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Aortic Haemostasis and Resuscitation: Advanced Resuscitative Endovascular Balloon Occlusion of the Aorta for non-compressible torso haemorrhage and reversal of haemorrhage-induced traumatic cardiac arrest in a swine model

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Abstract

Trauma is the leading cause of death in young people in the UK and US. Non-compressible torso haemorrhage (NCTH) is a major cause of potentially survivable trauma death. A large proportion of these patients are in traumatic cardiac arrest (TCA) on arrival at hospital, and survival rates after haemorrhagic TCA are extremely low. The limited data available suggest that closed chest compressions (CPR) are ineffective, and may be harmful, in haemorrhagic TCA. Resuscitative Endovascular Balloon Occlusion of the Aorta (REBOA) has shown promise in the setting of NCTH, although its utility in haemorrhagic TCA is unknown. Selective Aortic Arch Perfusion (SAAP) is an experimental intervention that has the potential to resuscitate haemorrhagic TCA, but it has not been compared to CPR or REBOA. The aim of this research was to evaluate SAAP against other interventions for the management of haemorrhagic TCA.

A large swine (70-90 kg) translational model of TCA secondary to NCTH and a controlled arterial haemorrhage was developed to answer hypotheses in three parts. First, a comparison of 60 minute survival between CPR, REBOA, SAAP with oxygenated lactated Ringer's solution (SAAP-LR), and SAAP with oxygenated fresh whole blood (SAAP-FWB), followed by surgical control and a three-hour critical care period; second, in a more severe model of TCA, comparison of SAAP-LR and SAAP-FWB, and an evaluation of extra-corporeal life support (ECLS) to mitigate the effects of aortic occlusion; and third, a translational paradigm of escalating endovascular intervention, from REBOA to SAAP, to ECLS.

In Part One, 40 animals were allocated to four groups; SAAP-FWB inferred a significant 60 minute survival advantage (90.0%, 95%CI 59.6-99.5) over CPR (10.0%, 95%CI 0.5-40.4), REBOA (0.0%, 95%CI 0.0-27.8), and SAAP-LR (30.0%, 95%CI 10.8-60.3), $p<0.001$. SAAP-FWB and CPR were observed to resuscitate cardiac electrical asystole; both of these are novel findings, but no asystolic animals survived to the end of the protocol. In Part Two, ECLS after SAAP catheter removal demonstrated a significant three-hour survival advantage over non-ECLS historical controls, $p<0.05$. The translational paradigm of escalating endovascular intervention was shown to be a feasible and efficacious method of resuscitating swine in haemorrhagic TCA in Part Three.

A novel swine donor pool supplied >700 units of FWB for the experiments, and was an effective way of obtaining large volumes of blood product whilst reducing overall animal use. Ventricular fibrillation was observed in animals in the SAAP-FWB group, and further development of the technique is needed prior to clinical implementation.

SAAP-FWB is capable of resuscitating swine in haemorrhagic cardiac electrical asystole, and infers a superior short-term survival compared to intervention with CPR, REBOA, and SAAP-LR, but further data are needed to ensure normocalcaemia during infusion of citrated blood products. ECLS has been demonstrated to prolong survival in the setting of cardiopulmonary dysfunction secondary to TCA and intra-aortic balloon occlusion. An escalating paradigm of endovascular intervention is a feasible and efficacious method of resuscitating large swine in haemorrhagic TCA that is translatable to clinical practice.

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List of publications

Barnard EBG, Morrison JJ, Madureira RM, et al. Resuscitative Endovascular Balloon Occlusion of the Aorta (REBOA): a population based gap analysis of trauma patients in England and Wales. *Emerg Med J* 2015;32:926-932.

Barnard EBG, Yates D, Edwards A, et al. Epidemiology and aetiology of traumatic cardiac arrest in England and Wales – A retrospective database analysis. *Resuscitation* 2017;110:90-94.

Barnard EBG, Hunt PF, Lewis PEH, et al. The outcome of patients in traumatic cardiac arrest presenting to deployed military medical treatment facilities – data from the UK Joint Theatre Trauma Registry. *J R Army Med Corps* 2018;164:150-154.

Barnard EBG, Manning JE, Smith JE, et al. A comparison of Selective Aortic Arch Perfusion and Resuscitative Endovascular Balloon Occlusion of the Aorta for the management of hemorrhage-induced traumatic cardiac arrest: A translational model in large swine. *PLoS Med* 2017;14(7):e1002349. doi: 10.1371/journal.pmed.1002349.

Barnard EBG, Smith JE. Non-compressible torso haemorrhage - the new holy grail for further improvement in trauma survival. *J R Nav Med Serv* 2018;104(2):107-114.

List of abstracts

Barnard EBG, Smith JE, Manning JE, et al. Selective Aortic Arch Perfusion for the reversal of haemorrhage-induced traumatic cardiac arrest in a swine model of non-compressible torso haemorrhage. *Emerg Med J* 2016;33:897.

Barnard EBG, Smith JE, Manning JE, et al. A descriptive paradigm of escalating endovascular intervention for the management of traumatic cardiac arrest in a swine model of non-compressible torso haemorrhage. *Emerg Med J* 2016;33:900-1.

Selected presentations and research prizes

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A descriptive paradigm of escalating endovascular intervention for the management of traumatic cardiac arrest in a swine model of non-compressible torso haemorrhage. *Royal College of Emergency Medicine Annual Scientific Conference, Bournemouth, UK* – September 2016.

NOST to ROSC: Endovascular Reanimation. *Royal Society of Medicine, London, UK* – September 2017.

Traumatic cardiac arrest – heroic or futile? *University of Antwerp Focused Trauma Conference, Antwerp, Belgium* – October 2017.

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List of abbreviations, 1 of 2

AAJT	Abdominal Aortic Junctional Tourniquet
ABG	Arterial blood gas
ACE-i	Angiotensin converting enzyme inhibitor
ACT	Activated clotting time
AHR	Aortic haemostasis and resuscitation
AIS	Abbreviated Injury Scale
BP	Blood pressure
CPP	Coronary perfusion pressure
CPR	Cardiopulmonary resuscitation (also closed chest compressions)
DoDTR	Department of Defense Trauma Registry (US military trauma registry)
DOW	Died of wounds (died after arrival at a medical treatment facility)
ECG	Electrocardiogram
ECLS	Extracorporeal life support
ECMO	Extracorporeal membrane oxygenation
ELSO	Extracorporeal Life Support Organization
EPR	Emergency preservation and resuscitation
ERC	European Resuscitation Council
EtCO2	End-tidal carbon dioxide
F_iO_2	Fraction of inspired oxygen
FWB	Fresh whole blood (<72 hours from donation)
H2S	Hydrogen sulphide
HVHF	High-volume haemofiltration
IABO	Intra-aortic balloon occlusion (commonly referred to as REBOA)
IACUC	Institutional Animal Care and Use Committee
IED	Improvised explosive device (home-made bomb)
ISS	Injury Severity Score
JTTR	Joint Theatre Trauma Registry (UK military trauma registry)
LOST	Low-output state in trauma
LR	Lactated Ringer's solution
MAC	Mean alveolar concentration of inhaled anaesthetic gas
MAP	Mean arterial pressure
MTF	Medical treatment facility (hospital with surgical capability)
NCTH	Non-compressible torso haemorrhage
NIRS	Near-infrared reflectance spectroscopy (StO_2 – see below)
NISS	New Injury Severity Score
NOST	No-output state in trauma
NTDB	National Trauma Data Bank (US civilian trauma registry)
PT	Prothrombin time
REBOA	Resuscitative endovascular balloon occlusion of the aorta (see IABO)
ROSC	Return of spontaneous circulation
RT	Resuscitative thoracotomy
R-SAAP	Rescue-SAAP (see below)
SAAP	Selective aortic arch perfusion
StO_2	Tissue oxygenation
TARN	Trauma Audit and Research Network (UK civilian trauma registry)
TBI	Traumatic brain injury

List of abbreviations, 2 of 2

TCA	Traumatic cardiac arrest
TEG	Thromboelastography (an *in vitro* analysis of clotting function)
UK	United Kingdom
US	United States of America
VA	Venoarterial (in reference to ECLS, see above)
VAV	Venoarterialveno (in reference to ECLS, see above)
VV	Venovenous (in reference to ECLS, see above)
VF	Ventricular fibrillation
Z1	Aortic Zone 1 (left subclavian artery to coeliac axis)
Z3	Aortic Zone 3 (renal vessels to aortic bifurcation)

PART ONE

CHAPTER ONE

THE TRAUMA BURDEN

1.1 Thesis introduction

This thesis explores current and evolving interventions for the management of traumatic cardiac arrest (TCA) in large animal translational models of haemorrhage. There are four background chapters to Part One that describe the relevance of this research area and frame the aims: 1) An overview of the burden of trauma in civilian and military settings that identifies the control of torso haemorrhage as the greatest opportunity to improve trauma survival; 2) an evaluation of the aetiology and epidemiology of torso haemorrhage that demonstrates that a high proportion of patients with this injury pattern are in TCA on arrival at hospital; 3) a review of TCA, including the management options for pre-hospital haemorrhagic TCA; and 4) a review of the existing knowledge and clinical practice in the pre-hospital management of non-compressible torso haemorrhage, including the use of intra-aortic balloon occlusion. The background chapters demonstrate that the greatest medical opportunity to improve trauma survival is via the pre-hospital management of haemorrhagic TCA, and identify a requirement to undertake a comparison of current and evolving interventions for this highly-lethal clinical scenario.

