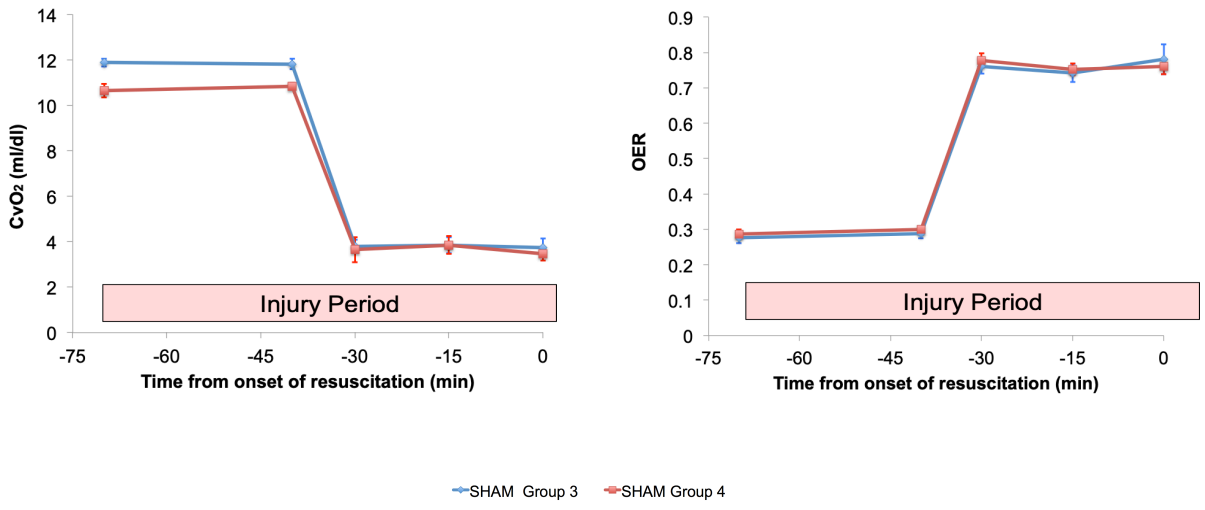


Figure 8-6. Effects of tissue injury and haemorrhagic shock on mixed venous oxygen content (CvO_2) and oxygen extraction ratio (OER) in the SHAM blast injury groups prior to resuscitation. Group 3 - planned pre-hospital blood component resuscitation. Group 4 - planned pre-hospital saline. Mean values ± SEM.

8.3.3 *Haematology – Injury Period (Sham Blast Groups)*

Haemoglobin and haematocrit levels both fell significantly during the injury and haemorrhage period (difference over time $P < 0.001$; Table 8-1 and Figure 8-7) with no difference between groups. A small significant drop in platelet count was also observed; there was no difference between groups (see Table 8-1 for *P* values).

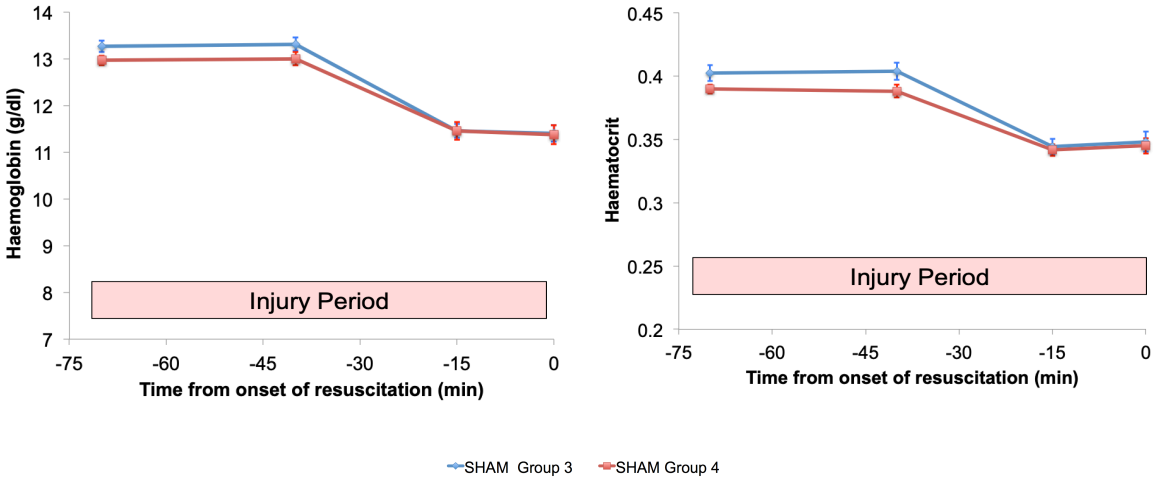


Figure 8-7. Effects of tissue injury and haemorrhagic shock on haemoglobin and haematocrit in the SHAM blast injury groups prior to resuscitation. Group 3 - planned pre-hospital blood component resuscitation. Group 4 - planned pre-hospital saline. Mean values ± SEM.

8.3.4 *Fluids – Injury Period (Sham Blast Groups)*

There was no significant difference between groups in the volume of fluid (0.9% saline) used in order to maintain a systolic blood pressure of 60mmHg in some animals during the Injury Period (Figure 8-8, $P = 0.344$).

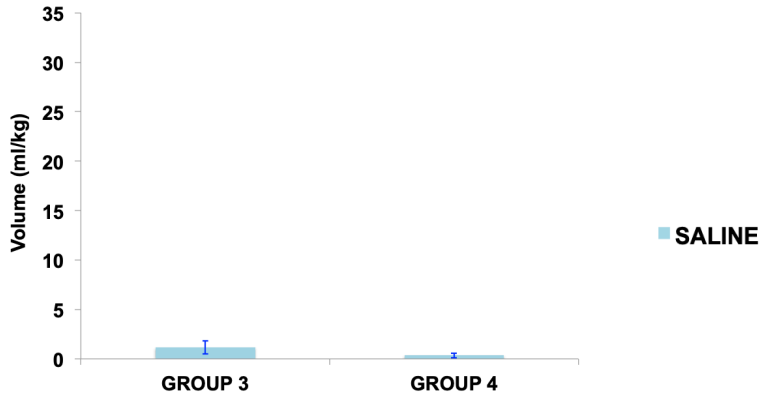


Figure 8-8. Total volume of fluids given to each group during the Injury Period for SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline. Mean values ± SEM.

8.3.5 Biochemistry – Injury Period (Sham Blast Groups)

There was a small, but significant decrease in calcium levels in both groups (difference over time $P < 0.001$) with no difference between groups. However, the decrease was not clinically significant (1.3 to 1.2; Table 8-1).

A significant rise in potassium levels was observed in both groups over the Injury Period (5.1 – 5.2 mM; Table 8-1 and Figure 8-9) with no difference between groups ($P = 0.826$).

Blunt injury and haemorrhage caused a small reduction in calcium levels and a clinically significant rise in potassium levels to reach a level at the upper end of normal.

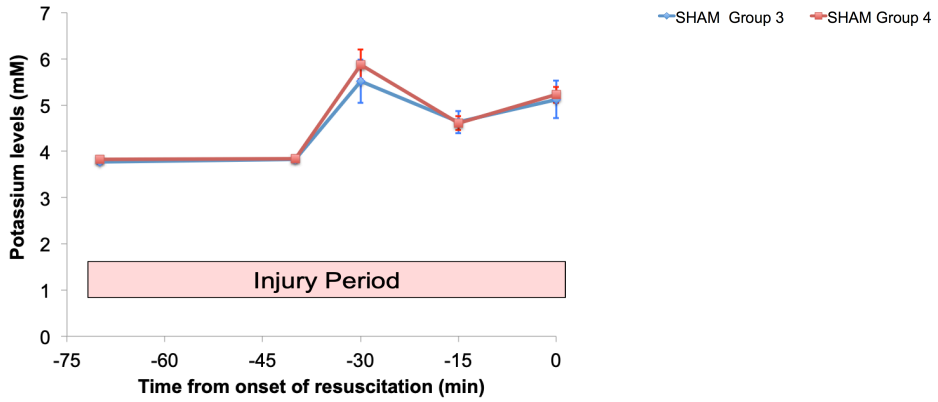


Figure 8-9. Effects of tissue injury and haemorrhage on potassium levels in the SHAM blast injury groups. Group 3 - planned pre-hospital blood component resuscitation. Group 4 - planned pre-hospital saline. Mean values ± SEM.

8.4 Primary Treatment Period – Sham Blast Groups

The effect of the two different pre-hospital treatment regimes on coagulation parameters is illustrated in Table 8-2. It contains data for R60 (end of 60 minutes pre-hospital phase) and R90 (additional 30 minutes in-hospital blood component resuscitation; the primary outcome time point and the end of the Primary Treatment Period). All remaining parameters are illustrated in Table 8-3.

8.4.1 Coagulation – Primary Treatment Period (Sham Blast Groups)

Group 3, having received pre-hospital blood components in the pre-hospital evacuation phase, had minimal changes in TEG values by the end of the pre-hospital phase (R60) and by the end of the Primary Treatment Period (R90) (Table 8-2 and Figure 8-10).

Group 4 however, having received only 0.9% saline in the pre-hospital phase, had a significantly elevated TEG R and K time compared to Group 3 at R60 (Table 8-2 and Figure 8-10). This difference persisted at R90, although K time was not significantly different between groups at this point. In contrast, TEG MA showed little change over time in either group and consequently there was no significant difference between them.

In common with the TEG R and K times, the saline-treatment Group 4 exhibited significant increases in both PT and aPTT over time, while Group 3 showed minimal changes in these parameters throughout this period. Consequently PT and aPTT were significantly higher in Group 4 when compared to Group 3 by R90 (Table 8-2 and Figure 8-11).

Fibrinogen levels for Group 4 (pre-hospital saline) at hospital admission (R60) and at R90 were significantly lower than Group 3 (P = 0.007 and 0.050 respectively; Figure 8-11) with a significant difference observed in pattern over time between groups (P < 0.001). The Fibrinogen levels at hospital admission (R60) in Group 4 dropped below 1.5 g/dl, which is markedly below the 95% reference range for this strain of pigs (1.77-3.36 g/l)[1]. Although this drop in fibrinogen can be explained to some extent by haemodilution (as measured by haematocrit), this is not the complete cause.

[1] Data based on samples taken after minimal surgery in Large White pigs of similar age that contributed to this and other studies in our laboratory (n=147). Data distribution was normalised with a 1/square root prior to determining the reference range.

			End of Pre-Hospital Phase (R60)		Statistical *P*	Effect of injury and haemorrhagic shock Statistical *P* between S30/R0 and R60			Primary Outcome Point (R90)		Statistical *P*
			Pre-Hosp PRBC:FFP	In-Hosp PRBC:FFP	Between groups at R60	Changes over time	Differences between groups	Differences in pattern over time between groups	Pre-Hosp PRBC:FFP	In-Hosp PRBC:FFP	Between groups at R90
			Group 3	Group 4					Group 3	Group 4	
Coagulation	TEG	R (sec)	17.5±2.9	40.1±9.5	0.012 *	0.011 °	0.036 °	0.097 °	17.8±2.3	41.7±11.0	0.044
		K (sec)	6.8±1.5	17.2±3.8	0.022	0.004 °	0.053 °	0.039 °	6.2±1.5	17.2±6.9	0.096
		MA (mm)	65.8±2.1	67.6±2.6	0.595	0.251	0.764	0.258	66.2±1.9	63.6±2.8	0.239 *
	PT (min)		13.2±0.3	15.2±0.4	0.001 *	< 0.001	< 0.001	0.009	13.8±0.4	15.0±0.3	0.021 *
	aPTT (min)		13.3±0.9	18.0±1.4	0.024 *	0.002	< 0.001	0.033	13.8±0.9	18.1±1.0	0.007
	Fibrinogen (g/dl)		1.85±0.09	1.43±0.10	0.007	< 0.001 °	0.258 °	< 0.001 °	1.78±0.11	1.53±0.08	0.05

Table 8-2. Animal data for coagulation parameters: R60 (end of Pre-Hospital Period) and R90 (primary outcome point) for SHAM blast injury groups.
* Comparison performed using Mann-Whitney U test. ° Comparison using logarithmic transformed data. Values are mean ±SEM.

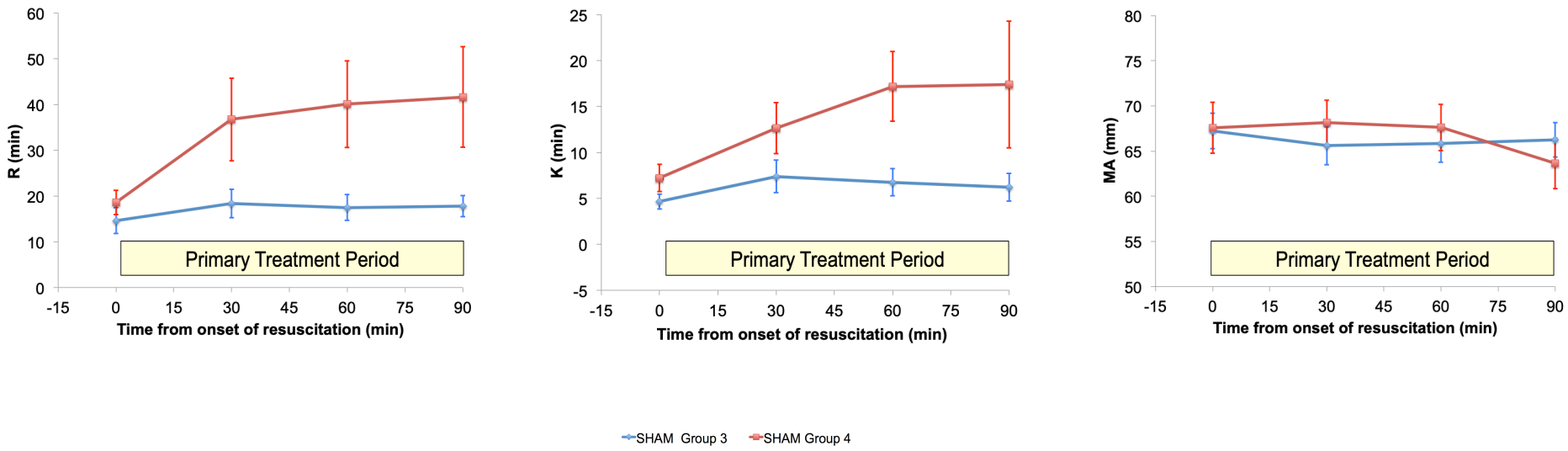


Figure 8-10. TEG R time (clot initiation), K time (clot dynamics) and MA (clot strength) in the SHAM blast injury groups, at end of 90 minutes of resuscitation (60 minutes pre-hospital and 30 minutes in-hospital). Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Mean values ± SEM.

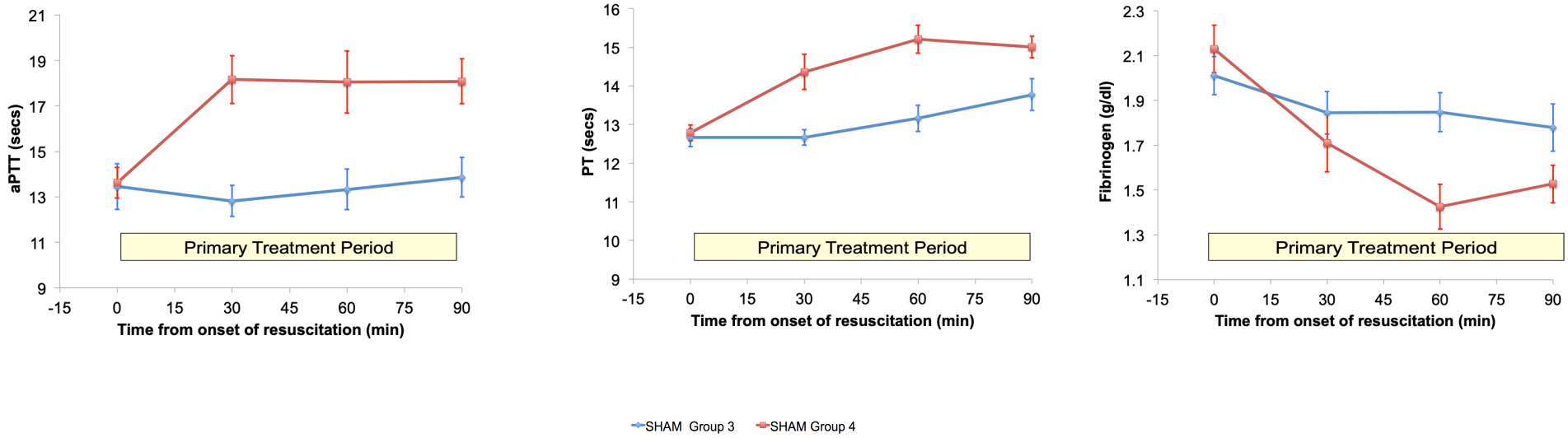


Figure 8-11. PT, aPTT and Fibrinogen in the SHAM blast injury groups during the Primary Treatment Period (90 minutes of resuscitation; 60 minutes pre-hospital and 30 minutes in-hospital).. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline. Mean values ± SEM.

The ratio of the proportional fall in fibrinogen levels from baseline, to the proportional fall in haematocrit from baseline, should be 1.0 if the reduction in fibrinogen levels is comparative to haemodilution. This ratio would be less than 1.0 if there is additional fibrinogen loss. The ratio at the end of the pre-hospital phase in Group 4 was 0.65 ± 0.03, suggesting there was loss of Fibrinogen over and above the effects of haemodilution.

These results illustrate that after combined blunt injury and haemorrhagic shock Group 4 (receiving pre-hospital saline) developed a change in coagulation parameters, suggestive of coagulopathy. By contrast the coagulation measured in the blood component treatment Group 3 changed little over the same period.

8.4.2 Physiology – Primary Treatment Period (Sham Blast Groups)

Systolic blood pressure (SBP) targets were maintained at the relevant level for each phase (80mmHg pre-hospital and 110 mmHg in-hospital) in both groups resulting in a significant elevation in SBP by R90 in both groups (Figure 8-12 and Table 8-3). In contrast to there being no difference between groups in SBP, mean arterial pressure in Group 3, having received pre-hospital blood components, was significantly higher than Group 4.

A corresponding rise in cardiac output (CO) was observed in both groups by R90. Group 4 (pre-hospital saline) is observed to have a lower CO throughout the pre-hospital phase, but once that group received in-hospital blood components CO was observed to increase faster than the other group and at R90 Group 4 CO is significantly higher than Group 3 (P = 0.0019). Both groups had a small non-significant drop in heart rate (HR) throughout the primary treatment period. Although there was no difference in HR at R90, it was noted that the pre-hospital saline group has a consistently lower HR than the pre-hospital blood component group throughout this period (Figure 8-12).

		Primary Outcome Point (R90)		Statistical *P*
		Pre-Hosp PRBC:FFP	In-Hosp PRBC:FFP	Between groups at R90
		Group 3	Group 4	
Cardiovascular	Systolic BP(mmHg)	117±3	111±2	0.477 *
	MAP (mmHg)	87±4	73±3	0.017
	Heart Rate (bpm)	222±12	200±8	0.124
	Cardiac Output (l/min)	5.58±0.42	7.27±0.47	0.019
Physiology	PaO2 (kPa)	17.0±1.0	17.8±0.7	0.496
	PaCO2 (kPa)	5.8±0.2	5.2±0.2	0.045
	Arterial pH	7.25±0.03	7.15±0.03	0.044
	ABE (mM)	-7.3±1.8	-13.7±1.3	0.012
	Lactate (mM)	11.5±1.4	14.0±0.7	0.104
	CvO2 (ml/dl)	8.3±0.5	7.8±0.2	0.340
	OER	0.50±0.08	0.37±0.03	0.309
Haematology	Hb (g/dl)	10.6±0.2	9.2±0.4	0.005 *
	Hct	0.33±0.01	0.28±0.01	0.001
	Platelet count (10/l)	244±19	188±18	0.056
Biochemistry	K (mM/l)	4.2±0.2	4.2±0.1	0.786
	Ca (mM/l)	1.08±0.03	1.04±0.03	0.267

Table 8-3. Animal data: R90 (primary outcome point) for SHAM blast injury groups.
* Comparison performed using Mann-Whitney U test. Values are mean ±SEM.

The resuscitation goals (based exclusively on systolic blood pressure) were achieved for both groups in the pre-hospital and early in-hospital phases, and were associated with a rise in cardiac output by R90 in both groups.

For both groups arterial base excess (ABE) continued to drop to its nadir and lactate rise to its peak at R60 - the end of the pre-hospital evacuation phase - with both then changing little during the remaining 30 minutes of the

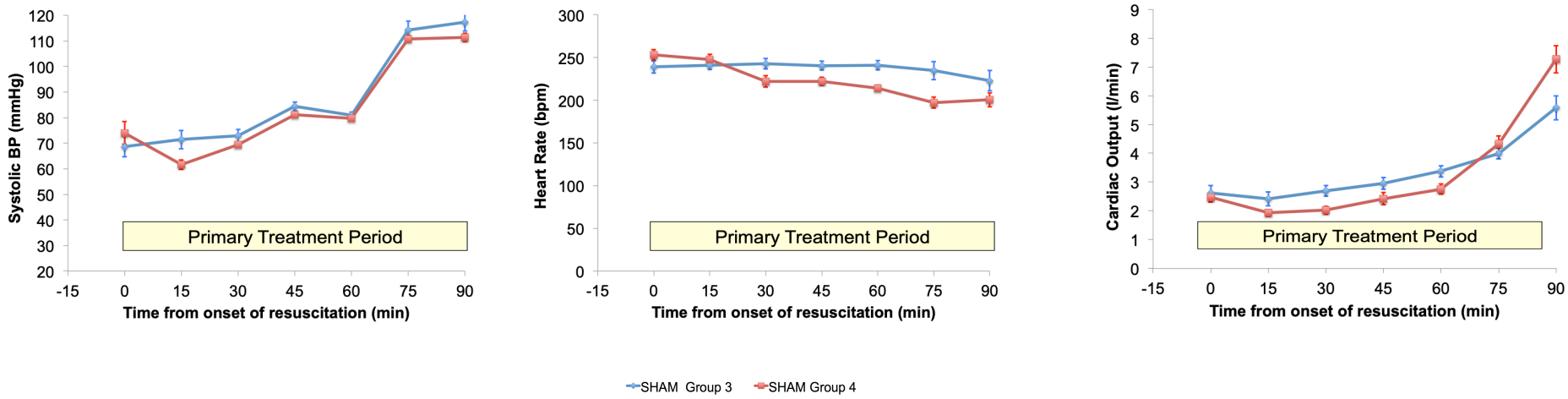


Figure 8-12. Systolic Blood Pressure (BP), Heart Rate and Cardiac Output in the SHAM blast injury groups during the Primary Treatment Period (90 minutes of resuscitation; 60 minutes pre-hospital and 30 minutes in-hospital). Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline. Mean values ± SEM.

Primary Treatment Period. By R90 both groups had a lower ABE and higher lactate than at S30 (Table 8-3 and Figure 8-13). There was a tendency for the pre-hospital blood component resuscitation group (Group 3) to have a lesser level of shock, so that at R90 ABE for Group 3 was significantly less negative than the saline group (4; $P = 0.012$), although the difference in lactate did not attain statistical significance ($P = 0.104$).

The saline-treatment Group 4 had a consistently significant lower $PaCO_2$ ($P = 0.045$ at R90) throughout the Primary Treatment Period, with a slightly higher PaO_2 that did not achieve significance at R90.

Both groups had a significant increase in mixed venous oxygen (CvO_2) and a significant drop in Oxygen Extraction Ratio (OER) by R60 so that at the end of the Primary Treatment Period (R90) both groups had a significantly higher CvO_2 and lower OER. There was no difference between groups (Table 8-3 and Figure 8-14).

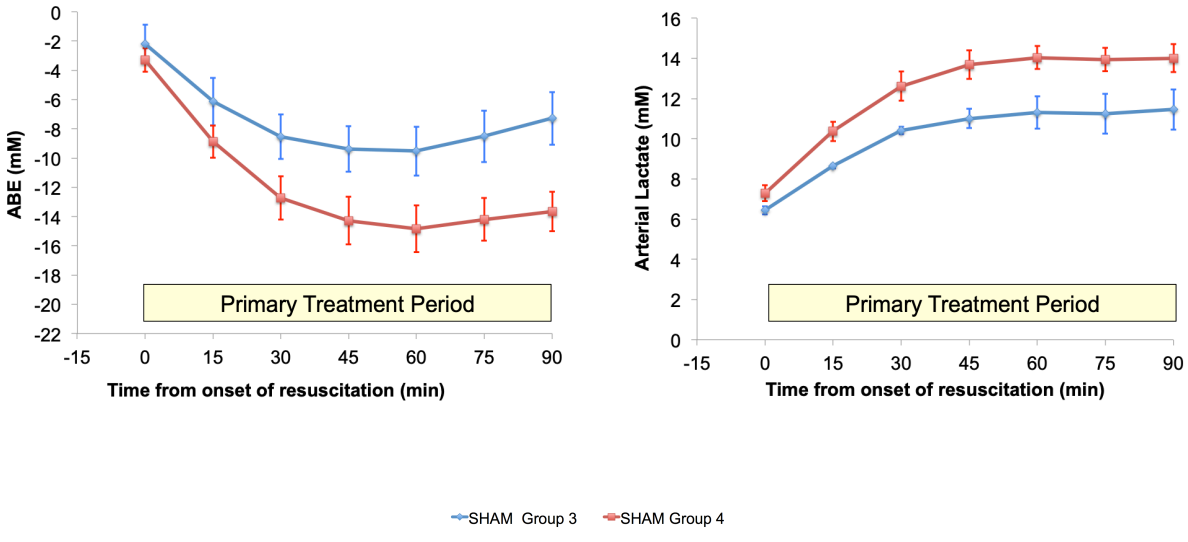


Figure 8-13. Arterial base excess (ABE) and arterial lactate in the SHAM blast injury groups during the Primary Treatment Period (90 minutes of resuscitation; 60 minutes pre-hospital and 30 minutes in-hospital). Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline. Mean values ± SEM.

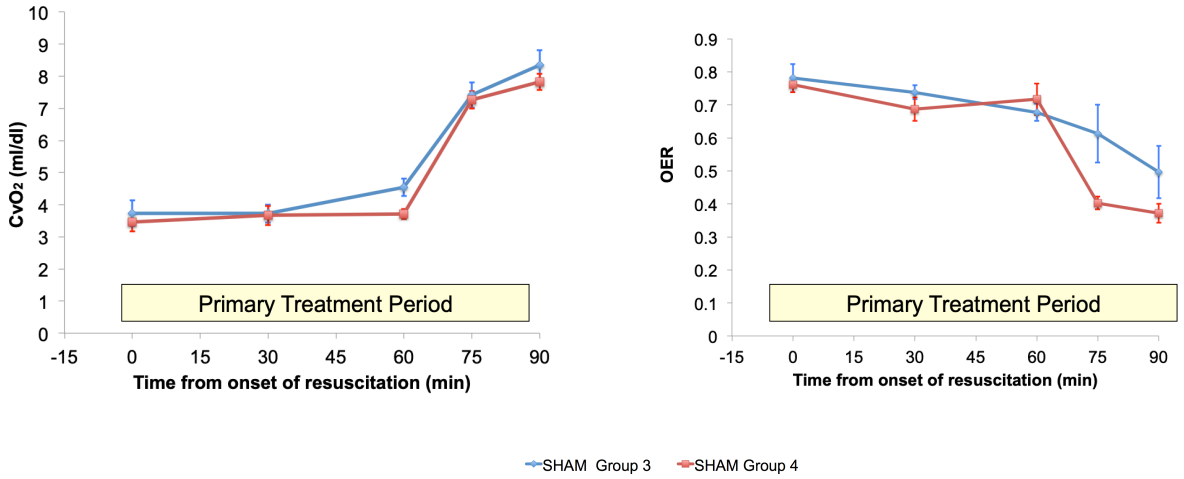


Figure 8-14. Mixed venous oxygen content (CvO2) and oxygen extraction ratio (OER) in the SHAM blast injury groups during the Primary Treatment Period (90 minutes of resuscitation; 60 minutes pre-hospital and 30 minutes in-hospital). Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline. Mean values ± SEM.

Pre-hospital and initial in-hospital resuscitation raised systolic blood pressure and cardiac output for both groups. Pre-hospital resuscitation in both groups stopped the drop in base excess and rise in lactate, and this effect continued into the initial in-hospital phase. Mixed venous content and oxygen extraction however, did not return toward baseline levels until the in-hospital phase. The pre-hospital saline group was noted to have a greater level of shock and lower $PaCO_2$.

8.4.3 Haematology – Primary Treatment Period (Sham Blast Groups)

Group 3, receiving pre-hospital blood components, had minimal change to haematocrit by R90 (Table 8-3 and Figure 8-15). Group 4, receiving pre-hospital saline, had a significant decrease in haematocrit by the end of the pre-hospital phase (R90) compared to Group 3 (P = 0.001).

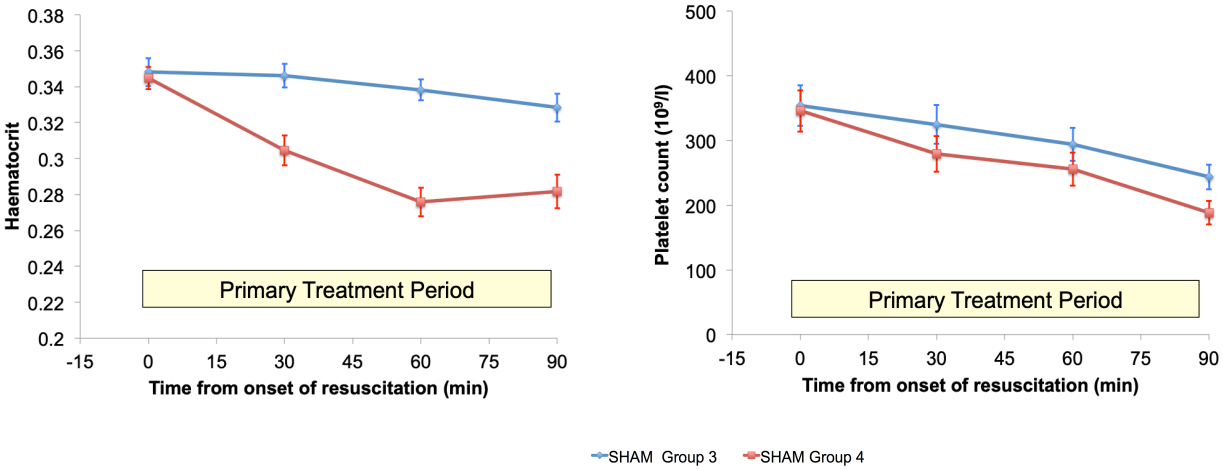


Figure 8-15. Haematocrit and platelet count (10^9/l) in the SHAM blast injury groups during the Primary Treatment Period (90 minutes of resuscitation; 60 minutes pre-hospital and 30 minutes in-hospital). Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline. Mean values ± SEM.

Platelet count dropped significantly for both groups by R90 with no difference between groups (*P* = 0.056; Table 8-3 and Figure 8-15).

At the end of the Primary Treatment Period, Group 3, receiving pre-hospital blood components had little change in haematocrit, in contrast to Group 4, which showed a significant drop. Platelet count fell in both groups.

8.4.4 Fluids – Primary Treatment Period (Sham Blast Groups)

The fluid used in each group for the different phases of the Primary Treatment Period is illustrated in Figure 8-16 and 8-17; sub-divided into the pre-hospital and (early) in-hospital phases. Compared to Group 3 (pre-hospital PRBC:FFP), the other Group (4; pre-hospital saline) required a greater volume of fluids in the pre-hospital phase (*P* = 0.002; Figure 8-16) and over the whole Primary Treatment Period up to R90 (*P* = 0.002; Figure 8-18).

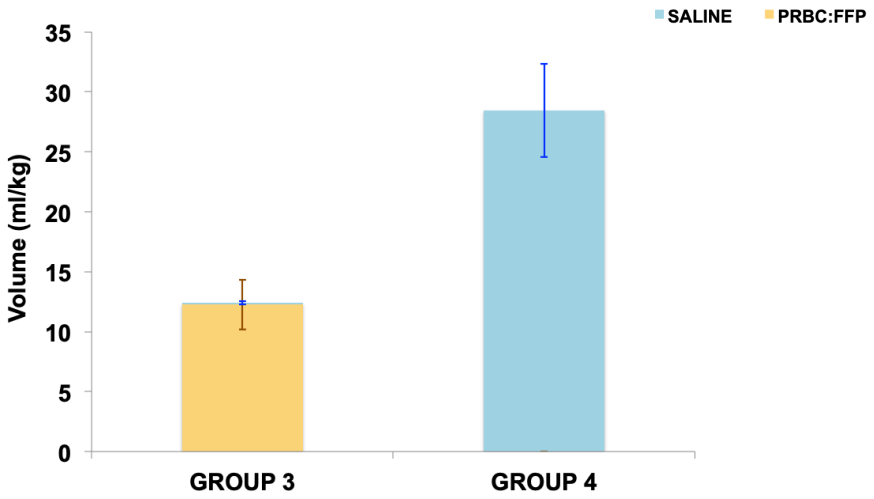


Figure 8-16. Total volume of fluids given to each group in the pre-hospital phase (between S30 and R60) for SHAM blast groups. Values are Mean ± SEM.

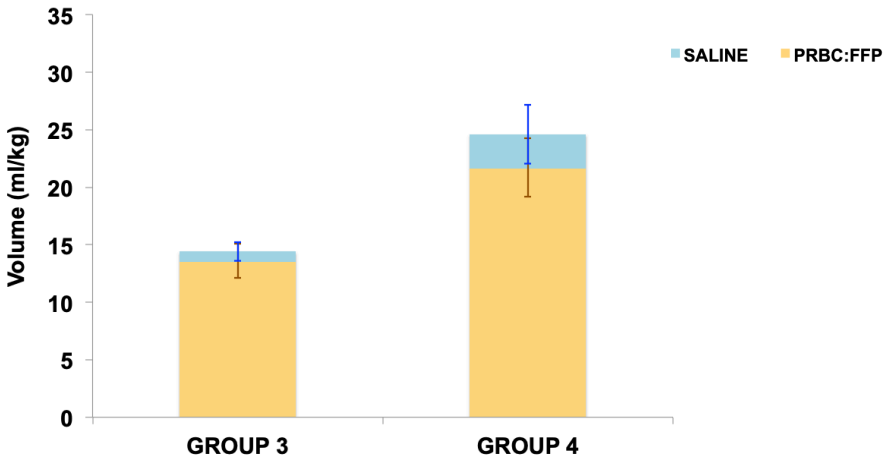


Figure 8-17. Total volume of fluids given to each group in the early in-hospital phase (between R60 and R90) for SHAM blast groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline. Values are Mean ± SEM.

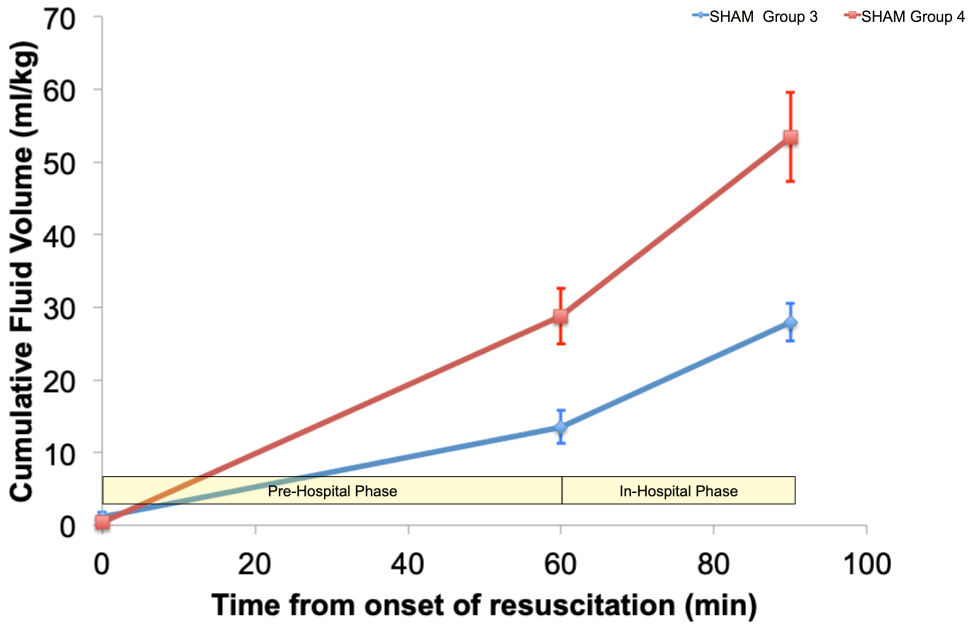


Figure 8-18. Cumulative volume of fluids given to each SHAM blast group in the Primary Treatment Period; pre-hospital and early in-hospital phases combined (S30/R0 = 0 min; R60 = 60 min; R90 = 90min). Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline Values are Mean ± SEM.

8.4.5 Biochemistry – Primary Treatment Period (Sham Blast Groups)

There was a reduction in calcium levels in both groups over time ($P < 0.001$) in the Primary Treatment Period with no difference between groups ($P = 0.267$; Table 8-3 and Figure 8-19).

Potassium levels however dropped from its previous high level in both groups by R60, with little change thereafter (Table 8-3). At R90 there was no difference in potassium levels (4.2 mM) between groups.

By R90, after the use of blood component resuscitation in both groups, calcium had dropped to the lower end of the normal range and potassium had dropped into the normal range in both groups.

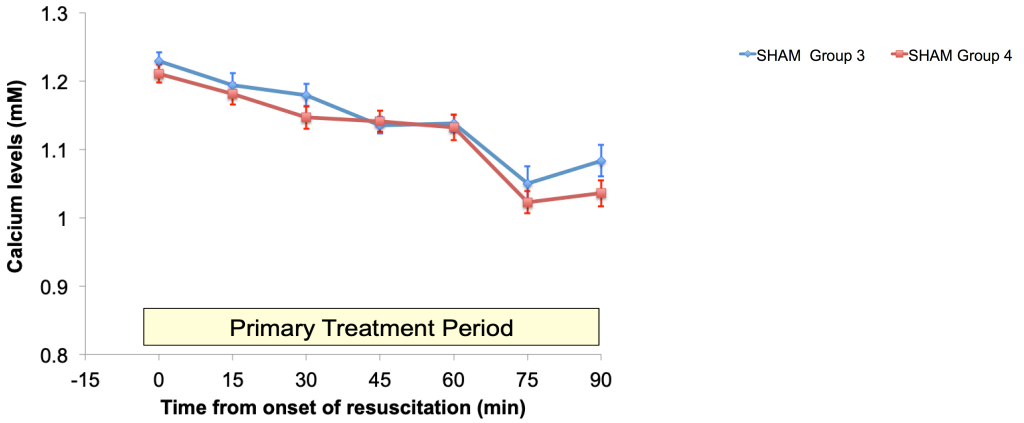


Figure 8-19. Calcium levels in the SHAM blast injury groups during the Primary Treatment Period (90 minutes of resuscitation; 60 minutes pre-hospital and 30 minutes in-hospital). Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline. Mean values ± SEM.

8.5 Final Treatment Period – Sham Blast Groups

The results for all parameters for the remaining (late) in-hospital phase is summarised in Table 8-4, which includes statistical analysis of the whole treatment period.

8.5.1 Coagulation – Final Treatment Period (Sham Blast Groups)

The abnormal TEG values (R and K time) for the pre-hospital saline-treatment Group 4 returned towards baseline levels so that by R210 there was little difference between groups (Table 8-4 and Figure 8-20). Both these parameters exhibited a significant difference over time (P = 0.001).

			End of In Hospital Phase (R210)		Effect of injury and haemorrhagic shock Statistical *P* between S30/R0 and R210		
			Pre-Hosp PRBC:FFP	In-Hosp PRBC:FFP	Changes over time	Differences between groups	Differences in pattern over time between groups
			Group 3	Group 4			
Coagulation	TEG	R (sec)	15.8±0.8	17.0±1.4	0.001 °	0.040 °	0.077 °
		K (sec)	5.5±0.4	6.6±1.3	0.001 °	0.064 °	0.014 °
		MA (mm)	66.0±2.0	64.8±1.6	0.544	0.213	0.511
	PT (min)		14.6±0.5	15.6±0.5	< 0.001	< 0.001	0.285
	aPTT (min)		12.6±0.4	15.1±0.8	< 0.001	< 0.001	0.025
	Fibrinogen (g/dl)		1.93±0.12	1.69±0.11	0.005 °	0.113 °	0.097 °
Cardiovascular	Systolic BP(mmHg)		117±3	117±2	< 0.001	0.099	0.096
	MAP (mmHg)		94±2	87±5	< 0.001	0.101	0.622
	Heart Rate (bpm)		198±15	208±14	< 0.001	< 0.001	0.005
	Cardiac Output (l/min)		5.88±0.60	5.80±0.37	< 0.001	0.203	0.038
Physiology	PaO2 (kPa)		18.4±1.5	17.8±1.0	< 0.001	0.006	0.765
	PaCO2 (kPa)		5.9±0.4	5.7±0.3	< 0.001	0.022	0.860
	Arterial pH		7.42±0.03	7.33±0.03	< 0.001	< 0.001	0.197
	ABE (mM)		3.7±1.2	-3.0±1.9	< 0.001	< 0.001	0.264
	Lactate (mM)		3.8±0.7	6.7±1.3	< 0.001	< 0.001	0.858
	CvO2 (ml/dl)		7.3±0.4	6.7±0.4	< 0.001	0.011	0.980
	OER		0.48±0.04	0.50±0.02	< 0.001	0.110	0.809
Haematology	Hb (g/dl)		10.4±0.3	9.8±0.3	< 0.001	< 0.001	< 0.001
	Hct		0.31±0.01	0.29±0.01	< 0.001	< 0.001	< 0.001
	Platelet count (10/l)		204±18	179±20	< 0.001	0.569	0.865
Biochemistry	K (mM/l)		4.4±0.2	4.8±0.3	< 0.001	< 0.001	0.131
	Ca (mM/l)		1.14±0.04	1.15±0.04	< 0.001	0.767	0.981

Table 8-4. Animal data; R210 (end of Late In-Hospital Phase and Final Treatment Period) for SHAM blast injury groups. ° Comparison using logarithmic transformed data. Values are mean ±SEM.