1.2 Civilian trauma

Trauma is a global public health problem. More than five million people die each year as a result of injury - responsible for more deaths per year globally than

tuberculosis, HIV, and malaria combined.[1] In middle and low-income countries, the number of people dying from trauma is increasing, with road traffic collisions predicted to be the seventh leading cause of death worldwide by 2030.[1] In the United Kingdom (UK) and United States (US), trauma is the leading cause of death in those aged between one and 35 years old,[2,3] and as such is responsible for the greatest number of life years lost. There are approximately 170,000 trauma deaths in the US per year, and 5,400 in England.[3,4]

A review of six US studies (1977-2001) identified traumatic brain injury (TBI) and haemorrhage as the leading causes of civilian trauma death.[5] Aggregation of the data demonstrates that TBI was the cause of death in 45%, and haemorrhage in 30%.[5] While TBI accounts for more trauma deaths in these studies, the scope for successful medical intervention to improve outcome are limited.[6,7] In contrast, haemorrhage is certainly amenable to medical intervention with current and evolving technology.[8]

1.2.1 Civilian trauma studies

A number of civilian trauma death analyses have been published in the last 15 years. Chiara et al undertook an epidemiological study of all trauma deaths (excluding hanging, suffocation, and drowning) over a 12 month period in an Italian urban setting.[9] In-hospital and post-mortem records were used to identify the causes of death, which were verified by inter-observer reliability kappa scores. Of the 255 deaths analysed, 112 (44%) were due to a combination of TBI and haemorrhage, 68 (27%) haemorrhage alone, and 55 (22%) TBI alone.[9] In the haemorrhage category, ten patients died before arrival at a medical facility. This

study raises two issues: first, trauma death often includes lethal injury to more than one body area, and as such there is a considerable cross-over of the two leading causes of death (TBI and haemorrhage); second, that examining in-hospital trauma mortality may give a biased view by not including those patients who die before arrival at hospital (and haemorrhage may be a large proportion of these).

A subsequent larger civilian trauma death analysis of 558 consecutive patients was undertaken by Tien et al at a Canadian trauma center.[10] Hospital medical records and post-mortem reports were used to assign the cause of death. In recognition that death from TBI is largely not amenable to medical intervention, the study aimed to identify potentially survivable deaths from haemorrhage. TBI was the leading cause of death (60%), followed by haemorrhage (15%), and a combination of TBI and haemorrhage (11% - inclusion criteria were met for both aetiologies). More detailed analysis of haemorrhagic deaths (n=86) were dichotomised into blunt and penetrating injury: the pelvis was the leading site of haemorrhagic death in blunt injury (39%), and the chest in penetrating injury (41%). The thorax was the most prevalent site of haemorrhage (n=26/86, 30%), and the combination of chest, abdomen, and pelvis (collectively 'torso haemorrhage') accounted for 55 of 86 (64%) haemorrhagic deaths. The authors judged that the greatest area for improving trauma survival was in those who died from a blunt pelvic injury – they identified in-hospital delays in treating haemorrhage in 34% of these cases.[9]

A 2007 study by Teixeira et al analysed 2,081 trauma deaths at a US trauma center between 1998 and 2005.[11] The aim of this study was to identify potentially preventable trauma deaths secondary to system and human errors. The authors identified 51 deaths as potentially preventable, of which haemorrhage was the leading cause (40%). In the haemorrhagic deaths 100% were secondary to torso haemorrhage (86% pelvis, 7% chest, 7% abdomen).[11] This study does not include patients who died pre-hospital and were not transported to hospital. The data may therefore be biased towards reporting injuries that are not rapidly lethal.

More recent analysis of civilian trauma deaths in the US, by Davis et al, focused on patients who died before arrival at hospital (including those who were not transported).[8] Post-mortem reports from a single US county during 2011 were examined by an expert panel of trauma surgeons with the aim of determining if trauma deaths were potentially survivable or non-survivable if optimal care had been immediately available. A total of 512 trauma deaths were analysed: the leading cause of death was TBI (36%), followed by haemorrhage (34%) – a combination of TBI and haemorrhage accounted for 15%.[8] 146 (29%) deaths were identified as potentially survivable, 54% of which were due to haemorrhage alone, and a further 10% in which haemorrhage was in part causal. The torso (chest and abdomen) was the most frequently injured anatomical area in potentially survivable trauma deaths, and the authors concluded that improving treatment of non-compressible torso haemorrhage may improve survival.[8]

1.2.2 Civilian Trauma Summary

TBI is the leading cause of civilian trauma death. However, there is no current or evolving medical intervention that is likely to substantially improve survival from devastating TBI. In these studies haemorrhage, either solely or as a contributing factor, was responsible for 26% to 71% of deaths.[8-10] Death from haemorrhage alone accounted for 15% to 40% of trauma deaths.[8-11] The studies that examined the site of lethal haemorrhage showed that the torso was the most prevalent anatomical location (64% to 100%).[10,11]

1.3 Combat Trauma

Owing to the inherent instability of armed conflict, accurately determining mortality, let alone morbidity, is extremely difficult.[12] The most contemporaneous estimate of deaths secondary to armed conflict is 378,000 deaths globally per year.[13] The number of civilian and local security forces deaths due to direct violence in the recent conflicts in Iraq and Afghanistan has come under close media and government scrutiny, and has been estimated to be approximately 20,000 and 4,000 per year respectively.[14,15] The estimation of these numbers is contentious, and furthermore there is a paucity of data on injury patterns and medical interventions – they are therefore of limited utility when examining methods to improve survival from combat trauma. However, part of the governance of the UK and US response to combat trauma during recent conflicts has been to examine causes of death and identify potential future survivors.[16,17]

1.3.1 The UK and US combat trauma experience

The UK military was continuously engaged in armed conflict in Iraq and Afghanistan between 2003-2014. During this period, 179 UK military personnel died in Iraq, and 450 died in Afghanistan.[18] The US military recorded 6,784 deaths in Iraq and Afghanistan in the same time period – a UK/US total of 7,413.[18] In 2012, the UK Care Quality Commission described front-line trauma care as 'exceptional'.[19] The ability to provide excellent care has been dependent on a large number of discrete and strategic changes to practice over a period of large casualty numbers.[20] An example of a discrete improvement was the 2007 introduction of limb tourniquets. Early data from these conflicts showed that extremity haemorrhage was a significant cause of potentially survivable death,[21] and comparison of combat trauma deaths in Vietnam, and pre-2007 Iraq and Afghanistan deaths has demonstrated that mortality from extremity haemorrhage had not improved in over thirty years; 7.4% and 7.8% respectively.[22,23] The subsequent widespread use of tourniquets has been attributed to an 85% reduction in mortality from extremity haemorrhage.[16] Other changes in practice, for example audit of trauma outcomes, critical event reporting, and weekly medical conferences, have allowed rapid evolution of medical practice. However, their individual effect on outcomes is difficult to quantify.[24]

During the conflicts in Iraq and Afghanistan, considerable effort has focused on medical developments to reduce morbidity and mortality. Despite this, the first conclusive evidence of an improvement in combat trauma survival was not published until 2015 – Penn-Barwell et al demonstrated that among UK military

personnel the New Injury Severity Score (NISS) associated with a 50% chance of survival increased year-on-year between 2003 and 2012 (from 32 to 60).[25] Furthermore, this substantial improvement in combat trauma survival raises questions about the ongoing relevance and utility of existing trauma scoring systems.[25] Penn-Barwell's report highlights another point – there has been no substantial improvement in survival since 2012. The reduction in casualty numbers since 2012 (4.3 per year since 2012, compared to a peak of 108 deaths in 2009) may have a part to play in this. However, it also suggests that a paradigm shift in medical intervention may be required to further effect an improvement in survival.

The audit of combat trauma outcomes relies on bespoke databases, termed trauma registries. The UK Joint Theatre Trauma Registry (JTTR), the US Department of Defense Trauma Registry (DoDTR) and other databases have been used during these conflicts to retrospectively describe causes of combat death. It is therefore possible to identify particular injury patterns towards which future medical interventions could be focused.

1.3.2 Combat trauma studies

The first description of combat deaths during the past 15 years examined the post-mortem records of 82 US personnel who died between 2001 and 2004.[26] The aim of this study was to identify those with potentially survivable injuries, with a bias towards inclusion in order to present the greatest opportunity to stimulate improvement in medical care. Prior to examination of the data, a consensus rule format was derived to determine whether particular injuries

should be classified as potentially survivable or non-survivable with reference to contemporary trauma care guidelines. An expert panel of forensic pathologists, trauma surgeons, a nurse, and a military medic reviewed the 24 (29%) cases where initial review suggested a potentially survivable injury. Twelve (15%) combat deaths were regarded by the panel as potentially survivable – the most prevalent injury (n=8, 67% of potentially survivable deaths) was torso haemorrhage; two of whom were alive on arrival at the military hospital.[26]

The next published evaluation of causes of combat death in US forces was published in 2008, and examines two discrete time periods – March 2003 to April 2004, and June to December 2006.[23] The aims of this study were to examine how perceived improvements in medical care, despite hypothesised increases in injury severity, had affected outcomes, and to evaluate potential areas for improvement in medical intervention. A similar methodology to that of Holcomb et al's[26] study was used – an expert panel decided if combat deaths were potentially survivable or non-survivable. The number of combat deaths per month doubled from the first to the second time period (35, compared to 71), but there was no statistical difference in the combat fatality rate (the percentage of deaths amongst all those injured – a crude measurement of lethality).[27] Overall, 982 combat deaths were included in the analysis (486 from the first time period, and 496 from the second). 232 (24%) deaths were classified as potentially survivable. The most prevalent injury type was haemorrhage (n=197, 85%), which included 115 patients with torso haemorrhage – 50% of the potentially survivable deaths.[23]

The first published UK military mortality review during this time period examined 76 trauma related deaths in both Iraq and Afghanistan between April 2006 and March 2007; 57 (75%) were due to hostile action.[28] This study utilised anatomical injury data stored in the UK JTTR: the abbreviated injury scale (AIS) for individual injuries (≥3 indicating severe injury, 6 indicating a non-survivable injury), and the injury severity score (ISS) and new injury severity score (NISS) for overall injury scoring. In the 57 hostile action deaths, head injury was the most prevalent severe injury (24/57, 42%), followed by thoracic injury (23/57, 40%). Furthermore 20 of the 24 severe head injury patients had a head AIS of 6 – indicating a non-survivable injury, compared to 11 of the 23 thoracic injuries. In this group of 57 hostile action deaths, anatomical injury scoring (NISS) identified 46 (81%) expected deaths (therefore 11 (19%) were anatomical unexpected deaths). Subsequent analysis of all 76 deaths was undertaken by independent expert panel review – this identified 69 as 'unsalvageable'. Of the remaining seven potentially survivable deaths, two were deemed unpreventable owing to tactical constraints (unable to gain safe access to the patient). In the remaining five, four were deemed potentially survivable if surgical control could have been achieved within 30 minutes.[28] Although the exact injury pattern in this group of four patients is not described it would be reasonable to conclude that these patients died from torso haemorrhage.