There is a significant difference between groups over the resuscitation period for R time (*P* = 0.040; Figure 8-22); which increased in the pre-hospital saline group during the pre-hospital phase and remained high into the in-hospital phase. K had a similar pattern in difference over the resuscitation period, which approached significance (*P* = 0.064; Figure 8-23).

In similarity with other periods MA width did not change in either group in the Final Treatment Period (Figure 8-20). The MA results at R210 were very similar to baseline levels and there was a significant difference in neither the MA result change over time (P =0.544), nor between groups (P = 0.213).

The aPTT times for Group 4 changed in a similar manner to the TEG K and R times with a trend towards returning to baseline levels by R210 (Table 8-4 and Figure 8-21). However in this case there was still a significant difference between groups (P < 0.001; Figure 8-24), as at R210 the pre-hospital saline group did not recover to the same level as the other group.

PT showed a different pattern of response over time in both groups (Figure 8-21 and 8-25). There was a significant rise in PT with the early (pre-hospital) elevation being significantly greater in the saline group resulting in a significant difference between groups (P = 0.001 at R60; Table 8-2). However, unlike the other parameters PT did not return towards baseline in either group during the in-hospital phase although the rate of rise did decline. The pre-hospital saline group (4) PT levels remained higher than Group 3 with a significant difference between the groups over the resuscitation period (P < 0.001). These elevated PT results for both groups were, however, only just outside the 95% reference range for this strain of pigs (12.6 to 12.9[1]). The final results equate to an INR of approximately 1.1.

Both groups showed a small elevation in fibrinogen levels back toward the normal range (Figure 8-21). Over the whole resuscitation period there was a significant difference in change over time (P = 0.005; Figure 8-26) with a marked drop in fibrinogen levels in the pre-hospital period for the saline resuscitation group. By R210 although the pre-hospital blood component group (3) had higher fibrinogen levels for the majority of the time, this difference did not reach significance (P = 0.113; Table 8-4 and Figure 8-26).

[1] The 95% confidence limit based on samples taken after minimal surgery in Large White pigs of similar age that contributed to this and other studies in our laboratory (n=149).

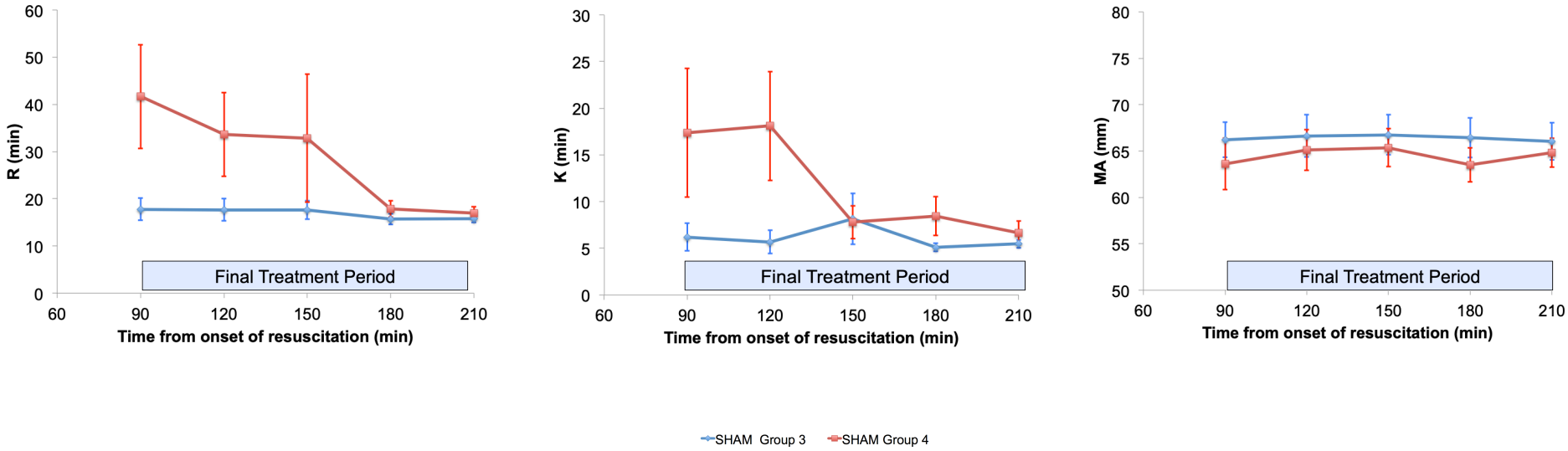


Figure 8-20. TEG R time (clot initiation), K time (clot dynamics) and MA (clot strength) in the SHAM blast injury groups for the Final Treatment Period. Group 3 – pre-hospital blood component resuscitation. Group 4 – pre-hospital saline resuscitation. Mean values ± SEM.

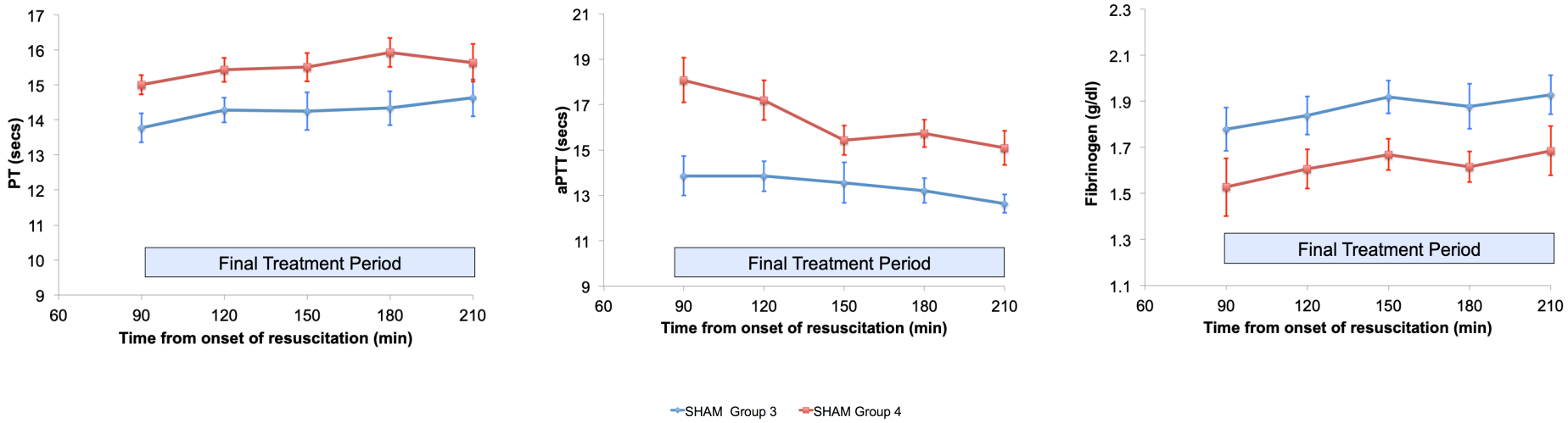


Figure 8-21. PT, aPTT and fibrinogen in the SHAM blast injury groups during the Final Treatment Period. Group 3 – pre-hospital blood component resuscitation. Group 4 – pre-hospital saline. Mean values ± SEM.

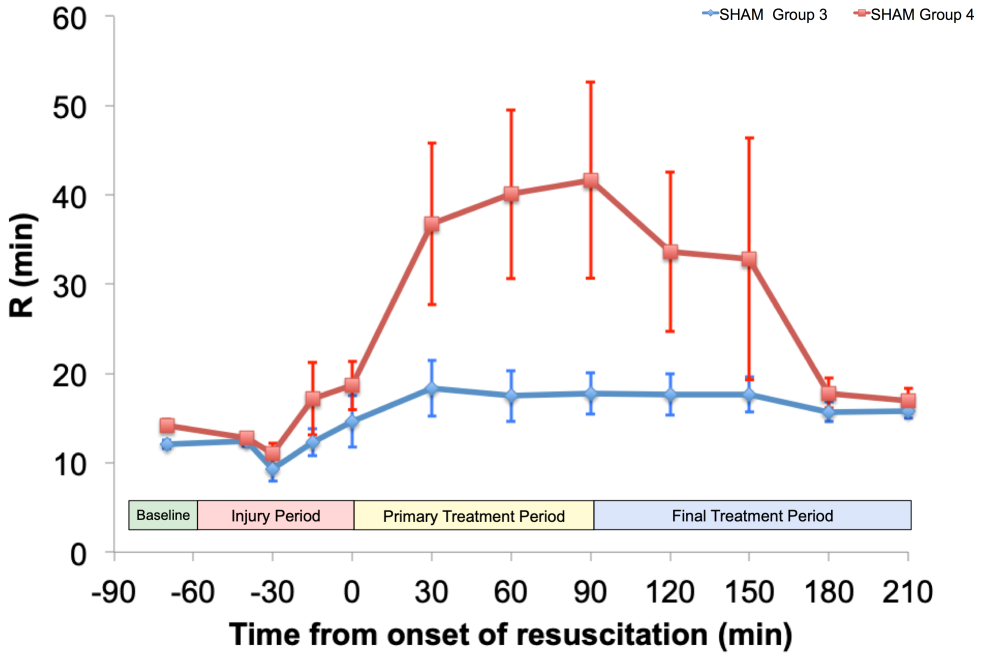


Figure 8-22. TEG R time (clot initiation) in the SHAM blast injury groups. Group 3 – pre-hospital blood component resuscitation. Group 4 – pre-hospital saline resuscitation. Mean values ± SEM.

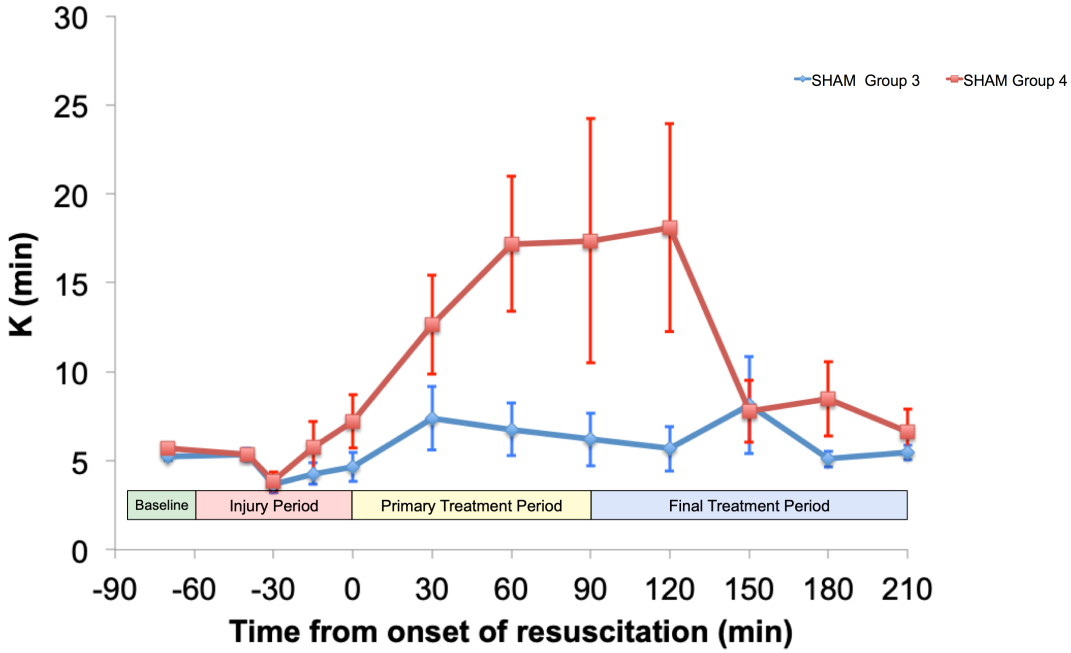


Figure 8-23. TEG K time (clot dynamics) in the SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Mean values ± SEM.

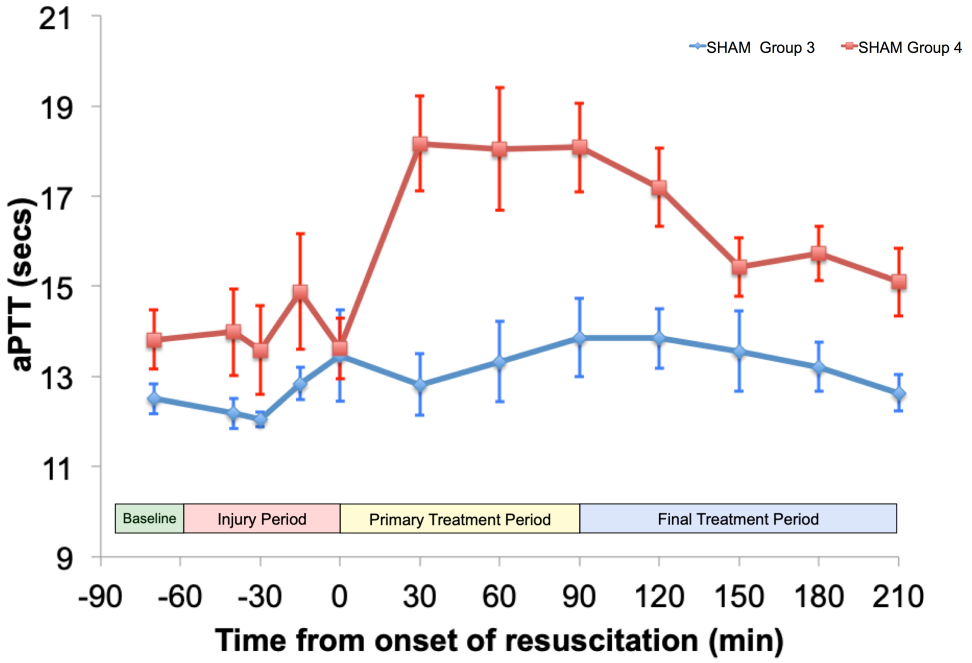


Figure 8-24. aPTT in the SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Mean values ± SEM.

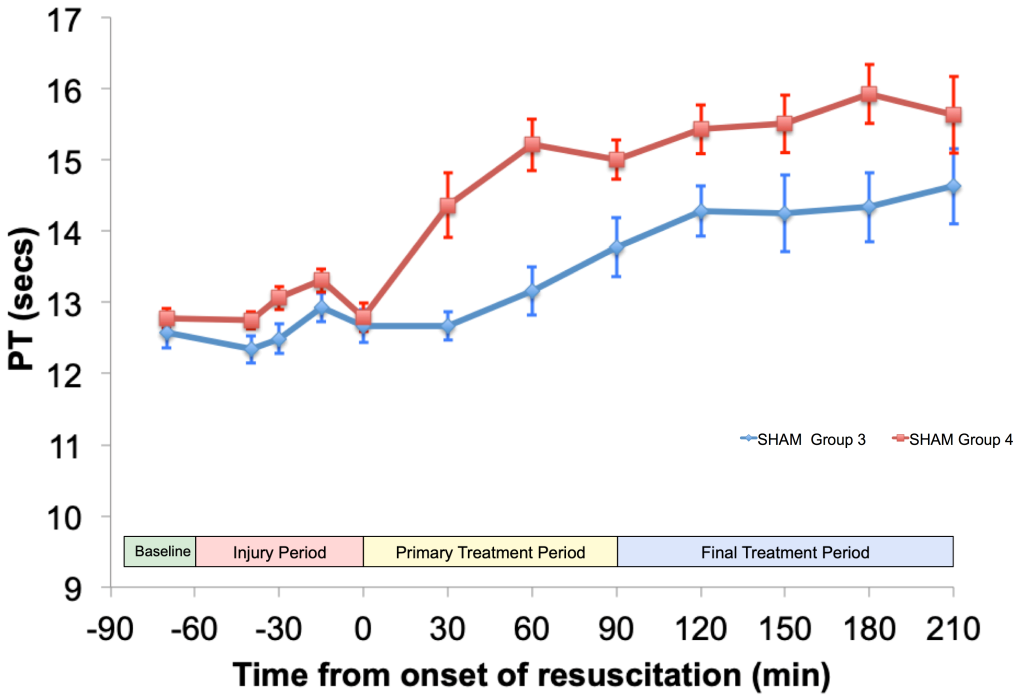


Figure 8-25. PT in the SHAM blast injury groups. Group 3 – pre-hospital blood component resuscitation. Group 4 – pre-hospital saline resuscitation. Mean values ± SEM.

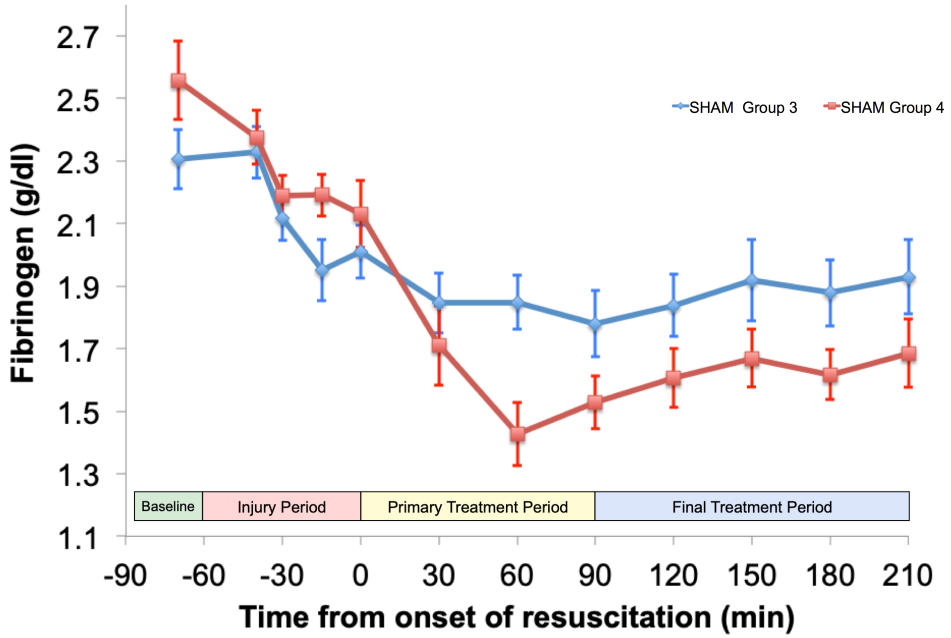


Figure 8-26. Fibrinogen in the SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Mean values ± SEM.

Changes in clotting, suggestive of a coagulopathy – as measure by TEG R and K times, aPTT and fibrinogen – was developed by Group 4 (pre-hospital saline) in the pre-hospital phase and lasted into the initial in-hospital phase, resolving after 60 to 90 minutes of in-hospital blood component resuscitation (Figure 8-22 to 8-26). These changes were significantly different from Group 3 and over time in the majority of these parameters. If no significance was obtained, there was a trend to change and significance was only not obtained secondary to the size of error bars. Clotting in Group 3, as measured by these parameters, remained stable throughout.

8.5.2 *Physiology – Final Treatment Period (Sham Blast Groups)*

Systolic blood pressure (SBP) targets were maintained for the final period of the investigation with no difference between groups ($P = 0.099$; Table 8-4 and Figure 8-27). Mean arterial pressure mirrored the SBP and, with cardiac output, the results for these parameters plateaued during this period, remaining at similar levels as those at R90 with no difference between groups ($P > 0.101$; Table 8-3 and Figure 8-28). There was a significant difference between groups in heart rate (HR) for the resuscitation period ($P < 0.001$; Figure 8-29) which, can be attributed to the lower HR observed for the majority of the Primary Treatment Period in the pre-hospital saline group.

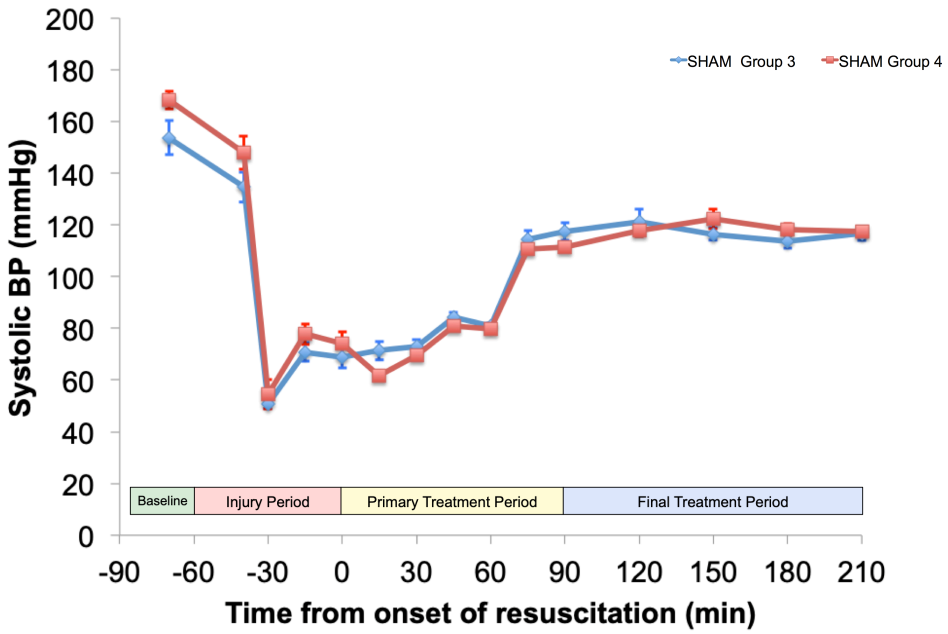


Figure 8-27. Systolic Blood Pressure in the SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Mean values ± SEM.

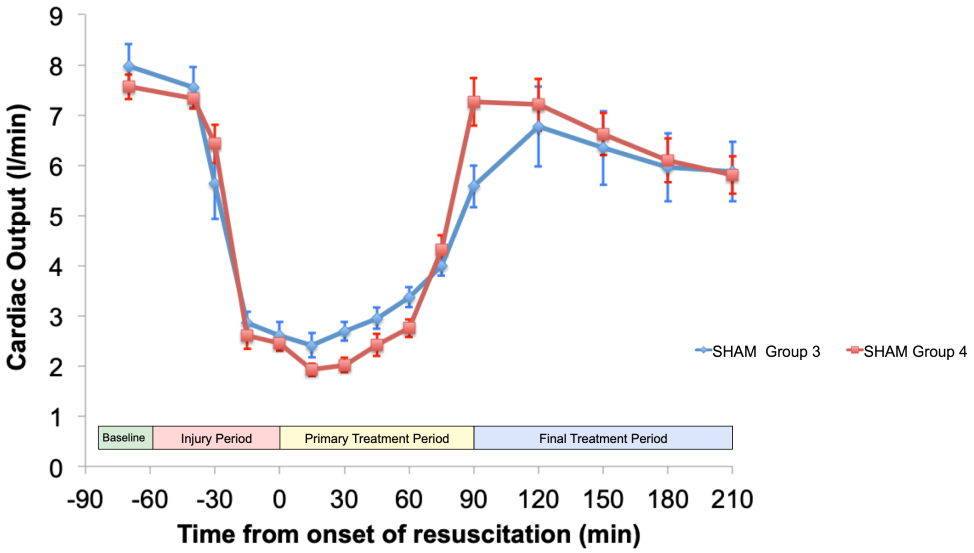


Figure 8-28. Cardiac Output in the SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Mean values ± SEM.

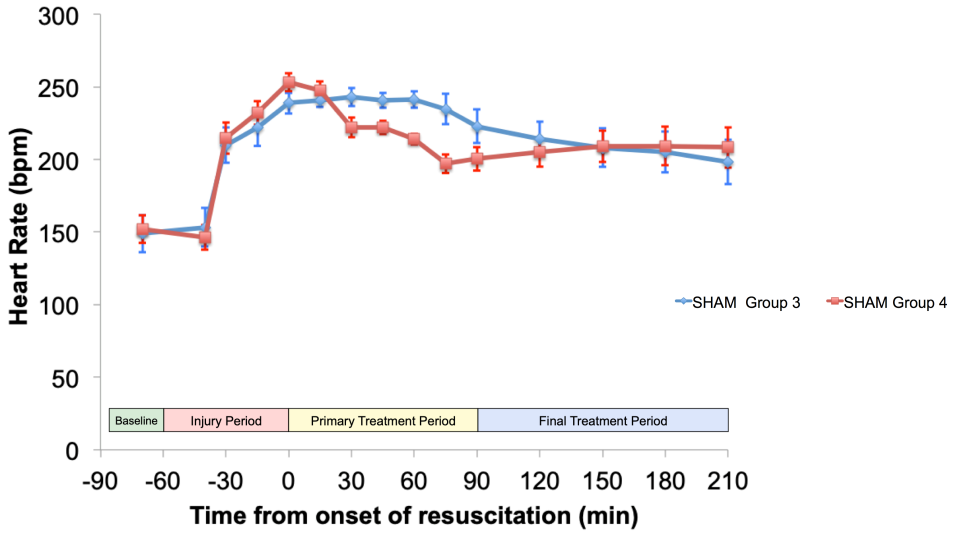


Figure 8-29. Heart Rate in the SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Mean values ± SEM.

Throughout the remaining in-hospital phase Group 3's (pre-hospital PRBC:FFP) trend for less shock endured (Figures 8-30 and 8-31). For the duration of the resuscitation period the pre-hospital saline group (4) had a significantly more negative ABE ($P < 0.001$) and a significantly lower lactate ($P < 0.001$) than the pre-hospital blood component group (Table 8-4).

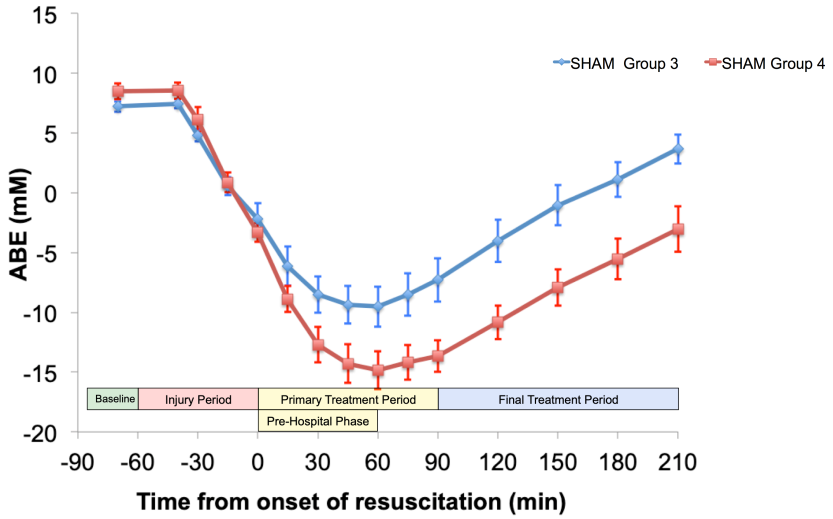


Figure 8-30. Arterial Base Excess in the SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Mean values ± SEM.

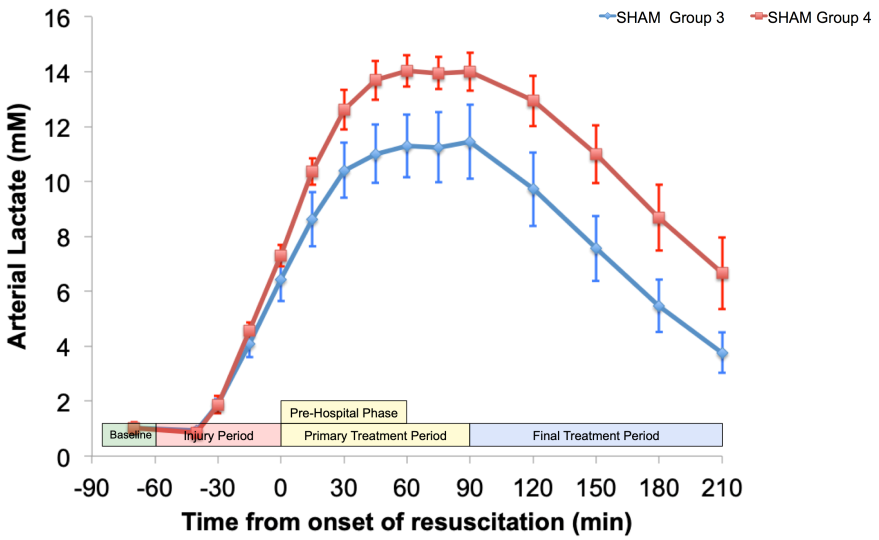


Figure 8-31. Arterial Lactate in the SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Mean values ± SEM.

At R210 the values of PaO_2 and $PaCO_2$ were similar between both groups, however over the whole resuscitation period there was a statistically significant difference between the groups in both parameters ($P < 0.023$) despite similar patterns over time ($P > 0.764$; Table 8-4). The majority of

this difference between groups occurred in the pre-hospital phase where Group 4 (pre-hospital saline) had a consistently higher PaO_2 and lower $PaCO_2$ (Figure 8-32). The difference in $PaCO_2$ persisted into the in-hospital phase and is probably caused by a degree of respiratory compensation in response to the comparatively greater metabolic acidosis in Group 4.

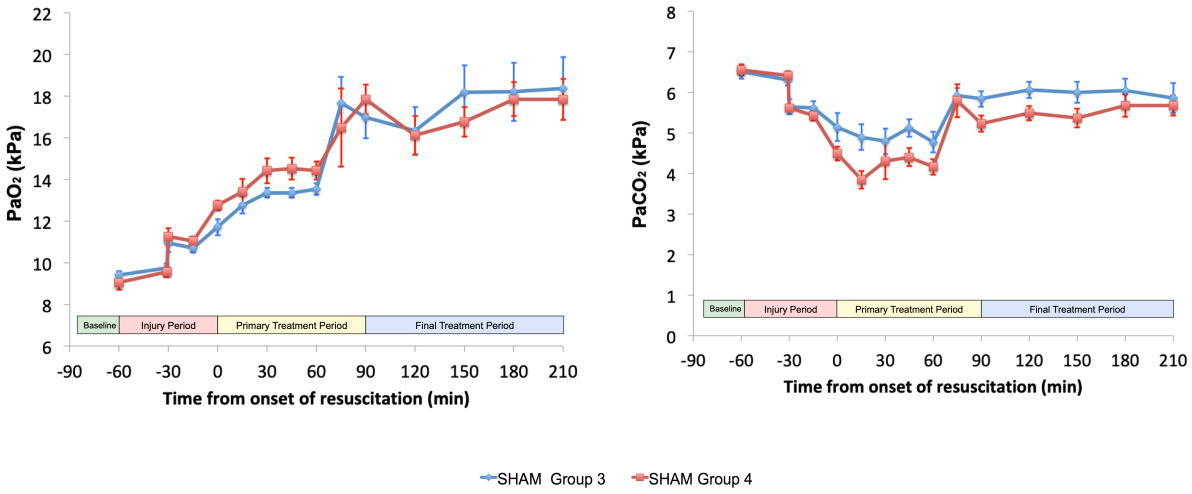


Figure 8-32. Partial pressure of arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$) for the SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Mean values ± SEM.

The OER and CvO_2 levels remained similar to that at R90 throughout the Final Treatment Period. Overall there was no difference between groups in OER (P = 0.110) with evidence of a difference in CvO_2 secondary to slightly higher levels in the pre-hospital group throughout the in-hospital phase (Table 8-4).

In-hospital blood component resuscitation to a normotensive goal, improved cardiac output and reduced the degree of shock. The group receiving pre-hospital saline displayed a greater degree of shock that although returned towards baseline levels remained greater than the other group throughout the remaining in-hospital period.

8.5.3 *Haematology – Final Treatment Period (Sham Blast Groups)*

During the remaining in-hospital phase with both groups receiving blood component resuscitation, there was little change in haemoglobin (Hb) and haematocrit (Hct) from R90. Both groups remained at a significantly lower level than baseline ($P < 0.001$, change over time) with a significant difference in pattern over time between groups ($P < 0.001$) secondary to the significant drop in both Hb and Hct in the pre-hospital phase for the saline resuscitated group (4). This difference remained throughout the in-hospital phase despite blood component resuscitation (Table 8-4 and Figure 8-33).

Platelet count remained lower than baseline in both groups (P < 0.001) with no difference between groups ($P = 0.569$; Table 8-4 and Figure 8-34).

Haemodilution cause a comparatively larger drop in the pre-hospital saline group for haemoglobin and haematocrit. Platelet count dropped steadily and similarly in both groups throughout the study period.

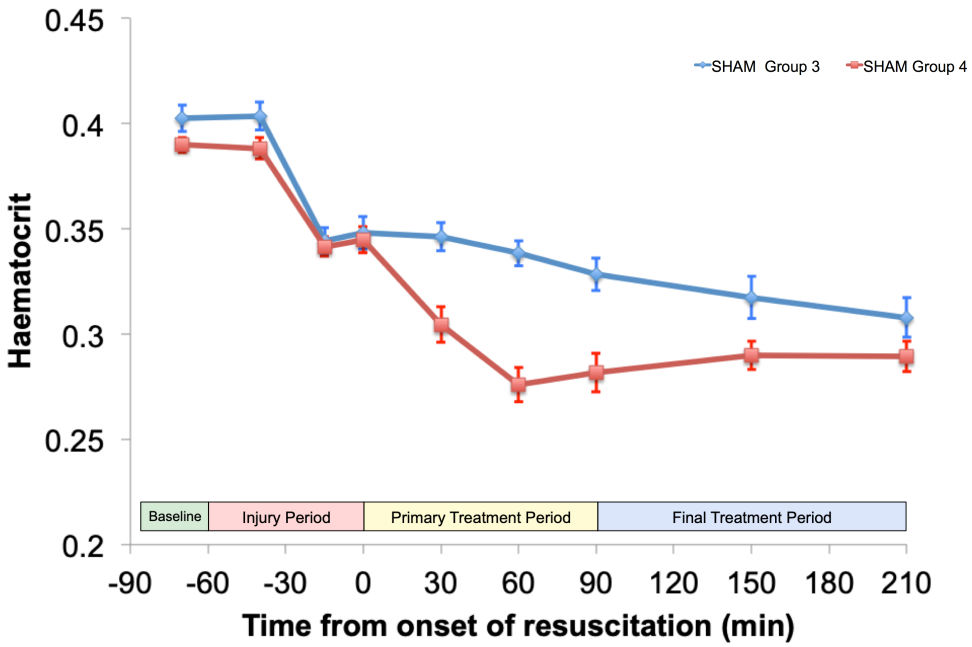


Figure 8-33. Haematocrit in the SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Mean values ± SEM.

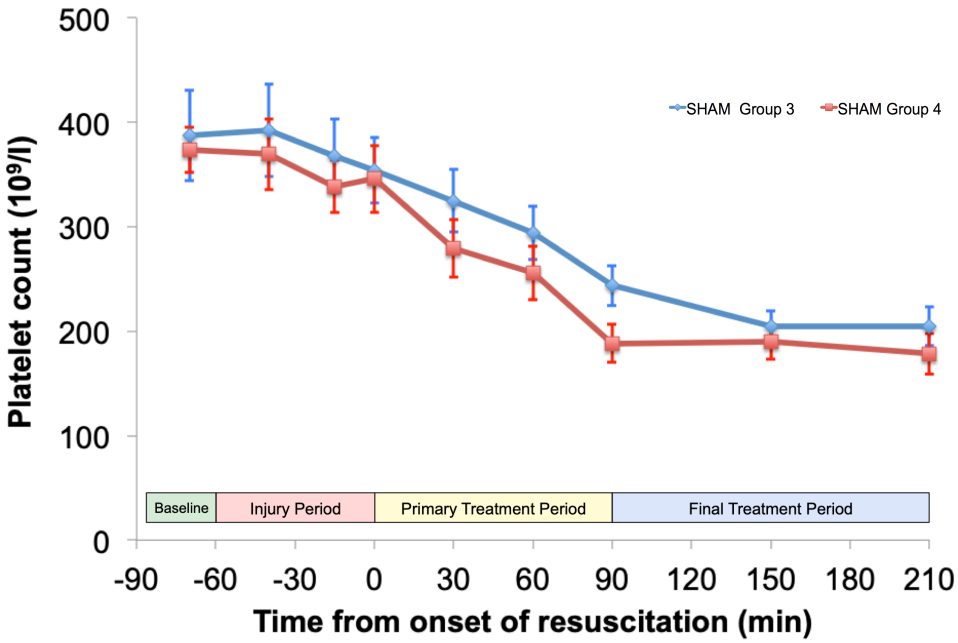


Figure 8-34. Platelet Count in the SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Mean values ± SEM.

8.5.4 Fluids – Final Treatment Period (Sham Groups)

The total fluids given in-hospital are illustrated in Figure 8-35 and the cumulative fluids given to each group are illustrated in Figure 8-36. For the Final Treatment Period, there was no significant difference between the volumes of fluid each group received ($P = 0.462$). For the whole resuscitation period the saline-treatment Group 4 received a significantly greater volume of fluids than Group 3 ($P = 0.011$), but the total volume of blood products received by both groups was similar ($P = 0.172$).

Group 4 (pre-hospital saline) received a significantly greater volume of fluids in total as a consequence of the volume of saline received in the pre-hospital phase.

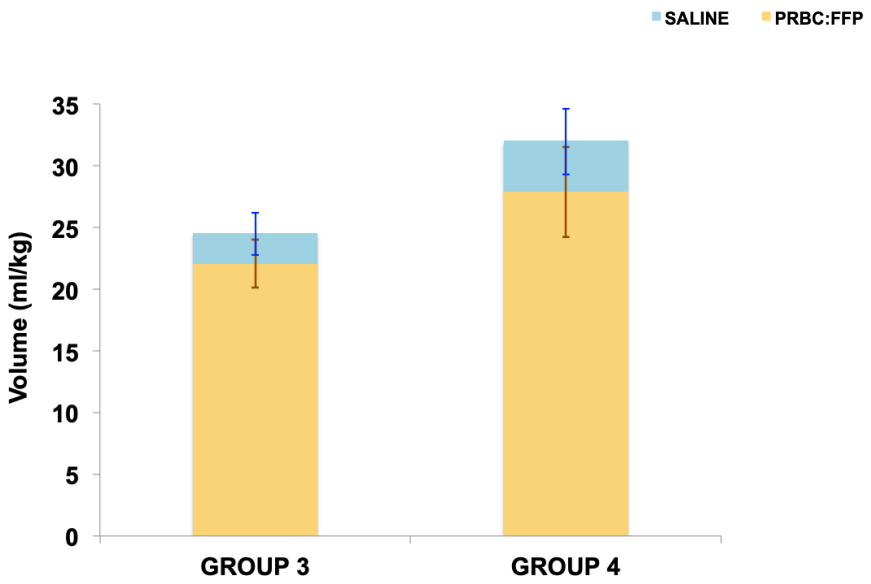


Figure 8-35. Total volume of fluids given in the in-hospital early and late phases for SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Values are Mean ± SEM.

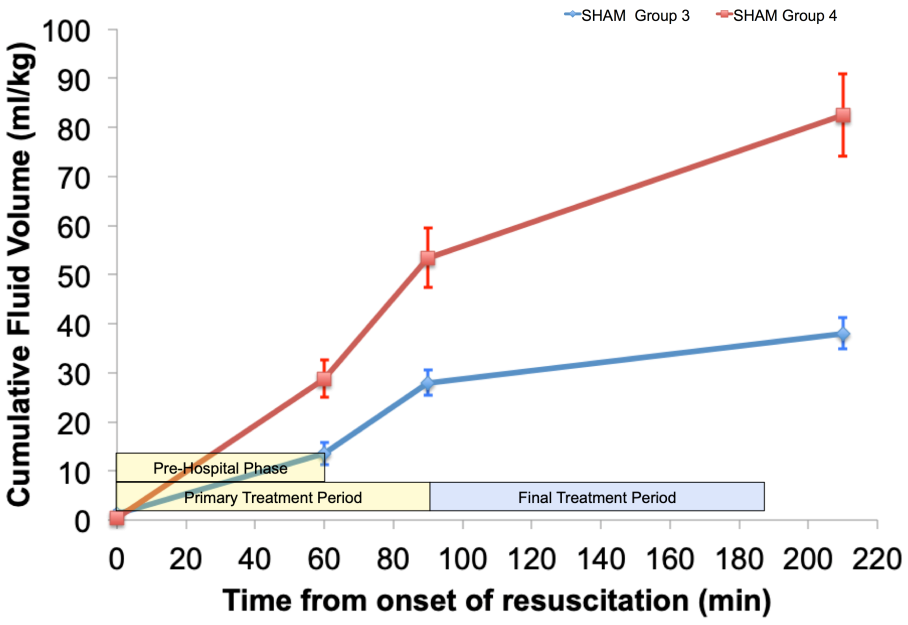


Figure 8-36. Cumulative volume of fluids given to each group for SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation Values are Mean ± SEM.