The most comprehensive review of mortality in Iraq and Afghanistan examined 4,596 US combat deaths between 2001 and 2011. The aim of this study was similar to that of the two previous studies – to identify opportunities to improve outcomes from combat trauma, but with specific emphasis on the pre-hospital

deaths.[16] This study utilised the same database (from the US Armed Forces Medical Examiner System) as the previous two, and as such includes patients already described. An expert panel reviewed the data, and in keeping with previous studies, classified each death as potentially survivable or non-survivable. Of the 4,596 combat deaths, 4,016 (87%) occurred pre-hospital. 976 combat deaths (24%) were classified as potentially survivable – the most prevalent injury type was haemorrhage (n=888, 91%), which includes 598 patients with torso haemorrhage – 61% of the potentially survivable deaths.[16] This study spans the introduction of arterial limb tourniquets, and it is therefore reasonable to suggest that the proportion of potentially survivable combat deaths due to torso haemorrhage was actually higher in the latter years of conflict. This paper also includes a limited analysis of non-survivable injuries (n=3,243) – TBI (42%), heart / thoracic injury (21%), and dismemberment (16%).[16] Severe TBI therefore is the most prevalent overall cause of death identified in this study – it is acknowledged that there is no current or evolving medical intervention that may move this injury type from non-survivable into the potentially survivable category.[29]

Russell et al undertook a further analysis of UK combat deaths (2002-2013) that utilised anatomical scores from the UK JTTR and an expert review panel to describe the Military Mortality Review Panel process.[30] This study identified 621 deaths, of which 517 were due to hostile action. The most prevalent fatal anatomical injury (AIS 6) area was head (220/621, 35%), followed by thorax (108/621, 17%). An AIS >3 was used to indicate a severe injury in this cohort of combat deaths. In this analysis, torso injury (a combination of thorax and

abdominal injury, but not including pelvis and lower limb) was the most prevalent injury type (540/621, 87%), followed by head (283/621, 46%). In the combat environment survivability analysis with more prompt or better clinical care should not be viewed in isolation from the tactical situation (i.e. the inability to provide this care owing to ongoing hostile action).[28] There is an equivalent civilian situation (for example restricted access to a trapped patient in a deformed vehicle), but it is likely to be less prevalent. Uniquely, this paper (and the earlier, smaller UK study) includes review of whether tactical constraints influenced the potentially survivability: the peer review panel deemed that 51 (10%) deaths were either definitely (3), potentially (13), or possibly (35) salvageable – 24 of these (47%) were affected by tactical constraints. Detailed analysis of the likely cause of death in these patients is not made, but suboptimal use of arterial limb tourniquets is cited as an influencing factor.

The initial mechanism of injury in Afghanistan was predominantly gunshot wound, but in the latter years of this conflict the signature injury was blast from an improvised explosive device (IED).[30] Singleton et al examined patients killed by IEDs in Afghanistan between November 2007 and August 2010, with the aim of identifying injury patterns and to inform future medical practice improvement.[29] Uniquely, this study used data from computed tomography post mortem to identify the cause of death. 146 UK personnel were killed by IED blast during the study period; the autopsy data was not available for one patient, and 24 patients had total body disruption – 121 were therefore included in the study group (79 were injured on foot (dismounted), and 42 inside vehicles (mounted)).[29] A total of 354 potentially fatal injuries were identified: 230 in the

79 mounted (2.9 per patient), and 42 in the 124 dismounted (3.0 per patient) groups. Overall, extremity and junctional haemorrhage was the most prevalent fatal injury (128/354, 36%), followed by TBI (105/354, 30%), and torso haemorrhage (88/354, 25%). In mounted patients, torso haemorrhage was the second most prevalent cause of death after TBI. This study does not make a distinction between potentially survivable and non-survivable injury patterns. As previously described, there is evidence that the introduction of arterial limb tourniquets has resulted in a paradigm shift in survival from extremity haemorrhage.[16] However, there is no distinction in Singleton et al's study between extremity and junctional haemorrhage (non-compressible bleeding in the neck, axilla, and groin). Junctional haemorrhage can arguably be placed in the torso haemorrhage group, and it is therefore difficult to determine how many patients in this study could be placed in a potentially survivable torso haemorrhage group.

A 2015 study from the UK military has described those patients who arrive at a medical facility alive, but subsequently die – 'died of wounds' (DoW).[27] Keene et al used the UK JTTR, post-mortem records, and clinical notes to identify all DoW UK personnel between 2003 and 2014. 71 combat related DoW were identified – isolated severe TBI accounted for 47% of this cohort, and severe TBI in conjunction with other injuries in a further 18% (a TBI total of 65%). Haemorrhage was identified as the cause of death in 21%.[31] This data supports two theories: the first is that in military trauma patients severe TBI is often, potentially universally, fatal even if a live patient is delivered to a hospital providing exceptional trauma care, and secondly that exsanguinating

haemorrhage is rapidly fatal, and therefore few of these patients arrive at hospital alive.

1.3.3 Combat trauma summary

There has been substantial improvement in trauma survival during the recent conflicts in Iraq and Afghanistan. There is no accepted standard of military combat death analysis, and the resulting heterogeneity of these studies makes comparison difficult. Individual combat deaths have also been included in multiple analyses of data. All but three of the studies examine small populations (<200 cases), and owing to the unprecedented battlefield survival in recent years of conflict only a small number of potential survivors whose injuries can be analysed.

However, it is clear that TBI and torso injuries account for the majority of modern combat trauma mortality. Severe TBI is the most prevalent cause of UK/US death in recent conflict but this injury is highly lethal, and potential improvements in survival do not lie in the medical domain, but should be focused at prevention (tactical awareness) and mitigation (improved vehicle design and head protection).[29,32] Consistently, throughout the available data, torso haemorrhage has been identified as the most prevalent 'potentially survivable' combat death.

1.4 Trauma Burden Summary

TBI and haemorrhage are the leading causes of both civilian and combat trauma death in recent published data. Analyses of potentially survivable trauma deaths

has identified that torso haemorrhage is the specific area in which the greatest increase in survival may be possible.[10,11,16,26] Therefore, an evaluation of torso haemorrhage epidemiology, aetiology, and mortality is presented in the next chapter.

CHAPTER TWO

NON-COMPRESSIBLE TORSO HAEMORRHAGE

2.1 Introduction

Examination of the aetiology of civilian and combat trauma has identified haemorrhage as the leading potentially preventable cause of mortality. Data from the US military has demonstrated that an 85% reduction in mortality of extremity haemorrhage is possible with the use of arterial limb tourniquets.[16] The remaining medical challenges in improving trauma survival are therefore in controlling torso (chest, abdomen, and pelvis) and junctional (axilla, and groin) haemorrhage. In potentially survivable combat trauma, the prevalence of torso haemorrhage death is over 3.5 times that of junctional heamorrhage,[16] and in some treatment situations junctional haemorrhage (particularly groin) can be considered as torso. The term 'non-compressible torso haemorrhage' (NCTH) has been coined as a term to describe the presence of a significant haemorrhagic injury within the torso - 'vascular disruption to axial torso vessels, solid organs, pulmonary parenchyma, and / or the bony pelvis, when accompanied by shock'.[33] Effective management of NCTH therefore presents the greatest opportunity to improve trauma survival.

2.2 Civilian NCTH

A number of civilian studies, described in Chapter One, demonstrate that haemorrhage alone accounts for between 15% and 40% of trauma deaths.[8-11] Two studies aimed at characterising traumatic death examined the anatomical

location of haemorrhage – Tien et al showed that 64% of haemorrhage deaths were from bleeding in the chest, abdomen, and or pelvis,[10] and Tiexereira et al showed that the most common cause of potentially preventable death was haemorrhage (39%), and that all missed injuries were torso haemorrhage,[11] but neither study aimed to specifically describe the prevalence of NCTH.

2.2.1 Civilian NCTH studies

There is a single published civilian study that aimed to describe the prevalence of NCTH. Kisat et al applied the previously described combat definition to interrogate the US National Trauma Data Bank to report the epidemiology of NCTH, and to examine associations between injury types and mortality.[34] Between 2007-2009 there were 1.8 million adult patients who were alive on arrival at a Level 1 Trauma Center in the registry – 20,414 (1%) had an NCTH. Univariate analysis of injury types demonstrated that pulmonary injury and torso vascular injury increased the odds of mortality in this group of patients with NCTH. It is unclear at what time interval mortality was measured, but the overall mortality in this cohort was 45%.[34] Owing to the inclusion criteria (alive on arrival at hospital) the actual civilian prevalence and mortality of NCTH is likely to be higher than quoted.