8.5.5 Biochemistry – Final Treatment Period (Sham Groups)

For the in-hospital phase Group 4 (pre-hospital saline) received blood components at a higher rate than the other group. As a consequence that group had a significantly higher potassium level ($P < 0.001$) and a lower calcium level than the Group 3, however the calcium levels difference did not achieve significance ($P = 0.767$; Table 8-4 and Figure 8-37).

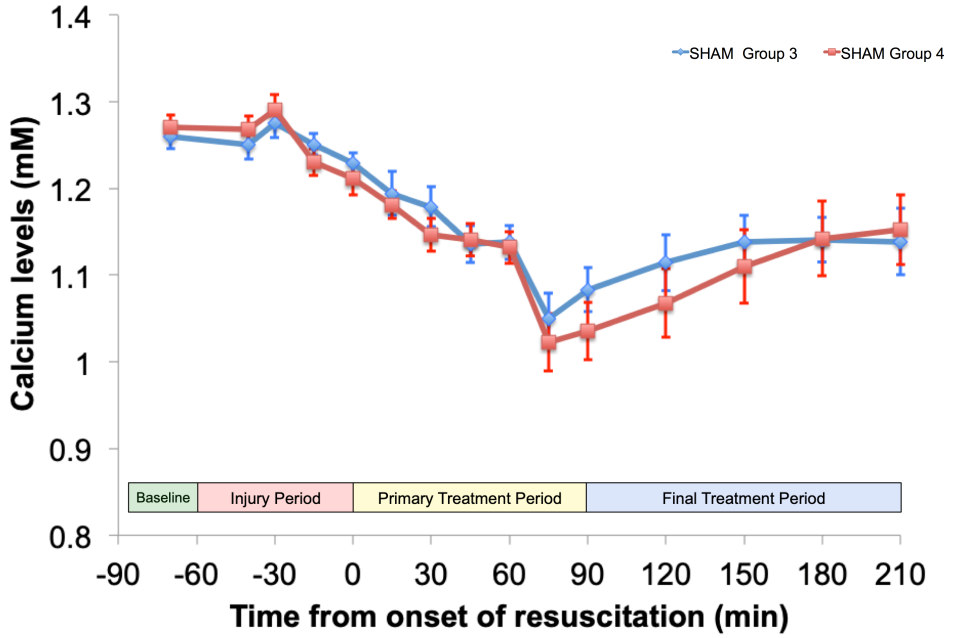


Figure 8-37. Calcium levels in the SHAM blast injury groups. Group 3 - pre-hospital blood component resuscitation. Group 4 - pre-hospital saline resuscitation. Mean values ± SEM.

8.6 Baseline Results – Blast Groups

All animals that survived to S30 (end of Injury Period) survived to the end of the investigation. Baseline data is shown in Table 8-5. There were no significant differences between groups in the initial parameters.

8.7 Injury Period – Blast Groups

The effect of blast injury, blunt injury and haemorrhagic shock – Injury Period – ending at S30, is illustrated in Table 8-5.

8.7.1 Coagulation – Injury Period (Blast Groups)

At S30 the overall clotting picture was broadly similar to that shown in sham blast groups with an initial fall followed by a rise in both R and K times, and in contrast to sham blast, also aPTT (Figures 8-38 and 8-39). Although the overall pattern of response during the injury/shock phase for R time was similar in the two treatment groups (P = 0.536), there was a significant difference in values by S30, although this appears to be of little physiological consequence.

The consequence of the later rise in aPTT during the shock phase meant there was a significant difference between treatment groups and a significant difference in the pattern of response during the injury/shock phase in this parameter. However, although the values of aPTT at the end of the shock phase showed a statistically significant difference, this was relatively small and is unlikely to be of clinical significance. PT did not show the early fall immediately after injury, but did show the later rise.

There was a small, statistically significant drop in fibrinogen observed in both groups; with no significant difference between groups (Table 8-1 and Figure 8-39).

			Baseline		Statistical *P*	S30/R0 (End of Injury Period)		Statistical *P*	Effect of injury and haemorrhagic shock Statistical *P* between baseline and S30/R0		
			Pre-Hosp PRBC:FFP	In-Hosp PRBC:FFP	Between groups at Baseline	Pre-Hosp PRBC:FFP	In-Hosp PRBC:FFP	Between groups at S30/R0	Changes over time	Differences between groups	Differences in pattern over time between groups
			Group 1	Group 2		Group 1	Group 2				
Demographics	No of animals		9	9	1.000						
	Body temp °C		38.1±0.3	38.4±0.2	0.076						
	Body weight Kg		52.7±0.6	51.9±0.5	0.369						
Coagulation	TEG	R (sec)	11.7±0.6	12.7±0.7	0.262	12.5±1.4	18.2±2.3	0.038 *	0.010	0.020	0.536
		K (sec)	5.1±0.3	5.3±0.4	0.733	4.7±0.6	7.1±1.3	0.233 *	< 0.001	0.175	0.063
		MA (mm)	66.1±2.0	67.9±1.2	0.331 *	67.6±1.7	68.5±1.5	0.683	< 0.001	0.384	0.866
	PT (min)		12.5±0.1	12.6±0.2	0.581	12.7±0.2	13.1±0.2	0.094	< 0.001	0.064	0.144
	aPTT (min)		12.6±0.4	12.6±0.5	0.895 *	12.4±0.2	13.7±0.5	0.046	< 0.001	< 0.001	0.016
	Fibrinogen (g/dl)		2.8±0.2	2.7±0.1	0.453	2.3±0.2	2.1±0.1	0.757	< 0.001	0.435	0.477
Cardiovascular	Systolic BP(mmHg)		150±6	159±9	0.421	69±4	60±3	0.081	< 0.001	0.104	0.513
	MAP (mmHg)		131±4	136±7	0.542	42±3	38±2	0.260	< 0.001	0.246	0.795
	Heart Rate (bpm)		139±5	162±12	0.171 *	234±7	220±7	0.366 *	< 0.001	0.959	0.165
	Cardiac Output (l/min)		8.12±0.35	8.64±0.85	0.578	2.85±0.27	2.27±0.25	0.064 *	< 0.001	0.520	0.468
Physiology	PaO2 (kPa)		9.1±0.3	9.4±0.2	0.410	8.5±0.5	9.1±0.5	0.477 *	< 0.001	0.943	0.576
	PaCO2 (kPa)		6.6±0.1	6.4±0.1	0.302	5.5±0.3	5.5±0.3	0.906	< 0.001	0.191	0.812
	Arterial pH		7.43±0.01	7.45±0.01	0.429	7.37±0.02	7.40±0.01	0.042 *	< 0.001	< 0.001	0.132
	ABE (mM)		8.1±0.6	7.8±0.4	0.667	-3.3±1.2	-7.7±0.8	0.006	< 0.001	< 0.001	0.007
	Lactate (mM)		0.8±0.1	0.9±0.1	0.658 *	7.6±0.5	9.4±0.6	0.043	< 0.001	0.001	0.074
	CvO2 (ml/dl)		11.4±0.6	11.4±0.5	1.000	4.1±0.5	4.4±1.4	0.817	< 0.001	0.347	0.990
	OER		0.28±0.03	0.28±0.02	0.959	0.73±0.05	0.77±0.02	0.175	< 0.001	0.030	0.874
Haematology	Hb (g/dl)		13.1±0.2	13.1±0.3	0.978	11.6±0.2	11.4±0.4	0.609	< 0.001	0.480	0.735
	Hct		0.40±0.01	0.40±0.01	0.815	0.36±0.01	0.35±0.01	0.657	< 0.001	0.533	0.569
	Platelet count (10/l)		387±43	333±38	0.358	338±38	300±31	0.444	0.008	0.646	0.527
Biochemistry	K (mM/l)		3.8±0.04	3.8±0.06	0.316	5.1±0.4	5.2±0.2	0.283	< 0.001	0.020	0.464
	Ca (mM/l)		1.3±0.02	1.3±0.01	0.485	1.2±0.01	1.2±0.02	0.913	0.058	0.421	0.600

Table 8-5. Initial animal data; Baseline and S30 (end of Injury Period) for BLAST injury groups. Values are mean ±SEM.

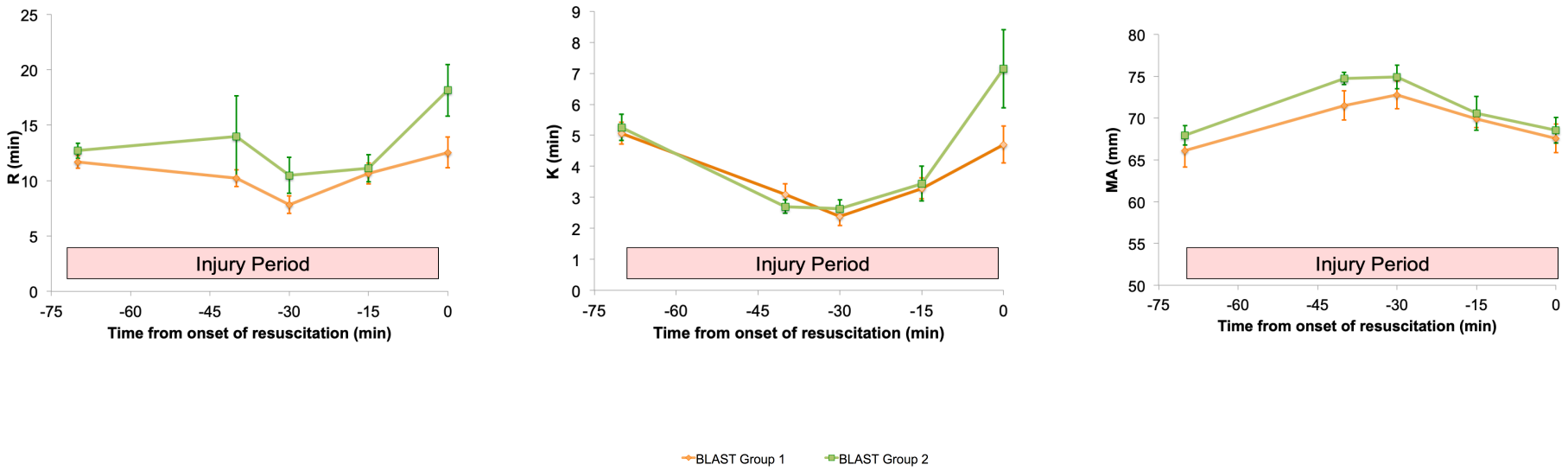


Figure 8-38. Effects of blast, tissue injury and haemorrhage on TEG R time (clot initiation), K time (clot dynamics) and MA (clot strength) in the BLAST injury groups, prior to resuscitation. Time 0 corresponds to S30. Group 1 - planned pre-hospital blood component resuscitation. Group 2 - planned pre-hospital saline. Mean values ± SEM.

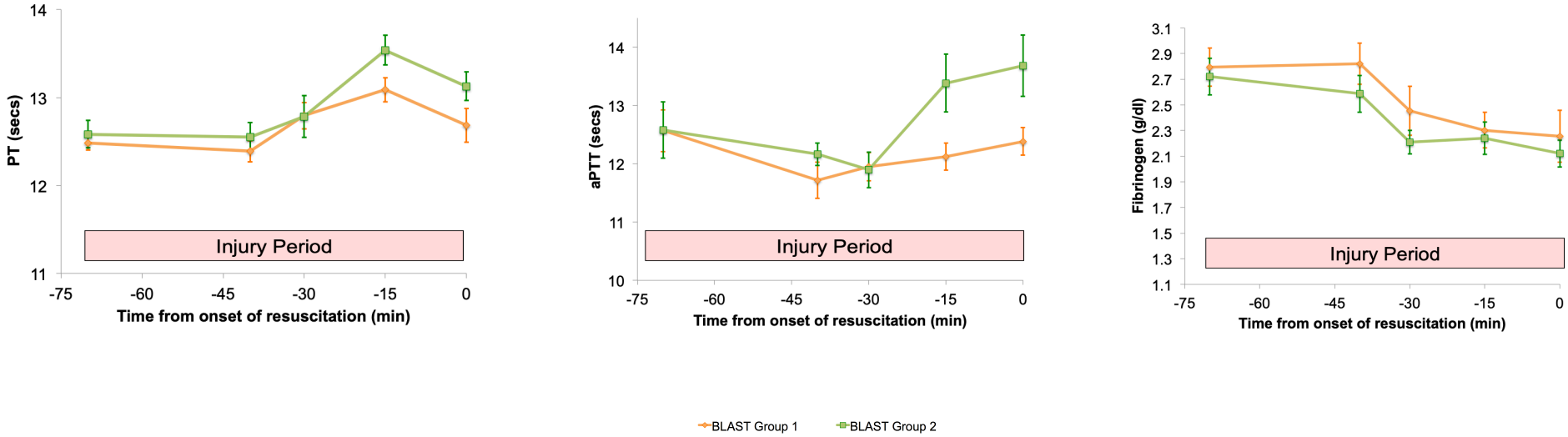


Figure 8-39. Effects of blast, tissue injury and haemorrhage on PT, aPTT and Fibrinogen in the BLAST injury groups, prior to resuscitation. Time 0 corresponds to S30. Group 1 - planned pre-hospital blood component resuscitation. Group 2 - planned pre-hospital saline. Mean values ± SEM.

Both groups therefore displayed a period of increased coagulation immediately after injury that appeared to resolve spontaneously prior to any resuscitation attempts.

8.7.2 Physiology – Injury Period (Blast Groups)

Blast injury, blunt injury and haemorrhage had a significant impact on the cardiovascular system in both groups. There was a marked and statistically significant drop in systolic blood pressure (SBP) and cardiac output (CO) and a significant increase in heart rate ([HR]; $P < 0.001$; for all parameters changing over time; Table 8-5). There was no significant difference in the cardiovascular response to haemorrhage between groups (see Table 8-5 for *P* values and Figure 8-40).

The degree of shock was evaluated by measuring changes in arterial base excess and lactate. Both groups showed a marked clinical and statistically significant fall in in arterial base excess (ABE) and a rise in lactate ($P < 0.001$). There was a significant difference observed between the groups ($P < 0.043$ for both parameters) at this time point, with the saline group at a level some investigators would describe as evidence of hypoperfusion (Brohi 2007a, Curry 2012). This difference is noted to be small when considering the over all change from the baseline (11mM compared to 14mM for ABE; Table 8-5 and Figure 8-41).

All animals exhibited an increase in respiratory effort (respiratory rate and tidal volume noted by simple observation) in response to injury, which resulted in a significant increase in arterial oxygen partial pressure (PaO_2) and a significant decrease in arterial carbon dioxide partial pressure ($PaCO_2$; $P < 0.001$ for change over time of both parameters). There was no difference between groups (Table 8-5).

Figure 8-40. Effects of blast, tissue injury and haemorrhagic shock on Mean Arterial Pressure, Heart Rate and Cardiac Output in the BLAST injury groups prior to resuscitation. Group 1 - planned pre-hospital blood component resuscitation. Group 2 - planned pre-hospital saline. Mean values ± SEM.

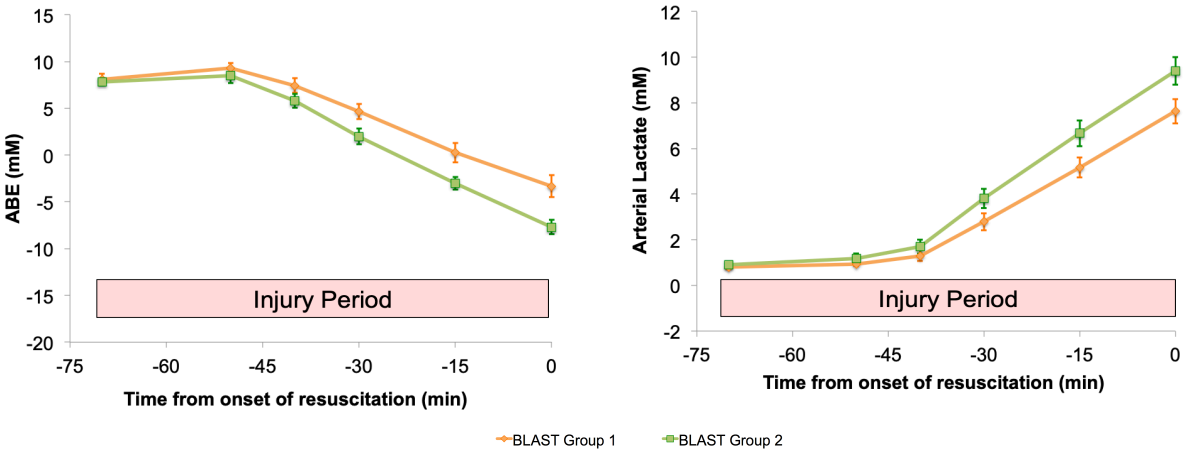


Figure 8-41. Effects of blast, tissue injury and haemorrhagic shock on arterial base excess (ABE) and arterial lactate in the BLAST injury groups prior to resuscitation. Group 1 - planned pre-hospital blood component resuscitation. Group 2 - planned pre-hospital saline. Mean values ± SEM

Mixed venous oxygen content (CvO_2) dropped significantly and the oxygen extraction ratio (OER) increased markedly for both groups with no difference between groups (Table 8-5 and Figure 8-42) by S30.

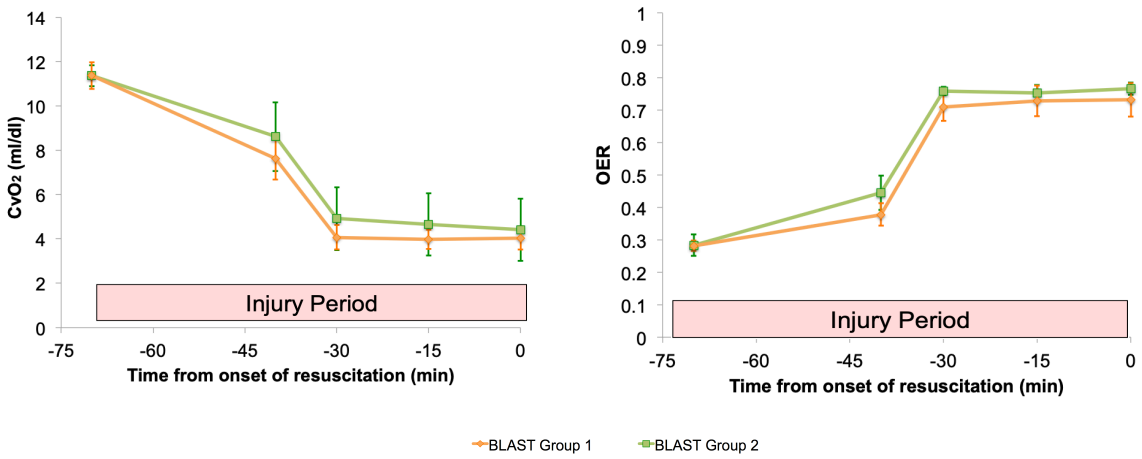


Figure 8-42. Effects of blast, tissue injury and haemorrhagic shock on mixed venous oxygen content (CvO2) and oxygen extraction ratio (OER) in the BLAST injury groups prior to resuscitation. Group 1 - planned pre-hospital blood component resuscitation. Group 2 - planned pre-hospital saline. Mean values ± SEM

By the end of the Injury Period, the injury and haemorrhage had caused equal physiological insult to the animals in both groups. They displayed a cardiovascular response to haemorrhage (rise in HR and drop in BP) and the level of shock was reflected by a developing acidosis and a marked rise in the peripheral extraction of oxygen.

8.7.3 Haematology – Injury Period (Blast Groups)

Haemoglobin and haematocrit levels both fell significantly during the injury and haemorrhage period ($P < 0.001$; Table 8-5 and Figure 8-43) with no difference between groups. A small drop in platelet count was also observed; there was no difference between groups (Table 8-5).

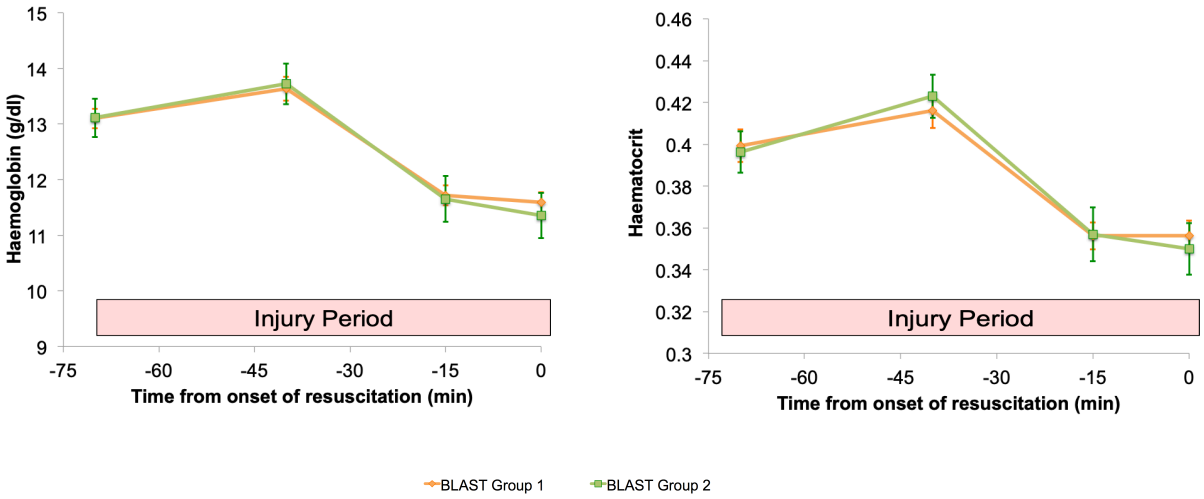


Figure 8-43. Effects of blast, tissue injury and haemorrhagic shock on Haemoglobin and Heamotocrit in the BLAST injury groups prior to resuscitation. Group 1 - planned pre-hospital blood component resuscitation. Group 2 - planned pre-hospital saline. Mean values ± SEM

Both treatment groups showed a rapid drop in haemoglobin in response to haemorrhage with a small drop in platelet count.

8.7.4 Fluids – Injury Period (Blast Groups)

There was no significant difference between groups in the volume of fluid (0.9% saline) used in order to maintain a systolic blood pressure of 60mmHg in some animals during the Injury Period (Figure 8-44, *P* = 0.271).

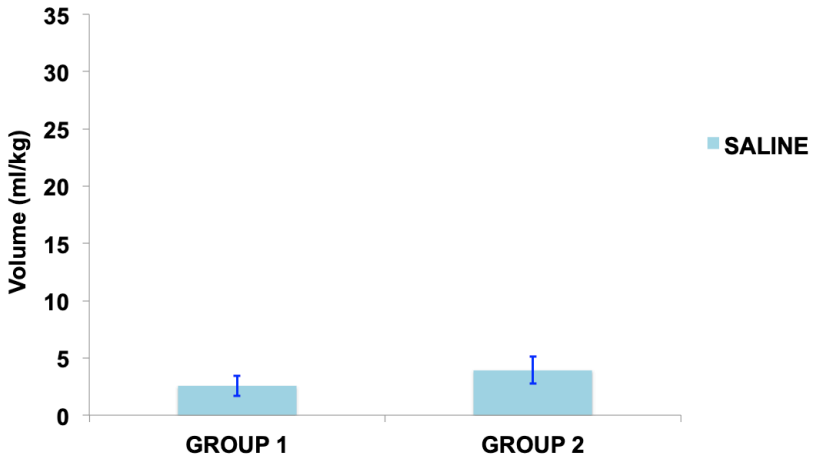


Figure 8-44. Total volume of fluids given to each group during the Injury Period for BLAST injury groups. Mean values ± SEM

8.7.5 Biochemistry – Injury Period (Blast Groups)

No difference was detected in the calcium levels between groups (*P* = 0.913) or over time in the Injury Period (*P* = 0.058; Table 8-5). A significant rise in potassium levels was observed in both groups over the Injury Period (*P* < 0.001; Table 8-5 and Figure 8-45) with no difference between groups (*P* = 0.283).

Blast, blunt injury and haemorrhage had no effect on calcium levels, but caused a rise in potassium levels to reach a level at the upper end of normal.

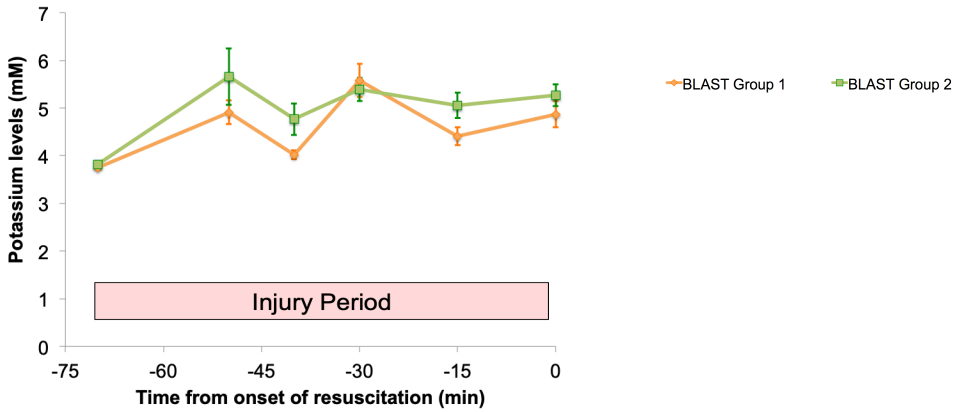


Figure 8-45. Effects of blast, tissue injury and haemorrhage on Potassium levels in the BLAST injury groups. Group 1 - planned pre-hospital blood component resuscitation. Group 2 - planned pre-hospital saline. Mean values ± SEM

8.8 Primary Treatment Period – Blast Groups

The effect of the two different pre-hospital treatment regimes on coagulation parameters is illustrated in Table 8-6. It contains data for R60 (end of 60 minutes pre-hospital phase) and R90 (additional 30 minutes in-hospital blood component resuscitation; the primary outcome time point and the end of the Primary Treatment Period). All remaining parameters are illustrated in Table 8-7.

8.8.1 Coagulation –Primary Treatment Period (Blast Groups)

After the onset of resuscitation there were significant changes in clotting as assessed by R and K times (Table 8-6, Figure 8-46). Both groups showed elevations in R and K times after the onset of resuscitation (Figure 8-46), in contrast to the situation in the sham blast injury strand (Figure 8-10) where only the saline-treatment strand displayed the elevation in R and K times. In the blast strand, the initial rate of rise of both R and K times appeared steeper in the saline-treated group compared to those given blood products

			End of Pre-Hospital Phase (R60)		Statistical *P*	Effect of injury and haemorrhagic shock Statistical *P* between S30/R0 and R60			Primary Outcome Point (R90)		Statistical *P*
			Pre-Hosp PRBC:FFP	In-Hosp PRBC:FFP	Between groups at R60	Changes over time	Differences between groups	Differences in pattern over time between groups	Pre-Hosp PRBC:FFP	In-Hosp PRBC:FFP	Between groups at R90
			Group 1	Group 2					Group 1	Group 2	
Coagulation	TEG	R (sec)	16.6±1.4	23.9±5.9	0.262	0.029 °	0.057 °	0.414 °	15.2±1.0	23.0±5.3	0.171
		K (sec)	5.7±0.5	9.8±2.9	0.209	0.007 °	0.094 °	0.890 °	5.3±0.4	8.3±2.3	0.218
		MA (mm)	65.4±1.3	66.7±1.5	0.535	0.315	0.698	0.737	66.4±1.5	66.7±1.0	0.659
	PT (min)		13.8±0.5	16.0±0.5	0.007	< 0.001	< 0.001	0.040	14.2±0.3	15.6±0.5	0.021 *
	aPTT (min)		14.1±0.5	16.5±1.3	0.095	0.001	0.280	0.412	12.9±0.5	15.2±1.0	0.027 *
	Fibrinogen (g/dl)		2.06±0.24	1.27±0.05	0.001	< 0.001 °	0.033 °	< 0.001 °	1.94±0.15	1.50±0.09	0.017

Table 8-6. Animal coagulation data; R60 (end of Pre-Hospital Period) and R90 (primary outcome point) for BLAST injury groups. * Comparison performed using Mann-Whitney U test. ° Comparison using logarithmic transformed data. Values are mean ±SEM.

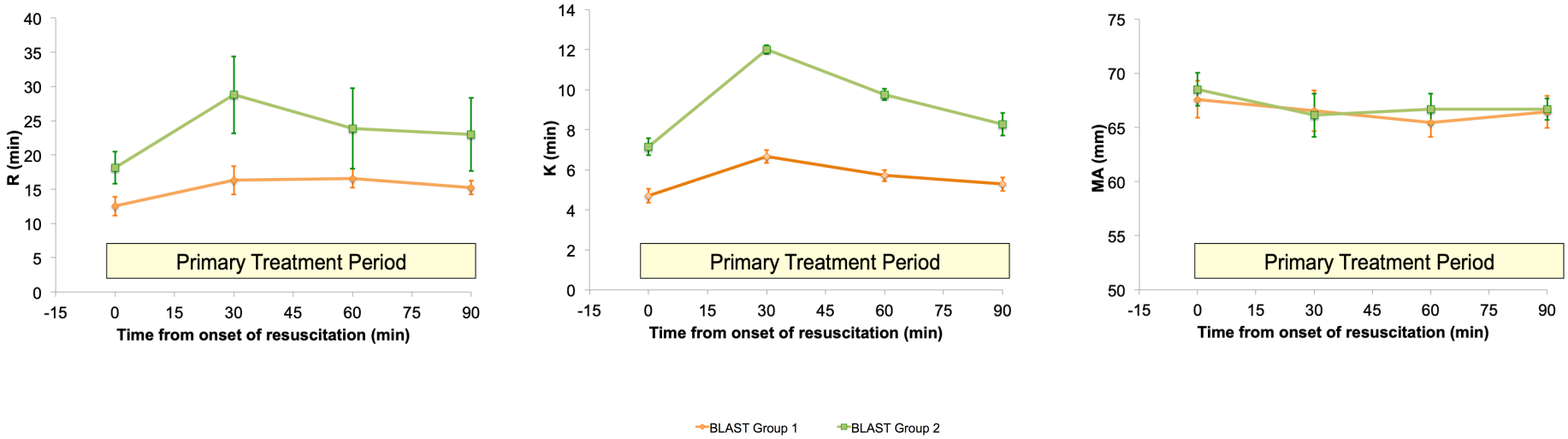


Figure 8-46. TEG R time (clot initiation), K time (clot dynamics) and MA (clot strength) in the BLAST injury groups, at end of 90 minutes of resuscitation (60 minutes pre-hospital and 30 minutes in-hospital). Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

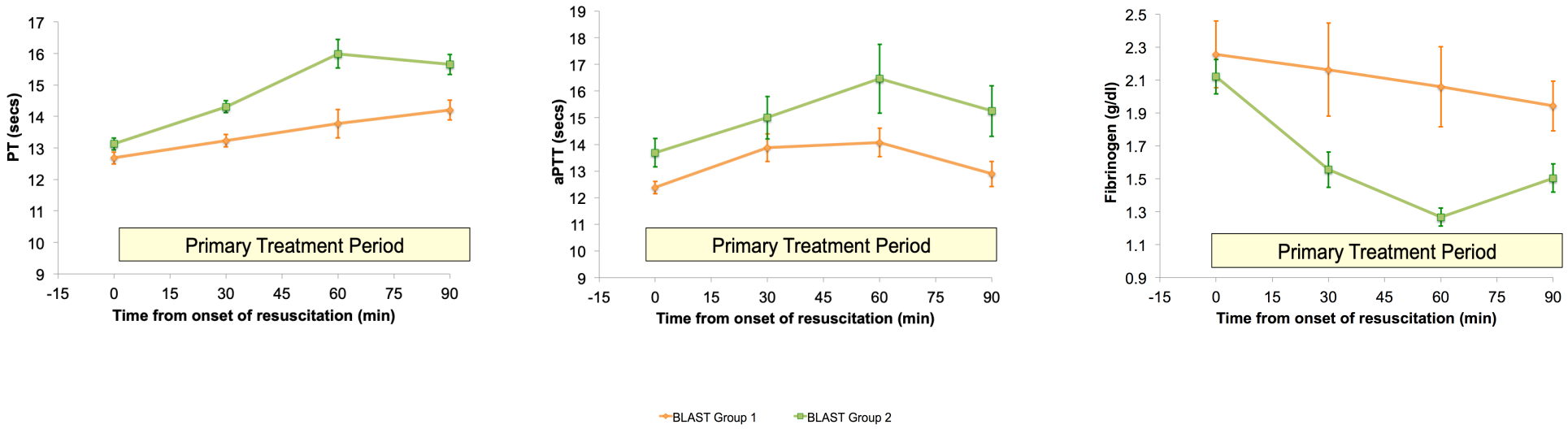


Figure 8-47. PT, aPTT and Fibrinogen (g/dl) in the BLAST injury groups during the Primary Treatment Period (90 minutes of resuscitation; 60 minutes pre-hospital and 30 minutes in-hospital).. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

(Figure 8-46) although there was no statistically significant difference between treatment groups in the general pattern of response over time (Table 8-6). Overall there was a trend for higher R and K times in the saline treated groups, but this did not attain statistical significance (Table 8-6).

Consequently, at R90 there was no significant difference in the absolute values of the R and K times between groups. This is probably explained by the small rise in these parameters in the blood treatment group (Group 1) and the wide variability in the saline group.

In common with the Sham groups MA changed little during this phase with no difference between the groups.

Conventional assessment of clotting (PT and aPTT) showed a similar overall picture to that seen for R and K times, although the difference between groups was statistically significant for PT, with the saline-treated Group 2 showing the greatest impairment in clotting. By R90 both PT and aPTT were significantly higher in Group 2 compared to Group 1 (Figure 8-47; *P* values in Table 8-6).

Fibrinogen levels for Group 2 (pre-hospital saline) at hospital admission (R60) dropped below 1.5 g/dl, which is markedly below the 95% reference range for this strain of pigs (1.77-3.36 g/l, derived as described in section 8.4.3). This drop in fibrinogen is only partly explained to some extent by haemodilution (as measured by haematocrit), as the ratio of the proportional fall in fibrinogen levels from baseline, to the proportional fall in Haematocrit from baseline, in Group 2 was 0.67 ± 0.02, suggesting there was loss of fibrinogen over and above the effects of haemodilution. Group 2 fibrinogen levels increased towards normal values by R90, however they remained significantly lower than Group 1 ($P = 0.017$; Figure 8-47).

A combined time-lapse example of the TEG trace for a subject in Group 2 is shown in Figure 8-48. This figure illustrates the change in R and K time over the initial injury period and pre-hospital phase.

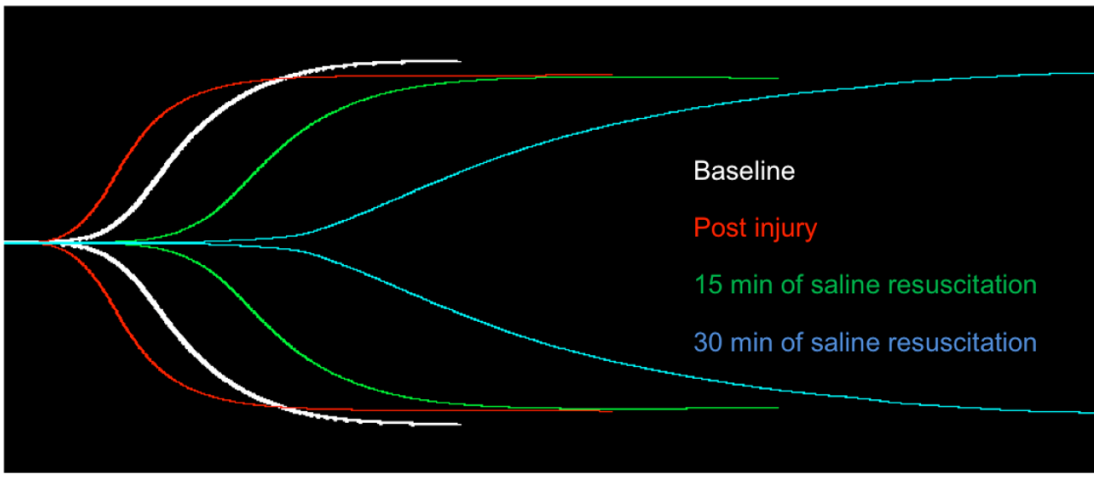


Figure 8-48. Initial time lapse illustration of TEG changes in one subject from Group 2 (receiving pre-hospital saline). The initial shortening of R and K times are seen after injury with subsequent development of coagulopathy in the pre-hospital phase during saline only resuscitation.

These results illustrate that after combined blast and tissue injury with haemorrhagic shock Group 2 (receiving pre-hospital saline) developed a change in clotting suggestive of coagulopathy. This clotting change was evident at the end of the pre-hospital period (R60) and persisted to R90 having received 30 minutes of hospital based blood component resuscitation. The coagulation measured in Group 1 (receiving pre-hospital PRBC:FFP) changed in a similar manner but to a much lesser extent than Group 2.

8.8.2 Physiology – Primary Treatment Period (Blast Groups)

Systolic blood pressure (SBP) targets were maintained at the relevant level for each phase (80mmHg pre-hospital and 110 mmHg in-hospital) in both groups resulting in a significant elevation in SBP by R90 in both groups, with no difference between groups ($P > 0.154$; Figure 8-49 and Table 8-7).

Resuscitation goals were therefore attained for both groups in the pre-hospital and early in-hospital phases.

		Primary Outcome Point (R90)		Statistical *P*
		Pre-Hosp PRBC:FFP	In-Hosp PRBC:FFP	Between groups at R90
		Group 1	Group 2	
Cardiovascular	Systolic BP(mmHg)	116±2	112±2	0.154
	MAP (mmHg)	74±3	75±4	0.986
	Heart Rate (bpm)	205±9	198±6	0.510
	Cardiac Output (l/min)	6.23±0.64	7.17±0.55	0.279
Physiology	PaO2 (kPa)	17.8±2.6	20.3±3.3	0.757 *
	PaCO2 (kPa)	5.54±0.41	5.51±0.27	0.625 *
	Arterial pH	7.24±0.04	7.22±0.03	0.658
	ABE (mM)	-8.9±1.3	-10.4±1.5	0.455
	Lactate (mM)	13.0±0.7	11.9±1.0	0.412
	CvO2 (ml/dl)	8.26±0.35	8.44±0.91	0.536 *
	OER	0.42±0.02	0.36±0.06	0.338
Haematology	Hb (g/dl)	10.8±0.1	9.3±0.3	0.001
	Hct	0.33±0.01	0.29±0.01	0.002
	Platelet count (10/l)	202±33	198±21	0.921
Biochemistry	K (mM/l)	3.89±0.11	4.42±0.15	0.010
	Ca (mM/l)	1.13±0.02	1.10±0.03	0.454

Table 8-7. Animal data: R90 (primary outcome point) for BLAST injury groups.
* Comparison performed using Mann-Whitney U test. Values are mean ±SEM.

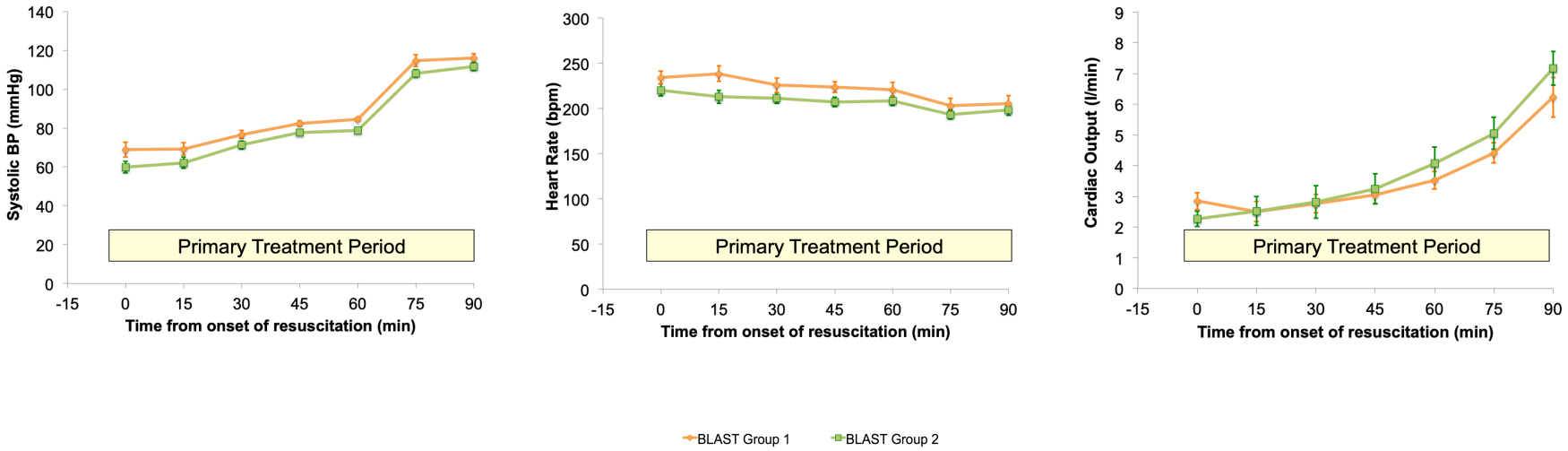


Figure 8-49. Systolic Blood Pressure (BP), Heart Rate and Cardiac Output in the BLAST injury groups during the Primary Treatment Period (90 minutes of resuscitation; 60 minutes pre-hospital and 30 minutes in-hospital). Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline. Mean values ± SEM.

A corresponding rise in cardiac output was observed in both groups by R90 with both showing a small drop in heart rate, with no difference between groups in either parameter ($P > 0.279$).

For both groups arterial base excess (ABE) continued to drop to its lowest point and lactate rise to its peak at R60 - the end of the pre-hospital evacuation phase - with both then plateauing during the remaining 30 minutes of the Primary Treatment Period. Group 2 (pre-hospital saline) ABE decreased to a lower level compared to Group 1 at R60 and remained below the other group up to R90. The difference had reduced by R90 and so there was not a significant difference between them at this time point ($P = 0.455$; Table 8-7 and Figure 8-50).

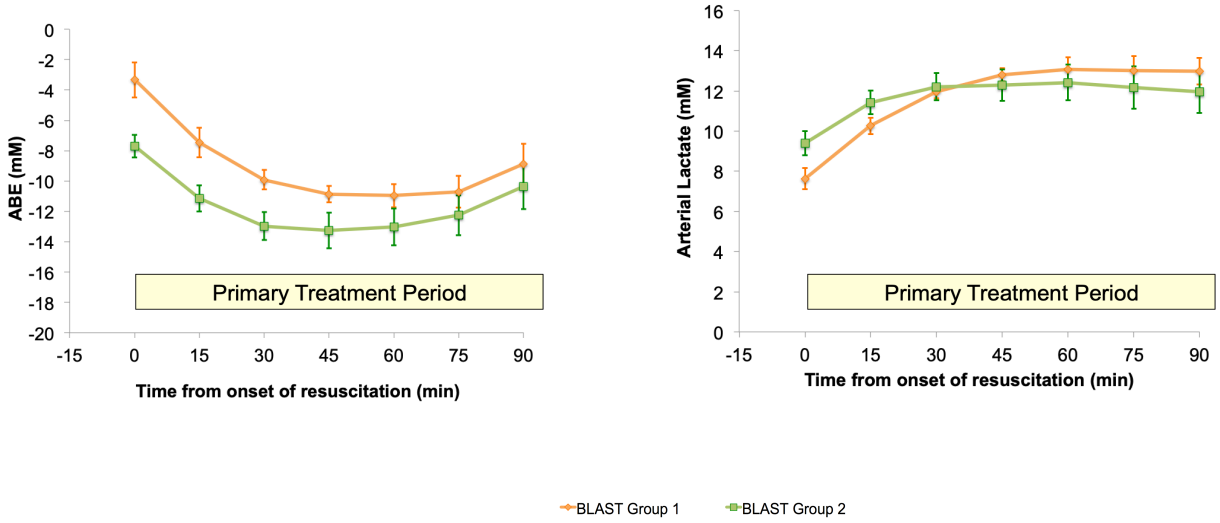


Figure 8-50. Arterial base excess (ABE) and arterial lactate in the BLAST injury groups during the Primary Treatment Period (90 minutes of resuscitation; 60 minutes pre-hospital and 30 minutes in-hospital). Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline. Mean values ± SEM.

Group 2 (pre-hospital saline) had a consistently lower $PaCO_2$ throughout the Primary Treatment Period. The difference reduced at R90 and as a consequence there was not a statistical difference between treatment groups at this time point (Table 8-7 and Figure 8-51). Both treatment groups had similar PaO_2 levels throughout this period.

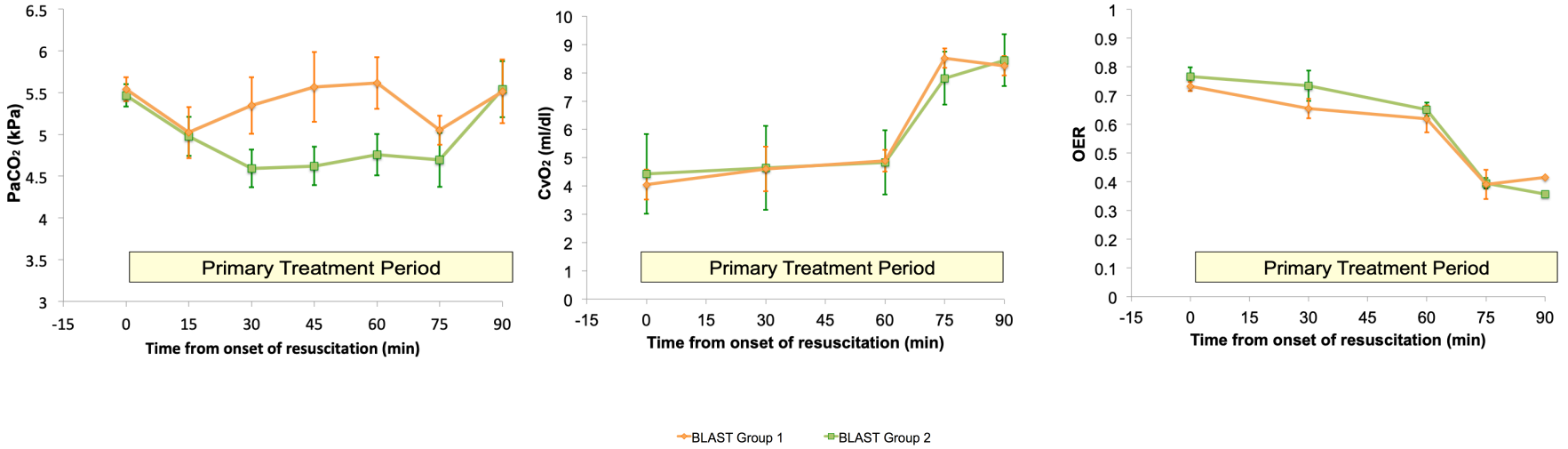


Figure 8-51. Partial pressure of arterial carbon dioxide ($PaCO_2$), mixed venous oxygen content (CvO_2) and oxygen extraction ratio (OER) in the BLAST injury groups during the Primary Treatment Period (90 minutes of resuscitation; 60 minutes pre-hospital and 30 minutes in-hospital). Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline. Mean values ± SEM.

There was a significant increase in mixed venous oxygen (CvO_2) and a significant drop in Oxygen Extraction Ratio (OER) after R60 so that at the end of the Primary Treatment Period (R90) both groups had a significantly higher CvO_2 and lower OER compared to the pre-hospital phase. There was no difference between groups (Table 8-7 and Figure 8-51).

Pre-hospital and initial in-hospital resuscitation raised systolic blood pressure and cardiac output for both groups. Pre-hospital resuscitation in both groups stopped the drop in base excess and rise in lactate, and this effect continued into the initial in-hospital phase. The pre-hospital saline group displayed a greater acidosis than the pre-hospital blood component group during the pre-hospital phase, but this difference did not attain statistical significance. Mixed venous content and oxygen extraction did not return toward baseline levels until the in-hospital phase.

8.8.3 Haematology – Primary Treatment Period (Blast Groups)

Group 1, receiving pre-hospital blood components, had a small drop in both haemoglobin and haematocrit by R90 (Table 8-7 and Figure 8-52).

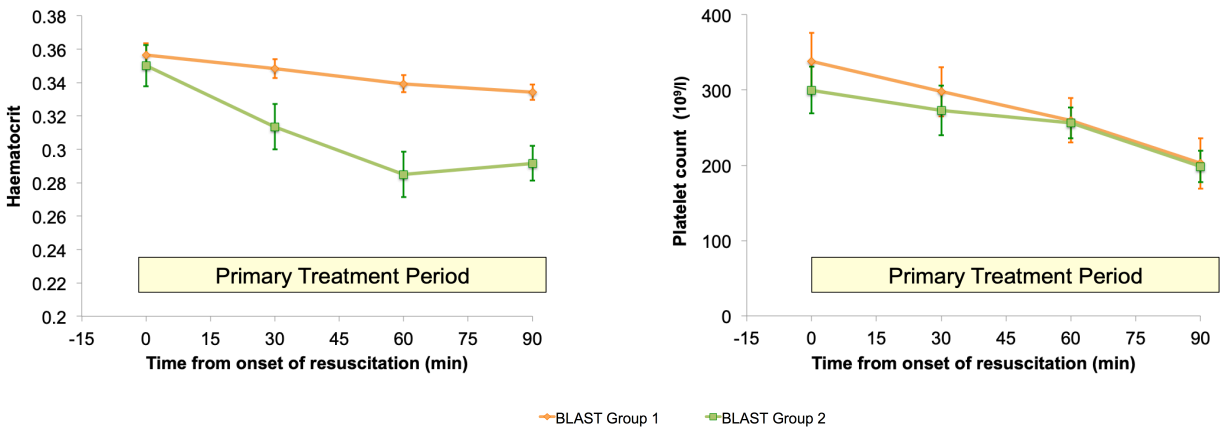


Figure 8-52. Haematocrit and platelet count (10^9/l) in the BLAST injury groups during the Primary Treatment Period (90 minutes of resuscitation; 60 minutes pre-hospital and 30 minutes in-hospital). Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline. Mean values ± SEM.

The saline-treatment Group 2, had a significant decrease in haematocrit by the end of the pre-hospital phase (R60) compared to Group 1, and this difference persisted at R90 (P = 0.002).

Platelet count dropped significantly for both groups by R90 with no difference between groups (P = 0.921; Table 8-7 and Figure 8-52). At the end of the Initial Treatment Period Group 1 receiving pre-hospital blood components had little change in haematocrit in contrast to Group 2, which showed a significant drop.

8.8.4 Fluids – Primary Treatment Period (Blast Groups)

The fluid used in each group for the different phases of the Primary Treatment Period is illustrated in Figure 8-53 and 8-55; sub-divided into the pre-hospital and (early) in-hospital phases. Compared to Group 1 (pre-hospital PRBC:FFP), the other Group (2; pre-hospital saline) required a greater volume of fluid in the pre-hospital phase (P = 0.007; Figure 8-53) and over the whole Primary Treatment Period up to R90 (P < 0.001; Figure 8-55).

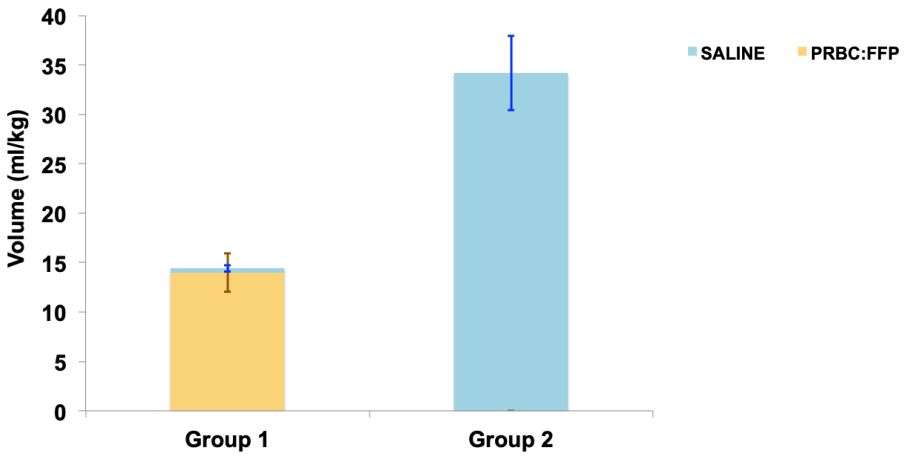


Figure 8-53. Total volume of fluids given to each group in the pre-hospital phase (between S30 and R60) for BLAST groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline. Values are Mean ± SEM.

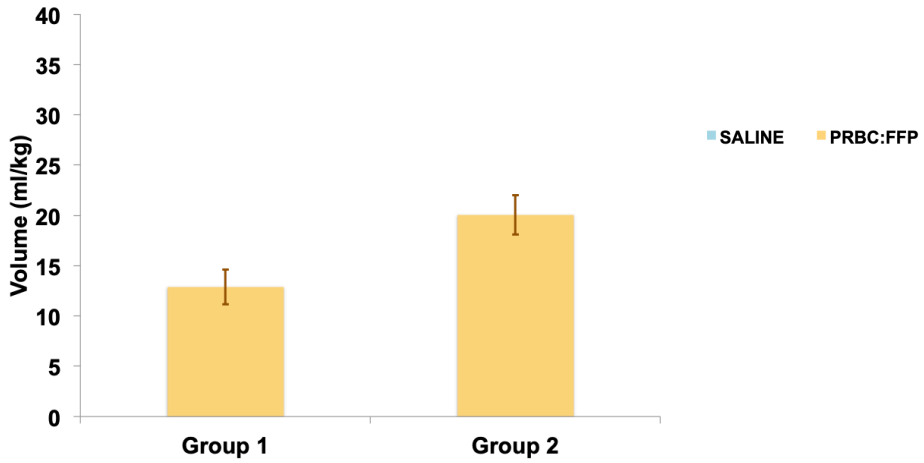


Figure 8-54. Total volume of fluids given to each group in the early in-hospital phase (between R60 and R90) for BLAST groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline. Values are Mean ± SEM.

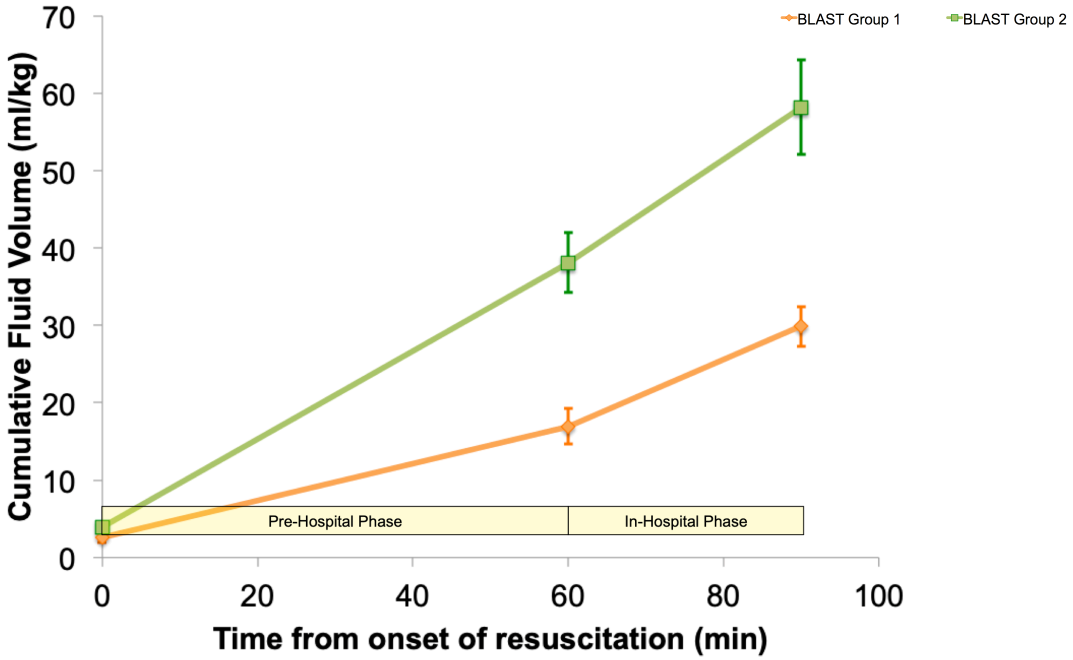


Figure 8-55. Cumulative volume of fluids given to each group in the Primary Treatment Period; pre-hospital and early in-hospital phases combined (S2 = 0 min; R60 = 60 min; R90 = 90min) for BLAST groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline. Values are Mean ± SEM.

8.8.5 Biochemistry – Primary Treatment Period (Blast Groups)

There was a (significant) reduction in calcium levels in both groups over time in the Primary Treatment Period with no difference between groups (*P* = 0.454; Table 8-7 and Figure 8-56). In the pre-hospital phase calcium dropped faster in Group 1 (pre-hospital blood components), with both groups illustrating a large drop once in the in-hospital phase (both receiving blood components; Figure 8-56).

Potassium levels dropped slightly in both groups by R90 (Table 8-7 and Figure 8-56). Both groups had potassium levels within the normal range, although there was a small but statistically significant difference between the groups.

By R90, after the use of blood component resuscitation in both groups, calcium had dropped to the lower end of the normal range and potassium had dropped into the high end of the normal range in both groups.

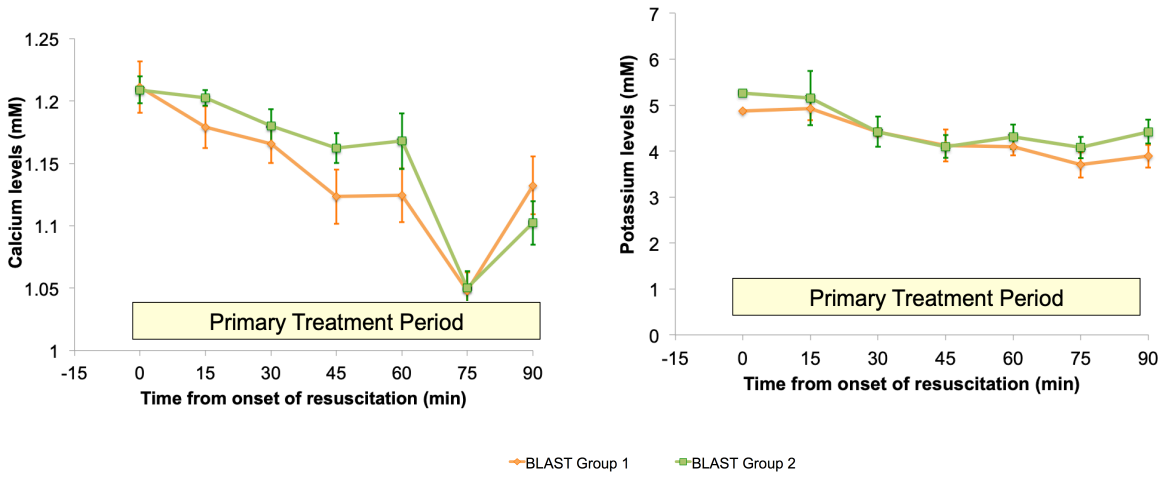


Figure 8-56. Calcium and Potassium levels in the BLAST injury groups during the Primary Treatment Period (90 minutes of resuscitation; 60 minutes pre-hospital and 30 minutes in-hospital). Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline. Mean values ± SEM.

8.9 Final Treatment Period – Blast Groups

The results for all parameters for the remaining (late) in-hospital phase is summarised in Table 8-8.

			End of In Hospital Phase (R210)		Effect of injury and haemorrhagic shock Statistical *P* between S30/R0 and R210		
			Pre-Hosp PRBC:FFP	In-Hosp PRBC:FFP	Changes over time	Differences between groups	Differences in pattern over time between groups
			Group 1	Group 2			
Coagulation	TEG	R (sec)	18.3±2.7	14.6±1.2	< 0.001 °	0.422 °	0.001 °
		K (sec)	8.2±1.6	4.6±0.6	< 0.001 °	0.593 °	0.001 °
		MA (mm)	61.3±2.5	67.0±1.6	0.245	0.057	0.339
	PT (min)		15.2±0.3	16.0±0.5	< 0.001	0.002	0.158
	aPTT (min)		12.7±0.4	13.2±0.7	< 0.001	0.714	0.132
	Fibrinogen (g/dl)		1.87±0.12	1.60±0.12	< 0.001 °	0.050 °	0.050 °
Cardiovascular	Systolic BP(mmHg)		120±2	118±3	< 0.001	0.009	0.873
	MAP (mmHg)		86±3	88±6	< 0.001	0.358	0.996
	Heart Rate (bpm)		194±12	195±11	< 0.001	0.013	0.978
	Cardiac Output (l/min)		6.08±0.66	5.73±0.43	< 0.001	0.006	0.773
Physiology	PaO2 (kPa)		16.2±1.1	17.1±2.0	< 0.001	0.330	0.999
	PaCO2 (kPa)		5.38±0.18	5.92±0.57	< 0.001	0.824	0.003
	Arterial pH		7.40±0.04	7.40±0.04	< 0.001	0.011	0.044
	ABE (mM)		2.7±1.8	0.3±2.0	< 0.001	0.675	0.957
	Lactate (mM)		4.9±1.4	5.2±1.5	< 0.001	0.001	0.831
	CvO2 (ml/dl)		7.32±0.47	7.36±1.15	< 0.001	0.528	0.944
	OER		0.46±0.03	0.51±0.03	< 0.001	0.492	0.560
Haematology	Hb (g/dl)		10.6±0.1	9.6±0.3	< 0.001	< 0.001	< 0.001
	Hct		0.31±0.01	0.29±0.01	< 0.001	< 0.001	< 0.001
	Platelet count (10/l)		194±26	175±20	< 0.001	0.318	0.784
Biochemistry	K (mM/l)		4.28±0.23	4.56±0.21	< 0.001	0.001	0.737
	Ca (mM/l)		1.18±0.03	1.21±0.04	< 0.001	0.076	0.912

Table 8-8. Animal data; R210 (end of Late In Hospital Phase and Final Treatment Period) for BLAST injury groups. Values are mean ±SEM.

8.9.1 Coagulation – Final Treatment Period (Blast Groups)

The abnormal clotting values for the saline-treatment Group 2 returned towards baseline levels by R210 (Table 8-8 and Figure 8-57 and 8-58). Higher times in the pre-hospital blood group for both K and R times at R210, meant that the difference between them was not significant secondary to one animal having an increase in both parameters in its last blood sample. That animal had achieved its targeted blood pressure and had recovered its acidosis and was not in receipt of a significant bolus of saline pre-blood sample, so this value cannot be easily explained. Despite this result there was noted to be a significant difference in pattern between the groups in both parameters for the resuscitation period ($P < 0.001$; Table 8-8 and Figures 8-59 and 8-60), with the saline-treatment group having longer times in the Primary Treatment Period. In similarity with other periods MA width did not change in either group (as per previous periods), with no difference between groups ($P = 0.057$).

The aPTT times for Group 2 (pre-hospital saline) changed in a similar manner to the TEG K and R times with a trend towards returning to baseline levels by R210 (Table 8-8 and Figure 8-61). In parallel with R and K times, although the levels in Group 2 were higher than Group 1 throughout, these differences did not achieve significance.

PT showed a different pattern of response over time in both groups (Figure 8-62). There was a significant rise in PT with the early (pre-hospital) elevation being significantly greater in the saline group resulting in a significant difference between groups ($P < 0.002$) over time. However, unlike the other parameters PT did not return towards baseline in either group during the in-hospital phase although the rate of rise did decline especially in the group previously treated with saline. Similar to the sham groups, these elevated PT results were just outside the 95% reference range for this strain of pigs and were equivalent to an INR of approximately 1.2.

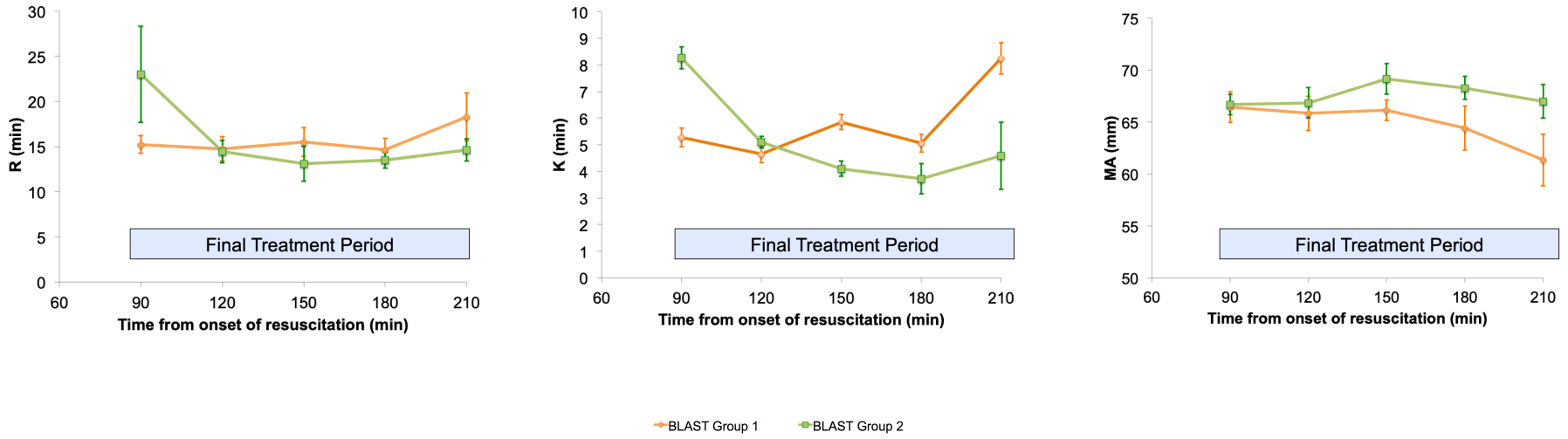


Figure 8-57. TEG R time (clot initiation), K time (clot dynamics) and MA (clot strength) in the BLAST injury groups for the Final Treatment Period. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

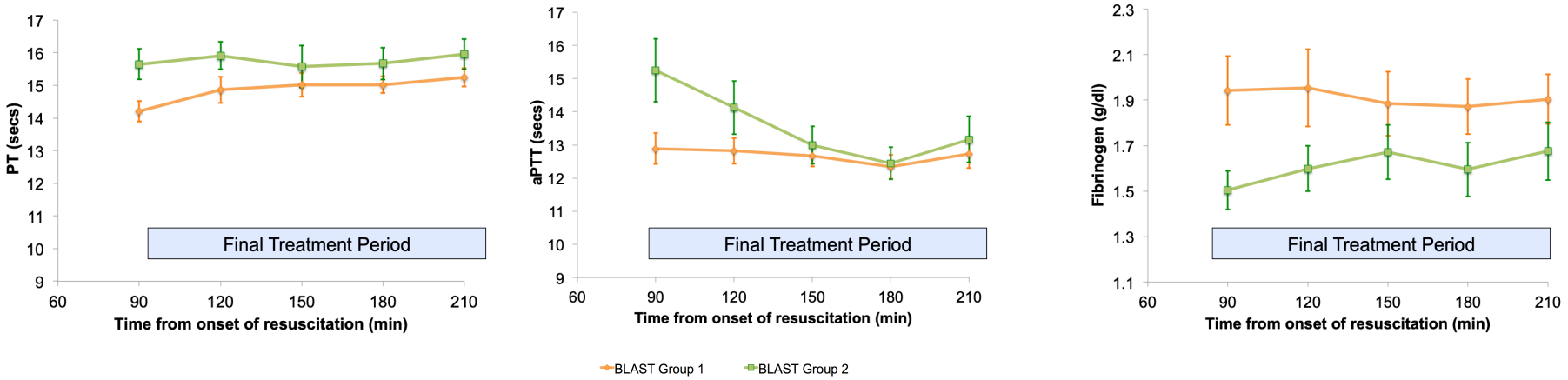


Figure 8-58. PT, aPTT and Fibrinogen in the BLAST injury groups during the Final Treatment Period. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline. Mean values ± SEM.

Group 1 (pre-hospital blood component resuscitation) had little change in fibrinogen throughout the final treatment period, in contrast to Group 2, which showed a small elevation in Fibrinogen levels back toward the normal range.

Despite continued blood component resuscitation the pre-hospital saline Group 2 fibrinogen levels remained less than the other group throughout the investigation with a significant difference between the groups in all statistical parameters ($P \leq 0.050$; Table 8-8 and Figure 8-63).

The clotting changes - as measure by TEG R and K times, aPTT and fibrinogen - that were developed by Group 2 (pre-hospital saline) in the pre-hospital phase and lasting into the initial in-hospital phase resolved after 60 to 90 minutes of in-hospital blood component resuscitation. These changes were significantly different from Group 1 as measured by difference in pattern over time between groups.

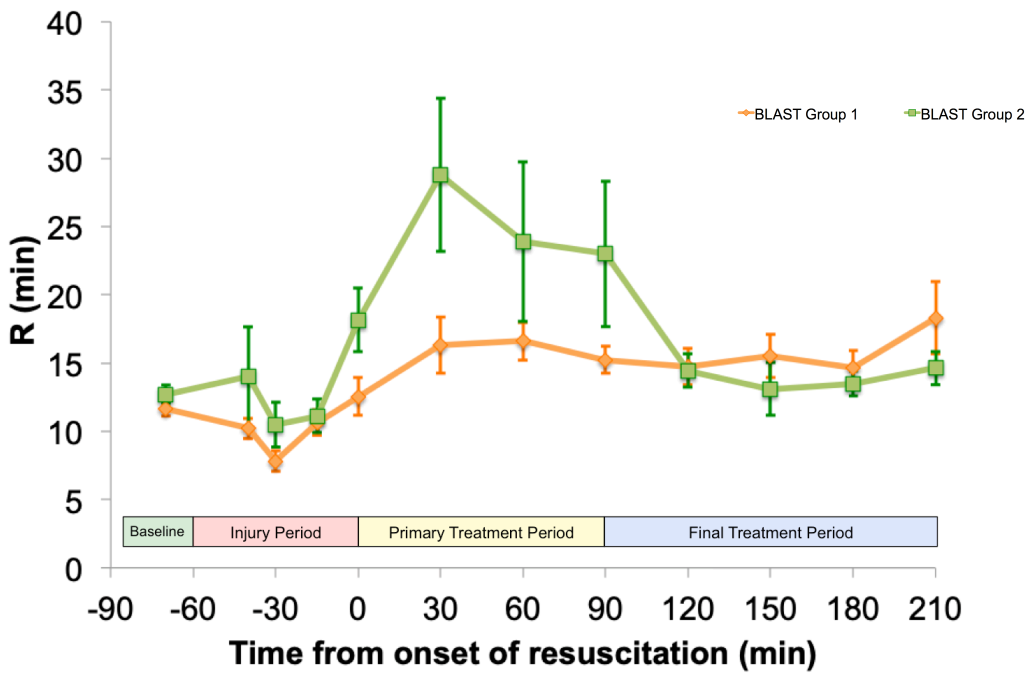


Figure 8-59. TEG R time (clot initiation) in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

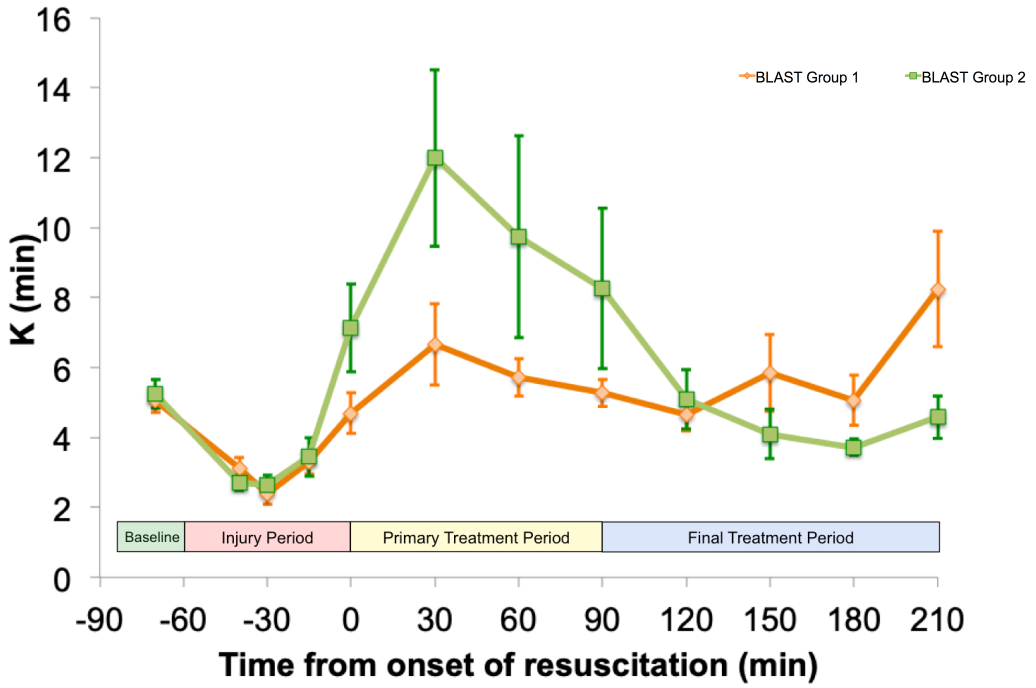


Figure 8-60. TEG K time (clot initiation) in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

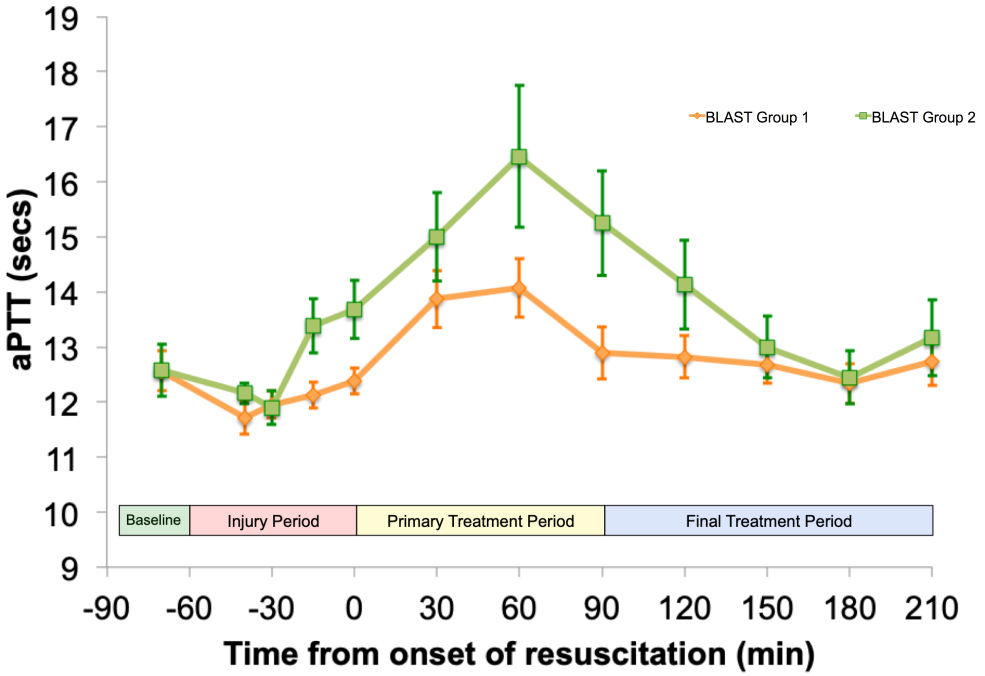


Figure 8-61. aPTT in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

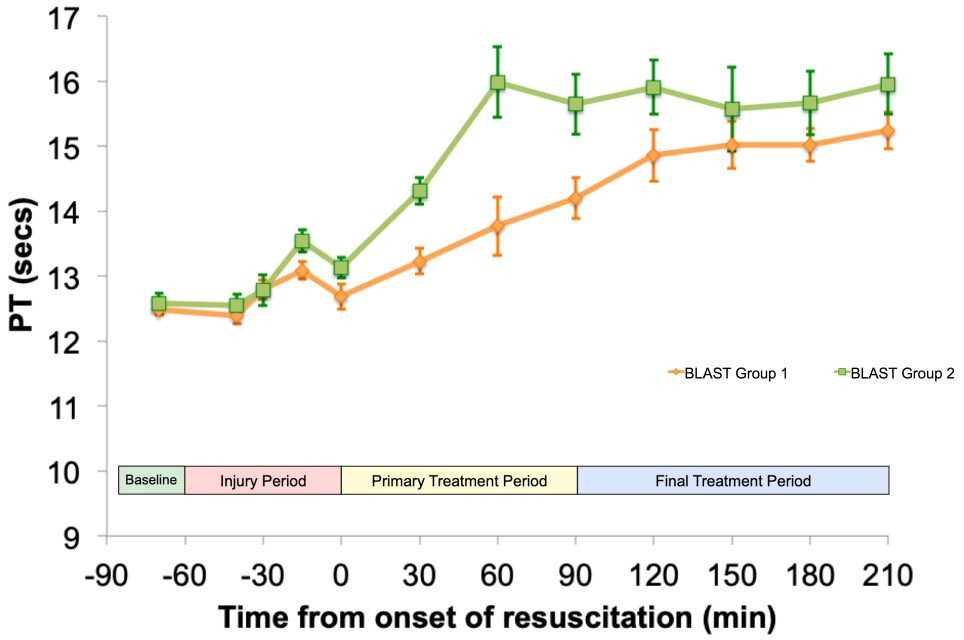


Figure 8-62. PT in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

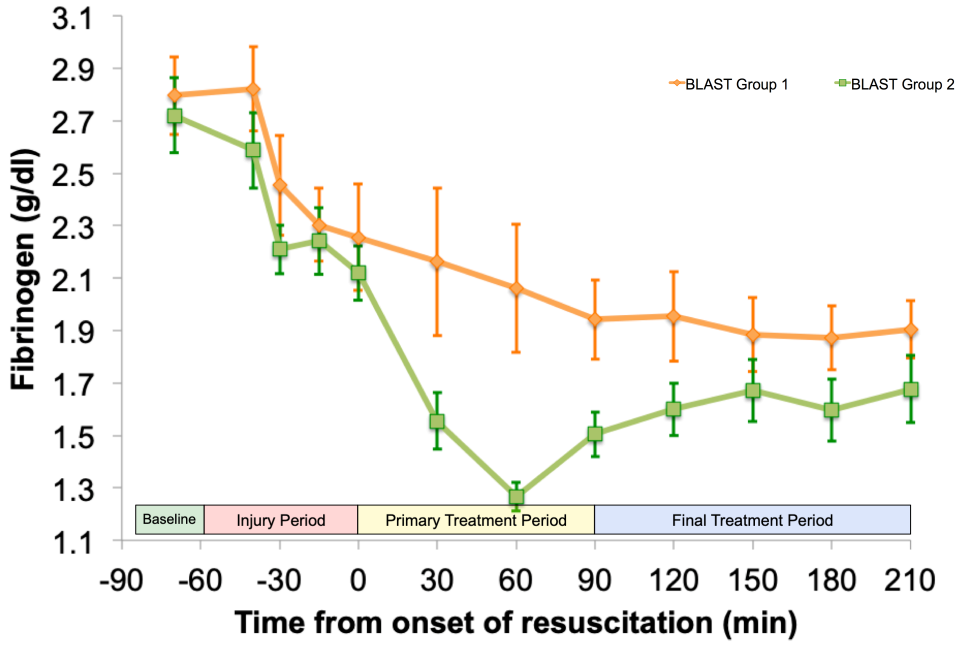


Figure 8-63. Fibrinogen in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

8.9.2 *Physiology – Final Treatment Period (Blast Groups)*

Blood pressure targets were maintained for the final period of the investigation (Table 8-8 and Figure 8-64). Despite being within 6mmHg of each other throughout the investigation, there was a statistically significant difference between the groups, which is probably not physiologically important. The remaining cardiovascular physiology measurements plateaued during this period and remained at the same levels as those at R90. There was a significant difference in heart rate (HR) for the resuscitation period (P = 0.013; Figure 8-65) which, can be attributed to the lower HR observed for the majority of the Primary Treatment Period in the pre-hospital saline group. Group 2 having started to receive blood components in-hospital exhibited a surge in cardiac output at this transition and consequently there was significant difference between groups for values (P = 0.006), although pattern was similar (P = 0.773; Figure 8-66).