Barnard et al examined the AIS scores of 72,677 trauma patients arriving at hospitals in England and Wales in 2012 and 2013.[35] This study identified that 3,543 (5%) of these patients had a non-compressible torso injury, of whom 397 (11%) had NCTH with no contraindications for intra-aortic balloon occlusion. This group of patients is small (just over 0.5% of the trauma population), but

they are young (median age of 43 years), and have a high mortality (32%).[35] However, this study did not include those patients who died before arrival at hospital, and as such is likely to underestimate the true mortality of civilian NCTH. Furthermore 40% of patients had physiological evidence of being in traumatic cardiac arrest on arrival at hospital.[35]

2.2.2 Civilian NCTH summary

There are limited data on the aetiology and epidemiology of civilian NCTH. Furthermore, both published studies only include patients who were transported to hospital, and therefore are likely to provide an underestimation of the prevelance and mortality. From the available data, civilian NCTH is a disease of the young, has a high mortality (32% to 45%), and a high proportion of patients are either peri-arrest or in traumatic cardiac arrest on arrival at hospital.

2.3 Combat NCTH

In the past 15 years there have been 7,413 UK and US combat deaths.[18] Both of these countries have bespoke combat trauma registries that accurately record injuries and medical interventions. Owing to the prevalence of torso haemorrhage identified in these registries, UK and US military surgeons developed a standard definition of NCTH that was used in the single civilian study by Kisat et al.[33,34] This has resulted in two studies that aim to describe the aetiology, epidemiology, and mortality of combat NCTH.

2.3.1 Combat NCTH studies

Stannard et al undertook the first examination of frequency and mortality of NCTH in recent combat.[36] This study used the US DoDTR to identify all US service personnel with NCTH between 2002 and 2010. This study only included those patients who were alive on arrival at the military hospital, and therefore represents a subset of combat NCTH – excluding those who died pre-hospital. The AIS scoring system was used to identify patients in the registry with a non-compressible torso injury (n=1,926, 13%). The NCTH cohort was derived from those with a non-compressible torso injury who had a systolic blood pressure <90 mmHg or required immediate surgical intervention for torso haemorrhage on arrival at the military hospital (n=331, 2%). The NCTH group had a mean ISS of 30, and a 30-day mortality of 19%.

Comparison of injury type (vascular injury types, solid abdominal organ injury, and pelvic fracture) between NCTH survivors and non-survivors demonstrated a higher incidence of major arterial injury and of pulmonary injury in non-survivors; 26% versus 9%, $p<0.001$, and 57% versus 38%, $p=0.03$ respectively.[36] Conversely, there was a higher incidence of splenic injury recorded in survivors (14%) versus 5% in non-survivors, $p=0.03$. Although this study is likely to underestimate the true incidence of NCTH and its mortality, it still demonstrates that one in eight US combat patients arriving at the military hospital had a non-compressible torso injury (for which there is currently no effective pre-hospital management), and that the mortality for patients with NCTH arriving at the military hospital is roughly one in every five.

The UK combat trauma registry (UK JTTR) includes data on those patients who die before arrival at the military hospital (killed in action) as well as those who died of wounds, and those who are wounded in action, thereby allowing comprehensive analysis of the aetiology and epidemiology of combat trauma.[24] Morrison et al undertook a retrospective review of the UK JTTR between 2002 and 2012 with the aim of classifying injury patterns in NCTH.[37] 296 patients sustained NCTH using previously defined criteria. During this ten year study 222 (75%) NCTH patients died before arrival at the military hospital. Of the 74 who were alive on arrival at hospital, a further 31 (11%) died of their wounds – the overall combat fatality rate of NCTH was 86%, with 88% dying pre-hospital. The authors further examined the mortality of NCTH, using three equally-sized cohorts over this time period, and found a non-significant trend of decreasing mortality: 2002-2008 (91%), 2008-2011 (83%), 2011-2012 (82%), p=0.16.[37]

2.3.2 Combat NCTH summary

These studies show that the mortality from combat NCTH is very high, the great majority of which is pre-hospital, and that despite significant overall improvement in survival, there has been no significant improvement in survival from combat NCTH between 2002 and 2012.[37]

2.4 NCTH Summary

There is limited published data on the aetiology, epidemiology, and mortality of NCTH, and only a single study that utilises both pre-hospital and in-hospital records to provide a complete picture of the mortality rate of NCTH. Morrison et al's combat study therefore provides the best estimate, albeit in a military setting

- a mortality of 86% - and 88% of these patients die before arrival at hospital.[37] The recent UK civilian analysis produced an under-estimate of 32% for NCTH mortality, and also suggests that 40% of patients are already in traumatic arrest on arrival at hospital.[35]

The current management of NCTH needs a patient to survive to reach hospital in order to receive formal surgery or interventional radiology to control haemorrhage – there is no established pre-hospital management option. It is therefore likely that a large proportion of patients with potentially survivable trauma die in the pre-hospital setting of haemorrhagic TCA. This data has identified a need to develop a torso haemorrhage control technique that is feasible pre-hospital and ideally effective in traumatic arrest.

CHAPTER THREE

TRAUMATIC CARDIAC ARREST

3.1 Introduction

TCA is fundamentally different to medical cardiac arrest, with different causes and underlying pathophysiology. In medical cardiac arrest the majority of adult patients have a primary cardiac cause,[38] whereas in TCA the leading causes are TBI and haemorrhage.[5] However, until recently protocols for the management of cardiac arrest have not differentiated between medical and traumatic aetiology.

3.1.1 Historical TCA studies

Shimazu et al retrospectively reviewed all trauma patients at a US trauma hospital who arrived in cardiac arrest between 1976 and 1981.[39] TCA was defined as a combination of no recordable blood pressure, no palpable pulse, no spontaneous respirations, and no electrocardiographic activity, following trauma. The authors state that similar pre-hospital care was given to all patients and included an airway, a variable amount of intravenous crystalloid, and closed chest compressions. In-hospital management included intravenous blood products, resuscitative thoracotomy to allow aortic cross-clamping and internal cardiac massage as indicated. Out of 267 TCA patients, 87 (33%) had a return of spontaneous circulation. However, 65 had a further cardiac arrest and died within 24 hours of admission. Six (2%) survived to hospital discharge, and four had a full neurological recovery.[39]

A subsequent retrospective study at a different US trauma hospital by Battistella et al defined TCA as the requirement for closed chest compressions following trauma, either pre-hospital or in-hospital.[40] Between 1991 and 1996, 16,724 trauma patients were admitted to hospital, of whom 604 (4%) were in TCA. Closed chest compressions were performed for a mean duration of 22 minutes, and 304 (50%) underwent resuscitative thoracotomy (all in-hospital). Sixteen (3%) patients survived – four following blunt, and 12 following penetrating trauma. In contrast to the inclusion criteria of Shimazu et al's study, some patients in this study had electrocardiographic (ECG) activity in TCA. No patients with a pre-hospital ECG of cardiac asystole or with a rate less than 40 per minute survived following blunt trauma. Two patients with an ECG rate less than 40 on arrival at the hospital survived – both had penetrating cardiac wounds and had recordable blood pressures pre-hospital.[40] The majority of survivors therefore had ECG activity greater than 40 beats per minute while in TCA.

3.1.2 The definition of TCA

These two historical papers highlight an important consideration in the pathophysiology of TCA. The contemporary definition of TCA is a patient who has sustained trauma with agonal or absent spontaneous respiration and absence of a central pulse.[41] This definition is appropriately simple in that it allows TCA to be rapidly clinically identified, followed by expedient treatment. Peripheral pulse character (normal versus weak) in pre-hospital trauma patients has been shown to be a differentiator of both adequate blood pressure and mortality.[42] Earlier editions of the Advanced Trauma Life Support course manual

proposed that if only the carotid pulse was palpable then the patient's systolic blood pressure was 60-70mmHg. However, the one small clinical study that evaluated this assertion demonstrated that from a sample of 20 hypovolaemic patients the Advanced Trauma Life Support criteria were not correct in 15 (75%).[43]

3.1.3 The spectrum of haemorrhage

Although the absolute blood pressure at which a central pulse cannot be palpated is debatable, and individually variable,[44] it is logical that along the spectrum of haemorrhage there will be a time period where there is ongoing cardiac output in the absence of a palpable pulse.[45] This has been described as a low-output state in trauma (LOST).[46] Patients in LOST, owing to their ongoing cardiac output, will have ECG activity and are not truly in cardiac arrest, and as such have a less severe injury than those included in Shimazu et al's study.[39] This fundamental difference in TCA definition may explain the extremely low survival in this report – an idea which is further supported by Battistella's observation that those with ECG activity over 40 per minute (who may therefore be in LOST) have a higher chance of survival than those with a lower cardiac electrical rate, or asystole.[40]

Traumatic cardiac arrest, especially haemorrhagic TCA, therefore represents a spectrum of disease – from those with ongoing cardiac function (LOST) to those with a no-output state in trauma (NOST).[46] While there is no rapid, reliable way to differentiate these conditions, those with no ECG activity will have no cardiac output and subsequently a lower chance of survival.[45] The inclusion of NOST

patients, and exclusion of LOST patients, in these and similar studies, led authors in the 1990s to suggest that attempted resuscitation of TCA was futile.[47]

3.2 TCA survival in modern trauma systems

Since 2005 there has been a paradigm shift in the reported survival of TCA.[48] The cause for this is unknown, but is likely to be a combination of a better understanding of the aetiology of TCA, a wider definition of TCA that includes LOST patients, and evolution in the standard of trauma care.