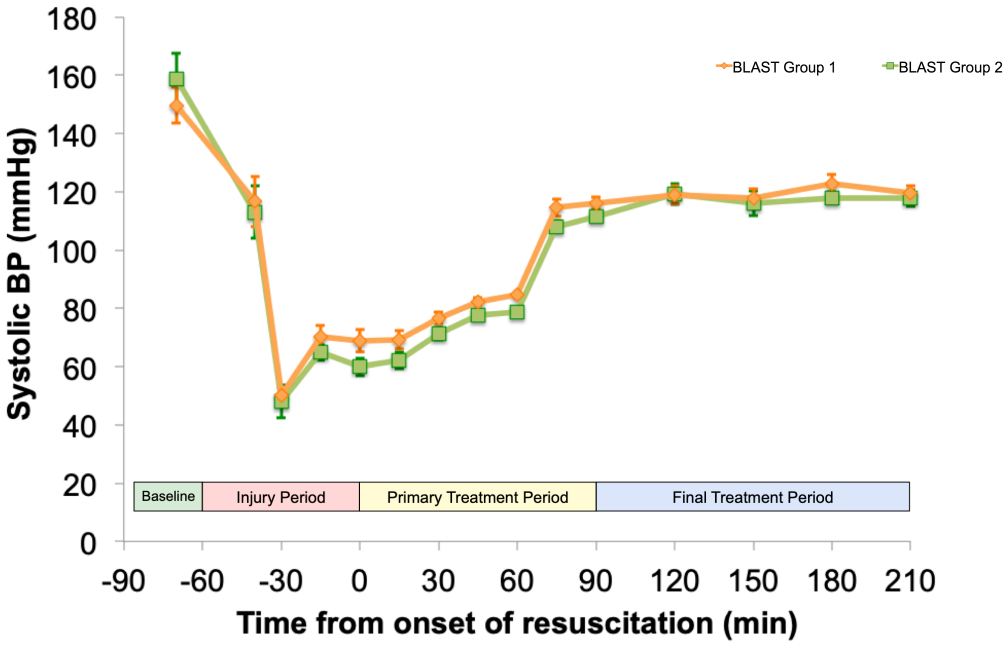


Figure 8-64. Systolic Blood Pressure in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

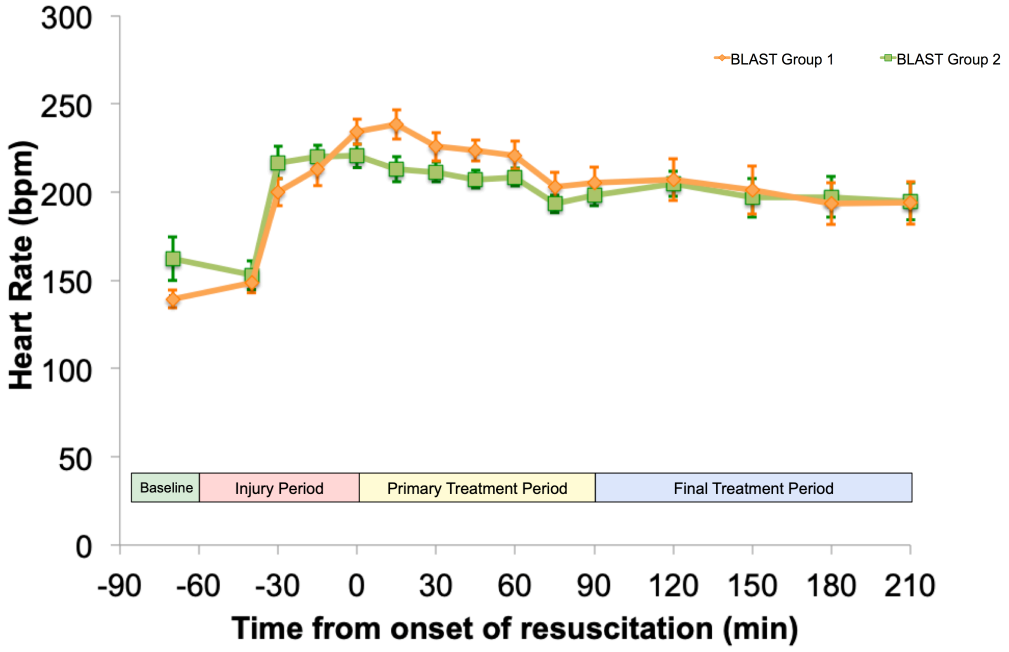


Figure 8-65. Heart Rate in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

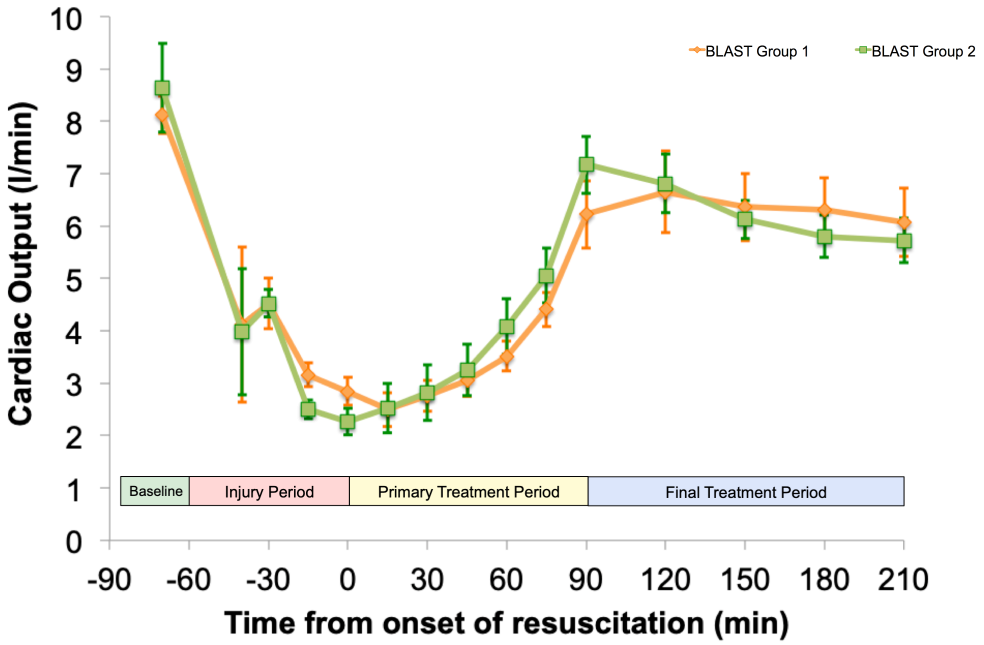


Figure 8-66. Cardiac Output in the SHAM injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

Throughout the remaining in hospital phase the trend for less negative ABE in Group 1 (pre-hospital PRBC:FFP) endured as both groups returned towards baseline levels, although the difference was not enough to obtain statistical significance (see Table 8-8 for *P* values and Figure 8-67). Lactate levels were significantly different, but not physiologically different for values with no statistical difference in the pattern between groups (*P* = 0.831; Figure 8-68).

At R210 the values of PaO_2 and $PaCO_2$ were similar between both groups, however over the whole resuscitation period there was a statistically significant difference in patterns over time between the groups in $PaCO_2$ (*P* < 0.003; Table 8-8). The majority of this difference occurred in the pre-hospital phase where the saline-treatment Group 2 had a consistently lower $PaCO_2$ (Figure 8-69) probably caused by a greater respiratory compensation in response to the developing metabolic acidosis.

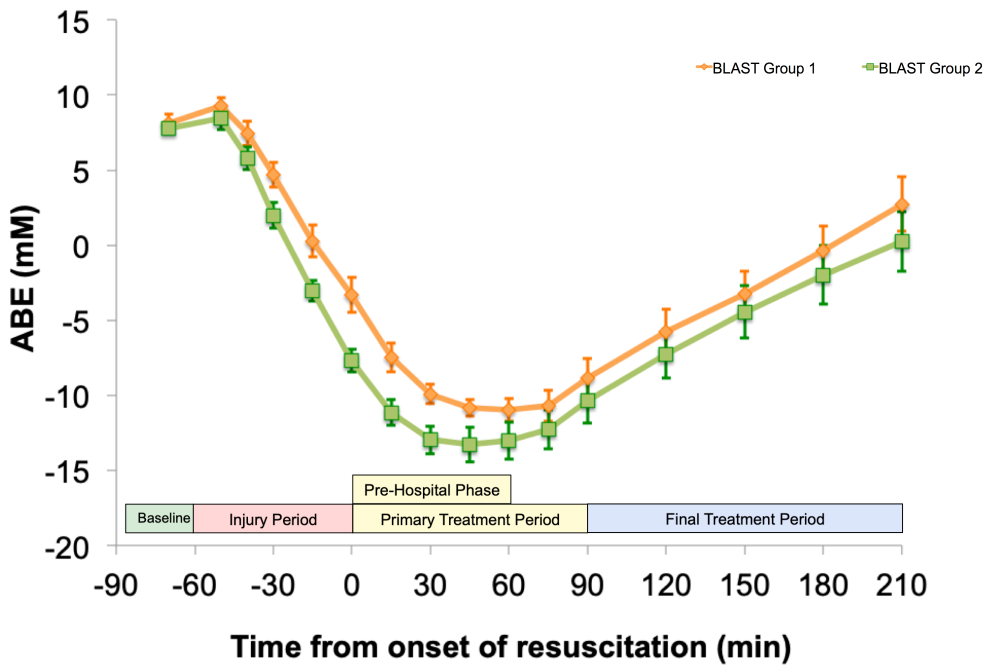


Figure 8-67. Arterial Base Excess in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

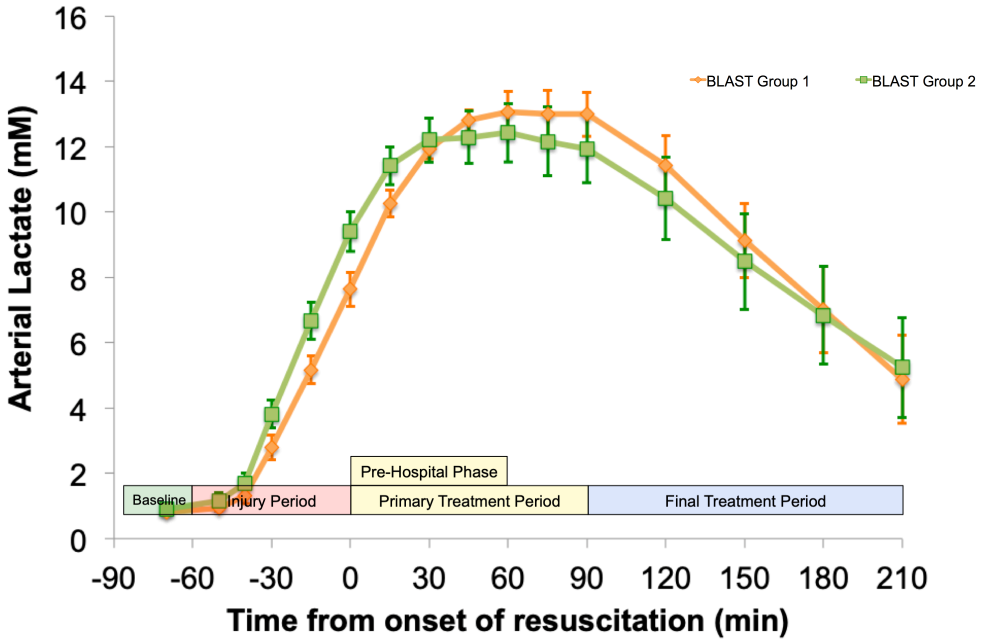


Figure 8-68. Arterial Lactate in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

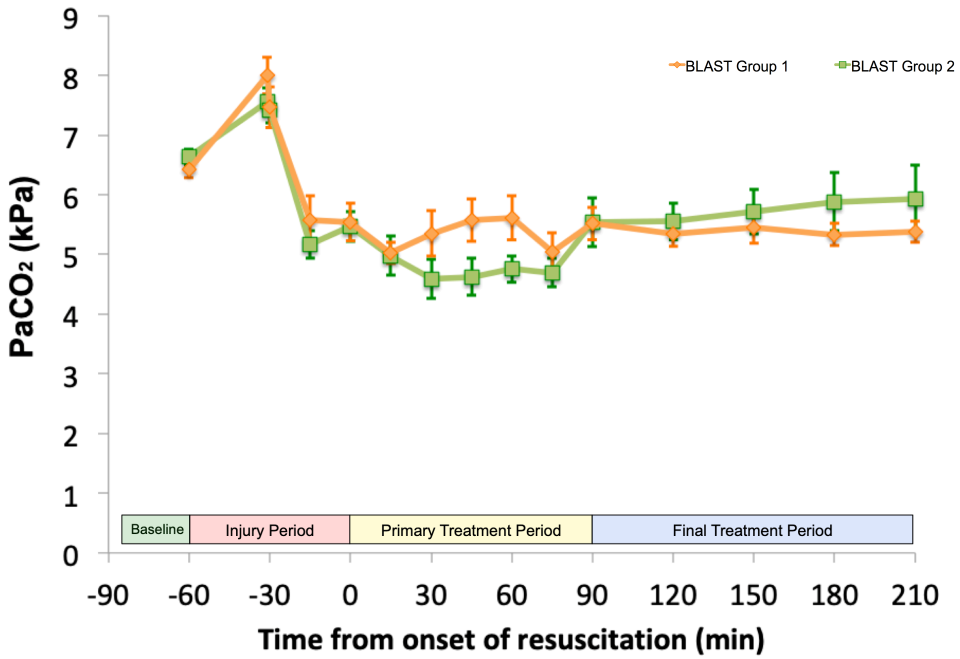


Figure 8-69. Partial pressure of arterial carbon dioxide ($PaCO_2$) in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

The OER and CvO_2 levels remained similar to that at R90 throughout the Final Treatment Period with no difference between groups in either parameter (Table 8-8 and Figure 8-70).

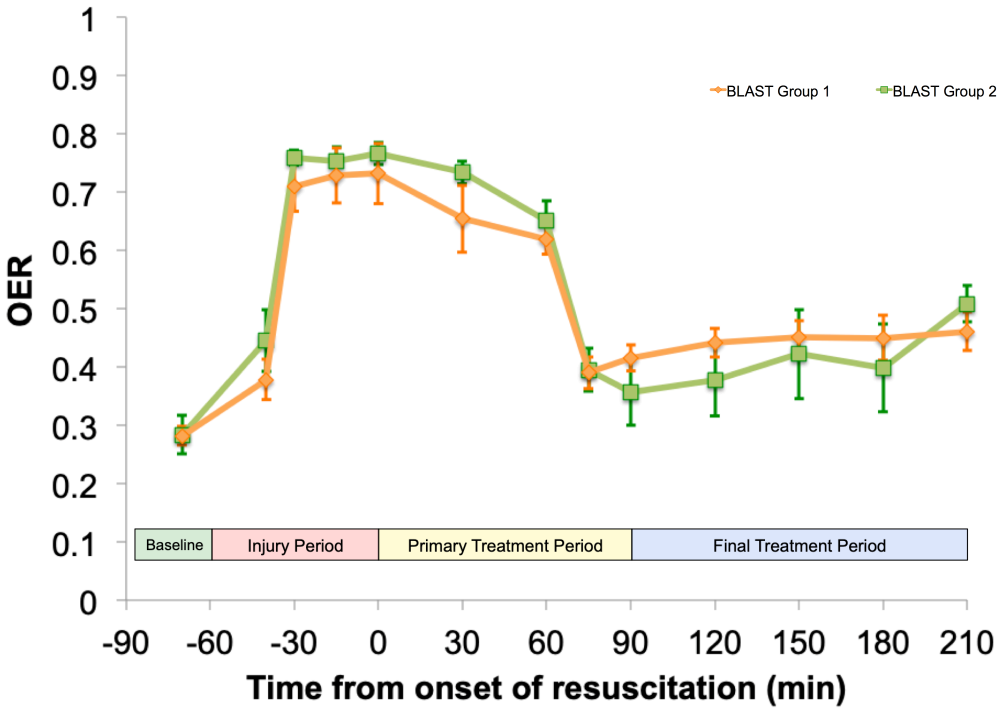


Figure 8-70. Oxygen Extraction Ratio in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

In-hospital blood component resuscitation to a normotensive goal improved cardiac output and reduced the degree of shock. The group receiving pre-hospital saline displayed a greater degree of shock that then returned towards baseline levels with little difference between groups by the end of the investigation.

8.9.3 *Haematology – Final Treatment Period (Blast Groups)*

During the remaining in hospital phase with both groups receiving blood component resuscitation, there was little change in haemoglobin (Hb) and haematocrit (Hct) from R90. Both groups remained at a significantly lower level than baseline ($P < 0.001$, change over time) with a significant difference in pattern over time between groups ($P < 0.001$) secondary to the significant drop in both Hb and Hct in the pre-hospital phase for the saline resuscitated group (2). This difference remained throughout the in-hospital phase despite blood component resuscitation (Figure 8-71).

Platelet count remained lower than baseline in both groups ($P < 0.001$) with no difference between groups ($P = 0.492$; Table 8-8 and Figure 8-72).

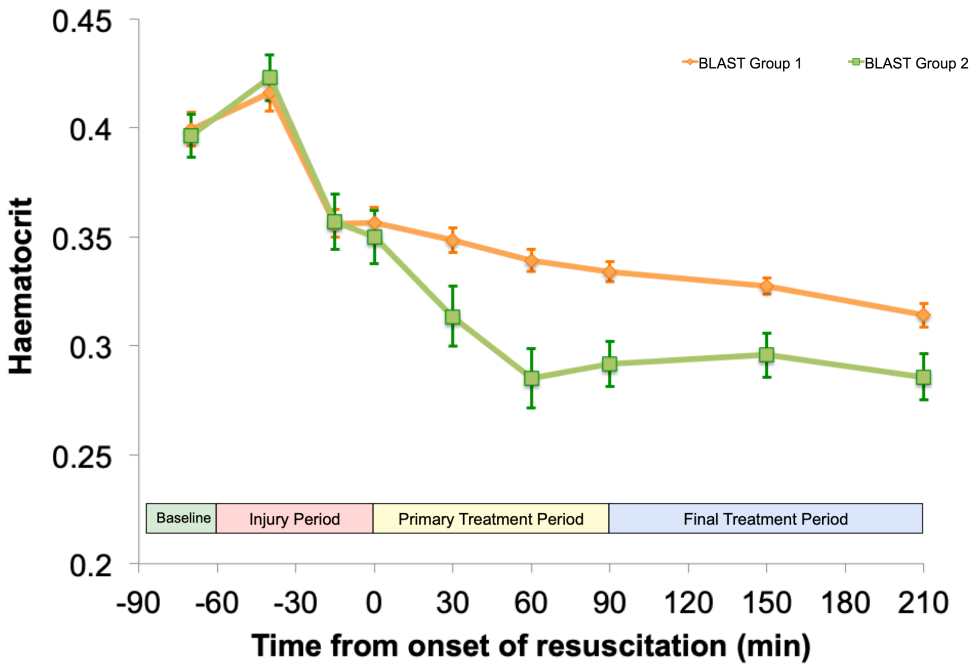


Figure 8-71. Haematocrit in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

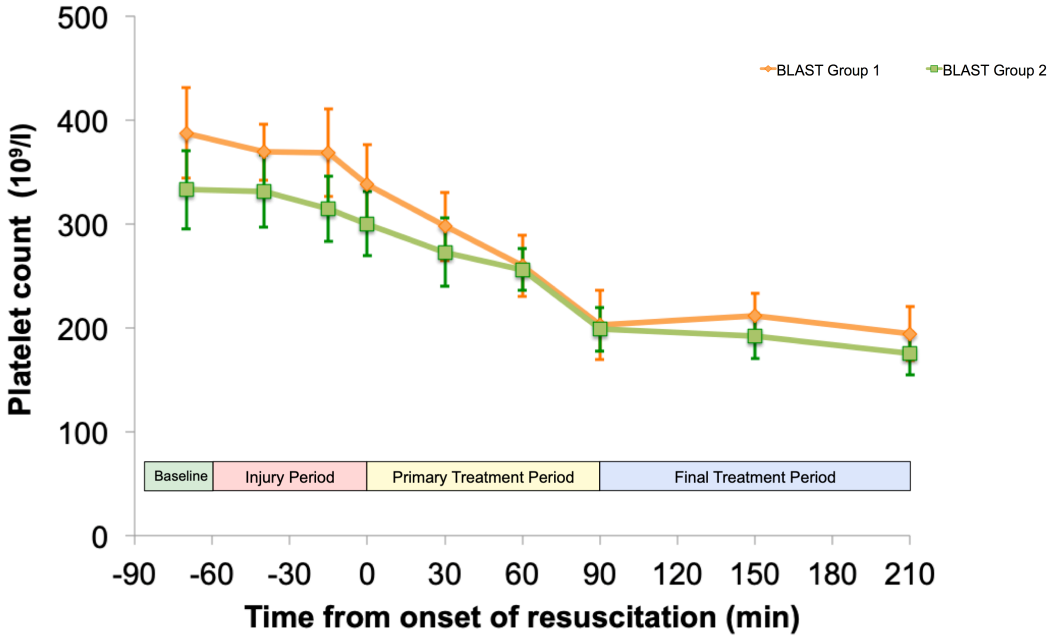


Figure 8-72. Platelet Count in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

Throughout the investigation both groups showed a drop in haematocrit and haemoglobin with the pre-hospital saline group having lower levels of both parameters throughout, particularly in the pre-hospital phase. Platelet count dropped steadily and similarly in both groups throughout the study period.

8.9.4 Fluids – Final Treatment Period (Blast Groups)

The total fluids given in hospital are illustrated in Figure 8-73 and the cumulative fluids given to each group are illustrated in Figure 8-74.

For the Final Treatment Period, there was no significant difference between the volumes of fluid each group received (P = 0.215). However, for the whole resuscitation period Group 2 (pre-hospital saline) received a

significantly greater volume of fluids than Group 1 ($P < 0.001$), but the total volume of blood products received by both groups was similar ($P = 0.392$).

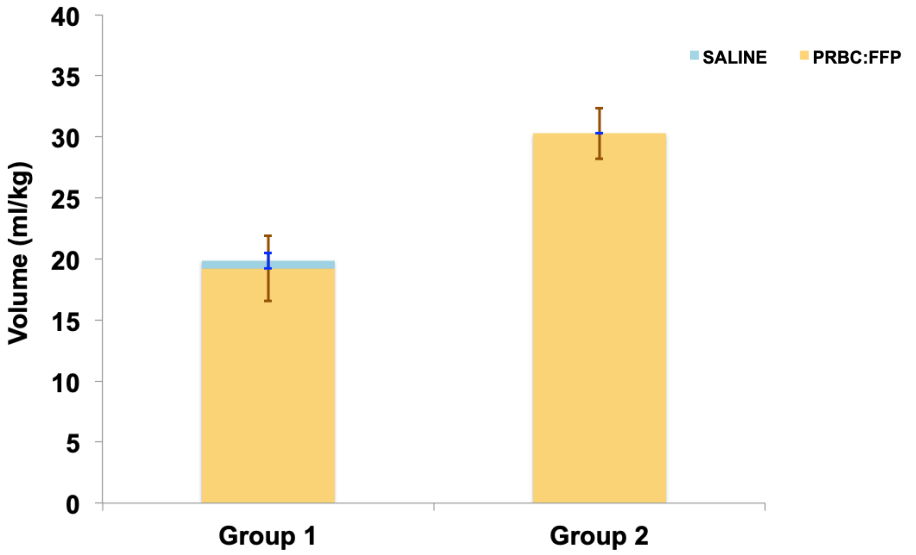


Figure 8-73. Total volume of fluids given in the in-hospital early and late phases for BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Values are Mean ± SEM.

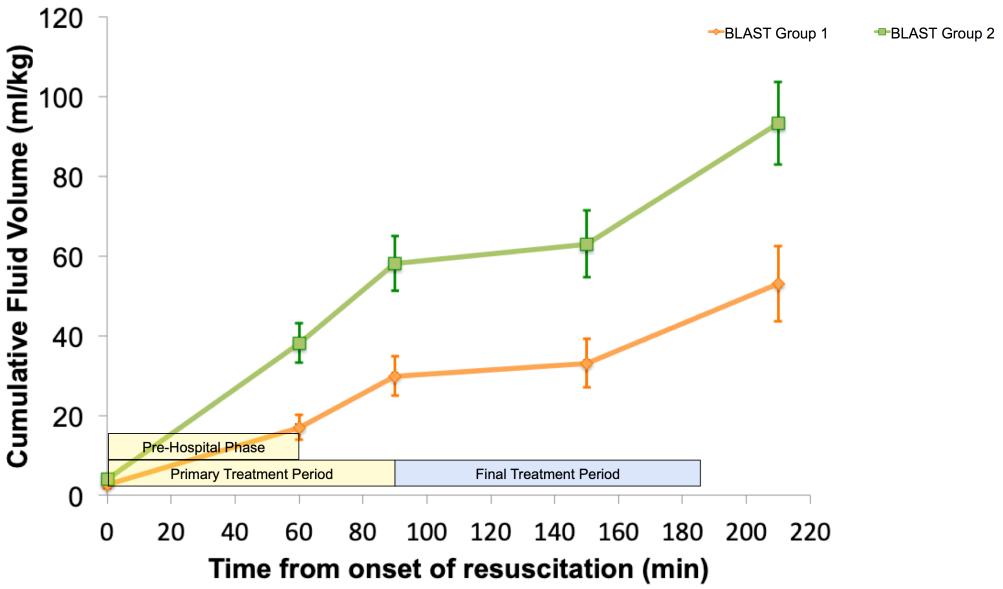


Figure 8-74. Cumulative volume of fluids given to each group for BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Values are Mean ± SEM.

Group 2 (pre-hospital saline) received a significantly greater volume of fluids in total as a consequence of the significantly greater (P = 0.007) volume of saline received in the pre-hospital phase.

8.9.5 Biochemistry – Final Treatment Period (Blast Groups)

For the in-hospital phase Group 2 (pre-hospital saline) received blood components at a higher rate than the other group. As a consequence that group had a significantly higher potassium level (P = 0.001) and a lower calcium level than the Group 1. The difference in calcium levels did not achieve significance (P = 0.076; Table 8-4 and Figure 8-75).

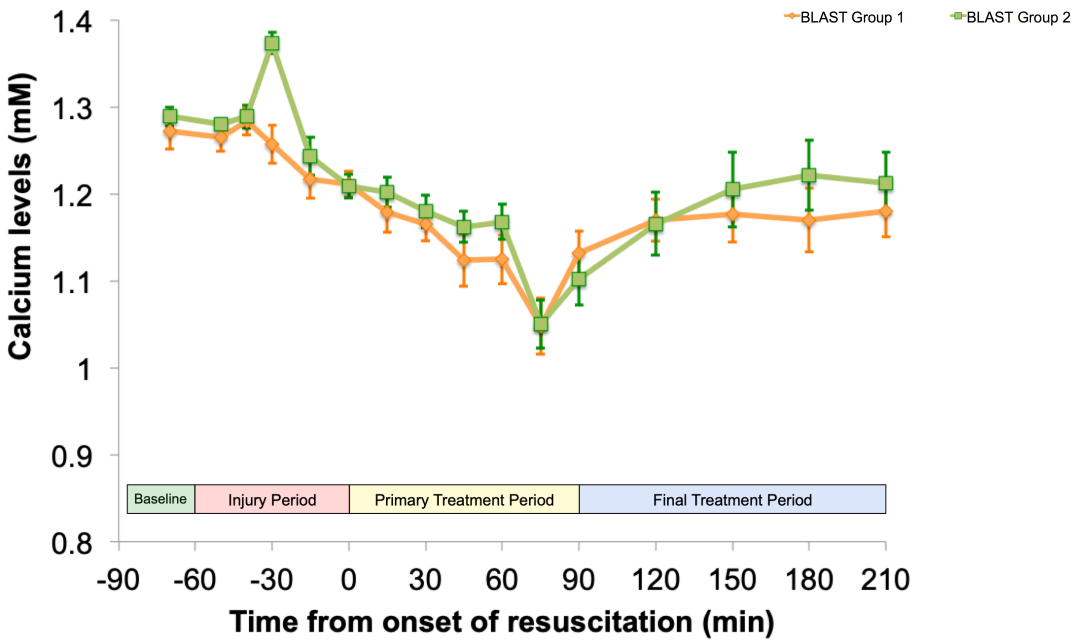


Figure 8-75. Calcium levels in the BLAST injury groups. Group 1 - pre-hospital blood component resuscitation. Group 2 - pre-hospital saline resuscitation. Mean values ± SEM.

Chapter 9. Discussion

This investigation produced an animal model of trauma coagulopathy in a severely injured, hypotensive casualty with evidence of shock that required pre-hospital resuscitation in order to sustain life on the way to the hospital. The injury sustained was a significant blunt tissue injury (representing a ballistic injury), controlled haemorrhage and haemorrhagic shock with or without a primary blast injury. Resuscitation was carried out successfully to set, clinically relevant goals in separate pre-hospital and in-hospital phases, resulting in 100% survival in all groups.

The principal finding of this study is that the use of blood components for pre-hospital resuscitation attenuates the coagulopathy developed by a group resuscitated with pre-hospital saline.

9.1 Coagulation

For severe injuries and haemorrhagic shock in the absence of blast, the pre-hospital blood component group developed no coagulopathy compared to the saline group as measured by viscoelastic techniques. A full 60 to 90 minutes of hospital based, aggressive, blood component resuscitation was required to return the coagulopathy developed by the pre-hospital saline group to normal. In contrast these injuries plus an additional primary blast injury had a significant impact on both resuscitation groups, with the pre-hospital blood component resuscitation group showing a tendency to a lesser coagulopathy.

Overall the impact of blood products is qualitatively similar in both blast and sham strands; it attenuates the coagulopathy seen in the saline treated

group, although the difference after blast is somewhat less due to the greater impact of blast on coagulation.

The coagulopathy developed was observed to be predominantly due to clot initiation and amplification (TEG R and K times) suggesting a potential deficiency in clotting factors and/or fibrinogen. aPTT levels were raised at the same time points; indicating a reduced level of clotting factors (FII, FV, FVIII, FIX, FX, FXI, FXII) or fibrinogen. A noticeable drop in fibrinogen was noted in all groups, with both pre-hospital saline groups having the greatest decline to a significantly lower sub-normal level. The nadir of the fibrinogen levels mirrored the timings of the worst phase of the coagulopathy.

In accordance with previous work on trauma coagulopathies, a reduction in fibrinogen is a consequence of the Acute Traumatic Coagulopathy (ATC) secondary to the actions of the activated protein C (APC) pathway (Brohi 2008, Floccard 2012). The lowest fibrinogen levels reached by the pre-hospital saline group were similar to the levels found in previous human studies on fibrinogen and ATC where it was associated with increased mortality (Rourke 2012).

The drop in fibrinogen levels and platelet counts observed in all groups approached clinically relevant levels by hospital admission and reinforces the guidance given in military (CGOs) and civilian (Rossaint 2016) resuscitation practice that both of these should be supplemented early in a trauma casualty's in-hospital resuscitation, particularly if that patient has received pre-hospital saline. There is a mounting argument for pre-hospital fibrinogen supplementation in severely injured patients.

The raised aPTT observed is in all probability secondary to reduced fibrinogen levels, rather than consequent to a lack of clotting factors. A factor deficiency would have been indicated by a change in both PT and aPTT. Although PT did change, this was initially small. A steady escalation in PT followed, reaching its maximum at the end of the investigation, when all groups' coagulation as measured by all other parameters had returned to

normal. If studied in isolation this would suggest there might have been a steady reduction in clotting factors in all groups, but this was not reflected in the other coagulation parameters. Although this would be a trend requiring monitoring, this appears to be a clinically unexceptional finding in view of the fact that the PT remained within or very close to the normal range at all times. As the continued rise in PT across the investigation period had no impact on the other coagulation parameters, this infers that if a clotting factor deficiency was present, its part in the coagulopathy was minor.

More remarkable was the results of a parallel investigation in the same model using pre-hospital PRBC only (Watts 2015), where it was found that the use of PRBC alone in the pre-hospital phase was as effective as PRBC:FFP in avoiding a coagulopathy. This finding is consistent with the concept that tissue hypoperfusion and reduced oxygen delivery cause an endotheliopathy, an early driver for Acute Traumatic Coagulopathy (Brohi 2007a, 2008, Naumann 2018).

9.2 Physiology

Secondary, but related findings pertain to the physiological state of the animals. All groups developed a significant degree of shock during the pre-hospital phase as illustrated by a distinct drop in base excess and elevated lactate levels. Oxygen extraction was maximal in all groups in the pre-hospital phase with an equally high oxygen extraction ratio (OER) and low mixed venous oxygen content in all groups, suggesting the low blood flow state that occurs in pre-hospital resuscitation is the limiting factor for oxygen delivery, rather than blood oxygen content. Again the parallel PRBC only investigation (Watts 2015) produced interesting results for non blast injuries where the shock burden was greater for PRBC only resuscitation compared to PRBC:FFP resuscitation; an ostensibly counter-intuitive result as the higher oxygen carrying capacity fluid was associated with a greater shock burden. Although oxygen delivery to reduce the effects of hypoperfusion is

vital, it is not the only component involved in reversing the coagulopathy. Plasma may have a beneficial effect on the microvasculature, feasibly via an action on the endothelial glycocalyx (Pati 2010, Kozar 2011, Torres 2013, 2014). This function of plasma, independent of individual clotting factors appears to reduce the endothelial inflammatory response in trauma, possibly by limiting neutrophil adhesion and leading to a restored glycocalyx within hours of plasma transfusion (Barelli 2018), resulting in better microvascular flow and hence delivery of oxygen. A recent study illustrating the mortality benefits of plasma only pre-hospital resuscitation (Sperry 2018) further supports this theory.

The results concur that both oxygen delivery *and* plasma derived endothelial protection is needed to reverse the Acute Traumatic Coagulopathy observed in severely injured patients. Timings aside it transpires from this study's results that both PRBC and FFP are essential and equal elements to damage control resuscitation in a 1:1 ratio. This model shows that pre-hospital blood component in a 1:1 ratio resuscitation confers benefit extending into relevant in-hospital phases (surgical decision making point) even when aggressive resuscitation strategies are used from the outset in the in-hospital phase.

Closer examination of the physiology data illustrates that in the absence of blast the effect of PRBC:FFP resuscitation has a long lasting effect, extending far beyond the coagulation benefit timelines. The pre-hospital saline group continued to have a base deficit and higher lactate beyond the period coagulation had returned to normal, despite 2 hours of in-hospital blood component resuscitation. The blast injury pre-hospital saline group again displayed a similar pattern in base deficit, but did not achieve statistical significance.

Cardiovascular responses were appropriate for the injury challenges confronted by each animal. At times mean arterial pressure (MAP) was significantly different between treatment groups despite no difference in systolic blood pressure (SBP). This was most evident in the pre-hospital

phase when the sham pre-hospital saline group had a significantly lower MAP in in association with a lower cardiac output. That group in receipt of a larger volume of fluid may have had a reduced arterial vasoconstriction response to haemorrhage, although if this hypothesis was correct peripheral perfusion should have been better within this group. This was not the case as acidosis (arterial base excess) was worse in this group, unless there was a local microvascular disturbance present that was not necessarily reflected in gross haemodynamics. The systolic blood pressure targets were achieved and the response in heart rate was largely appropriate. Of interest was the significantly lower heart rate in both pre-hospital saline groups in the pre-hospital phase, despite similar or lower cardiac outputs. The assumption is that this was secondary to the greater volume of fluid both saline groups received during this period.

The sham pre-hospital saline group developed a greater shock burden compared to the blood component resuscitation group, the timing of which correlated with the coagulopathy. The acidosis that developed in the pre-hospital phase in all groups was also associated with a reduced carbon dioxide partial pressure ($PaCO_2$). This is a classic respiratory response to a metabolic acidosis: the acidosis stimulates the peripheral chemoreceptors, leading to increased ventilation and a reduced $PaCO_2$, and consequently a partial respiratory compensation for the metabolic acidosis. There was a significant difference in $PaCO_2$ between treatment groups in both sham and blast strands. Both pre-hospital saline groups had a comparatively lower $PaCO_2$ in the pre-hospital phase, at a time associated with a comparatively higher level of acidosis in that group, which is consistent with the explanation given above. In the sham strand the saline-treatment group's greater acidosis persisted into the in-hospital phase and concurrent with that, a comparatively lower $PaCO_2$ endured.

9.3 Effect of Blast

The effect of blast on trauma casualties is not only relevant to military physicians, but in an uncertain and increasingly high-risk terror related World it is a pertinent subject that civilian physicians need to be cognisant of. The evidence from this study is that blast produces a patient that is considerably worse physiologically. Despite clinically relevant blood pressure goals and the use of blood components, those animals exposed to blast were worse off, as measured by coagulation and physiological parameters. Not only did this lead to an inability to observe a clear statistical difference between groups, but it does raise the question of what relevance does this have.

It could be argued that because of the reduced difference between treatment groups, and the increased variability due to the blast element of the model, this aspect of the study was under-powered. The obvious way to resolve this problem would have been to increase the group sizes in the blast strand of the study. However, after a cost-benefit evaluation (cost in terms of animal use and benefit in terms of improving treatment of human casualties), it was decided that the additional use of animals was not justified. This was because the effects of blood products in the blast strand were qualitatively similar to those seen in the sham blast strand (where a clear, statistically and clinically significant, effect was seen); after blast there was an indicative trend suggesting that blood products were beneficial in exactly the same way as that seen in the sham blast strand, and no suggestion of a detrimental effect. Consequently, it is arguable that the current study, taken in its entirety, supports the necessity to give blast-injured as well as non-blast injured patient groups blood components as early as possible. This conclusion is emphasized by the clinical observation that the majority of blast-related casualties are more likely to be suffering penetrating ballistic injury from blast, rather than a primary blast injury. Unfortunately in the pre-hospital environment there is no way of distinguishing the two types of blast casualty except in the most severe of circumstances. Giving blast casualties blood components early will at worst

bring with it either a minimal or, at best, clear beneficial consequence and as such it would be imprudent not to state that blood products should be recommended for blast and non-blast casualties.

9.4 Hypercoagulation

One feature of our model was the presence of a self-resolving increased coagulation immediately after injury lasting for approximately 30 minutes. Hypercoagulability is well recognised in trauma, but normally only considered after 24 hours in humans (Selby 2009), although blunt trauma casualties have been found to have evidence of pro-coagulation at 2 hours (Park 2012). Animal models have shown it is present after trauma, but again usually later in the injury model; 1 hour after injury (Riha 2013, Prat 2015), 4 to 72 hours after injury (Mulier 2012). One swine study did find similar findings to ours with a reduction in fibrinogen and reduced TEG R time within minutes of injury (Duan 2014). This is an understandable reaction by the coagulation system in order to form an adequate clot rapidly in response to injury, but there is a suggestion that it may not be appropriate in all cases. There is evidence that ballistic and blast casualties have a high incidence (9.3%) of abnormal pulmonary thrombus (Lundy 2013). The incidence of later diagnosed Pulmonary Embolus is similar in certain populations (13% in military amputees; Palm 2012). This study's results suggest a foundation for this thrombo-embolic occurrence and so we must be wary of the pharmacological manipulation of the fibrinolytic system in trauma without good cause.

9.5 Comparative Studies

The results of our study are in agreement with a recent observational clinical study that compared pre-hospital transport platforms, one of which

used plasma and red cells in a 1:1 ratio, the other using crystalloids (Holcomb 2015a). The patients receiving pre-hospital blood components had a statistically significantly improved physiological status (less base deficit) and a reduced volume of pre-hospital fluids required. The investigators found no difference in coagulation status, as measured by TEG, however. An explanation for this is the degree of shock their patients were in was not pronounced (ABE of -3 mM) and much less than in our study (-10 to -15) and less than that found in Helmand (-5 to -14; Morrison 2013a).

Equal ratios of plasma to red cells have been investigated in the hospital setting showing an improved 6-hour survival (PROMMTT – Prospective Observational Multicentre Major Trauma Transfusion; Holcomb 2013, del Junco 2013) and fewer deaths from exsanguination at 24 hours (PROPPR – Pragmatic Randomized Optimal Platelet and Plasma Ratio; Holcomb 2015b), but evidence of clear longer term mortality benefits have been lacking in part due to trial constraints and timing of death in severe trauma. Our study did not investigate the longer-term mortality benefits, but concentrated on the early benefits of blood products and is therefore in agreement with these other studies findings and comments, despite them being from a civilian hospital setting.

Military trauma casualties treated for a potentially prolonged time in the pre-hospital space are arguably at greater risk of death. This study was carried out to investigate a set pre-hospital time and challenges that can occur in this environment. A more military relevant investigation has been completed recently, the findings of which are in agreement with our findings. It was a retrospective cohort study of pre-hospital blood product transfusion of over 500 military combat casualties in Afghanistan (Shackelford 2017) and therefore is the most relevant translational study associated with this animal study. Timelines, capability, blood component or saline resuscitation and indeed treatment by MERT-E are all features of Shackelford's study. By matching non-recipients of blood components to balance factors such as injury severity with recipients, they were able to show that pre-hospital blood

product transfusion was associated with a greater 24-hour and 30-day survival.

9.6 Model Limitations

9.6.1 Coagulation Differences

It is acknowledged that the coagulopathy observed in this model is not identical to that observed in humans. There are key similarities with variation in R times and aPTT (and to some extent PT) in addition to a drop in fibrinogen. Overt fibrinolysis was not observed, nor the reduction in MA width one would expect (despite a drop in fibrinogen) with Acute Traumatic Coagulopathy (ATC). The greater proportion of similarities though suggest the model produced an abnormal coagulation state secondary to the trauma, rather than any other potential cause, such as dilution. The resuscitation was conducted to meet defined, clinically relevant, hypotensive targets in the pre-hospital phases and limit the volume of fluid infused to a minimum required to sustain life. During model development failure to provide this fluid was incompatible with survival. Crystalloid was used in the immediate post-injury shock phase and in the control groups for the pre-hospital phase because this is still in widespread use. This produced a degree of haemodilution (drop in haematocrit), which is valid, as it represents the dilution present in current pre-hospital crystalloid resuscitation regimes. This dilution must be interpreted in context with other animal models. In Group 3 (sham, pre-hospital saline) it amounted to approximately ⅔ of the remaining blood volume. By contrast a recognised model published by Cho (2009) infused over 4 times the end haemorrhage blood volume. The coagulopathy observed in this study may have an element of dilution, but comparatively it is not an all-consuming degree of dilution. It is likely that the coagulopathy observed had a dilutional element present, but the volume of fluid given was kept to a minimum compatible with sustaining survival using relevant hypotensive resuscitation targets.

The results of this study are therefore relevant to a situation where a severely injured casualty demands limited resuscitation to sustain life prior to evacuation to a hospital and is therefore relevant to not only military battlefield casualties but also some civilian trauma victims.

9.6.2 Biochemical Differences

The biochemical changes that occurred in all groups did not reflect those commonly observed in damage control resuscitation. Potassium was observed to rise after injury, but did not make significant changes with blood component resuscitation. This difference from human clinical practice may be partly secondary to the short storage time for the blood used (48-72 hours). Calcium dropped steadily with resuscitation reaching levels of almost 1.0 mM by initial hospital admission. The resultant rise in levels despite ongoing blood component resuscitation in all groups was unexpected. It is possible porcine calcium homeostasis is more efficient than humans and the levels observed in this study were a reflection of rate of resuscitation and the resulting homeostatic balance of calcium levels. When confronted with a high level of infusion (end of pre-hospital and start of in-hospital phases), calcium levels dropped more rapidly. Calcium levels rose again once infusion rates diminished again (remaining in-hospital phase). The cause of calcium reduction in relation to pre-hospital saline resuscitation cannot be easily explained.