3.2.1 TCA studies

Lockey et al undertook a ten-year retrospective database review of TCA in a mature physician-led pre-hospital medical service. Between 1994 and 2004 12,086 trauma patients were attended by the service, of which 909 (8%) required cardiopulmonary resuscitation.[49] 131 (14%) survived to Emergency Department discharge, and 68 (8%) patients survived to hospital discharge. After more detailed review, 11 (16%) of the long-term survivors were suspected to have had a primary medical cardiac arrest leading to or following trauma, nine (13%) had penetrating trauma, 27 (40%) had blunt trauma, and 21 (31%) were secondary to burn injury, electrocution, or drowning.[49] Of the nine penetrating trauma survivors, eight had cardiac tamponade at pre-hospital thoracotomy, and one was secondary to hypovolaemia. This paper suggests that those with cardiac tamponade, who receive pre-hospital thoracotomy, have a good chance of survival, whereas survival from haemorrhagic TCA is rare.

TCA data from the German Trauma Society Trauma Registry has reported a higher survival rate. Huber-Wagner et al examined 757 trauma patients who received closed chest compressions either pre-hospital or in-hospital between 1993 and 2004. Survival to hospital discharge was 17%, and in the 38% in whom data were available, 10% had a moderate or good neurological outcome. A logistic regression model was completed for 226 cases of TCA with complete data – pre-hospital tube thoracostomy was the only variable associated with improved survival.[50] The authors do not include a specific analysis of haemorrhagic TCA.

Leis et al reported TCA survival data from a pre-hospital medical service in Madrid. Between 2006 and 2009 the service recorded 167 trauma patients who received 'advanced life support' – a return in spontaneous circulation was recorded in 82 (49%) patients, and 11 patients (7%) survived to hospital discharge with a complete neurological recovery. An evaluation of response time showed that no patients had a complete neurological recovery if the first ambulance took more than ten minutes to arrive at the scene.[51] Initial ECG rhythm analysis demonstrated a significantly higher chance of survival in ventricular fibrillation (VF) (36%) compared to pulseless electrical activity (7%), and asystole (3%). VF is a rare cardiac rhythm in TCA,[45,52] and the survival described in this study may reflect a proportion of patients with primary medical cardiac arrest who then sustained trauma (for example a road traffic collision, or a fall from height). This pre-hospital system has two types of ambulances (basic and advanced), and there was a trend towards improved survival if an advanced

ambulance was the first to arrive – 9% had a complete neurological recovery compared to 4%.[51]

Barnard et al used the Trauma Audit Research Network (TARN) database to undertake the first analysis of epidemiology and aetiology of TCA in England and Wales.[46] Between 2009 and 2015, 227,944 patients were included in the TARN database; 705 had TCA either pre-hospital, in the Emergency Department, or both. The 30-day survival was 8%, and 63% of these had a moderate neurological disability or a good outcome. Those with a pre-hospital TCA had a significantly higher chance of survival compared to those with TCA in the ED (12% versus 4% respectively, $p<0.02$). The majority of patients with TCA had severe TBI and/or severe haemorrhagic injury (87%). A smaller proportion had severe haemorrhage without evidence of severe TBI (36%). 60% received closed chest compressions, 27% were attended by a pre-hospital doctor, and 14% underwent a resuscitative thoracotomy.

There have been two reports on military TCA outcomes, both from UK experience in Iraq and Afghanistan (2003-2014). Tarmey et al prospectively collected data on all adult TCA at the UK military hospital in Afghanistan from November 2009 to June 2010. Fifty-five patients were included, of which 14 (27%) had a return of spontaneous circulation, and four (8%) had a good neurological recovery.[53] Procedures performed in the four neurologically-intact survivors included release of pericardial tamponade, non-anatomical lung resection, and direct compression of the proximal aorta to effect distal haemorrhage control (all had a resuscitative thoracotomy).

Barnard et al undertook a more comprehensive analysis of TCA during the Iraq and Afghanistan conflicts.[54] The JTTR contained 424 TCA patients (44 in Iraq, 387 in Afghanistan) with physiological evidence of TCA who were transported to a UK military hospital between 2003 and 2014. The overall survival to hospital discharge was 11%, with a trend of increasing survival during the study period. In one six-month period (May to October 2009) the survival was 25% - coinciding with a relatively high proportion of explosive mechanism of injury compared to gunshot wounds (2:1). Those who died were more likely to have an AIS >3 head injury than those who survived. Conversely those who survived were more likely to have an AIS >3 lower limb injury than those who died. The addition of a severe head injury to the other three most commonly severely injured body regions (thorax, abdomen, lower limbs) reduced the composite survival from 13% to 6% - further highlighting the lethality of severe TBI in the military setting of TCA. Conversely those in TCA with a severe lower limb or abdominal injury had a survival of 16%. These survivors all had a primary haemorrhagic aetiology. However, there were 69 TCA patients with a severe lower limb or abdominal injury (in the absence of severe thoracic or head injury) who died. Over 80% of these patients were estimated to have had a primary haemorrhagic TCA.[54]

3.2.2 Summary of TCA studies

These studies are heterogeneous in their inclusion criteria and definitions. They report a TCA survival of between 7% and 17%, and a combined survival of 10.4% (95%CI 9.3-11.5). The outlier amongst these studies is the study by Huber-Wagner et al, that reports a 17.2% survival.[50] The mean systolic blood

pressures (66 mmHg pre-hospital and 80 mmHg on arrival at hospital) may explain the higher survival, in that a proportion of included patients may have either had a palpable central pulse (and therefore not been in TCA), or that there was a high-proportion of LOST patients with TCA.

However, there has been a paradigm shift in reported TCA survival from the mid-1990s onwards. Although this may in part be due to different definitions of TCA (to include patients with a low-output state in trauma), there is some evidence within these studies that earlier intervention, the presence of pre-hospital physicians, and the use of resuscitative thoracotomy may improve outcomes.

3.3 TCA management protocols

Survival from TCA has historically been reported as extremely rare, leading to the question of futility of resuscitation in this group of patients.[47] This, together with the failure to recognise that the aetiology of TCA is different to that of medical cardiac arrest, has resulted in a lack of specific guidance that addresses the potential reversible causes of TCA, and a lack of recognition that neurologically intact survivors are possible. Practice guidelines are by necessity based upon the existing evidence – this paradox has been broken by international variation in medical practice resulting in an evolution of understanding of TCA.

This is well illustrated by the maturation of the European Resuscitation Council (ERC) guidelines from 2005 to 2015: in 2005, the group examined eleven studies (1983-2004) including 2,839 TCA patients, of whom 64 survived – a survival of

2.3% (95%CI 1.7-2.9).[55] This guideline recommends standard cardiopulmonary resuscitation with exclusion of reversible causes. In 2010, the group analysed a further seven studies (2005-2007), including 2,378 TCA patients, of whom 230 survived – a survival of 9.7% (95%CI 8.5-10.9).[48] This is a significant increase in survival, with survival odds post-2005 of 4.6 (95%CI 3.5-6.2), $p<0.001$; this remains significant even after removal of the outlier 17.2% survival reported by Huber-Wagner et al.[50] This guideline recommends standard cardiopulmonary resuscitation as the immediate treatment of TCA, but places more emphasis on reversible causes, listing aetiologies that may be amenable to specific interventions.[48] The 2015 ERC guideline acknowledges the better understanding of TCA by saying that while cardiopulmonary resuscitation remains the standard of immediate care, management of reversible aetiology should take priority. There is also recognition in 2015 that neurological outcome following TCA is better than previously quoted, and may even be better than after medical cardiac arrest.[41]

There are three other published TCA guidelines: the UK Resuscitation Council, London's Air Ambulance, and the Greater Sydney Area Helicopter Emergency Medical Service.[56-58] All of these guidelines recognise the need for resuscitative thoracotomy in penetrating TCA, but their emphasis on closed chest compressions following blunt trauma is variable.

3.3.1 Closed chest compressions

George Washington Crile (1864-1943) was a pioneering US surgeon who published numerous reports on haemorrhagic shock and cardiac arrest.[59] In

1903 he was the first person to describe the successful application of closed chest compressions in medical cardiac arrest. Despite this, the first comprehensive description of compressions was not published for a further 60 years; Kouwenhoven demonstrated compressions as a method of maintaining adequate circulation in cardiac arrest in 1960.[60] In his study, ventricular fibrillation was induced in a canine model, and these animals received compressions for up to 30 minutes before successful external defibrillation (a technology his team also developed). This report also contains a description of five cases of the successful use of chest compressions in humans. Following this experimental work, the American Heart Association introduced a new education programme: the start of 'CPR' training.

The mechanisms by which chest compressions effect blood flow remain controversial.[61] The cardiac model, in which the heart is compressed between the sternum and the veterbrae, was put forward by Kouvenhoven in 1960.[60] The thoracic model, in which the increase in intrathoracic pressure during compressions produces flow, was first postulated by Rudikoff and colleagues in 1980.[62] It is likely that compressions in normovolaemia influence flow by a combination of these two models, dependent on the force of the compression, the compliance of the heart muscle, the behaviour of the mitral valve, and positive pressure ventilation.[61,63]

3.3.1.1 Closed chest compressions studies

Compressions in normovolaemic cardiac arrest produce a forward flow of blood that equates to 10-15% of physiological normal, and importantly for ROSC, a

coronary perfusion pressure (CPP) of greater than 15mmHg.[64] Compressions are a universally accepted treatment for medical cardiac arrest.[65,66] This has been extrapolated to TCA management in the absence of good quality evidence of positive effect.[45] At the start of this research project, there was a single published study that evaluated the efficacy of compressions in hypovolaemia.[67] This study of three large baboons examined the efficacy of compressions in three models (sequentially in the same animals): cardiac tamponade, hypovolaemia, and normovolaemic cardiac arrest. Initially cardiac tamponade was created by instilling crystalloid solution into the pericardium; palpable femoral artery pulsation was lost at approximately 40 mmHg, and compressions were then initiated, resulting in a mean blood pressure (BP) of 57/8 mmHg. The tamponade was then relieved before the animals were bled until femoral pulsation was lost (again 40 mmHg), equivalent to 35-40% of total blood volume; compressions resulted in a mean BP of 64/4 mmHg. Of note, restoration of vascular volume rapidly effected a mean BP increase to baseline levels (105/55 mmHg). In the last phase of this experiment the now normovolaemic baboons had a barbiturate-induced cardiac arrest, after which compressions resulted in a mean BP of 108/12 mmHg.[67]

This small study suggests that compressions are more effective at increasing BP in normovolaemia compared to hypovolaemia, and that restoration of blood volume is more effective than compressions in increasing BP in hypovolaemia. Coronary blood flow predominantly occurs during diastole – compressions in hypovolaemia in this study produced a diastolic BP of 8 mmHg.[67] Cardiac arrest studies have repeatedly shown that a CPP (the difference between aortic

diastolic pressure and left ventricular end-diastolic pressure) of between 15 and 25 mmHg is required for ROSC.[64] Therefore, the pressures obtained with compressions in this model of hypovolaemia are unlikely to generate sufficient CPP to obtain ROSC.[61,67] It also shows that relief of cardiac tamponade, in relative normovolaemia, is more effective at producing ROSC than compressions.[67] In 2016, after the completion of the large animal work of this thesis, Jeffcoach et al published an evaluation of closed chest compressions and intravenous filling in hypovolaemic canines.[68] The main finding of this study reiterated that of Luna et al; demonstrating that restoration of vascular volume in LOST TCA is a more effective intervention than compressions alone, and is further described in relation to the findings of this thesis in Section 8.10.

The use of compressions in TCA is widespread, illustrated by the fact that 17 of the 18 clinical studies examined by the authors of the 2010 ERC guideline used 'CPR' as the sole inclusion criteria.[48] Mattox et al reported on 100 consecutive cases of TCA following thoracic and abdominal trauma in 1982 – all received compressions pre-hospital, and despite other interventions in hospital (for example resuscitative thoracotomy, RT) none survived to hospital discharge.[69] The authors concluded that compressions are not an effective treatment in TCA following thoracic and abdominal trauma, and instead advocated rapid transport to a hospital for surgical intervention.[69]

The recommendation to commence compressions in TCA is complicated: on one hand, there is good evidence that in normovolaemic TCA (for example secondary to TBI) they would be effective, but on the other hand there is very limited evidence that in TCA secondary to cardiac tamponade or hypovolaemia compressions is an effective management strategy. In the same vein, it is likely that in TCA secondary to a tension pneumothorax, compressions would also be ineffective (owing to the obstruction of venous return). Instead, in these specific causes of TCA, the management should focus on treating the reversible cause (i.e. RT for cardiac tamponade, replacement of intravascular volume in hypovolaemia, and thoracostomy for tension pneumothorax). However, there is no clear guidance on how to rapidly identify the cause of TCA, and furthermore it is estimated that 3% of pre-hospital medical cardiac arrests occur in vehicles (potentially masquerading as TCA),[41] and first responders (the public and paramedics) are unlikely to have the required skills and equipment to undertake interventions to address the reversible causes of TCA.

In the military setting, TCA is more likely to be secondary to haemorrhage than in civilian settings.[46,53,54] It would therefore make sense to de-emphasise the use of compressions in military TCA. Compressions are particularly unlikely to be effective in haemorrhagic TCA, primarily as by definition there is insufficient blood volume to generate pressure. Translation of military experience to civilian practice in this instance is not necessarily appropriate, but it should be remembered that in all types of TCA, compressions may be harmful to resuscitation efforts due to a reduction in the rate of transfusion (by increasing

intra-thoracic pressure), an increase in difficulty of performing procedures such as endotracheal intubation, reduced access to the chest to undertake thoracostomy (to relieve a tension pneumothorax), reduced access to the chest for resuscitative thoracotomy (to relieve cardiac tamponade or to occlude the thoracic aorta to minimise ongoing distal blood loss and increase cardiac afterload), increased risk to medical personnel (by increased risk of needle-stick injury, and physical injury from carrying out compressions), and increased time to definitive care.[46]

3.3.2 Resuscitative Thoracotomy

Surgical opening of the chest for resuscitation (thoracotomy) was first described in an animal model by physiologist Moritz Schiff in 1874.[70] Schiff undertook thoracotomy to perform internal cardiac massage in a medical cardiac arrest induced by chloroform overdose; he also noted that cross-clamping the aorta increased the likelihood of successful resuscitation.[70] This technique translated into clinical practice in the early 20th century in order to relieve cardiac tamponade and to suture wounds of the heart.[71] The modern term resuscitative thoracotomy (RT) refers to thoracotomy performed at the first patient contact, without anaesthetic, in patients who are in TCA.[72] The technique of clamshell RT has been described by Wise et al to relieve cardiac tamponade following TCA as a result of penetrating chest injury.[73]

3.3.2.1 RT studies

The single clinical case series of pre-hospital RT is contained within Lockey et al's report of TCA at London's Air Ambulance.[49] It does not include a specific analysis of RT, but ten out of 68 survivors (15%) underwent pre-hospital RT. Nine had penetrating TCA (with a 9% survival): eight had cardiac tamponade, and one had thoracic haemorrhage. There was a single survivor following RT for blunt thoracic, non-haemorrhagic, trauma. Indeed, in the whole data set of 909 patients, there was only one survivor from blunt haemorrhagic TCA.[49]

Rhee et al published a systematic review of 24 in-hospital RT studies between 1974 and 1998.[74] Only studies that reported both penetrating and blunt mechanism injury were included, and patients who underwent an operating theatre RT were excluded. There were 24 papers, from three countries, including 4,620 patients. The overall survival was 7% (341 patients), 9% in penetrating trauma and 1% in blunt trauma. When examined by body region injured, thoracic injury had the highest survival following RT (11%), followed by abdominal (5%) – if the thoracic injury was primarily cardiac the survival was 20%. Patients with signs of life on arrival at hospital (i.e. did not have a pre-hospital TCA) had a 12% survival rate, compared to 3% for those without signs of life on arrival.[74]

More recently Keller et al studied 448 patients who underwent RT at a US trauma center between 2000 and 2010.[75] 37 (8%) survived to hospital discharge, 98% of whom had sustained penetrating trauma (65% GSW, 33% stab wounds). The most common mode of transportation in survivors was police

(46%), followed by ambulance (35%), and private vehicle (19%). It is not possible to calculate the survival from blunt TCA as the total number of blunt trauma patients is not reported.[75]

There are two reports of military RT over the past 14 years. Tarmey et al described 52 consecutive TCA patients at a UK combat hospital during a seven month period in 2009 and 2010.[53] RT was undertaken on 12 (23%) TCA patients, of whom nine (75%) had a ROSC, and four (8% of the total) survived to hospital discharge. The four survivors were all injured by blast; one had an in-hospital TCA and three had pre-hospital TCA. Atypically, compared to civilian reports of RT, 75% of survivors had a haemorrhagic TCA. Morrison et al undertook an analysis of a larger military dataset – 8,402 consecutive trauma admissions to the UK combat hospital in Afghanistan between 2006 and 2011.[52] This study therefore includes the patients reported in the previous paper. Sixty-five (1%) patients underwent RT following TCA; before medical evacuation (15%), en route to hospital (45%), and in-hospital (40%). ROSC was recorded in 33 (51%) patients, and 14 (22%) survived to hospital discharge. The time from TCA to RT was significantly lower in the group that survived (six minutes versus 18 minutes, $p<0.001$). None of the patients with TCA before medical evacuation had a ROSC, whereas 10% of those with TCA en route to the hospital and 42% of those with TCA in the emergency department were long-term survivors. The highest survival was associated with pulseless electrical activity on ECG (no patients in asystole or ventricular fibrillation survived). The majority of patients (88.2%) died in the medical treatment facility (MTF) operating theatre; all died due to haemorrhage.[52]

In summary, RT is an effective intervention in penetrating TCA, and in cases of cardiac tamponade has a reported survival of almost one in five patients. Survival after RT is associated with an in-hospital TCA (compared to pre-hospital), and minimal delay between cardiac arrest and intervention. The survival following blunt injury is considerably lower. Cardiac tamponade following blunt trauma is exceedingly rare, occurring in less than 1:2,000 trauma patients, and is often not clinically recognised.[72] The main indication for RT in blunt TCA is to allow manual compression of the descending aorta, thereby reducing further haemorrhage below the diaphgram.[73,76] This technique has been described in Morrison et al's analysis of RT in military trauma,[52] but there is no high-quality data of its effectiveness, and as such it is not standard practice. Civilian and military studies report that a high proportion of patients with non-compressible torso haemorrhage are in TCA on arrival at hospital,[35,37] but the current capability to deliver pre-hospital RT is extremely limited. Furthermore, RT is not a good solution for this clinical situation – ideally vascular control would be achieved before TCA, but RT requires an unconscious patient (normally secondary to cardiac arrest). There is therefore a need to develop a technique that can be used in pre-hospital non-compressible torso haemorrhage to both prevent and successfully resuscitate haemorrhagic TCA.