9.6.3 Equipment Used

In this investigation thromboelastography was the method of choice for clotting assessment for a number of reasons. Viscoelastic testing is considered by many to be superior to conventional laboratory tests (PT, aPTT) in both pigs (Martini 2008) and humans (Veigas 2016, Roullet 2018) when investigating haemorrhagic shock and trauma related coagulation abnormalities. The ROTEM®*delta* is the Defence Medical Services prime

choice in this role; its inflexibility of operation precludes ease of use in laboratory based research. Thromboelastography fulfilled this role in our investigation facilitating the ability to investigate different activation compounds in the pilot study. Reassuringly however aPTT gave a similar pattern (although not always statistically significant) to that seen with the TEG parameters.

9.6.4 Animal Model

There are limitations to the study described in this thesis. It is animal-based, and differences as well as similarities to the human response need to be acknowledged. The hemodynamic response to trauma is broadly similar between humans and pigs and as such it is a widely acknowledged model for human trauma, with the porcine splenectomy removing the capacity to autotransfuse splenic blood. Although there are similarities in clotting between pigs and humans, there are also important differences, e.g., uninjured pigs are hypercoagulable compared with humans. These differences are important when evaluating some of the specific component therapies, but they do not exclude the general deductions that can be concluded regarding patterns of changes described in this report. Caution must always be exercised when comparing between species; patterns of response rather than absolute numerical values should be compared. It is notable in the present study that the coagulopathy was characterised on the basis of clot initiation and dynamics, but no change was seen in clot strength despite a fall in fibrinogen and a marked coagulopathy developing based on the other parameters. The solution to these limitations of animal-based studies is to triangulate results between species and always make comparisons to translational human data whenever it is available.

9.7 Model Improvements

The inability to find a significant difference between the different groups in the blast arm for coagulation parameters despite the saline resuscitation group showing a trend for a coagulopathy was unsatisfactory. Data from one animal was missing as a consequence to equipment failure. The impact of this missing data meant a lack of power in the statistical analysis and significance was not achieved. Keeping the number of animals to the minimum number required in order to complete the study is a valid and ethical decision. However the potential for equipment failure or other complications must be kept in mind and consideration of investigating additional animals to allow for this should be heeded.

The coagulation parameters investigated could be enhanced so that specific aspects of Acute Traumatic Coagulopathy can be fully examined. Use of ROTEM and its additional assays could provide additional information on fibrinogen (FIBTEM) and fibrinolysis (APTEM). The addition of simple additional tests could elucidate further information on fibrinolysis such as fibrin degradation product (FDP) measurement. Assessing specific clotting factors levels could assist in the determination of the specific causes of elevated PT despite all other coagulation parameters improving.

9.8 Future Studies

There has been a recent change in military campaigning with a move away from sustained conflict by large numbers of troops to smaller Units deploying to more isolated locations. The impact on medical care will be the need to care for severely injured casualties in a resource-constrained environment for prolonged periods. Henceforth it is imperative to investigate how these patients can be resuscitated and their life sustained for prolonged periods prior to surgical intervention. Can adequate pre-hospital resuscitation take place for 3 or more hours with a severely injured

casualty and how can the medical team realistically monitor the effectiveness of their resuscitation strategy? If logistical support is inhibited, the fluids and medications used in that resuscitation need to have a reduced logistical impact, being free from temperature constraints. Resuscitation using only stable, easily stored compounds should be investigated; lyophilised plasma and fibrinogen concentrate with appropriate adjuncts such as tranexamic acid or rFVIIa. Indeed a valid austere logistically contested study would be to look at the effects of using tranexamic acid and fibrinogen (Fibrinogen Concentrate) only as a resuscitation regime.
In view of the current and increasing interest in its use in trauma, there should also be a place to investigate the use of fresh whole blood (FWB) as either an adjunct or as a sole resuscitation fluid. There is no better storage medium for blood components than within the donor, and its use would result in the removal of a significant logistical burden.

A follow up study should be performed using the animal model developed for this study and adapt it to encompass the effects of prolonged pre-hospital timings, the use of basic markers to aid resuscitation (arterial base excess) and using resuscitation regimes that are purely lyophilised or use FWB as a sole resuscitation fluid, compared to PRBC and FFP in a 1:1 ratio. The potential investigation of inflammatory biomarkers may give an indication of subsequent complications related to the inflammatory system such as multiple organ failure and sepsis.

9.9 Summary

The impact of blood products appears to be qualitatively similar in both blast and sham blast casualties in that it attenuates the coagulopathy observed in a saline treated ballistic injury group with the use of blood pre-hospital components. The difference after blast is somewhat less due to the greater impact of blast on coagulation.

The findings of this study are relevant to civilian pre-hospital providers particularly if they can overcome the logistical challenges of providing blood components outside of the hospital environment. In conclusion, this study provides evidence that the use of pre-hospital blood products (PRBC and FFP in a 1:1 ratio) confers an advantage compared with the old standard of care (limited crystalloid administration). This resuscitation regime supports a treatment strategy that was started pragmatically by clinicians (MERT) in Afghanistan and remains extant as the gold standard for military trauma resuscitation.

Bibliography

AAGBI. Association of Anaesthetists of Great Britain and Ireland. Recommendations for standards of monitoring during anaesthesia and recovery 2015. Anaesthesia 2016; 71: 85-93.

ACS. American College of Surgeons. Advanced Trauma Life Support (ATLS): the Ninth Edition. Chicago: American College of Surgeons, 2012

Adamzik M, Gorlinger K, Peters J, Hartmann M. Whole blood impedance aggregometry as a biomarker for the diagnosis and prognosis of severe sepsis. Critical Care 2012; 16(5): R204.

Agarwal S, Coakley M, Reddy K, Riddell A, Mallett S. Quantifying the effect of antiplatelet therapy: a comparison of the platelet function analyzer (PFA-100) and modified thromboelastography (mTEG) with light transmission platelet aggregometry. Anaesthesiology. 2006; 105: 676-683.

Allen C, Valle E, Jouria J, et al. Differences between blunt and penetrating trauma after resuscitation with hydroxyethyl starch. J Trauma 2014; 77(6): 859-64.

Allen C, Ruiz X, Meizoso J, et al. Is Hydroxyethyl Starch safe in penetrating trauma patients? Mil Med 2016; 181(S5): 152-155.

Alson R, Braithwaite S. Role of TXA in management of traumatic hemorrhage in the field. International Trauma Life Support, TXA Resource Document. www.itrauma.org 2014.

American Society of Anesthesiologists Task Force on Perioperative Blood Transfusion and Adjuvant Therapies: Practice guidelines for perioperative blood transfusion and adjuvant therapies: an updated report by the American Society of Anesthesiologists Task Force on Perioperative Blood Transfusion and Adjuvant Therapies. Anesthesiology 2006, 105: 198-208.

Anderson L, Quasim I, Steven M, Moise SF, Shelley B, et al. Interoperator and intraoperator variability of whole blood coagulation assays: a comparison of thromboelastography and rotational thromboelastometry. J Cardiothorac Vasc Anesth 2014; 28(6): 1550-1557.

Angell James J. The effects of altering mean pressure, pulse pressure and pulse frequency on the impulse activity in baroreceptor fibres from the aortic arch and right subclavian artery in the rabbit. J Physiol (Lond) 1971; 214: 65–88

Angle N, Hoyt DB, Coimbra R, et al. Hypertonic saline resuscitation diminishes lung injury by suppressing neutrophil activation after hemorrhagic shock. Shock 1998; 9(3): 164–170.

Annecke T, Fischer J, hartmann H, Tschoep J, et al. Shedding of the coronary endothelial glycocalyx: effects of hypoxia/reoxygenation vs ischaemia/reperfusion injury. Br J Anaesth 2011; 107(5): 679-86.

Arnold JL, Halperin P, Tsai MC, Smith- line H. Mass casualty terrorist bombings: a comparison of outcomes by bombing type. Ann Emerg Med 2004; 43: 263-73.

Astrup T. The haemostatic balance. Thromb Diath Haemorrh 1958; 2: 347-357

Avidan M, Alcock E, Da Fonseca J, Ponte J, et al. Comparison of structured use of routine laboratory tests or near-patient assessment with clinical judgement in the management of bleeding after cardiac surgery. Br J Anaesth 2004; 92: 178-86

Aylwin C, König T, Brennan N, Shirley P, et al. Reduction in critical mortality in urban mass casualty incidents: analysis of triage, surge, and resource use after the London bombings on July 7, 2005. Lancet 2006; 368(9554): 2219-2225.

Baraniuk S, Tilley BC, del Junco DJ, Fox EE, et al. Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) Trial: design, rationale and implementation. Injury 2014; 45(9): 1287–95.

Barcroft H, Edholm O, McMichael J, Sharpey Schafer EP. Post haemorrhagic fainting: study by cardiac output and forearm blood flow. Lancet 1944; 1: 489 – 91

Barelli S, Alberio L. The role of plasma transfusion in massive bleeding: protecting the endothelial glycocalyx. Frontiers in Medicine 2018; 5 (Article 91): 1-9.

Bates S, Grand'Maison A, Johnston M, et al. A latex D-dimer reliably excludes venous thromboembolism. Arch Intern Med 2001; 161(3): 447-453.

BCSH. Practical Guidelines for the Management of Those With, Or at Risk of Major Haemorrhage (2014). British Committee for Standards in Haematology. 2014.

Bellamy RF. *Combat trauma overview*. In: Zajtchuk R, Grande CM, eds. Textbook of Military Medicine, Anesthesia and Perioperative Care of the Combat Casualty. Falls Church, VA: Office of the Surgeon General, United States Army 1995:1– 42.

Benson G. Rotational thromboelastometry and its use in directing the management of coagulopathy in the battle injured trauma patient. J Perioper Pract. 2014; 24(1–2): 25–8.

Bickell W, Wall M, Pepe P, Martin R, et al. Immediate versus delayed fluid resuscitation for hypotensive patients with penetrating torso injuries. NEJM 1994; 331: 1105-9.

Blackbourne L, Baer D, Eastridge B, Kheirabadi B, et al. Military medical revolution: prehospital combat casualty care. J Trauma 2012; 73(6S5): S372-7.

Blood Transfusion Task Force (Duguid J Chair). Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryosupernatant. BJ Haematology 2004; 126(1): 11-28.

Bluth M, Kasuk J. Mechanistic Links in Trauma-Induced Coagulopathy: A Tale of Two Cities. Ann Surg 2011; 254(1): 20-21.

Bochsen L, Wiinberg B, Kjelgaard-Hansen M, Steinbrüche ID, Johansson P. Evaluation of the TEG platelet mapping assay in blood donors. Thromb J 2007; 5(3).

Boffard K, Riou B, Warren B, et al. Recombinant factor VIIa as adjunctive therapy for bleeding control in severely injured trauma patients: two parallel randomized, placebo-controlled, double-blind clinical trials. J Trauma. 2005; 59: 8–15.

Bolliger D, Görlinger K, Tanaka K. Pathophysiology and Treatment of Coagulopathy in Massive Hemorrhage and Hemodilution. Anesthesiology 2010; 113: 1205-19.

Borgman MA, Spinella PC, Perkins JG, Grathwohl KW, et al. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. J Trauma 2007, 63: 805-813

Born GV. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 1962; 194: 927–929.

Bowbrick VA, Mikhailidis DP, Stansby G. Value of thromboelastography in the assessment of platelet function. Clin Appl Thromb Hemost 2003; 9: 137-142.

Bowley D, Davis N, Ballard M, Orr L, Eddlestone J. Military assistance to the civil authority: medical liaison with the Manchester clinicians after the Arena bombing. JRAMC 2018; doi: 10.1136/jramc-2018-000944

Bouma BN, Meijers JC. Thrombin-activatable fibrinolysis inhibitor (TAFI, plasma procarboxypeptidase B, procarboxypeptidase R, procarboxypeptidase U). J Thromb Haemost 2003; 1: 1566-1574.

Bozzette A, Aeron-Thomas A. Reducing trauma deaths in the UK. Lancet. 2013; 382(9888):208.

Branco B, Inaba K, Ives C, et al. Thromboelastogram evaluation of the impact of hypercoagulability in trauma patients. Shock 2014; 41(3): 200-7.

Brockamp T, Nienaber U, Mutschler M, Wafaisade A, et al. Predicting on-going hemorrhage and transfusion requirement after severe trauma: a validation of six scoring systems and algorithms on the Trauma Register DGU. Crit Care 2012; 16(4): R129.

Brohi K, Singh J, Mischa H, Coats T. Acute traumatic coagulopathy. J Trauma 2003; 54(6): 1127-1130.

[a]Brohi K, Cohen M, Ganter M, et al. Acute traumatic coagulopathy: initiated by hypoperfusion: modulated through the protein C pathway? Ann Surg 2007; 245: 812-8.

[b]Brohi K, Cohen J, Davenport R. Acute coagulopathy of trauma: mechanism, identification and effect. Curr Opin Crit Care 2007; 13: 680-5.

Brohi K, Cohen M, Ganter M, Schultz M, et al. Acute coagulopathy of trauma: hypoperfusion induces systemic anticoagulation and hyperfibrinolysis. J Trauma 2008; 64(5): 1211-1217.

Brohi, K. Trauma induced coagulopathy. JRAMC 2009; 155 (4): 320–322.

Brown J, Cohen M, Minei J, Maier R, et al. Goal directed resuscitation in the prehospital setting: a propensity-adjusted analysis. J Trauma 2013; 74(5): 1207–12

[a]Brown J, Sperry J, Fombona A, et al. Pre-trauma center red blood cell transfusion is associated with improved early outcomes in air medical trauma patients. J Am Coll Surg 2015; 220: 797-808.

[b]Brown J, Cohen M, Minei J, Maier R, et al. Pretrauma center red blood cell transfusion is associated with reduced mortality and coagulopathy in severely injured patients with blunt trauma. Ann Surg 2015; 261(5): 997-1005.

[c]Brown J, Neal M, Guyette F, Peitzman A, et al. Design of the Study of Tranexamic Acid during Air Medical Prehospital Transport (STAAMP) trial: addressing the knowledge gaps. Prehosp Emerg Care 2015; 19: 79–86.

Brown LM, Call MS, Margaret Knudson M, et al. A normal platelet count may not be enough: the impact of admission platelet count on mortality and transfusion in severely injured trauma patients. J Trauma 2011; 71(2 S3): S337–42

Brown R, Cooper G, Maynard R. The ultrastructure of rat lung following acute primary blast injury. Int J Exp Pathol 1993; 74: 151-162.

Brunkhorst FM, Engel C, Bloos F, Meier-Hellmann A, et al. Intensive insulin therapy and pentastarch resuscitation in severe sepsis. N Engl J Med 2008; 358(2): 125–39.

Bulger E, Cuschieri J, Warner K, Maier R. Hypertonic resuscitation modulates the inflammatory response in patients with traumatic hemorrhagic shock. Ann Surg 2007; 245(4): 635-641.

Bulger E, May S, Brasel K, Schreiber M, et al. Out-of-hospital hypertonic resuscitation following severe traumatic brain injury: a randomized controlled trial. JAMA. 2010; 304(13): 1455–64.

Bulger E, May S, Kerby J, Emerson S, et al. Out-of-hospital hypertonic resuscitation after traumatic hypovolemic shock: a randomized, placebo controlled trial. Ann Surg. 2011; 253(3): 431–41.

Bunn F, Roberts I, Tasker R, Akpa E. Hypertonic versus near isotonic crystalloid for fluid resuscitation in critically ill patients. Cochrane Database Syst Rev 2004; 3: CD002045.

Burris D, Rhee P, Kaufmann C, Pikoulis, et al. Controlled resuscitation for uncontrolled hemorrhagic shock. J Trauma 1999; 46(2): 216-223.

Butler F, Hagman J, Butler E. Tactical combat casualty care in special operations. Mil Med 1996; 161: 3-16

Butler F, Holcomb J, Schreiber M, et al. Fluid resuscitation for hemorrhagic shock in tactical combat casualty care. J Spec Ops Med 2014; 14(3): 13-38.

Butt M, Zacharias N, Velmahos G. Penetrating abdominal injuries: management controversies. Scand J Trauma Resusc Emerg Med 2009; 17: 19.

Bux J, Dickhörner D, Scheel E. Quality of freeze-dried (lyophilized) quarantined single-donor plasma. Transfusion 2013; 53: 3203-3209.

Calderbank P, Woolley T, Mercer S, et al. Doctor on board? What is the optimal skill-mix in military pre-hospital care? EMJ 2011; 28: 882-883.

Callum J, Pinkerton P: *Blood Easy 2. A Guide to Transfusion Medicine*. 2nd edition. Toronto: Helen Stevenson Savattuq Inc.; 2005.

Callum J, Karkouti K, Lin Y. Cryoprecipitate: the current state of knowledge. Transfus Med Rev 2009; 23: 177 – 88

Campbell D. Trauma doctors reveal horror of knife crime “epidemic”. Guardian 21 Oct 2018.

Cap A, Beckett A, Benov A, Borgman M, et al. Whole blood transfusion. Mil Med 2018; 183: 44-51.

Capone A, Safar P, Stezoski W, Tisherman, Peitzman A. Improved outcome with fluid restriction in treatment of uncontrolled hemorrhagic shock. J Am Coll Surg 1995; 180(1): 49-56.

Cardenas J, Zhang X, Fox E, Cotton B, et al. Platelet transfusions improve hemostasis and survival in a substudy of the prospective, randomized PROPPR trial. Blood Adv 2018; 2(14): 1696-1704.

Cardinal D, Flower R. The electronic aggregometer: a novel device for assessing platelet behaviour in blood. J Pharmacol Methods. 1980; 3: 135–158.

Carey J. Physiological hemodilution: interrelationships between haemodynamics and blood volume after acute blood loss. Ann Surg 1973; 178(1): 87-94.

Carrick M, Morrison C, Tapia N, Leonard J, et al. Intraoperative hypotensive resuscitation for patients undergoing laparotomy or thoracotomy for trauma: early termination of a randomized prospective clinical trial. J Trauma 2016; 80(6): 886-896.

Carroll R. *Body Fluid Distribution* in Elsevier's Integrated Physiology, Ed Carroll R. Elsevier Inc. 2007.

Cesarman-Maus G, Hajjar K. Molecular mechanisms of fibrinolysis. Br J Haem 2005; 129(3): 307-321.

CGOs. Clinical Guidelines for Operations Joint Services Publication 999. Policy 10. Massive Transfusion Protocol. 2012.

CGOs. Clinical Guidelines for Operations, Joint Services Publication 999. 2017.

Chaiwat O, Lang J, Vavilala M, et al. Early packed red blood cell transfusion and acute respiratory distress syndrome after trauma. Anesthes 2009; 110: 351-60.

Chakrabarti R, Hocking E, Fearnley G. Reaction pattern to three stresses: electroplexy, surgery and myocardial infarction; of fibrinolysis and plasma fibrinogen. J Clin Pathol 1969; 22(6): 659-662.

Champion H, Bellamy R, Roberts P, Leppaniemi A. A profile of combat injury. J Trauma 2003; 54: S13-19.

Chandler W. Procoagulant Activity in Trauma Patients. Am J Clin Path 2010; 134(1): 90-96.

Chapman C, Stainsby D, Jones H, et al. Ten years of hemovigilance reports of transfusion-related acute lung injury in the United Kingdom and the

impact of preferential use of male donor plasma. Transfusion 2009; 49: 440–52
Chappell D, Jacob M, Hofmann-Kiefer K, Bruegger D, et al. Hydrocortisone Preserves the Vascular Barrier by Protecting the Endothelial Glycocalyx Anesthesiology 2007; 107:776–84.

Charles A, Shaikh A, Walters M, et al. Blood transfusion is an independent predictor of mortality after blunt trauma. Am Surg 2007; 73(1): 1-5.

Chaudry I, Bland K. Cellular mechanisms of injury after major trauma. Br J Surg 2009; 96(10): 1097-1098.

Chesebro, Rahn P, Carles M, et al. Increase in activated protein C mediates acute traumatic coagulopathy in mice. Shock 2009; 32: 659-65.

Cho S, Holcomb J, Tieu B, Englehart M, et al. Reproducibility of an animal model simulating complex combat-related injury in a multiple-institution format. Shock 2009; 31(1): 87-96.

Ciavarella D, Reed R, Counts R, Baron L, et al. Clotting factor levels and the risk of diffuse microvascular bleeding in the massively transfused patient. Br J Haematol 1987; 67: 365-8.

Claridge J, Crabtree T, Pelletier S, Butler K, et al. Persistent occult hypoperfusion is associated with a significant increase in infection rate and mortality in major trauma patients. J Trauma 2000; 48(1): 8-14.

Claridge J, Sawyer R, Schulman A, et al. Blood transfusions correlate with infetcions in trauma patients in a dose-dependent manner. Am Surg 2002; 68(7): 566-72.

Cochrane Injuries Group Albumin Reviewers. Human albumin administration in critically ill patients: systematic review of randomised controlled trials. Brit Med J. 1998; 317(7153):235–40.

Cohen D. EMA calls for hydroxyethyl starch solutions to be taken off market. Brit Med J 2018; 360: k225

Cohen M, Brohi K, Calfee C, Rahn P, et al. Early release of high mobility group box nuclear protein 1 after severe trauma in humans: role of injury severity and tissue hypoperfusion. Crit Care 2009; 13(6): R174-83.

Cohen M, Call M, Nelson M, et al. Critical role of activated protein C in early coagulopathy and later organ failure, infection and death in trauma patients. Ann Surg 2012; 255: 379-85.

Cohen M, Kutcher M, Redick B, et al. Clinical and mechanistic drivers of acute traumatic coagulopathy. J Trauma 2013; 75: S40-7.

Cole E, Davenport R, Willett K, Brohi K. Tranexamic acid use in severely injured civilian patients and the effects on outcomes: a prospective cohort study. Ann Surg 2015; 261: 390–4.

Cooper D, Myles P, McDermott F, Murray L, et al. Prehospital hypertonic saline resuscitation of patients with hypotension and severe traumatic brain injury: a randomized controlled trial. JAMA. 2004; 291(11): 1350–7.

Cooper G, Maynard R, Cross N, Hill J. Casualties from terrorist bombings. J Trauma 1983; 23: 955-967.

Cooper G, Pearce B, Sedman A, Bush I, Oakley C. Experimental evaluation of a rig to simulate the response of the thorax to blast loading. J Trauma 1996; 40(3): S38-S41.

Cosgriff N, Moore E, Angela S, Kenny-Moynihan M, et al. Predicting life-threatening coagulopathy in the massively transfused trauma patient: hypothermia and acidosis revisited. J Trauma 1997; 42(5): 857-862.

Cothren CC, Moore EE, Hedegaard HB, Meng K. Epidemiology of urban trauma deaths: a comprehensive reassessment 10 years later. World J Surg 2007; 31(7): 1507–11.

Cotton B, Gunter O, Isbell J, Au B, et al. Damage control hematology: the impact of a trauma exsanguination protocol on survival and blood product utilization. J Trauma 2008; 64(5): 1177-1183.

Cotton B, Harvin J, Kostousouv V, Minei K, et al. Hyperfibrinolysis at admission is an uncommon but highly lethal event associated with shock and prehospital fluid administration. J Trauma Acute Care Surg 2012; 73(2): 365-370.

Council of Europe. Guide to the preparation, use and quality assurance of blood components, 2006. 12th Edn. Strasbourg, Council of Europe Publishing.

Counts R, Haisch C, Simon T, Maxwell N, et al. Haemostasis in massively transfused trauma patients. Ann Surg 1979; 190: 91-9.

CRASH-2 Trial Collaborators. Shakur H, Roberts I, Bautista R, Caballero J, Coats T, et al. Effects of tranexamic acid on death, vascular occlusive events, and blood transfusion in trauma patients with significant haemorrhage (CRASH-2): a randomised, placebo-controlled trial. Lancet. 2010; 376(9734): 23-32

CRASH 2 collaborators. The importance of early treatment with tranexamic acid in bleeding trauma patients: an exploratory analysis of the CRASH-2 randomised controlled trial. Lancet 2011; 377(9771): 1096-1101.

Cross J, Gruber D, Burchard K, et al. Hypertonic saline fluid therapy following surgery: a prospective study. J Trauma 1989; 29(6): 817-825.

Curry N, Davenport R, Hunt B, Stanworth S. Transfusion strategies for traumatic coagulopathy. Transf Rev 2012; 26(5): 223-32.

Curry N, Rourke C, Davenport R, Stanworth S, Brohi K. Fibrinogen replacement in trauma haemorrhage. Scand J Trauma Resusc and Emerg. 2014; 22(S1): A5-A6

Curry N, Rourke C, Davenport R, Beer S, et al. Early cryoprecipitate for major haemorrhage in trauma: a randomised controlled feasibility trial. Br J Anaesth. 2015; 115(1): 76–83.

Daban J-L, Deshayes A, Clapson P, Batjom E, et al. Le plasma cryodesséché: un produit stable et rapidement disponible pour les opérations militaires. Société française d'anesthésie réanimation. Congress (51;2009;Paris). Ann Fr Anesth Reanim 2009, 28: S141-S144.

Daban J-L, Clapson P, Ausset S, Deshayes A, Sailliol A. Freeze dried plasma: a French army specialty. Crit Care 2010; 14: 412.

Dahlback B. Blood coagulation. Lancet 2000; 355: 1627-1632.

Danes AF, Cuenca LG, Bueno SR, et al. Efficacy and tolerability of human fibrinogen concentrate administration to patients with acquired fibrinogen deficiency and active or in high-risk severe bleeding. Vox Sang 2008; 94: 221-6.

[a]Davenport R, Khan S. Management of major trauma haemorrhage: treatment priorities and controversies. BJH 2011; 155: 537-548.

[b]Davenport, R., Manson, J., De'Ath, H., et al. Functional definition and characterization of acute traumatic coagulopathy. Critical care medicine 2011; 39 (12): 2652–2658

[c]Davenport R, Curry N, Manson J, De'ath H, Coates A, Rourke C, Pearse R, Stanworth S, Brohi K. Hemostatic effects of fresh frozen plasma may be maximal at red cell ratios of 1:2. J Trauma 2011; 70(1): 90-95.

Dawes R, Thomas G. Battlefield resuscitation. Curr Opin Crit Care 2009; 15(6): 527-535.

De Caterina R, Lanza M, Manca G, et al. Bleeding time and bleeding: an analysis of the relationship of the bleeding time test with parameters of surgical bleeding. Blood 1994; 84 (10): 3363–3370.

Deitch EA, Shi HP, Feketeova E, et al. Hypertonic saline resuscitation limits neutrophil activation after trauma-hemorrhagic shock. Shock 2003; 19(4): 328–333.

Dente C, Shaz B, Nicholas J, Harris R, et al. Improvements in early mortality and coagulopathy are sustained better in patients with blunt trauma after institution of a massive transfusion protocol in a civilian level I trauma center. J Trauma 2009; 66(6): 1616–24.

DePalma, Burris D, Champion H, Hodgson M. Blast Injuries. NELM 2005; 352: 1335-42.

Deusch E, Gamsjäger T, Kress H-G, et al. Binding of Hydroxyethyl Starch Molecules to the Platelet Surface. Anesth Analg 2003; 97(3): 680-683.

Dieterich H, Weissmuller T, Rosenberger P, Eltzschig H. Effect of hydroxyethyl starch on vascular leak syndrome and neutrophil accumulation during hypoxia. Crit Care Med 2007; 34: 1775–82.

Dirkmann D, Hanke A, Görlinger K, Peters J. Hypothermia and acidosis synergistically impair coagulation in human whole blood. Anesth Analg 2008; 106(6): 1627-32.

Djaldetti M, Fishman P, Bessier H, Chaimoff C. pH-induced platelet ultrastructural alterations. A possible mechanism for impaired platelet aggregation. Arch Surg 1979; 114(6): 707-10.

Doran C, Woolley T, Midwinter M. Feasibility of using rotational thromboelastometry to assess coagulation status of combat casualties in a deployed setting. J Trauma 2010; 69(S1): S40-8.

Doran C, Doran C, Woolley T, Carter A, et al. Targeted resuscitation improves coagulation and outcome. J Trauma 2012; 72(4): 835-843.

Drobin D, Hahn RG. Volume kinetics of Ringer's solution in hypovolaemic volunteers. Anesthesiology 1999; 90: 81–91.

Duan K, Yu W, Lin Z, Tan S, et al. A time course study of acute traumatic coagulopathy prior to resuscitation: from hypercoagulation to hypocoagulation caused by hypoperfusion? Transfus Apher Sci 2014; 50(3): 399-406.

Dunbar N, Chandler W. Thrombin generation in trauma patients. Transfusion 2009; 49(12): 2652-60.

Dubick M, Shek P, Wade C. ROC trials update on prehospital hypertonic saline resuscitation in the aftermath of the US-Canadian trials. Clinics 2013; 68(6): 883-886.

Duchesne J, Kavitha M, Marr A, et al. Current evidence based guidelines for factor VIIa use in trauma: the good, the bad and the ugly. Amer Surg 2008; 74(12): 1159-1165.

Dutton R, Mackenzie C, Scalea T. Hypotensive resuscitation during active haemorrhage: impact on in hospital mortality. J Trauma 2002; 52(6): 1141-1146.

Dutton R, Carson J. Indications for early red blood cell transfusion. J Trauma 2006; 60(6): S35-S40.

Dutton R. Current concepts in hemorrhagic shock. Anesth Clin 2007; 25: 23-34.

Dutton R. Haemostatic resuscitation. Br J Anaesth 2012; 109(S1): i39-i46.

Dzik, W. Predicting hemorrhage using preoperative coagulation screening assays. Curr Hematol Rep 2004; 3: 324-30.

Eastridge B, Salinas J, McManus J, Blackburn L, et al. Hypotension begins at 110mmHg: redefining “hypotension” with data. J Trauma 2007; 63: 291-299.

Eastridge B, Mabry R, Seguin P, Cantrell J, et al. Death on the battlefield (2001-2011): Implications for the future of combat casualty care. J Trauma 2012; 73(6 S5): S431-S437.

Eder AF, Herron R, Strupp A, Dy B, et al. Transfusion related acute lung injury surveillance (2003-2005) and the potential impact of the selective use of plasma from male donors in the American Red Cross. Transfusion. 2007; 47: 599-607.

Elgjo GI, Knardahl S. Low-dose hypertonic saline (NaCl 8.0%) treatment of uncontrolled abdominal hemorrhage: effects on arterial versus venous injury. Shock 1996; 5(1): 52-58.

Emerson CP, Ebert RV. A Study of Shock in Battle Casualties: Measurements of the Blood Volume Changes Occurring in Response to Therapy. Ann Surg. 1945; 122(5): 745–72.

Esmon C. The protein C pathway. Chest 2003; 124(S3): 26S-32S.

Etulain J, Negretto S, Carestia A, Pozner R, et al. Acidosis down regulates platelet haemostatic functions and promotes neutrophil proinflammatory responses mediated by platelets. Thromb Haemostat 2012; 107(1): 99-110.

Evans PA, Glenn JR, Heptinstall S, Madira W. Effects of gelatin-based resuscitation fluids on platelet aggregation. Br J Anaesth 1998; 81: 198–202.

Evans R, Ludbrook J, Potocnik S. Intracisternal naloxone and cardiac nerve blockade prevent vasodilatation during simulated haemorrhage in awake rabbits. J Physiol 1989; 409: 1-14.

Fackler M. Gunshot wound review. Ann Emerg Med 1996; 28: 194-203.

Faller D. Endothelial cell responses to hypoxic stress. Clin Exp Pharmacol Physiol 1999; 26(1): 74-84.

Fenger-Eriksen C, Lindberg-Larsen M, Christensen AQ, et al. Fibrinogen concentrate substitution therapy in patients with massive haemorrhage and low plasma fibrinogen concentrations. Br J Anaesth 2008; 101: 769-73.

Fenger-Erikson C, Ingerslev J, Sørenson B. Fibrinogen concentrate - a potential universal haemostatic agent. Expert Opin Biol Ther 2009; 9: 1325-33.

FDA. United States Food and Drug Administration. FDA Safety Communication: Boxed Warning on increased mortality and severe renal injury, and additional warning on risk of bleeding, for use of hydroxyethyl starch solutions in some settings. 2013.

Finfer S, Bellomo R, Boyce N, French J, Myburgh J, Norton R, et al. A comparison of albumin and saline for fluid resuscitation in the intensive care unit. N Engl J Med 2004; 350(22): 2247–56.

Fisher B, Thomas D, Peterson B. Hypertonic saline lowers raised intracranial pressure in children after head trauma. Journal of Neurosurgical Anesthesiology 1992; 4(1): 4-10.

Fischer PE, Bulger EM, Perina DG, et al. Guidance document for the prehospital use of tranexamic acid in injured patients. Prehosp Emerg Care 2016; 20: 557–9.

Flamm M, Diamond S. Multiscale systems biology and physics of thrombosis under flow. Ann Biomed Eng 2012; 40: 2355-2364.

Floccard B, Rugeri L, Faure A, Denis M, Boyle E, et al. Early coagulopathy in trauma patients: An on-scene and hospital admission study. Injury 2012; 43(1): 26-32.

Foëx B. Systemic responses to trauma. B Med Bulletin 1999; 55(4): 726-743.

Friedlander F. The diffraction of sound pulses; diffraction by a semi-infinite plane. Proc R Soc Lond A Math Phys Sci. 1946; 186(1006): 322-44.

Fries D, Innerhofer P, Perger P, et al. Coagulation management in trauma-related massive bleeding. Recommendations of the Task Force for Coagulation (AGPG) of the Austrian Society of Anesthesiology, Resuscitation and Intensive Care Medicine (OGARI). Anasthesiol Intensivmed Notfallmed Schmerzther 2010; 45: 552–61.

Frink M, Pape HC, van Griensven M, Krettek C, Chaudry IH, Hildebrand F. Influence of sex and age on MODS and cytokines after multiple injuries. Shock 2007; 27: 151–156.

Frith D, Goslings J, Gaarder C, Maegele M, et al. Definition and drivers of acute traumatic coagulopathy: clinical and experimental investigations. J Thromb Haem 2010; 8: 1919-25.

Frith D, Davenport R, Brohi K. Acute Traumatic Coagulopathy. Curr Opin Anesthesiol 2012; 25: 229-234.

Frost R. Forensic Pathology of Firearm Wounds. Medscape Pathology. 2015. www.emedicine.medscape.com

Frykberg E, Tepas J, Alexander R. The 1983 Beirut airport terrorist bombing. Injury patterns and implications for disaster management. The American Surgeon 1989; 55(3): 134-141.

Furie B, Furie BC. Mechanisms of thrombus formation. N Eng J Med 2008; 359(9): 938-49.

Gando S, Sawamura S, Hayakawa M. Trauma, shock, and disseminated intravascular coagulation. Ann Surg 2011; 254: 10–9.

Gando S, Wada H, Thachl J, et al. Differentiating disseminated intravascular coagulation (DIC) with the fibrinolytic phenotype from coagulopathy of trauma and acute coagulopathy of trauma-shock (COT/ACOTS). J Thromb Hameostasis 2013 11: 826-835.

Ganter M, Hofer C. Coagulation monitoring: current techniques and clinical use of viscoelastic point-of-care coagulation devices. Anesthesia and Analgesia 2008; 106 (5): 1366–1375.

Ganter M, Pittet J. New insight into acute coagulopathy in trauma patients. Best Pract Res Clin Anesthesiol 2010; 24: 15–25.

Garner J, Watts S, Parry C, Bird J, Kirkman E. Development of a large animal model for investigating resuscitation after blast and hemorrhage. World J Surg 2009; 33 (10): 2194-2202.

Garner J, Watts S, Parry C, Bird J, et al. Prolonged permissive hypotensive resuscitation is associated with poor outcome in primary blast injury with controlled hemorrhage. Ann Surg 2010; 251(6): 1131-1139.

Garrigue D, Godier A, Glacet A, Labreuche J, et al. French lyophilized plasma versus fresh frozen plasma for the initial management of trauma-induced coagulopathy: a randmonized open-label trial. J Thromb Haem 2017; 16(3): 481-489.

Gebhard F, Pfetsch H, Steinbach G, Strecker W, et al. Is interleukin 6 an early marker of injury severity following major trauma in humans? Arch Surg 2000; 135: 291–295.

Giannoudis P, Harwood P, Loughenbury P, Van Griensven M, et al. Correlation between IL-6 levels and the systemic inflammatory response score: can an IL-6 cutoff predict a SIRS state? J Trauma. 2008; 65(3): 646-652.

Giannoudis M, Harwood P. Damage control resuscitation: lessons learned. Eur J Trauma Emerg Surg 2016; 42: 273-282.

Gill M. The TEG®6s on shaky ground? A novel assessment of the TEG®6s performance under a challenging condition. J Extra Corpor Technol 2017; 49(1): 26-29.

Gilstad C, Roschewski M, Wells J, et al: Fatal transfusion-associated graft-versus-host disease with concomitant immune hemolysis in a group A combat trauma patient resuscitated with group O fresh whole blood. Transfusion 2012; 52(5): 930–5.

Goldsmith H, Kaufer E, McIntosh F. Effect of hematocrit on adenosine diphosphate-induced aggregation of human platelets in tube flow. Biorheology 1995; 32(5): 537-52.

Gorbunov N, McFaul S, januszkiewicz A, Atkins J., Int J Immunopathol Pharmacol 2005;18: 547-56.

Gorbunov N, Asher L, Ayyagari V, Atkins J. Inflammatory leukocytes and iron turnover in experimental hemorrhagic lung trauma. Exp Mol Pathol 2006; 80: 11-25.

Görlinger K, Jambor C, Hanke AA, Dirkmann D, et al. Perioperative coagulation management and control of platelet transfusion by point-of-care platelet function analysis. Transfus Med Hemother 2007; 34(6): 396–411.

Görlinger K, Fries D, Dirkmann D, Weber CF, et al. Reduction of fresh frozen plasma requirements by perioperative point of care coagulation management with early calculated goal-directed therapy. Transfus Med Hemother. 2012; 39(2): 104–13.

Griffiths N. Factors affecting the fibrinolytic response to surgery. Ann R Coll Surg Engl 1979; 61(1): 12-16.

Gross D, Landau EH, Assalia A, KrauszMM. Is hypertonic saline resuscitation safe in ‘uncontrolled’ hemorrhagic shock. J Trauma 1988; 28: 751–6.

Gross D, Landau EH, Klin B, Krausz MM. Quantative measurement of bleeding following hypertonic saline therapy in “uncontrolled” hemorrhagic shock. J Trauma 1989; 29(1): 79-83.

Gruen R, Brohi K, Schreiber M, Balogh Z, et al. Haemorrhage control in severely injured patients. Lancet 2012; 380(9847): 1099-1108.

Guidet B, Martinet O, Boulain T, Philippart F, Poussel JF, Maizel J, et al. Assessment of hemodynamic efficacy and safety of 6 % hydroxyethylstarch 130/0.4 vs. 0.9 % NaCl fluid replacement in patients with severe sepsis: the CRYSTMAS study. Crit Care 2012; 16(3): R94.

Gunter O Jr, Au B, Isbell J, Mowery N, et al. Optimizing outcomes in damage control resuscitation: identifying blood product ratios associated with improved survival. J Trauma 2008, 65: 527-534.

Gushchin V, Stegalkina S, Alam H, et al. Cytokine expression profiling in human leukocytes after exposure to hypertonic and isotonic fluids. J Trauma. 2002; 52: 867–871.

Gutierrez de Caballos J, Turegano Fuentes F, Perez Diaz D, Sanz Sanchez M, et al. Casualties treated at the closest hospital in the Madrid, March 11, terrorist bombings. Crit Care Med 2005; 33: S107-112

Guy RJ, Glover MA, Cripps NP. The pathophysiology of primary blast injury and its implications for treatment. Part I: the thorax. J R Nav Med Serv 1998;84:79-86.

Haas T, Fries D, Tanaka K, Asmis L, Curry N, Schöchl H. Usefulness of standard plasma coagulation tests in the management of perioperative bleeding: is there any evidence? BJA 2015; 114(2): 217-224.

Haemonetics Corporation. TEG® 5000 Haemostasis analyzer system. www.haemonetics.com. 2011.

Hagemo J, Viksmoen Y, Gaarder C, Naess P, et al. Comparing TEG and ROTEM as point-care devices in trauma patients. Poster presentation: 1st International Congress of the ISBT (International Society of Blood Transfusion) 2010.

Hagemo J, Christiaans S, Stanworth S, Brohi K, Johansson P, et al. Detection of acute coagulopathy and massive transfusion requirements by means of rotational thromboelastometry: an international prospective validation study. Crit Care 2015; 19: 97.

Haljamae H, Dahlqvist M, Walentin F. Artificial colloids in clinical practice: pros and cons. Bailliere’s Clinical Anaesthesiology 1997; 11: 49–791.

Hallet J, Lauzier F, Mailloux O, Trottier V, et al. The use of higher platelet:RBC transfusion ratio in the acute phase of trauma resuscitation: a systematic review. Crit Care Med. 2013; 41(12): 2800–11.

Hamilton G, Van P, Differding J, Kremenevskiy I, et al. Lyophilized plasma with ascorbic acid decreases inflammation in hemorrhagic shock. J Trauma 2011; 71: 292-7.

Hampton DA, Fabricant LJ, Differding J, Diggs B, Underwood S, DeLaCruz D, Holcomb JB, Brasel KJ, et al. Prehospital intravenous fluid is associated with increase survival in trauma patients. J Trauma 2013; 74 (7 S1); S9-S15.

Hannon J, Bossone C, Rodkey W. Splenic Red-Cell Sequestration and Blood-Volume Measurements in Conscious Pigs. Am J Physiol 1985; 248(3): R293-R301.

Hardaway R, Bredenberg C. *Monitoring hematology laboratory values* in Care of Wounded in Vietnam. Manhattan, Kansas: Sunflower University Press 1988; 139-220.