3.4 TCA summary

TCA management protocols have been driven by the available evidence – there is a clear argument for the need for RT in TCA caused by penetrating chest injury, but the effectiveness of RT in blunt trauma, and the rationale for compressions,

is less clear, particularly in hypovolaemic TCA. This clinical dilemma is further complicated by the inability to rapidly differentiate the underlying aetiology of the arrest. Futhermore, haemorrhagic TCA is a spectrum of disease, from LOST to NOST. While there are theoretical criteria (cardiac electrical rate, end-tidal carbon dioxide, cardiac motion on ultrasound) for differentiating LOST and NOST, these methods of evaluation are not in routine clinical use for this purpose. This is of particular importance in TCA secondary to NCTH, where in LOST, haemorrhage control adjuncts alone may lead to ROSC. There is therefore a need to evaluate the effect of compressions and intravenous volume replacement in a more severe model of haemorrhage (NOST), and to compare this intervention to the evolving and future potential management strategies for NCTH that are described in the next chapter.

CHAPTER FOUR

STRATEGIES FOR THE MANAGEMENT OF NCTH

4.1 Introduction

NCTH is the leading cause of potentially preventable trauma death, and a significant proportion of patients with this injury are in haemorrhagic TCA on arrival at hospital.[35,37] Interventions to improve trauma survival should therefore be aimed at managing NCTH with and without TCA, in the pre-hospital setting.

4.2 NCTH management options

The use of RT in TCA has been discussed in Chapter Three, and in summary is a well-proven intervention in managing cardiac tamponade secondary to penetrating trauma, but its place in managing all other types of NCTH is less clear (Section 3.3.2). There are a number of other potential management options, which are discussed in this chapter.

4.2.1 The abdominal aortic junctional tourniquet

The abdominal aortic junctional tourniquet (AAJT) is an external compression device, placed around the abdomen with the aim of occluding the aorta.[77] The AAJT can potentially provide proximal vascular control of the aorta (albeit infra-renal) in a similar way to RT and manual compression of the aorta, and could be applied to a conscious patient. The first published report of AAJT use was in 1961, described as a technique to reduce the whole-body exposure to toxic

chemotherapy. The author also describes the use of an intra-aortic occlusion balloon for the same purpose.[78] More recently studies on healthy human volunteers have demonstrated the AAJT's ability to occlude blood flow to the femoral vessels.[77,79] There has also been a case report of successful use of the AAJT to control lower limb haemorrhgae following bilateral lower limb amputation in Afghanistan.[80] However, there are no published case series or clinical trials of the AAJT.

There are two studies of AAJT use in large animal models of haemorrhage. Kheirabadi et al studied the effects of AAJT application on 45-55 kg swine.[81] Eighteen swine underwent a controlled haemorrhage of 25% of circulating volume, followed by a free haemorrhage from the femoral artery while the AAJT was applied. In all animals, the AAJT controlled the femoral arterial bleed, and the mean arterial pressure returned to pre-injury levels within a few minutes of application. On release of the AAJT the six (spontaneously breathing) swine all had a respiratory arrest – biochemical analysis demonstrated an acidaemia and hyperkalaemia; mechanically ventilated swine had a less pronounced physiological response to AAJT removal. Rall et al used a more severe model of haemorrhage in larger swine to evaluate the haemodynamic effects of AAJT application.[82] Four groups of ten 70-90 kg swine were assigned to either the presence of absence of a controlled haemorrhage of 40% of total blood volume, and either AAJT application or none. In the two treatment groups, the AAJT effectively prevented femoral artery blood flow, and there was no detrimental effect on cardiac output with compression of the inferior vena cava. However,

the study ended before the AAJT was removed, so the authors were unable to report the physiological effects of AAJT removal.[82]

The limited available data on the AAJT therefore suggest that it could be a suitable pre-hospital haemorrhage control adjunct, but only for haemorrhage inferior to the abdomen. Its use in pelvic haemorrhage associated with fracture has not been assessed, but it is a relative contraindication, as the force with which the AAJT is applied could further distract a pelvic fracture.

4.2.2 Intra-abdominal foam

In 2010, in recognition of the high-burden of combat NCTH, the US military launched the Wound Stasis Program - a team at Harvard medical school tested 1,300 different foams, and came up with a mixture of a polyol phase and an isocyanate phase.[83] Polyurethanes are one of the most biologically compatible materials,[84] and when percutaneously injected into the abdomen they can expand to 40 times their original volume. Multiple large animal translational studies have demonstrated the efficacy of intra-abdominal foam. Duggan et al created a severe intra-abdominal haemorrhage in 27 swine (40-45 kg). Eleven of the 12 non-treated controls died in less than one hour. Of the 15 swine who received 150 ml of foam ten minutes after injury, all were alive at one hour, and 11 (73%) were alive at three hours.[85] The polymer was easily removed in less than a minute at laparotomy, and the only organ abnormality observed were small areas of pressure necrosis of the bowel. While intra-abdominal foam may control haemorrhage in the abdomen, it does not control more distal

haemorrhage (for example to the pelvis). There are currently no reports of clinical use.

4.3 Intra-aortic balloon occlusion

In recent years, intra-aortic balloon occlusion (IABO) has generated the greatest number of both large animal studies and clinical reports with respect to potential pre-surgical management options for NCTH. This interest in IABO has almost certainly been stimulated by the high mortality of NCTH during the 15 years of recent conflict, described in Chapter Two.

IABO is categorised by the anatomical zone in which it is placed. Aortic zones are demarcated by arterial branches: Zone 1 (Z1) extends from the left subclavian artery to the coeliac artery, Zone 2 (Z2) from the coeliac axis to the caudal renal artery, and Zone 3 (Z3) from the caudal renal artery to the aortic bifurcation.[86] In general, Z1 occlusion is used to control NCTH below the diaphragm, and Z3 for bleeding associated with pelvic fracture (and lower limb haemorrhage).

4.3.1 Historical IABO studies

This approach to NCTH management is not novel. The first reported translational research into the use of IABO for haemorrhage control was in 1953, during the Korean War. Edwards et al cite 'the pressing need for a method of controlling massive intra-abdominal haemorrhage, especially from wounds of warfare' as the rationale for their canine experiments.[87] The authors concluded that IABO was very effective at reducing intra-abdominal bleeding. However, Z1 occlusion (superior to the coeliac axis) after a period of haemorraghic shock was

almost universally fatal despite restoring blood volume, compared to Z2 occlusion (between the coeliac axis and the renal arteries) after which the animals used in the experiments made an uneventful recovery.[87] The authors postulated that intestinal congestion and hepatic anoxia were possible mechanisms to explain this mortality difference, and concluded that IABO in humans was not a feasible solution to manage NCTH.

Despite this, the first clinical report of IABO use was published the next year, in 1954. Lieutenant Colonel Hughes, a US Army surgeon in Korea, had observed the beneficial effects of direct aortic compression during laparotomy in exsanguinating abdominal haemorrhage. Hughes described the use of a Dotter-Lukas balloon catheter in two 'moribund' patients with abdominal haemorrhage, whose BP could not be maintained despite receiving ten units of whole blood.[88] The balloon position was estimated to be at the diaphragm (Z1) by measuring the catheter on the outside of the patient. Both patients were in haemorrhagic TCA prior to balloon inflation. The first patient had a ROSC on balloon inflation, but subsequently died on the operating table. The second patient did not have ROSC despite receiving a further 2000 ml of whole blood after the aortic balloon was placed. Hughes highlighted some important considerations with respect to IABO: the need to inflate a balloon earlier in the clinical course of haemorrhage, and certainly before TCA, and the potential complications of liver, renal, and gut ischaemia following prolonged proximal aortic occlusion.[88]

The next published report of IABO use in trauma was not for over 30 years. Low et al described the use of the Percluder intra-aortic balloon in a total of 23

patients (including 15 trauma cases) in 1986.[89] Two (13%) patients survived (one had a gunshot wound to the heart, and the other a gunshot wound to the aorta and liver) – both had IABO for 30 minutes. A third patient had balloon occlusion for 90 minutes and subsequently died of ischaemic damage to multiple organs below the level of the balloon. A rise in systolic BP was noted in every patient who had organised cardiac electrical activity, but the effect of IABO during closed chest compressions was not reported. The other eight (non-trauma) patients in Low et al's report were cases of ruptured abdominal aortic aneurysm and gastrointestinal haemorrhage.[89] A few years later Gupta et al published a clinical series of IABO in 21 consecutive patients with penetrating abdominal injury.[90] Five patients were in haemorrhagic TCA – despite four out of the five demonstrating a rise in systolic BP to 80 mmHg after IABO, none survived. There was a 50% survival in those who received balloon occlusion prior to laparotomy, and a 40% survival of those who received balloon occlusion during laparotomy.[90] Three patients had major complications – one had an ischaemic leg after catheter removal, and in two others the catheter initially exited the aorta through the traumatic injury. There was no conventional therapy comparison group.

Non-trauma use of IABO, as described in Low et al's case series,[89] has been developed over a similar time period. The first use of IABO in the management of abdominal aortic aneurysm was published in 1962 by Hesse et al.[91] Although this index patient died shortly after surgery, further advances in vascular surgery have resulted in multiple successful clinical descriptions of IABO for this condition.[92] Similarly, IABO in gynaecological haemorrhage was first described

in the 1990s,[93] and is now established as an effective adjunct in the management of placenta previa and percreta.[94] However, in trauma the technique of IABO was again forgotten until research teams in Texas, USA, and Marseille, France undertook a series of large animal translational NCTH studies.