Harban F, Kirkman E, Kenward C, Watkins P. Primary thoracic blast injury causes acute reduction in cardiac function in the anaesthetized pig. J Physiol Lond 2001; 533: P81.

Harr J, Moore E, Ghasabyan A, et al. Functional fibrinogen assay indicates that fibrinogen is critical in correcting abnormal clot strength following trauma. Shock 2013; 39(1): 45-9.

Harrison P. Platelet function analysis. Blood Rev 2005; 19(2): 111-23.

Hartert H. Blutherinnungsstudien nit der thromboelastographie, einem neuen untersuchungsverfarhren. Klinische Wochenschrift 1948; 26(37/38): 577-83.

Härtl R, Ghajar J, Hochleuthner H, Mauritz W. Hypertonic/hyperoncotic saline reliably reduces ICP in severely head-injured patients with intracranial hypertension. Acta Neuroch Supp (Wien)1997; 70: 126-9.

Harvin J, Peirce C, Mims M, Hudson J, Podbielski J, Wade C, et al. The impact of tranexamic acid on mortality in injured patients with hyperfibrinolysis. J Trauma 2015; 78: 905–9.

Hasler R, Nuesch E, jüni P, Bouamra O, et al. Systolic blood pressure below 110mmHg is associated with increased mortality in blunt trauma patients: Multicentre cohort study. Resuscitation 2011; 82(9): 1202-1207.

Hauser C, Boffard K, Dutton R, et al. Results of the CONTROL trial: efficacy and safety of recombinant activated factor VII in the management of refractory traumatic hemorrhage. J Trauma 2010; 69(3): 489-500.

Hayakawa M, Sawamura A, Gando S, Kubota N, Uegaki S, Shimojima H, Sugano M, Ieko M. Disseminated intravascular coagulation at an early phase of trauma is associated with consumption coagulopathy and excessive fibrinolysis both by plasmin and neutrophil elastase. Surgery 2011; 149: 221–30.

Hayakawa M, Sawamura A, Gando S, Jesmin S, et al. A low TAFI activity and insufficient activation of fibrinolysis by both plasmin and neutrophil elastase promote organ dysfunction in disseminated intravascular coagulation associated with sepsis. Thromb Res 2012; 130(6): 906-913.

Helm M, Lampl L, hauke J, Bock K. Accidental hypothermia in trauma patients. Is it relevant to preclinical emergency treatment? Anaesthetist 1995; 44(2): 101-7.

Hess J, Holcomb J, Hoyt D. Damage control resuscitation: the need for specific blood products to treat the coagulopathy of trauma. Transf 2006; 46(5): 685-686.

Hess J, Holcomb J. Transfusion practice in military trauma. Transf Med 2008; 18(3): 143-50.

Hess J, Lindell A, Stansbury L, Dutton R, Scalea T. The prevalence of abnormal results of conventional coagulation tests on admission to a trauma center. Transf 2009; 49: 340-9.

Hewson J, Neame P, Kumar N, Ayrton A, et al. Coagulopathy related to dilution and hypotension during massive transfusion. Crit Care Med 1985; 13(5): 387-91.

Hiippala S, Myllyla G, Vahtera E. Hemostatic factors and replacement of major blood loss with plasma-poor red cell concentrates. Anesth Analg. 1995; 81(2): 360–5.

Hiippala S. Replacement of massive blood loss. Vox Sang, 1998; 74(S2): 399-407.

Hillman K, Bishop G, Bristow P. The crystalloid versus colloid controversy: present status. Bailliere's Clinical Anaesthesiology 1997;11:1–13.

Himmelseher S. Hypertonmic saline solutions for treatment of intracranial hypertension. Curr Opin Anaesth 2007; 20(5): 414-426.

Hirshberg A, Dugas M, Banez EI, Scott BG, Wall MJ, Jr, Mattox KL: Minimizing dilutional coagulopathy in exsanguinating hemorrhage: a computer
simulation. J Trauma 2003, 54:454-463

Ho AM, Dion PW, Cheng CA, Karmakar MK, Cheng G, Peng Z, Ng YW: A mathematical model for fresh frozen plasma transfusion strategies during

major trauma resuscitation with ongoing hemorrhage. Can J Surg 2005, 48: 470-478.

Hodgetts T, Mahoney P. ABC to <C>ABC: redefining the military trauma paradigm Emerg Med J 2006; 23: 745-746

Hodgetts T, Mahoney P, Kirkman E. Damage Control Resuscitation. JRAMC 2007; 153(4): 299-300.

Hoffbrand V, Moss P, Pettit J. Essential Haematology, 6th Edition. Wiley-Blackwell Publishing 2011.

Hoffman M, Monroe DM, 3rd. A cell-based model of hemostasis. Thromb Haemost 2001; 85: 958–65

Holcomb J. Fluid resuscitation in modern combat casualty care: lessons learned from Somalia. J Trauma. 2003; 54(5 Suppl): S46–51.

Holcomb J, Jenkins D, Rhee P, Johannigman J, et al. Damage control resuscitation: directly addressing the early coagulopathy of trauma. J Trauma 2007; 62: 307-10.

Holcomb JB, Wade CE, Michalek JE, Chisholm GB, et al. Increased plasma and platelet to red blood cell ratios improves outcome in 466 massively transfused civilian trauma patients. Ann Surg 2008, 248:447-458.

Holcomb J, Minei K, Scerbo M, et al. Admission rapid thrombelastography can replace conventional coagulation tests in the emergency department: experience with 1974 consecutive trauma patients. Ann Surg 2012; 256(3): 476-86.

Holcomb J, Junco J, Fox E, et al. The Prospective, Observational, Multicenter Major Trauma Transfusion (PROMMT) Study, Comparative Effectiveness of a Time-Variable Treatment With Competing Risks. JAMA Surg 2013; 148(2): 127-136.

[a]Holcomb J, Donathan D, Cotton B, del Junco D, et al. Prehospital transfusion of plasma and red cells in trauma patients. Prehosp Emerg Care 2015; 19(1): 1-9.

[b]Holcomb JB, Tilley B, Baraniuk S, Fox E, et al. Transfusion of Plasma, Platelets and Red Blood Cells in a 1:1:1 vs a 1:1:2 Ratio and Mortality in Patients With Severe Trauma, The PROPPR Randomized Clinical Trial. JAMA. 2015; 313(5): 471-482.

Holcroft JW. The hypertonic saline trial: a possible downside to the gold standard of double blinding. Ann Surg. 2011; 253(3): 442–3.

Hollerman J, Fackler M, Coldwell D, Ben-Menachem Y. Gunshot wounds: 1. Bullets, ballistics, and mechanisms of injury. AJR 1990; 155: 685-690.

Holmsen H, Holmsen I, Bernhardsen A. Microdetermination of adenosine diphosphate and adenosine triphosphate in plasma with the reyluciferase system. Anal Biochem 1966; 17: 456–473

Hopkinson D, Marhsall T. Firearm injuries. Br J Surg 1967; 54: 3444-353.

Horrocks C. Blast injuries: biophysics, pathophysiology and management principles. JRAMC 2001; 147(1): 28-40.

Horton S, Augustin S. Activated Clotting Time (ACT). Methods Mol Bol 2013; 992: 155-67.

Howell S. Advances in trauma care: a quiet revolution. Brit J Anaes 2014; 113(2): 201-202.

Hranjec T, Swenson BR, Dossett LA, Metzger R, Flohr TR, Popovsky KA et al. Diagnosis-dependent relationships between cytokine levels and survival in patients admitted for surgical critical care. J Am Coll Surg 2010; 210: 833–844, 845–846.

Huerta-Alardin A, Varon J, Marik P. Bench-to-bedside review: Rhabdomyolysis – an overview for clinicians. Crit Care 2004; 9(2) : 158-169.

Hunt B, Allard S, Keeling D, Norfolk D, Stanworth SJ, Pendry K. A practical guideline for the haematological management of major haemorrhage. Brit J Haematol 2015; 170(6): 788-803

Hunt H, Stanworth S, Curry N, Woolley T, Cooper C, et al. TEG and ROTEM for diagnosing trauma-induced coagulopathy (disorder of the clotting system) in adult trauma patients with bleeding. Cochrane Database of Systematic Reviews 2015, Issue 2. Art. No.: CD010438.

Huskisson L. Intravenous volume replacement: which fluid and why? Archives of Disease in Childhood 1992; 67:649-653.

Inaba K, Karamanos E, Lustenberger T, Schochl H, Shulman I, et al. Impact of fibrinogen levels on outcomes after acute injury in patients requiring a massive transfusion. J Am Coll Surg. 2013; 216: 290–7.

[a]Inaba K, Branco BC, Rhee P, Blackbourne L, et al. Impact of plasma transfusion in trauma patients who do not require massive transfusion. J Am Coll Surg 2010; 210: 957–65.

[b]Inaba K, Lustenberger T, Rhee P, Holcomb JB, et al. The impact of platelet transfusion in massively transfused trauma patients. J Am Coll Surg. 2010; 211(5): 573–9.

Inaba K. Freeze-dried plasma. J Trauma 2011; 70(S5): S57-8.

Innerhoffer P, Fries D, Mittermayr M, Innerhoffer N, et al. Reversal of trauma-induced coagulopathy using first-line coagulation factor concentrates or fresh frozen plasma (RETIC): a single-centre, parallel-group, open-label, randomised trial. Lancet Haem 2017; 4(6): 258-271.

Iorio A, Basileo M, Marchesini E, Materazzi M, Marchesi M, Esposito A, Palazzesi GP, Pellegrini L, Pasqua BL, Rocchetti L, Silvani CM: The good use of plasma. A critical analysis of five international guidelines. Blood Transfus 2008, 6:18-24.

Irwin R, Lerner M, Bealer J, Mantor P, et al. Shock after blast wave injury is caused by a vagally mediated reflex. J Trauma 1999; 47: 105-110.

Ismali F, et al. Scan J Trauma Resusc Emerg Med 2015; 23: 68-75

Ives C, Inaba K, Branco B, Okoye O, Schöchl H, et al. Hyperfibrinolysis elicited via thromboelastography predicts mortality in trauma. J Am Coll Surg. 2012; 215(4): 496–502.

Jacobsen J, Søfelt S, Sheikh S, Warberg J, Secher N. Cardiovascular and endocrine responses to haemorrhage in the pig. Acta Physiol 1990; 138(2): 167-173.

Jacobsen J, Secher N. Heart rate during haemorrhagic shock. Clin Physiol Funct Imaging 1992; 12(6): 659-666.

Jacoby R, Owings J, Holmes J, Battistella F, et al. Platelet activation and function after trauma. J Trauma 2001; 51(4): 639–47.

Jackson D, Batty C, Ryan J, McGregor W. The Falklands war: Army Field Surgical experience. Annals R Coll Surg Eng 1983; 65: 281-285.

Jalbert LR, Rosen ED, Moons L, et al. Inactivation of the gene for anticoagulant protein C causes lethal perinatal consumptive coagulopathy in mice. J Clin Invest 1998; 102: 1481-1488

Jámbor C, Weber CF, Gerhardt K, Dietrich W, et al. Whole blood multiple electrode aggregometry is a reliable point of care test of aspirin-induced platelet dysfunction. Anesth Analg. 2009; 109(1): 25–31.

James M, Michell W, Nicol A, et al. Resuscitation with hydroxyethyl starch improves renal function and lactate clearance in penetrating trauma in a randomized controlled study: the FIRST trial (Fluids in Resuscitation of Severe Trauma). Brit J Anaes 2011; 107(5): 693-702.

Jansen J, Thomas R, Loudon M, Brooks A. Damage control resuscitation for patients with major trauma. BMJ 2009; 338: 1436-1440.

Jansen J, Luke D, Davies E, Spencer P, et al. Temporal changes in ROTEM®-measured coagulability of cotrated blood samples from coagulopathic trauma patients. Injury 2013; 44(1): 36-9.

Jastrow KM III, Gonzalez EA, McGuire MF, Suliburk JW,Kozar RA, Iyengar S et al. Early cytokine production risk stratifies trauma patients for multiple organ failure. J Am Coll Surg 2009; 209: 320–331.

Johannson P, Bochsen L, Andersen S, Viuff D. Investiigation of the effect of kaolin and tissue factor-activated citrated whole blood, on clot forming variables, as evaluated by thromboelastography. Transfusion 2008; 48: 2377-2383.

[a]Johansson P, Stissing T, Bochsen L, Ostrowski S. Thrombelastography and tromboelastometry in assessing coagulopathy in trauma. Scand J Trauma Resusc Emerg Med 2009; 17(45).

[b]Johansson PI, Stensballe J: Effect of haemostatic control resuscitation on mortality in massively bleeding patients: a before and after study. Vox Sang 2009, 96:111-118.

Johansson P, Ostrowski S. Acute coagulopathy of trauma: balancing progressive catecholamine induced endothelial activation and damage by fluid phase anticoagulation. Med Hypotheses 2010; 75: 564–7.

Johansson P, Stensballe J, Rasmussen L, Ostrowski S. A high admission syndecan-1 level, a marker of endothelial glycocalyx degradation, is associated with inflammation, protein C depletion, fibrinolysis, and increased mortality in trauma patients. Ann Surg 2011; 254: 194–200.

[a]Johansson P. Coagulation monitoring of the bleeding traumatized patient. Curr Opin Anaesthesiol 2012; 25(2): 234-41.

[b]Johansson P, Oliveri R, Ostrowski S. Hemostatic resuscitation with plasma and platelets in trauma. J Emerg Trauma Shock 2012; 5(2): 120-125.

Johansson P, Stensballe J, Ostrowski S. Shock induced endotheliopathy (SHINE) in acute critical illness – a unifying pathophysiologic mechanism. Crit Care 2017; 21(1): 25.

Joint Trauma System Clinical Practice Guideline; Damage Control Resuscitation (CPG ID: 18). 2017.

de Jongee, Levi M. Effects of different plasma substitutes on blood coagulation: a comparative review. Crit Care Med 2001; 29: 1261-7.

JPAC. Joint UK Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee (JPAC). Guidelines fro the Blood Transfusion Services. 8th Ed, 2013.

JRCALC. Association of Ambulance Chief Executives (AACE). Joint Royal Colleges Ambulance Liaison Committee (JRCALC) Clinical Practice Guidelines 2016.

del Junco D, Holcomb J, Fox E, Brasel K, et al. The PROMMTT Study Group. Resuscitate early with plasma and platelets or balance blood products gradually: findings from the PROMMTT study. J Trauma 2013; 75 (1 S1): S24–S30.

Junger W, Coimbra R, Liu F, et al. Hypertonic saline resuscitation: a tool to modulate immune function in trauma patients? Shock. 1997; 8: 235–241.

Jungheinrich C, Neff T. Pharmacokinetics of Hydroxyethyl Starch. Clin Pharm 2005; 44(7): 481-499.

Jurk K, Kehrel B. Platelets: physiology and biochemistry. Semin Thromb Hemost 2005; 31(4): 381-92.

Jurkovich G, Greiser W, Luterman A, Curreri P. Hypothermia in trauma victims: an ominous predictor of survival. J Trauma 1987; 27(9): 1019-24.

Kaafarani H, Velmahos G. Damage Control resuscitation in trauma. Scan J Surg 2014; 103: 81-88.

Kalantzi KI, Tsoumani ME, Goudevenos IA, Tselepis AD. Pharmacodynamic properties of antiplatelet agents: current knowledge and future perspectives. Expert Rev Clin Pharmacol 2012; 5(3): 319–36.

Kanani AN, Hartshorn S. NICE clinical guideline NG39: Major trauma: assessment and initial management. Arch Dis Child Educ Pract Ed. 2017. 102(1): 20–3.

Kang Y, Martin D, Marquez J, et al. Intraoperative changes in blood coagulation and thrombelastographic monitoring in liver transplantation. Anesth Analg 1985; 64(9).

Karoutsos S, Nathan N, Lahrimi A, Grouille D, Feiss P, Cox DJA. Thromboelastogram reveals hypercoagulability after administration of gelatin solution. Br J Anaesth 1999; 82: 175–7.

Kashuk J, Moore E, Millikan J, Moore J. Major abdominal vascular trauma – a unified approach. J Trauma 1982; 22(8): 672-9.

Kashuk J, Moore E, Johnson J, et al. Postinjury life threatening coagulopathy: is 1:1 fresh frozen plasma: packed red blood cells the answer? J Trauma. 2008; 65: 261–271.

[a]Kashuk J, Moore E, Sawyer M, et al. Postinjury coagulopathy management: goal directed resuscitation via POC thrombelastography. Ann Surg 2010; 251(4): 604-14.

[b]Kashuk JL, Moore EE, Sawyer M, Wohlauer M, et al. Primary fibrinolysis is integral in the pathogenesis of the acute coagulopathy of trauma. Ann Surg. 2010; 252: 434–42.

Katz E, Ofek B, Adler J, Abramowitz H, Krausz M. Primary blast injury after a bomb explosion in a civilian bus. Ann Surg 1989; 209(4): 484-8.

Kaufmann C, Dwyer K, Crews J, Dols S, Trask A. Usefulness of thrombelastography in assessment of trauma patient coagulation. J Trauma 1997; 42(4): 716-22.

Kehoe A, Jones A, Marcus S, Nordmann G, et al. Current controversies in military pre-hospital critical care. JRAMC 2011; 157(3S1): S305-309.

Kembell-Cook G, Tuddenham E, McVey J. "*Normal Haemostasis*" in Postgraduate Haematology, 5th edn, V. Haffbrand, D. Catovsky, & E. Tuddenham, eds., 2005. Blackwell Publishing, Oxford, pp. 783-808

Kerwin A, Schinco M, Tepas J 3rd et al. Hypertonic saline for the management of elevated intracranial pressure in patients with severe traumatic brain injury: a pilot study. Journal of Trauma 2009; 67(2): 277-82.

Kessler U, Grau T, Gronchi F, Berger S, Brandt S, et al. Comparison of porcine and human coagulation by thromboelastometry. Thromb Res 2011; 128(5): 477-482.

Khan S, Brohi K, Chana M, Raza I, et al. Hemostatic resuscitation is neither hemostatic nor resuscitative in trauma hemorrhage. J Trauma 2014; 76(3): 561–7.

[a]Kirkman E, Watts S, Cooper G. Blast injury research models. Phil Trans R Soc B 2011; 366: 144-159.

[b]Kirkman E, Watts S. Characterization of the response to primary blast injury. Phil Trans Royal Soc B 2011; 366: 286-90.

Klabunde R. *Hemorrhagic Shock* in Cardiovascular Physiology Concepts, 2nd Edition. Ed Klabunde R. Lippincott, Williams and Wilkins. 2012.

Kluger Y, Nimrod A, Biderman P, Mayo A, Sorkin P. The quinary pattern of blast injury. Am J Disaster Med 2007; 2(1): 21-5.

Knapp J, Sharp R, Beatty R, Medina F. Blast trauma in a child. Pediatr Emerg Care 1990; 6(2): 122-6.

Kobbe P, Yoram V, Kaczorowski D, Mollen D, et al. Patterns of cytokine release and evolution of remote organ dysfunction after bilateral femur fracture. Shock 2008; 30(1): 43-47.

Koko K, McCauley B, Gaughan J, Nolan R, et al. Kaolin-based hemostatic dressing improves hemorrhage control from a penetrating inferior vena cava injury in coagulopathic swine. J Trauma 2017; 83(1): 71-76.

Kolarova H, Ambruzova B, Svihalkova Sindlerova L, Klinke A, Kubala L: Modulation of endothelial glycocalyx structure under inflammatory conditions. Mediators Inflamm 2014: 694312.

Kolsen-Petersen JA. Immune effect of hypertonic saline: fact or fiction? Acta Anaesthesiol Scand. 2004; 48:667– 678.

Komblith LZ, Kutcher ME, Redick BJ, Calfee CS, Vilardi RF, Cohen MJ. Fibrinogen and platelet contributions to clot formation: implications for trauma resuscitation and thromboprophylaxis. J Trauma 2014; 76: 255–63

Kortbeek J, Al Turki S, Ali J, Antoine A, et al. Advanced Trauma Life Support, 8th Edition, the evidence for change. J Trauma: Inj, Inf and Crit Care. 2008; 64(6): 1638-50.

Kozar RA, Peng Z, Zhang R, Holcomb JB, Pati S, Park P, et al. Plasma restoration of endothelial glycocalyx in a rodent model of hemorrhagic shock. Anesth Analg 2011; 112(6): 1289–95.

Kramer G, English T, Gunther R, Holcroft J. Physiological mechanisms of fluid resuscitation with hyperosmotic/hyperoncotic solutions. Prog Clin Biol Res. 1989; 299: 311–20.

Kramer G. Hypertonic resuscitation: physiological mechanisms and recommendations for trauma care. J Trauma 2003; 54(5): S89-S99.

Krausz M, Landau E, Klin B, Gross D. Hypertonic saline treatment of uncontrolled hemorrhagic shock at different periods from bleeding. Arch Surg. 1992; 127(1): 93–96.

Krausz M. Initial resuscitation of hemorrhagic shock. World J Emerg Surg 2006; 1: 14.

Kreimeier U, Brückner U, Niemczyk S, Messmer K. Hyperosmotic saline dextran for resuscitation from traumatic hemorrhagic hypotension: effect on regional blood flow. Circ Shock 1990; 32(2): 83-99.

Kumar P, Clark M. Clinical Medicine, 9th Edition. Edited by Kumar P, Clark M. Elsevier, Jul 2016.

Kushimoto S, Kudo D, Kawazoe Y. Acute traumatic coagulopathy and trauma-induced coagulopathy: an overview. J Int Care 2017; 5: 6-12.

Kutcher ME, Redick BJ, McCreery RC, et al. Characterization of platelet dysfunction after trauma. J Trauma 2012; 73: 13–9.

LAA News. London's Air Ambulance News. https://londonsairambulance.co.uk/our-service/news/2018/10/landmark-study-to-save-hundreds-more-lives. 2018.

Laffan M, Manning R. *Investigation of Hameostasis* in Dace and Lewis Practical Haematology 12th Edition, Elsevier Ltd., 2017: 366-409.

Lang T, von Depka M. Possibilities and limitations of thrombelastometry-graphy. Hamostaseologie 2006; 26(3 S1): S20–9.

Larsen OH, Fenger-Eriksen C, Christiansen K, Ingerslev J, S.rensen B. Diagnostic performance and therapeutic consequence of thromboelastometry activated by kaolin versus a panel of specific reagents. Anesthesiology 2011; 115(2): 294–302.

Laxenaire MC, Charpentier C, Feldman L. Anaphylactoid reactions to colloid plasma substitutes: incidence risk factors mechanisms. A French multicenter prospective study. Annales Francais d'Anesthesie et Reanimation 1994; 13: 301–10.

Lee T, Van P, Spoerke N, Hamilton G, et al. The use of lyophilized plasma in a severe multi-injury pig model. Transf 2013; 53: 72S-79S.

Lee WL, Slutsky AS: Sepsis and endothelial permeability. N Engl J Med 2010, 363: 689–691.

Leemann H, Lustenberger T, Talving P, Kobayashi L, et al. The role of rotation thromboelastometry in early prediction of massive transfusion. J Trauma. 2010; 69(6): 1403–8.

Lenz A, Franklin GA, Cheadle WG. Systemic inflammation after trauma. Injury 2007; 38: 1336–1345.

Levi M, ten Cate H, van der Poll T. Endothelium: Interface between coagulation and inflammation. Crit Care Med 2002; 30(5): S220-S224.

Levi M, van der Poll T. Inflammation and coagulation. Crit Care Med 2010; 38: S26-34.

Levrat A, Gros A, Rugeri L, Inaba K, et al. Evaluaion of rotational thromboelastography for the diagnosis of hyperfibrinolysis in trauma patients. Brit J Anaes 2008; 100(6): 792-7.

Levy JH, Dutton R, Hemphill JC, et al. Multidisciplinary approach to the challenge of hemostasis. Anesth Analg 2010; 110: 354-364.

Levy JH, Welsby I, Goodnough LT. Fibrinogen as a therapeutic target for bleeding: a review of critical levels and replacement therapy. Transfusion 2013; 54: 1389–405.

Ley EJ, Clond MA, Srour MK, Barnajian M, Mirocha J, Margulies DR, Salim A. Emergency Department Crystalloid Resuscitation of 1.5L or More is Associated With Increased Mortality in Elderly and Nonelderly Patients. J Trauma. 2011; 70: 398-400.

Lier H, Bottiger BW, Hinkelbein J, Krep H, Bernhard M. Coagulation management in multiple trauma: a systematic review. Intensive Care Med 2011; 37: 572–82.

LITES Network, Linking Investigations in Trauma and Emergency Services Network, Task Order 2. www.litesnetwork.org/about/task-orders/ . 2018.

Liman S, Kuzucu A, tastepe A, Ulasan G , Topcu S. Chest injury due to blunt trauma. Eur J Cardiothorac Surg. 2003; 23(3): 374-8.

Lindoff GA, Palmer JHM. Assessment of the thermal safety of microwave warming of crystalloid fluids. Anaesthesia 2000; 55: 251-254.

Ling G, Bandak F, Armondo R, Grant G, Eckland J. Explosive blast neurotrauma. J Neurotrauma 2009; 26(6): 836-9
Lissauer M, Chi A, Kramer M, Scalea T, Johnson S. Association of 6% hetastarch resuscitation with adverse outcomes in critically ill trauma patients. Am J Surg 2011; 202(1): 53-58.

Little R, Randall P, Redfern W, Stoner H, Marshall H. Components of injury (haemorrhage and tissue ischaemia) affecting cardiovascular reflexes in man and rat. Q J Exp Physiol 1984; 69: 753–62

Little R, Marshall H, Kirkman E. Attenuation of the acute cardiovascular responses to haemorrhage by tissue injury in the conscious rat. Q J Exp Physiol 1989; 74: 825 – 33

Little R, Kirkman E. *Cardiovascular control after injury* in Scientific foundations of trauma. Cooper G, et al Eds. Butterworth Heineman, Oxford. 1997.

Liu J, Pedersen LC. Anticoagulant heparan sulfate: structural specificity and biosynthesis. Appl Microbiol Biotechnol 2007; 74: 263–72

Lockey D. Improved Trauma Outcomes after the Introduction of a Trauma System in England. EClinicial Medicine 2-3 2018; 3-4.

Lockyer A, Oram R, Lopez G, et al. Pattenrs of injury in penetrating sharp trauma in a Provincial KwaZulu-Natal Hospital. Afr J Emerg Med 2013; 3(2): 67-70.

Lonner J, Koval K. Polytrauma in the elderly. Clin Orth Rel Res 1995; 318: 136-143.

Loomis WH, Namiki S, Hoyt DB, et al. Hypertonicity rescues T cells from suppression by trauma-induced anti-inflammatory mediators. Am J Physiol Cell Physiol. 2001; 281: C840–C848.

Loscalzo J, Schafer A. Thrombosis and Hemorrhage, 3rd Edition. Baltimore, Williams and Wilkins. 2003.

Lundy J, Oh, J, Chung K, Ritter J, et al. Frequency and relevance of acute peritraumatic pulmonary thrombus diagnosed by computed tomographic imaging in combat casualties. J Trauma 2012; 75 (2): S215-S220

Lupu C, Lupu F, Dennehy U, Kakkar VV, Scully MF. Thrombin induces the redistribution and acute release of tissue factor pathway inhibitor from specific granules within human endothelial cells in culture. Arterioscler Thromb Vasc Biol. 1995; 15: 2055–2062.

Lyon R, Sausmarez E, McWhirter E, Wareham, et al. Pre-hospital transfusion of packed red blood cells in 147 patients from a UK helicopter emergency medical service. Scand J Trauma 2017; 25: 12.

Mackie I, Kitchen S, Machin S, Lowe G. Guidelines on Fibrinogen Assays. British J Haematology 2003; 121: 394-404

Mackway-Jones K, Foëx B, Kirkman E, Little R. Modification of the cardiovascular response to hemorrhage by somatic afferent nerve stimulation with special reference to gut and skeletal muscle blood flow. J Trauma 1999; 47: 481–5

MacLaren R, Weber L, Brake H, et al. A multicenter assessment of recombinant factor VIIa off-label usage: clinical experiences and associates outcomes. Transf 2005; 45(9): 1434-1442.

MacLeod J, Lynn , McKenney M, Cohn S, Murtha M. Early coagulopathy predicts mortality in trauma. J Trauma 2003; 55(1): 39-44.

Madjdjpour C, Dettori N, Frascarolo P, et al. Molecular weight of hydroxyethyl starch: is there an effect on blood coagulation and pharmacokinetics? Brit J Anaesth 2005; 94(5): 569-576.

Maegele M, Lefering R, Yucel N, Tjardes T, et al. Early coagulopathy in multiple injury: an analysis from the German Trauma Registry on 8724 patients. Injury 2007; 38(3): 298-304.

Maegele M, Lefering R, Paff rath T, Tjardes T, et al. Working Group on Polytrauma of the German Society of Trauma Surgery (DGU): Red blood-cell to plasma ratios transfused during massive transfusion are associated with mortality in severe multiple injury: a retrospective analysis from the Trauma Registry of the Deutsche Gesellschaft für Unfallchirurgie. Vox Sang 2008, 95: 112-119.

Maegele M, Brockamp T, Nienaber U, Probst C, et al. Predictive models and algorithms for the need of transfusion including massive transfusion in severely injured patients. Transfus Med Hemother 2012; 39(2): 85–97.

Maegele M, Schochl H, Cohen MJ. An update on the coagulopathy of trauma. Shock 2014; 41 (suppl 1): 21–5.

Magnotti L, Zarzur B, Fischer P, et al. Improved survival after hemostatic resuscitation: does the emperor have no clothes? J Trauma 2011; 70(1): 97-102.

Maier V. Pathogenesis of multiple organ dysfunction syndrome- endotpxin, inflammatory cells, and their mediators: cytokines and reactive oxygen species. Surg Inf 2004; 1(3).

Malhotra A, Rifat L, Fabian T, Rao I et al. Multiplicity of solid organ injury: influence on management and outcomes after blunt abdominal trauma. J Trauma 2003; 54(5): 925-929.

Malone D, Dunne J, Tracy K, et al. Blood transfusion, independnat of shock severity, is associated with worse outcome in trauma. J Trauma 2003; 54(5): 898-907.

[a]Mann KG, Brummel K, Butenas S. What is all that thrombin for? J Thromb Haemost. 2003; 1: 1504-1514.

[b]Mann KG. Thrombin formation. Chest 2003 124: 4S-10S.

[c]Mann, K. G., Butenas, S. & Brummel, K. The dynamics of thrombin formation. Arterioscler Thromb Vasc Biol 2003; 23: 17-25.

Mapstone J, Roberts I, Evans P. Fluid resuscitation strategies: a systematic review of animal trials. J Trauma 2003; 55(3): 571-589.

Mardel SN, Saunders FM, Allen H, et al. Reduced quality of clot formation with gelatin-based plasma substitutes. Br J Anaesth 1998; 80: 204–7.

Marik P, Corwin H. Efficacy of red blood cell transfusion in the critically ill: a systematic review of the literature. Crit Car Med 2008; 36(9): 2667-2674.

Martin C, Boisson C, Haccoun M, Thomachot L, Mege J. Patterns of cytokine evolution (tumor necrosis factor-alpha and interleukin-6) after septic shock, hemorrhagic shock, and severe trauma. Crit Care Med 1997; 25(11): 1813-9.

Martini W, Holcomb J. Acidosis and coagulopathy: the differential effects on fibrinogen synthesis and breakdown in pigs. Ann Surg 2007; 246(5): 831-5.

Martini W, Cortez D, Dubick M, Park M, et al. Thromboelastography is better than PT, aPTT, and activated clotting time in detecting clinically

relevant clotting abnormalities after hypothermia, hemorrhagic shock and resuscitation in pigs. J Trauma 2008; 65(3): 535-543.

Martinaud C, Ausset S, Deshayes AV, Cauet A, et al. Use of freeze-dried plasma in French intensive care unit in Afghanistan. J Trauma 2011; 71:1761-4.

Martini W. Coagulopathy by hypothermia and acidosis: mechanisms of thrombin generation and fibrinogen availability. J Trauma 2009; 67(1): 202-8.

Matsuoka T, Hildreth J, Wisner DH. Liver injury as a model of uncontrolled hemorrhagic shock: resuscitation with different hypertonic regimens. J Trauma. 1995; 39(4): 674–680.

Mattox K, Maningas P, Moore E, et al. Prehospital hypertonic saline/dextran infusion for post-traumatic hypotension. The USA multicenter trial. Ann Surg 1991; 213(5): 482-491.

Matzinger P. The danger model: a renewed sense of self. Science 2002; 296: 301–305.

McLellan S, McClelland D, Walsh T. Anaemia and red blood cell transfusion in the critically ill patient. Blood Rev 2003; 17: 195-208.

McSwain N, Champion H, Fabian T, et al. State of the art fluid resuscitation 2010: prehopital and immediate transition to the hospital. J Trauma 2011; 70(5): S2-S10.

Mediavilla-Varas J, Philippens M, Meijer S, van den Berg A, et al. Physics of IED blast shock tube simulations for mTBI research. Front Neurol 2011; 2: 58.

Meledeo M, Peltier G, McIntosh C, Voelker C, et al. Functional stability of the TEG 6s hemostatsis analyzer under stress. J Trauma 2018; 84(6 S1), S83-88.

Mellor S, The pathogenesis of blast injury and its management. Br J Hosp Med. 1988; 39(6):536-9.

Meng Z, Wolberg A, Monroe D 3rd, Hoffman M. The effect of temperature and pH on the activity of factor VIIa: implications for the efficacy of high-dose factor VIIa in hypothermic and acidotic patients. J Trauma 2003; 55(5): 886-91.

Mengoli C, Franchini M, Marano G, Pupella S, et al. The use of fibrinogen concentrate for the management of trauma-related bleeding: a systematic review and meta-analysis. Blood Transfusion 2017; 15: 318-24.

Michelson AD. Evaluation of platelet function by flow cytometry. Pathophysiol Haemost Thromb 2006; 35: 67–82.

Miller R, Robbins T, Tong M, Barton S. Coagulation defects associated with massive blood transfusions. Ann Surg 1971; 174: 794-801.

Mitra B, Mazur S, Cameron P, Bernard S, Burns B, et al. Tranexamic acid for trauma: Filling the 'GAP' in evidence. EMA 2014; 26: 194-7.

Mock C, Lormand JD, Goosen JD, Joshipura M, Peden M. Guidelines for essential trauma care. World Health Organisation, Geneva, 2004.

Modesti PA, Rapi S, Paniccia R, Bilo G, et al. Index measured at an intermediate altitude to predict impending acute mountain sickness. Med Sci Sports Exerc. 2011; 43(10): 1811–8.

Monroe DM, Hoffman M, Roberts HR. Platelets and thrombin generation. Arterioscler Thromb Vasc Biol 2002; 22: 1381-1389.

Monroe DM, Hoffman M. What does it take to make the perfect clot? Arterioscler Thromb Vasc Biol 2006; 26: 41-48.

Moore E, Cogbill T, Jurkovich G, Shackford S, et al. Organ Injury Scaling - Spleen and Liver [1994 Revision]. J Trauma 1995; 38(3): 323-4.

Moore H, Moore E, Gonzalez E, Chapman M, et al. Hyperfibrinolysis, physiologic fibrinolysis, and fibrinolysis shutdown: The spectrum of postinjury fibrinolysis and relevance to antifibrinolytic therapy. J Trauma Acute Care Surg 2014; 77(6): 811-7.

Moore E, Moore H, Gonzalez E, Chapman M, et al. Postinjury fibrinolysis shutdown: rationale for selective tranexamic acid. J Trauma 2015; 78(S1): S65-69.

Moran C, Lecky F, Bouamra O, Lawrence T, et al. Changing the System – Major Trauma Patients and Their Outcomes in the NHS (England) 2008-17. EClinical Medicine 2-3 2018; 13-21.

Morrison C, Carrick M, Norman M, Scott B. Hypotensive resuscitation strategy reduces transfusion requirements and severe postoperative coagulopathy in trauma patients with hemorrhagic shock: preliminary results of a randomized controlled trial. J Trauma 2011; 70(3): 652-663.

Morrison J, Dubose J, Rasmusson T, Midwinter M. Military Application of Tranexamic Acid in Trauma Emergency Resuscitation (MATTERs) Study. Arch Surg 2012; 147(2): 113-9.

[a]Morrison J, Ross J, Poon H, Midwinter M, Jansen J. Intra-operative correction of acidosis, coagulopathy and hypothermia in combat casualties with severe haemorrhagic shock. Anaesthesia 2013; 68: 846-850.

[b]Morrison J, Ross J, Dubose J, Jansen J, et al. Association of cryoprecipitate and tranexamic acid with improved survival following wartime injury: findings from the MATTERs II Study. JAMA Surg 2013; 148(3): 218–25

MTN. National Peer Review Report: Major Trauma Networks 2014. 22 April 2014.

Mulier K, Greenberg J, Bellman G. Hypercoagulability in porcine hemorrhagic shock is present early after trauma and resuscitation. J Surg Res 2012; 174(1): e31-e35.

Mutschler M, Nienaber U, Münzberg M, Wölfl C, et al. The Shock Index revisited – a fast guide to transfusion requirement? A retrospective analyss on 21,853 patients derived from the TraumaRegister DGU®. Crit Care 2013 17(4): R172.

Mutschler M, Brockamp T, Wafaisade A, Lipensky A, et al. 'Time to TASH': how long does complete score calculation take to assess major trauma hemorrhage? Transfus Med. 2014; 24(1): 58–9.

Myburgh J, Cooper DJ, Finfer S, Bellomo R, Norton R, Bishop N, et al. Saline or albumin for fluid resuscitation in patients with traumatic brain injury. N Engl J Med. 2007; 357(9): 874–84.

Myburgh JA, Finfer S, Bellomo R, Billot L, Cass A, Gattas D et al. Hydroxyethyl starch or saline for fluid resuscitation in intensive care. N Engl J Med. 2012;367(20):1901–11.

Nakayama S, Sibley L, Gunther R, et al. Small-volume resuscitation with hypertonic saline (2,400 mOsm/liter) during hemorrhagic shock. Circ Shock 1984; 13(2): 149-159.

Napolitano LM, Ferrer T, McCarter RJ Jr, Scalea TM. Systemic inflammatory response syndrome score at admission independently predicts mortality and length of stay in trauma patients. J Trauma 2000; 49: 647–652.

Napolitano L, Cohen M, Cotton B, Schreiber M, Moore E. Tranexamic acid in trauma: how should we use it? J Trauma Acute Care Surg 2013; 74(6): 1575-1586.

Nascimento B, Callum J, Tien H, Peng H, et al. Fibrinogen in the initial resuscitation of severe trauma (FiiRST): a randomized feasibility trial. Brit J Anaesth 2016; 117(6): 775-82.

Naumann DN, Hazeldine J, Dinsdale RJ, Bishop JR, Midwinter MJ, Harrison P, et al. Endotheliopathy is associated with higher levels of cell-free DNA following major trauma: a prospective observational study. *PLoS One* 2017; 12(12): e0189870.

Naumann DN, Hazeldine J, Davies DJ, Bishop J, et al. Endotheliopathy of trauma is an on-scene phenomenon, and is associated with multiple organ dysfunction syndrome: a prospective observational study. Shock 2018; 49(4): 420–8.

NCEPOD. Trauma: Who Cares? National Enquiry into Perioperative Deaths, UK. 2007. http://www.ncepod.org.uk/2007report2/Downloads/SIP_report.pdf

Neeki M, Dong F, Vaezazizi R, Powell J, et al. Efficacy and safety of tranexamic acid in prehospital traumatic hemorrhagic shock: outcomes of the Cal-PAT study. West J Emrg Med 2017; 18(4): 673-683.

Nessen S, Eastridge B, Cronk D, Craig R, et al. Fresh Whole Blood Use by Forward Surgical Teams in Afghanistan is Associated with Improved Survival Compared to Component Therapy Without Platelets. Transfusion 2013; 53: 107S-113S

Nguyen C, Bordes J, Cungi P-J, Esnault P, et al. Use of French lyophilized plasma transfusion in severe trauma patients is associated with an early plasma transfusion and early transfusion ration improvement. J Trauma 2018; 84(5): 780-785.

NICE Technology Appraisal Guidance 74. National Institute for Health and Care Excellence. Pre-hospital initiation of fluid therapy in trauma. 2004.

NICE Guideline 24 (NG 24). National Institute for Health and Care Excellence (NICE) Guideline 24; Blood Transfusion. 2015.

NICE Guideline 39 (NG 39). National Institute for Health and Care Excellence (NICE) Guideline 39; Major trauma: assessment and initial management. 2016.

NICE Guideline 40 (NG 40). National Institute for Health and Clinical Excellence (NICE) Guideline 40; Major Trauma: Service Delivery. 2016.

Nielsen V. A comparison of the Thrombelastograph and the ROTEM. Blood Coagul Fibrinolysis 2007; 18: 247-52.

Nieuwdorp M, Meuwese MC, Mooij HL, van Lieshout MH, et al. Tumor necrosis factor-alpha inhibition protects against endotoxin-induced endothelial glycocalyx perturbation. Atherosclerosis 2009; 202(1): 296-303.

Niles S, McLaughlin D, Perkins J, Wade C, et al. Increased mortality associated with the early coagulopathy of trauma in combat casualties. J Trauma 2008; 64(6): 1463-5.

Nolan J. Fluid Replacement. British Medical Bulletin1999; 55(4): 821-843.

Nolan J. Fluid Resuscitation for the trauma patient. Resuscitation 2001; 48: 57-69.

Norda R, Tynell E, Åkerblom O. Cumulative risks of early fresh frozen plasma, cryoprecipitate and platelet transfusion in Europe. J Trauma 2006; 60: S41–S45.

Nordmann G. Adapted excerpt from personal diary. Operation Herrrick, Camp Bastion, Afghanistan. October 2010

Nordmann G, Jones A, Park C, Mahoney P. Acute Traumatic Coagulopathy is evident in severely injured military casualties within 40 minutes of injury. Anaesthesia 2016; 71(S2): 68.

Öberg B, Thorén P. Increased activity in left ventricular receptors during hemorrhage or occlusion of caval veins in the cat – a possible cause of the vago-vagal reaction. Acta Physiol 1972; 85(2): 164-173.

O'Brien SF, Yi QL, Fan W, Scalia V, Kleinman SH, Vamvakas EC: Current incidence and estimated residual risk of transfusion-transmitted infections in donations made to Canadian Blood Services. Transfusion 2007, 47:316-325.

O'Connell KA, Wood JJ, Wise RP, Lozier JN, Braun MM. Thrombo- embolic adverse events after use of recombinant human coagulation factor VIIa. JAMA. 2006; 295: 293–298

Ogilvie M, Pereira B, McKenney M, McMahon P, et al. First report on safety and efficacy of hetastarch solution for initial fluid resuscitation at a level 1 trauma center. J Am Coll Surg 2010; 210(5): 870–80, 80–2.

Ohnishi M, Kirkman E, Marshall H, Little R. Morphine blocks the bradycardia associated with severe haemorrhage in the anesthetized rat. Brain Research 1997; 763: 39-46.

Ohnishi M, Kirkman E, Watkins P. Effects of atropine on the bradycardia associated with primary thoracic blast injury in the anesthetized rat. Br J Pharmacol 1998; 123: U60.

Ohnishi M, Kirkman E, Guy R, Watkins P. Reflex nature of the cardiovascular response to primary thoracic blast injury in the anaesthetized rat. Experimental Physiol 2001; 86: 357-64.

Oktavec W Jr, Smetana E. Lyophilized normal human plasma control in the prothrombin-time clotting test. Am J Clin Pathol 1954; 24: 250.

Olde Engberink RH, Kuiper GJ, Wetzels RJ, et al. Rapid and correct prediction of thrombocytopenia and hypofibrinogenemia with rotational thromboelastometry in cardiac surgery. J Cardiothorac Vasc Anesth. 2014; 28(2): 210–6.

ONS. Office for National Statistics. Deaths registered in England and Wales : 2017. www.ons.gov.uk July 2018.

Ortega-Gonzalez M. Anaesthesia for trauma patients. S Afr Fam Pract 2012; 54(3 S1): S2-6.

O'Shaughnessy DF, Atterbury C, Bolton Maggs P, Murphy M,et al; British Committee for Standards in Haematology, Blood Transfusion Task Force: Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryosupernatant. Br J Haematol 2004, 126:11-28.

[a]Ostrowski SR, Johansson PI. Endothelial glycocalyx degradation induces endogenous heparinization in patients with severe injury and early traumatic coagulopathy. J Trauma 2012; 73: 60–6

[b]Ostrowski S, Sørensen A, Windeløv N, Perner A, et al. High levels of High levels of soluble VEGF receptor 1 early after trauma are associated with shock, sympathoadrenal activation, glycocalyx degradation and inflammation in severely injured patients: a prospective study. Scand J Trauma, Resusc and Emerg Med 2012; 20: 27- 34.

Ostrowski SR, Henriksen HH, Stensballe J, Gybel-Brask M, Cardenas JC, Baer LA, et al. Sympathoadrenal activation and endotheliopathy are drivers of hypocoagulability and hyperfibrinolysis in trauma: a prospective observational study of 404 severely injured patients. J Trauma 2017; 82(2): 293-301

Oyetunji T, Chang D, Crompton J, Greene W, et al. Redefining hypotension in the elderly. Arch Surg 2011; 146(7): 865-869.

Palm K, Apodoca A, Spencer D, Costanza G, et al. Evaluaion of military trauma system practices related to complicatiosn after injury. J Trauma 2012; 73: S465-S471.

Pape A, Weber C, Stein P, Zacharowski K. ROTEM and multiplate – a suitable tool for POC? ISBT Science Seires 2010; 5(n1): 161-168.

Park M, Taniguchi LU, Noritomi DT, Braga AL, Maciel AT and Cruz-Neto LM. Clinical utility of standard base excess in the diagnosis and interpretation of metabolic acidosis in critically ill patients. Brazil J Med Biol Res 2008; 41: 241-249.

Park M, Owen B, Ballinger B, Sarr M, et al. Quantification of hypercoagulable state after blunt trauma: microparticle and thrombin generation are increased relative to injury severity, while standard markers are not. Surgery 2012; 151(6): 831-836.

Patel H, Bouamra O, Woodford M, King A, et al. Trends in head injury outcome from 1989 to 2003 and the effect of neurosurgical care: an observational study. Lancet 2005; 366: 1538-44.

Pati S, Matijevic N, Doursout MF, Ko T, Cao Y, Deng X, et al. Protective effects of fresh frozen plasma on vascular endothelial permeability, coagulation, and resuscitation after hemorrhagic shock are time dependent and diminish between days 0 and 5 after thaw. J Trauma 2010; 69(S1): S55–63.

Pati S, Potter D, Baimukanova G, Farrel D, et al. Modulating the endotheliopathy of trauma: Factor concentrate versus fresh frozen plasma. J Trauma Acute Care Surg 2016; 80(4): 576-84.

Patrick DA, Moore EE, Moore FA, Biffl WL, Barnett CC Jr. Release of anti-inflammatory mediators after major torso trauma correlates with the development of postinjury multiple organ failure. Am J Surg 1999; 178: 564–569

Patrick J, Cauet A, Deshayes A-V, Demazeau N, et al. Clinical use of freeze dried plasma (FDP) in deployed operations: Analysis of 72 episodes of transfusion. European J Anaesth 2011; 28: 91.

Peng Z, Pati S, Potter D, et al. Fresh frozen plasma lessens pulmonary endothelial inflammation and hyperpermeability after hemorrhagic shock and is associated with loss of syndecan-1. Shock 2013; 40(3): 195-202.

Penn-Barwell J, Roberts S, Midwinter M, Bishop J. Improved survival in UK combat casualties from Iraq and Afghanistan: 2003-2012. Trauma Acute Care Surg 2015; 78(5): 1014-1020.

Pereira A. Cryoprecipitate versus commercial fibrinogen concentrate in patients who occasionally require a therapeutic supply of fibrinogen: risk comparison in the case of an emerging transfusion-transmitted infection. Haematologica 2007; 92: 846-9.

Perel P, Roberts I, Kerr K. Colloids versus crystalloids for fluid resuscitation in critically ill patients. Cochrane Database Syst Rev 2013; 28(2): CD000567

Perkins J, Cap A, Spinella P, Blackbourne L, et al. An evaluation of the impact of apheresis platelets used in the setting of massively transfused trauma patients. J Trauma 2009;66(4 Suppl):S77–84.

Perner A, Haase N, Guttormsen AB, Tenhunen J, et al. Hydroxyethyl starch 130/0.42 versus Ringer's acetate in severe sepsis. N Engl J Med. 2012;367(2):124–34.

Petäjä J. Inflammation and coagulation. An overview. Thromb Res 2011; 127(S2): S34-37.

Petroianu G, Liu J, Maleck W, et al. The Effect of *In Vitro* Hemodilution with Gelatin, Dextran, Hydroxyethyl Starch, or Ringer's Solution on Thrombelastograph®. Anesth Analg 2000; 90(4): 795-800.

Pierce A, Pittet JF. Inflammatory response to trauma: implications for coagulation and resuscitation. Curr Opin Anaesthesiol 2014, 27:246–252.

Plotkin A, Wade C, Jenkins D, Smith K, et al. A reduction in clot formation rate and strength assessed by thromboelastographyis indicative of transfusion requirements in patients with penetrating injuries. J Trauma 2008; 64(S2): S64-8.

Pluthero F, Rand M, Blanchette V, Kahr W. Rapid assessment of platlet function using thromboelastography and small volumes of citrated whole blood. Blood 2005; 106: 3995-9.

Poels P, Gabreëls F. Rhabdomyolysis: a review of the literature. Clin Neurol and Neurosurg 1993; 95(3): 175-192.

Poller L. International Normalized Ratios (INR): The first 20 years. J ThrombHaemost 2012; 2: 849–860.

Popovsky M. Pulmonary consequences of transfusion: TRALI and TACO. Transfus Apher Sci 2006, 34:243-244.

Prat N, Montgomery R, Cap A, et al. Comprhensive evaluation of coagulation in swine subjected to isolated primary blast injury. Shock 2015; 43(6): 598-603.

Proctor R, Rapaport S. The partial thromboplastin time with kaolin. A simple screening test for first stage plasma clotting factor deficiencies. Am J Clin Pathol 1961; 36: 212-9.

Pusateri A, Weiskopf R , Bebarta V, et al. Tranexamic acid and trauma: current status and knowledge gaps with recommended research priorities. Shock 2013; 39: 121–6.

Quest J, Gebber G. Modulation of baroreceptor reflexes by somatic afferent nerve stimulation. Am J Physiol 1972; 222(5): 1251-6.

Raber MN. *Coagulation Tests*, Chapter 157 in Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd Edition. Eds Walker HK, Hall WD, Hurst JW. Butterworths, 1990.

Rady M, Little R, Edwards J, Kirkman E, Faithful S. The effect of nociceptive stimulation on the changes in hemodynamics and oxygen transport induced by hemorrhage in anesthetized pigs. J Trauma 1991; 31: 617–21.

Rady M, Kirkman E, Cranley J, Little R. A comparison of the effects of skeletal muscle injury and somatic afferent nerve stimulation on the response to hemorrhage in anesthetized pigs. J Trauma 1993; 35(5): 756-761.

Rahbar E, Cardenas JC, Baimukanova G, Usadi B, Bruhn R, Pati S, et al. Endothelial glycocalyx shedding and vascular permeability in severely injured trauma patients. J Transl Med 2015; 13: 117.

Rahe-Meyer N, Pichlmaier M, Haverich A, Solomon C, et al. Bleeding management with fibrinogen concentrate targeting a high-normal plasma fibrinogen level: a pilot study. Br J Anaesth 2009; 102(6): 785-92.

Rai R, Tuddenham E, Backos M, Jivraj S, et al. Thromboelastography, whole-blood haemostasis and recurrent miscarriage. Hum Reprod 2003; 18: 2540-3.

The Red Book. Guidelines for the Blood Transfusion Services in the UK. 8th Edition. NHS Blood and Transfusion Services [NHSBT]. 2013.

Redfern W, Little R, Stoner H, Marshall H. Effect of limb ischaemia on blood pressure and the blood pressure-heart rate reflex in the rat. Q J Exp Physiol 1984; 69: 763 – 79

Reed R, Bracey A, Hudson J, Miller T, Fischer R. Hypothermia and blood coagulation: dissociation between enzyme activity and clotting factor levels. Circ Shock 1990; 32(2): 141-52.

Reed R, Johnston T, Chen Y, Fischer R. Hypertonic saline alters plasma clotting times and platelet aggregation. J Trauma 1991; 31: 8–14.

Rehm M, Bruegger D, Christ F, et al. Shedding of the endo- thelial glycocalyx in patients undergoing major vascular surgery with global and regional ischemia. Circulation 2007; 116: 1896–906

Rehn M, Weaver A, Eshelby S, et al. Pre-hospital transfusion of red blood cells in civilian trauma patients. Transf Med 2017; 28(4): 277-283.

Reitsma S, Slaaf D, Vink H, van Zandvoort M, Oude Egbrink M. The endothelial glycocalyx: composition, functions, and visualization. Pflugers Arch 2007; 454: 345–59

Reilly P, Bulkley G. Vasoactive mediators and splanchnic perfusion. Crit Care Med 1993; 21(S2): S55-68.

Rider P, Voronov E, Dinarello R, Cohen I. Alarmins: Feel the stress. J Immunol 2017; 198(4): 1395-1402.

Riha GM, Kunio NR, Van PY, Kremenevskiy I, et al. Uncontrolled hemorrhagic shock results in a hypercoagulable state modulated by initial fluid resuscitation regimens. J Trauma 2013; 75(1): 129-34.

Rijken D, Lijnen H. New insights into the molecular mechanisms of the fibrinolytic system. J Thromb Haem 2009; 7: 4-13.

Risberg B. Fibrinolysis and trauma. Eur J Surg 1978; 10: 373– 81

Risberg B, Medegård A, Heideman M, Gyzander E, et al. Early activation of humoral proteolytic systems in patients with multiple trauma. Crit Care Med 1986; 14(11): 917-925.

Ritenour A, Toney B. Primary blast injury: update on diagnosis and treatment. Crit Care Med 2008; 36(7): S311-S317.

Rivard G, Brummel-Ziedins K, Mann K, Fan L, et al. Evaluation of the profile of thrombin generation during the process of whole blood clotting as assessed by thromboelastography. J Thromb Haemostat 2005; 3(9): 2039-43.

Rizoli S, Rhind S, Shek P, Inaba K, Filips D, Tien H, et al. The Immunomodulatrory Effects of Hypertonic Saline Resuscitation in Patients Susutaining Traumatic Hemorrhagic Shock. Annals of Surg 2006; 243(1): 47-57.

Rizoli S, Scarpelini S, Callum J, Nasciemento B, et al. Clotting Factor Deficiency in Early Trauma-Associated Coagulopathy. J Trauma 2011; 71(5 S1): S427-434.

Roberts HR, Hoffman M, Monroe DM. A cell-based model of thrombin generation. Semin Thromb Haemost 2006; 32 (Suppl 1): 32-38.

Roeloffzen W, Kluin-Nelemans HC, Bosman L, de Wolf J. Effects of red blood cells on hemostasis. Transfusion 2010; 50(7): 1536-44.

Rohrer M, Natale A. Effect of hypothermia on the coagulation cascade. Crit Care Med 1992; 20(10): 1402-5.

Rossaint R, Bouillon B, Cerny V, Coats t, Duranteau J, et al. Management of bleeding following major trauma: an updated European guideline. Crit Care 2010, 14: R52.

Rossaint R, Bouillon B, Cerny V, Coats T, et al. The European guideline on management of major bleeding and coagulopathy following trauma: fourth edition. Crit Care 2016; 20: 100

Rotondo M, Schwab C, McGonigal M, Phillips G 3rd, et al. “Damage Control”: an approach for improved survival in exsanguinating penetrating abdominal injury. J Trauma 1993; 35(3): 375-82.

Rotstein OD. Modeling the two-hit hypothesis for evaluating strategies to prevent organ injury after shock/resuscitation. J Trauma 2003; 54(suppl): 203–206.

Roullet S, Maistre E, Ickx B, Blais N, et al. Position of the French Working Group on Perioperative Haemostasis (GIHP) on viscoelastic tests: What

role for which indication in bleeding situations? Anaesth Crit Care Pain 2018 https://doi.org/10.1016/j.accpm.2017.12.014

Rourke C, Curry N, Khan S, Taylor R, et al. Fibrinogen levels during trauma hemorrhage, response to replacement therapy, and association with patient outcomes. J Thromb Haemos 2012; 10(7): 1342-1351.

Rugeri L, Levrat A, David JS, Delecroix E, et al. Diagnosis of early coagulation abnormalities in trauma patients by rotation thrombelastography. J Thromb Haemost. 2007; 5(2): 289–95.

Russell R, Clasper J, Jenner B, Hodgetts T, Mahoney P. Ballistic Injury. BMJ 2014; 348: bmj.g1143

Sadler JM, Horsey PJ. The new generation gelatins. A review of their history, manufacture and properties. Anaesthesia 1987; 42: 998–1004.

Sakles J, Sena M, Knight D, Davis J. Effect of immediate fluid resuscitation on the rate, volume and duration of pulmonary vascular hemorrhage ina sheep model of penetrating thoracic trauma. Ann Emrg Med 1997; 29(3): 392-399.

Salmon J, Mythen M. Pharmacology and physiology of colloids. Blood Rev 1993; 7(2): 114-120.

Savage S, Zarzaur B, Croce M, Fabian T. Time matters in 1:1 resuscitations: concurrent administration of blood: plasma and risk of death. J Trauma 2014; 77(6): 833–7.

Sawdon M, Ohnishi M, Watkins P, Kirkman E. The effects of primary thoracic blast injury and morphine on the response to haemorrhage in the anaesthetised rat. Exp Physiol 2002; 87(6): 683-688.

Scalea TM, Bochicchio KM, Lumpkins K, Hess JR, Dutton R, Pyle A, Bochicchio GV: Early aggressive use of fresh frozen plasma does not improve outcome in critically injured trauma patients. Ann Surg 2008, 248:578-584.

Schmand J, Ayala A, Morrison M, et al. Effects of hydroxyethyl starch after trauma hemorrhagic shock: restoration of macrophage integrity and prevention of increased circulating interleukin 6 levels. Crit Care Med 1995; 23: 806-14.

Schmidt E, Lee W, Zemans R, Yamashita C, Downey G. On, around, and through: neutrophil-endothelial interactions in innate immunity. Physiology (Bethesda) 2011; 26(5): 334-47.

Schnuriger B, Inaba K, Abdelsayed G, Lustenberger T, et al. The impact of platelets on the progression of traumatic intracranial hemorrhage. J Trauma. 2010; 68(4): 881–5.

Schöchl H, Frietsch T, Pavelka M, Jámbor C. Hyperfibrinolysis after major trauma: differential diagnosis of lysis patterns and prognostic value of thromboelastometry. J Trauma 2009; 67(1): 125-131

Schöchl H, Nienaber U, Hofer G, et al. Goal-directed coagulation management of major trauma patients using thromboelastometry (ROTEM)-guided administration of fibrinogen concentrate and prothrombin complex concentrate. Crit Care 2010; 14(2): R55.

Schöchl H, Cotton B, Inaba K, Nienaber U, et al. FIBTEM provides early prediction of massive transfusion in trauma. Crit Care 2011; 15: R265.

Schouten HJ, Geersing GJ, Koek HL, et al: Diagnostic accuracy of conventional or age adjusted D-dimer cut-off values in older patients with suspected venous thromboembolism: systematic review and meta-analysis. BMJ 2013; 346: f2492.

Schreiber M, Meier E, Tisherman S, Kerby J, et al. A controlled resuscitation strategy is feasible and safe in hypotensive trauma patients: results of a prospective randomized pilot trial. J Trauma Acute Care Surg 2015; 78(4): 687-697.

Secher N, Bie P. Bradycardia during reversible haemorrhagic shock—a forgotten observation? Clin Physiol 1985; 5: 315 – 23

Segal J, Dzik W. Paucity of studies to support that abnormal coagulation test results predict bleeding in the setting of invasive procedures: an evidence-based review. Transfusion 2005; 45: 1413-25.

Selby R, Geerts W, Ofosu F, Craven S, et al. Hypercoagulability after trauma: Hemostatic changes and relationship to venous thromboembolism. Thromb Res 2009; 124: 281-7

Sena M, Douglas G, Gerlach T, Grayson J, et al. A pilot study of the use of kaolin-impregnated gauze (combat gauze) for packing high-grade hepatic injuries in a hypothermic coagulopathic swine model. J Surg Res 2013; 183(2): 704-9.

Shackelford S, del Junco D, Powell-Dunford N, et al. Association of pre-hospital blood product transfusion during medical evacuation of combat casualties in Afghanistan with acute and 30-day survival. JAMA 2017; 318(16): 1581-1591.

Shafer S. Editor's note: notices of retraction. Anesth Analg 2011; 112(5): 1246-1247.

Shapiro NI, Schuetz P, Yano K, Sorasaki M, Parikh SM, Jones AE, Trzeciak S, Ngo L, Aird WC: The association of endothelial cell signaling, severity of illness, and organ dysfunction in sepsis. Crit Care 2010, 14: R182.

Shapiro S, Sherwin B, Gordimer H. Postoperative Thrombo-Embolization: The Platelet Count and the Prothrombin Time After Surgical Operations: A Simple Method For Detecting Reductions and Elevations of the Prothrombin Concentration (or Activity) of the Blood Plasma. Ann Surg 1942; 116: 175-83.

Shaz B, Winkler A, James A, Hillyer C, MacLeaod J. Pathophysiology of Early Trauma Induced Coagulopathy: Emerging Evidence for Hemodilution and Coagulation Factor Depletion. J Trauma 2011; 70(6): 1401-7.

Shehata N, Blajchman M, Heddle N: Coagulation factors in FFP and cryosupernatant. Transfus Med 2001. 11:391-401

Shen L, Lorand L. Contribution of fibrin stabilization to clot strength. J Clin Invest 1983; 71: 1336-1341.

Shi J, Shi Y, Waehrens LN, et al. Lactadherin detects early phosphatidylserine exposure on immortalized leukemia cells undergoing programmed cell death. Cytometry A. 2006; 69: 1193–1201.

Shlaifer A, Siman-Tov M, Radomislensky I, Kobi P, et al. Prehospital administration of freeze-dried plasma, is it the solution for trauma casualties? J Trauma Acute Care Surg 2017; 83(4): 675-682.

Shore-Lesserson L, Manspeizer H, Deperio M, Francis S, et al. Thromboelastography-guided transfusion algorithm reduces transfusions in complex cardiac surgery. Anesth Analg 1999; 88: 312-9.

(SHOT) Serious Hazards of Transfusion (SHOT) Annual Report. 2012. Available from http://www.shotuk.org/wp-content/uploads/ 2012/07/SHOT-ANNUAL-REPORT_FinalWebVersionBookmarked_ 2012_06_22.pdf

Shuja F, Shults C, Duggan M, Tabbara M, et al. Development and testing of freeze-dried plasma for the treatment of trauma associated coagulopathy. J Trauma 2008; 65: 975-85.

Sihler KC, Napolitano LM. Complications of massive transfusion. Chest 2010; 137: 209–220.

Sikorski R, Koerner A, Fouche-Weber L, Galvagno S. Choice of general anaesthetic for trauma patients. Curr Anesthesiol Rep 2014; 4: 225-232.

Siller-Matula J, Plasenzotti R, Spiel A, Quehenberger P, Jilma B. Interspecies differences in coagulation profile. Thromb Haemostat 2008; 100: 397-404.

Simmons R, Collins J, Heisterkamp C, et al. Coagulation disorders in combat casualties. I. Acute changes after wounding. II. Effects of massive transfusion. III. Post-resuscitative changes. Ann Surg 1969; 169(4): 455-82.

Smail N, Wang P, Cioffi W, Bland K, Chaudry I. Resuscitation after uncontrolled venous hemorrhage: does increased resuscitation volume improve regional perfusion. J Trauma 1998; 44(4): 701-708.

Smith G, Kramer G, Perron P, et al. A comparison of several hypertonic solutions for resuscitation of bled sheep. J Surg Res. 1985; 39(6): 517–28

Smith J. The epidemiology of blast lung injury during recent military conflicts: a retrospective database review of cases presenting to deployed military hospitals, 2003–2009. Phil Trans Royal Soc B 2011; 366 (1562); 291-294.

Snyder C, Weinberg J, McGwin G, Jr, et al. The relationship of blood product ratio to mortality: survival benefit or survival bias? J Trauma 2009, 66:358-362.

Solomon, C., Traintinger, S., Ziegler, B., et al. Platelet function following trauma. A multiple electrode aggregometry study. Thrombosis and haemostasis 2011; 106 (2): 322–330.

Solomonov E, Hirsh M, Yahiya A, Krausz MM. The effect of vigorous fluid resuscitation in uncontrolled hemorrhagic shick after massive splenic injury. Crit Care Med 2000; 28(3): 749—54.

Sondeen J, Coppes V, Holcomb J. Blood pressure at which rebleeding occurs after resuscitation in swine aortic injury. J Trauma 2003; 54: S110-117.

Sørensen B, Ingerslev J. Tailoring haemostatic treatment to patient requirements - an update on monitoring haemostatic response using thrombelastography. Haemophilia 2005; 11 (S1): 1-6.

Sørensen B, Fenger-Eriksen C, Christiansen K, Ingerslev J. Evaluation of coagulation kinetics using thromboelastometry – methodological influence of activator and test medium. Ann Hematology 2010; 89(11): 1155-1161.

Spahn D, Bouillon B, Cerny V, Coats T, et al. Management of bleeding and coagulopathy following major trauma: an updated European guideline. Crit Care 2013; 17: R76

Sparrow RL. Red blood cell storage duration and trauma. Transfus Med Rev. 2015; 29: 120–126.
Sperry J, Guyette F, Brown J, Yazer M, et al. Prehospital plasma during air medical transport in trauma patients at risk for hemorrhagic shock. NEJM 2018; 379: 315-326.

Spiess B, Gillies B, Chandler W, Verrier E. Changes in transfusion therapy and reexploration rate after institution of a blood management program in cardiac surgical patients. J Cardiothorac Vasc Anesth 1995; 9: 168-73

[a]Spinella P, Perkins J, Grathwohl K, Beekley A, et al. Effect of plasma and red blood cell transfusions on survival in patients with combat related traumatic injuries. J Trauma 2008, 64(2 Suppl): S69-S77.

[b]Spinella P, Perkins J, McLaughlin D, et al. The effect of recombinant activated factor VII on mortality in combat-related casualties with severe trauma and massive transfusion. J Trauma 2008; 64: 286-294.

Spinella PC, Perkins JG, Grathwohl KW, et al: Warm fresh whole blood is independently associated with improved survival for patients with combat-related traumatic injuries. J Trauma 2009; 66(4 Suppl): S69–76.

Spinella PC, Cap AP: Whole blood: back to the future. Curr Opin Hematol 2016; 23(6): 536–42.

Spoerke N, Zink K, Cho D. Lyophilized plasma for resuscitation in a swine model of severe injury. Arch Surg 2009; 144(9): 829-834.

Staab D, Sorensen V, Fath J, Raman S, Horst H, Obeid F. Coagulation defects resulting from ambient temperature-induced hypothermia. J Trauma 1994; 36(5): 634-8.

Stainsby D, Maclennan S, Thomas D, Isaac J, Hamilton P. Guidelines on the management of massive blood loss. Br J Haematol 2006; 135: 634-41.

Stamatos CA. Geriatric trauma patients: initial assessment and management of shock. J Trauma Nurs 1994; 1(2): 45-56

Stansbury L, Hess A, Thompson K, Kramer B, et al. The clinical significance of platelet counts in the first 24 hours after severe injury. Transfusion 2013; 53: 783–9

Stansbury L, Hess J: The 100th anniversary of the first blood bank. Transfusion 2017; 57(11): 2562–3.

Stanworth SJ, Brunskill SJ, Hyde CJ, McClelland DB, Murphy MF: Is fresh frozen plasma clinically effective? A systematic review of randomized controlled trials. Br J Haematol 2004, 126:139-152.

Stanworth SJ, Hyde CJ, Murphy MF. Evidence for indications of fresh frozen plasma. Transfus Clin Biol. 2007; 14: 551–556.

Starkey K, Keene D, Morrison J, Doughty H, et al. Impact of high ratios of plasma-to-red cell concentrate on the incidence of acute respiratory distress syndrome in UK transfused combat casualties. Shock 2013; 40: 15–20.

Stein M and Hirshberg A. Trauma Care in the New Millenium: Medical Consequences of Terrorism, the Conventional Weapon Threat. Surgical Clinics of North America 1999; 79 (6).

Stern S, Dronen S, Birrer P, Wand X. Effect of blood pressure on hemorrhage volume and survival in a near-fatal hemorrhage model incorporating a vascular injury. Ann Emrg Med 1993; 22(2): 155-163.

Stinger H, Spinella P, Perkins J, Grathwohl K, et al. The ratio of fibrinogen to red cells transfused affects survival in casualties receiving massive transfusions at an army combat support hospital. J Trauma 2008; 64(2 Suppl): S79–85.

Sunde G, Vikenes B, Strandenes G, Flo K, Hervig T, et al. Freeze dried plasma and fresh red blood cells for civilian prehospital hemorrhagic shock resuscitation. J Trauma 2015; 78(6 S1): S26-30.

Tapia N, Chang A, Norman M, et al. TEG-guided resuscitation is superior to standardized MTP resuscitation in massively transfused penetrating trauma patients. J Trauma 2013; 74(2): 378-86.

Tarmey NT, Woolley T, Jansen JO, Doran CM, et al. Evolution of coagulopathy monitoring in military damage-control resuscitation. J Trauma 2012; 73(6 Suppl 5): S417–22.

Tauber H, Innerhofer P, Breitkopf R, Westermann I, et al. Prevalence and impact of abnormal ROTEM(R) assays in severe blunt trauma: results of the 'Diagnosis and Treatment of Trauma-Induced Coagulopathy (DIA-TRE-TIC) study'. Br J Anaesth 2011; 107(3): 378–87.

Teixeira P, Inaba K, Shulman I, Salim A, et al. Impact of plasma transfusion in massively transfused trauma patients. J Trauma 2009, 66:693-697.

Theodoulou A, Berryman J, Nathwani A, Scully M. Comparison of cryoprecipitate with fibrinogen concentrate for acquired hypofibrinogenaemia. Transfus Apher Sci 2012; 46: 159–62.

Theusinger OM, Wanner GA, Emmert MY, Billeter A, Eismon J, Seifert B, et al. Hyperfibrinolysis diagnosed by rotational thromboelastometry (ROTEM) is associated with higher mortality in patients with severe trauma. Anesth Analg. 2011; 113: 1003–12.

Theusinger O, Felix C, Spahn D. Strategies to reduce the use of blood products: a European perspective. Curr Opin Anaesthesiol 2012; 25(1): 59-65.

Theusinger OM, Baulig W, Seifert B, Muller SM, Mariotti S, Spahn DR. Changes in coagulation in standard laboratory tests and ROTEM in trauma patients between on-scene and arrival in the emergency department. Anesth Analg. 2015; 120: 627–35.

Thomas D, Wee M, Clyburn P, et al. Blood transfusion and the anaesthetist: management of massive haemorrhage. Anaesthesia 2010; 65: 1153–61.

Thomas G, Dutton R, Hemlock B, et al. Thromboembolic complications associated with factor VIIa administration. J Trauma 2007; 62(3): 564-569.

Thorarinsdottir H, Sigurbjornsson F, Hreinsson K, et al. Effects of fibrinogen concentrate administration during severe hemorrhage. Acta Anaesthesiol Scand 2010; 54: 1077-82.

Torres L, Sondeen J, Dubick M, Filho I. Systemic and microvascular effects of resuscitation with blood products after severe haemorrhage in rats. J Trauma 2014; 77(5): 716-723.

Tóth O, Calatzis A, Penz S, Losonczy H, Siess W. Multiple electrode aggregometry: a new device to measure platelet aggregation in whole blood. Thromb Haemost. 2006; 96(6): 781–8.

Toulon P, Ozier Y, Ankri A, Fléron M, et al. Point-of-care versus central laboratory testing during haemorrhagic surgery. A multicenter study. Thromb Haemostat 2009; 101(2): 394-401.

Traverso L, Bellamy R, Hollenbach S, et al. Hypertonic sodium chloride solutions: effect on hemodynamics and survival after hemorrhage in swine. J Trauma. 1987; 27(1): 32–39.

Treib J, Haass A, Pindur G, Grauer MT, Wenzel E, Schimrigk K. All medium starches are not the same: influence of the degree of hydroxethyl substitution of hydroxethyl starch on plasma volume, haemorrhagic conditions and coagulation. Transfusion 1996; 36: 450-55.

Trieb J, Baron JF. Hydroxyethyl starch: effects on haemostasis. Ann Fr Anesth Réanim 1998; 17: 72-81.

Tripodi A, Chantarangkul V, Mannucci P. Acquired coagulation disorders: revisited using global coagulation/anticoagulation testing. Br J Haematol 2009; 147: 77-82.

Tschoeke SK, Hellmuth M, Hostmann A, Ertel W, Oberholzer A. The early second hit in trauma management augments the proinflammatory immune response to multiple injuries. J Trauma 2007; 62: 1396–1403.

Tsukamoto T, Chanthaphavong RS, Pape HC. Current theories on the pathophysiology of multiple organ failure after trauma. Injury 2010; 41: 21–26.

Turrito V, Weiss H, Baumgartner H. The effect of shear rate on platelet interaction with subendothelium exposed to citrated human blood. Microvasc Res 1980; 19(3): 352-365.

UK Statutory Instrument 2005 No. 50. Health and Safety. The Blood Safety and Quality Regulations. 2005. www.legislation.gov.uk

Ushiyama A, Kataoka H, Iijima T. Glycocalyx and its involvement in clinical pathophysiologies. J Intensive Care (2016) 4(1): 59.

Valeri C, Khuri S, Ragno G. Nonsurgical bleeding diathesis in anemic thrombocytopenic patients: role of temperature, red blood cells, platelets, and plasma-clotting proteins. Transfusion 2007; 47(4S): 206S-248S.

Valle E, Allen C, Van Haren R, et al. Do all trauma patients benefit from tranexamic acid? J Trauma 2014; 76: 1373–8.

Van Beek J, Mushkudiani N, Steyerberg E, Butcher I, et al. Prognostic value of admission laboratory parameters in traumatic brain injury: results from the IMPACT study. J Neurotrauma 2007; 24(2): 315–28.

Varicoda E, Poli de Figueiredo L, Cruz R Jr, et al. Blood loss after fluid resuscitation with isotonic or hypertonic saline for the initial treatment of uncontrolled hemorrhage induced by spleen rupture. J Trauma. 2003; 55(1): 112–117.

Vassar M, Fischer R, O'Brien P, Chambers J, Hoyt D, Holcroft J. A multicenter trial for resuscitation of injured pateints with 7.5% sodium chloride. The effect of added dextran 70. The Multicenter Group for the Study of Hypertonic Saline in Trauma Patients. Arch Surg 1993; 128(9): 1003-11.

Veigas P, Callum J, Rizoli S, Nascimento B, da Luz L. A systematic review on the rotational thrombelastometry (ROTEM®) values for the diagnosis of coagulopathy, prediction and guidance of blood transfusion and prediction of mortality in trauma patients. Emerg Med 2016; 24: 114

Velasco I, Pontieri V, Rocha e Silva M, et al. Hyperosmotic NaCl and severe hemorrhagic shock. Am J Physiol 1980; 239(5): H664-673.

Wade C, Tillman F, Lovejoy J, et al. Effect of Dehydration on Cardiovascular Responses and Electrolytes Following Hypertonic Saline Dextran Treatment for Moderate Hemorrhage. Div Mil Trauma Research; Institute Report No 426. 1989.

[a]Wade CE, Grady JJ, Kramer GC. Efficacy of hypertonic saline dextran (HSD) in patients with traumatic hypotension: meta-analysis of individual patient data. Acta Anaesthesiol Scand. 1997; 110: 77–79.

[b]Wade C, Grady J, Kramer G, et al. Individual patient cohort analysis of the efficacy of hypertonic saline/dextran in patients with traumatic brain injury and hypotension. J Trauma 1997; 42: S61–65.

[c]Wade C, Kramer G, Grady J, et al. Efficacy of hypertonic 7.5% saline and 6% dextran-70 in treating trauma: a meta-analysis of controlled clinical studies. Surgery 1997; 122: 609–616.

Wade C, Grady J, Kramer G. Efficacy of hypertonic saline dextran fluid resuscitation for patients with hypotension from penetrating trauma. J Trauma 2003; 54(5): S144-S148.

Wafaisade A, Lefering R, Maegele M, Helm P, Braun M, Paffrath T, et al. Recombinant factor VIIa for the treatment of exsanguinating trauma patients: a matched-pair analysis from the Trauma Registry of the German Society for Trauma Surgery. Unfallchirurg 2013; 116(6): 524–30.

Wafaisade A, Lefering R, Bouillon B, Böhmer AB, et al. Trauma Register DGU. Prehospital administration of tranexamic acid in trauma patients. Crit Care 2016; 20: 143.

Wang S, Overman R. A neurogenic factor in experimental traumatic shock: a summayr of recent studies including observations on procainized and spinal dogs. Ann Surg 1949; 129(2): 207-222.

Watenpaugh DE, Gaffney FA. Measurement of the net whole-body transcapillary fluid transport and effective vascular compliance in humans. J Trauma 1998; 45: 1062 – 8.

Watson G, Sperry J, Rosengart M, et al. Fresh frozen plasma is independently associated with a higher risk of multiple organ failure and acute respiratory distress syndrome. J Trauma 2009; 67: 221–230.

Watts S, Nordmann G, Brohi K, Midwinter M, et al. Evaluation of Prehospital Blood Products to Attenuate Acute Coagulopathy of Trauma in a Model of Severe Injury and Shock in Anesthetized Pigs. Shock 2015; 44 (S1): 138–48.

Weaver A, Eshelby S, Norton J, Lockey D. The introduction of on-scene blood transfusion in a civilian physician-led pre-hospital trauma service. Scan J Trauma Resusc Emerg Med 2013; 21(S1): S27.

Wei S, Rodriguez E, Chang R, Holcomb J. Elevated Sndecan-1 after Trauma and Risk of Sepsis: A Secondary Analysis of Patients from the PROPPR Trial. J Am Coll Surg 2018. https://doi.org/10.1016/j.jamcollsurg.2018.09.003

WHO. World Health Organization. Injuries and violence: the facts. www.who.int/violence_injury_prevention/en/ 2010.

Wilder D, Reid T, Bakaltcheva I. Hypertonic resuscitation and blood coagulation: in vitro comparison of several hypertonic solutions for their action on platelets and plasma coagulation. Thromb Res 2002; 107(5): 255-261.

Wiles MD. ATLS: Archaic Trauma Life Support? Anaesthesia 2015; 70: 893-897.

Wilkes M, Navickis R. The Boldt affair: a quandry for meta-analysts. Anesthesiology News. 2013 April.

Wohlauer M, Moore E, Thomas S, Sauaia A, et al. Early platelet dysfunction: an unrecognized role in the acute coagulopathy of trauma. J Am Coll Surg 2012; 214: 739–46.

Wohner N, Sotonyi P, Machovich R, et al. Lytic resistance of fibrin containing red blood cells. Arterioscler Thromb Vasc Biol 2011; 31: 2306–2313.

Wolberg A, Meng Z, Monroe D 3rd, Hoffman M. A systematic evaluation of the effect of temperature on coagulation enzyme activity and platelet function. J Trauma 2004; 56(6): 1221-8.

Wolf S, Bebarta V, Bonnett C, Pons P, Cantrill S. Blast injuries. Lancet 2009; 374(9687): 405-15.

Woolley T, Midwinter M, Spencer P, Watts S, Doran C, Kirkman E. Utility of interim ROTEM® values of clot strength, A5 and A10, in predicting final assessment of coagulation status in severely injured battle patients. Injury 2013; 44(5): 593-9.

Woolley T, Thomson P, Kirkman E, Reed R, et al. Trauma Haemostasis and Oxygenation Research Network position paper on the role of hypotensive resuscitation as part of remote damage control resuscitation. J Trauma 2018; 84(6S): S3-S13.

Wound Data and Munitions Effectiveness Team (WDMET). *The WDMET Study.* Uniformed Services University of the Health Sciences, Bethesda. 1970.

Wu W-T, Lin N-T, Subeq Y-M, Lee R-P, Chen I-H, Hsu B-G. Erythropoietin protects severe haemorrhagic shock-induced organ damage in conscious rats. Injury 2010; 41: 724–30

Xu D-Z, Lu Q, Adams C, Issekutz A, Deitch E. Trauma hemorrhagic shock-induced up-regulation of endothelial cell adhesion molecules is blunted by mesenteric lymph duct ligation. Crit Care Med 2004; 32: 760–5

Yamamoto K, Yamaguchi A, Sawano M, Matsuda M, et al. Pre-emptive administration of fibrinogen concentrate contributes to improve prognosis in patients with severe trauma. Trauma Surg Acute Care Open 2016; 1: 1-5

Yazer M, Cap A, Spinella P. Raising the standards on whole blood. J Trauma 2018; 84(6S1): S14-S17.

Younes R, Aun F, Accioly C, Casale L, Szajnbok I, Birolini D. Hypertonic solutions in the treatment of hypovolemic shock: a prospective, randomized

study in patients admitted to the emergency room. Surgery 1992; 111(4): 380-5.

Yuan S, Ferrell C, Chandler W. Comparing the prothrombin time INR versus the APTT to evaluate the coagulopathy of acute trauma. Thromb Res 2007; 120(1): 29-37.

Yukizawa Y, Inaba Y, Watanabe S, Yajima S, Kobayasha N, et al. Association between venous thromboembolism and plasma levels of both soluble fibrin and plasminogen-activator inhibitor 1 in 170 patients undergoing total hip arthroplasty. Acta Orthop 2012; 83(1): 14-21.

Ziegler B, Schimke C, Marchet P, St.germüller B, Schöchl H, Solomon C. Severe pediatric blunt trauma successful ROTEM guided hemostatic therapy with fibrinogen concentrate and no administration of fresh frozen plasma or platelets. Clin Appl Thromb Hemost 2013; 19(4): 453-9.

Zink K, Sambasivan C, Holcomb J, Chisholm G, Schreiber M. A high ratio of plasma and platelets to packed red blood cells in the first 6 hours of massive transfusion improves outcomes in a large multicenter study. Am J Surg 2009, 197: 565-570.

Zunic G, Pavlovic R, Malicevic Z, Savic V, Cernak I. Pulmonary blast injury increases nitric oxide production, disturbs arginine metabolism, and alters the plasma free amino acid pool in rabbits during the early posttraumatic period. Nitric Oxide 2000; 4: 123-128.