4.3.2 Translational models of IABO in trauma

In recognition that NCTH is the leading cause of potentially preventable death in recent military conflicts, in 2011 White et al designed a study to compare IABO with the more traditional approach of thoracotomy with aortic clamping.[95] A total of 18 swine (70-95 kg) underwent a controlled haemorrhage of 35% of total blood volume from the iliac artery over 20 minutes, followed by a 10 minute 'transport' phase. The animals were then randomised into three groups of n=6 for surgical intervention (laparotomy and vascular control of the iliac vessel): no aortic occlusion (control), thoracotomy and Z1 aortic clamp, and Z1 IABO. Following vascular repair, the IABO and aortic clamps were removed, and animals observed during a six-hour resuscitation phase, where they received intravenous fluid and norepinephrine to maintain a mean arterial pressure greater than 60 mmHg. There was no statistical difference in overall survival, but the aortic clamp and IABO animals had a higher BP, carotid blood flow, and brain oxygenation compared to the control group. However, the IABO animals required less resuscitation fluid and norepinephrine compared to the aortic clamp group. The IABO group also demonstrated less physiological derangement (higher pH, lower lactate, higher base excess) than the aortic clamp group.[95] In summary, IABO was comparable to aortic clamping in haemodynamic

profile, but required a lower volume of resuscitation fluid, and was associated with less physiological derangement.

Avaro et al used a model of splenic trauma (NCTH) in large swine (median weight of 77 kg) to compare no aortic occlusion, 40 minutes IABO (with and without splenectomy, followed by balloon deflation), and 60 minutes IABO followed by splenectomy and balloon deflation.[96] 25 animals had an uncontrolled NCTH secondary to the spleen being severed into two parts. When the mean arterial pressure was below 60 mmHg, resuscitation was commenced and all animals received a 30 ml/kg bolus of intravenous 0.9% saline, after which the animals were randomised to four groups. Further unlimited boluses of intravenous saline were administered to maintain a mean arterial pressure greater than 60 mmHg and a heart rate less than 120 beats/minute. Animals were observed for a maximum of two hours after the start of the injury. All (nine) control animals were dead 80 minutes after injury, two (25%) of eight animals who received 60 minutes of IABO and splenectomy survived for two hours, as did one (25%) of the four animals who received 60 minutes of IABO (with no splenectomy). All animals who received 40 minutes IABO followed by splenectomy survived to the end of the protocol. IABO was effective in haemorrhage control of this NCTH (there was an immediate increase in BP, and a reduction in intravenous fluid requirement in all balloon occlusion groups), and there was no evidence of ischaemic injury to the bowel or kidney.[96] However, there was a clear difference in lactate and potassium between 40 and 60 minutes of IABO, that resulted in the rapid death of 60 minute IABO animals after balloon deflation. This large animal study therefore shows that while IABO is effective

for haemorrhage control in NCTH, 60 minutes of occlusion resulted in a non-survivable re-perfusion injury. Avaro et al successfully inserted and inflated all Z1 balloon catheters without imaging – an important consideration for pre-hospital use.

These initial large animal studies have all described the use of Z1 IABO. In 2012 Morrison et al reported on the comparison of Z3 IABO and haemostatic gauze in a translational model of (compressible) iliac artery haemorrhage.[97] 21 swine (median weight of 81 kg) underwent 45 seconds of uncontrolled haemorrhage from the iliac artery. All animals then received a 500 ml bolus of intravenous colloid before being assigned to one of three experimental groups (n=7): no intervention, wound packing with a haemostatic gauze, and inflation of a Z3 IABO. 100 ml boluses of lactated Ringer's solution were given, up to a maximum of ten litres per animal, if the mean arterial pressure was lower than 65 mmHg. The experiment was stopped 180 minutes after the start of the haemorrhage (without IABO removal). Mortality in the no treatment group was 100% after 15 minutes, whereas all of the gauze and IABO animals survived to the end of the protocol (180 minutes). However, base excess and clotting function were both statistically better in the gauze group compared to the IABO group at 180 minutes. In order to evaluate these intervention groups in a model of coagulopathy, two further experiments were done. The animals had 60% of their circulating blood volume replaced with a colloid, resulting in a pre-injury INR of between 1.4 and 1.6. In this model, there was a 72% (five out of seven) mortality in the gauze group, but all IABO animals survived. This study therefore demonstrates that Z3 IABO is effective for compressible groin haemorrhage, but

is only superior to haemostatic gauze in the presence of an induced coagulopathy. While this study has limited generalisability to NCTH (the injury was compressible) it can be translated to the use of IABO for more proximal non-compressible haemorrhage in the pelvis.

In response to Avaro et al's earlier swine study that showed Z1 IABO for greater than 40 minutes resulted in a severe reperfusion injury,[96] Markov et al undertook a translational model with the aim of better describing the physiological consequences of prolonged IABO.[98] A total of 24 animals had a controlled haemorrhage of 35% of total blood volume over 20 minutes from the iliac artery. In order to achieve a state of class IV shock, haemorrhage was continued at a rate of 0.15 ml/kg/minute for a further 10 minutes, but paused if the mean arterial pressure was lower than 30 mmHg. Animals were then randomised into one of four (n=6) groups: 30 minutes with no intervention, 30 minutes of Z1 IABO, 90 minutes with no intervention, and 90 minutes of Z1 IABO. This was followed by six hours of resuscitation, initially using the previously shed fresh whole blood, and after this was exhausted using boluses of lactated Ringer's solution and norepinephrine; followed by 48 hours of observation (the animals remained anaesthetised). The key finding of this study is that IABO effected a higher mean arterial pressure and higher cerebral tissue oxygenation than controls, but this was at the expense of high serum lactate measurements after balloon deflation (that was more marked in the 90 minute IABO group compared to the 30 minute IABO group). Furthermore the 90 minute IABO group had histological evidence of renal and hepatic damage. While the physiological state of all surviving animals had returned to near baseline levels

by 48 hours, prolonged (90 minute) IABO caused significantly more derangement than 30 minutes of IABO (and no IABO groups). This raises two questions: in a less well controlled injury would this additional physiological burden be lethal, and in an uncontrolled NCTH would all animals have died without IABO?

A subsequent large swine study by Scott et al provides further data on the physiological consequences of 60 minute IABO.[99] Sixteen animals (median weight of 78 kg) underwent a controlled haemorrhage via the femoral artery using the same methodology as the previous study by Morrison et al. Animals then had one of two types of REBOA catheters inserted and inflated in Z1 for 60 minutes (conventional and fluoroscopy-free). Following controlled balloon deflation, boluses of fresh whole blood and 0.9% saline (up to 20 ml/kg) were administered intravenously, followed by norepinephrine as required to maintain a mean arterial blood pressure above 60 mmHg. The animals were then maintained on ketamine and midazolam infusions for 48 hours of observation under anaesthesia. In all animals, IABO resulted in an increase in mean arterial pressure, cerebral tissue oxygenation, and carotid blood flow. Immediately following balloon deflation, mean lactate was above 10 mmol/l and pH was below 7.20 – both returned to baseline levels after 24 hours of observation. The serum potassium at balloon deflation was not reported in this study, but it was noted to remain elevated after 48 hours of observation. This study therefore concludes that 60 minutes of IABO in a large swine model of controlled haemorrhage produces a survivable metabolic acidosis, and that a fluoroscopy-

free IABO catheter can be effectively placed,[99] increasing its suitability for pre-hospital use.

The first large animal model that examines the use of IABO with adherence to the modern trauma resuscitation principles of damage control resuscitation was reported by Morrison et al in 2014.[100,101] It is also the first to investigate the use of intermittent, compared to continuous, IABO. The model was additionally more severe than those previously reported: 24 swine (weight range of 70-90 kg) underwent a laparoscopic liver resection that resulted in grade V shock (mean systolic BP between 28 and 48 mmHg) – these animals were therefore either in, or approaching, haemorrhagic TCA. For intervention, the animals were assigned to one of three groups (n=8): no IABO, continuous IABO for 60 minutes, or intermittent IABO for 60 minutes (the balloon was fully deflated for 60 seconds, 20 and 40 minutes after initial inflation). Following the 60 minute intervention phase, surviving animals underwent resuscitation with whole blood (from donor animals), laparotomy, and control of the liver injury (surgery phase). This was followed by a four hour critical care observation phase. All of the no IABO animals were dead 35 minutes after injury, whereas seven of eight (88%) continuous IABO and six of eight (75%) intermittent IABO animals survived to the surgery phase. Overall, 11 (69%) of the sixteen IABO animals survived to the end of the protocol (with improving pH and serum lactate). There was no reported physiological advantage in intermittent IABO compared to continuous. More importantly this study demonstrates that the physiological derangement associated with 60 minutes of IABO in a severe model of

haemorrhage can be managed with aggressive resuscitation using whole blood.[101]

In summary, these large animal studies have demonstrated that IABO significantly increases survival in NCTH,[96,101,102] significantly increases BP,[96,99,101] causes less physiological derangement and reduced fluid requirements compared to RT and aortic cross clamping,[95] and increases brain oxygenation and carotid flow compared to controls.[98,99] There is also evidence that balloon occlusion is superior to the use of haemostatic gauze in the control of otherwise lethal groin haemorrhage.[97] The placement of IABO has been demonstrated without imaging,[99] making pre-hospital placement feasible, but while Morrison's 2014 study uses a more severe model of NCTH (in which some animals may have been in LOST TCA), there are no reports of the efficacy of IABO in severe TCA. There is therefore a need to evaluate IABO (with fluid resuscitation according to modern trauma management principles) in a more severe model of haemorrhagic TCA.

4.3.3 Clinical studies of IABO in trauma

The clinical descriptions of IABO use in trauma between 1954 and 1989 have already been described – the subsequent development of trauma systems limits their generalisability to current practice. The first modern clinical description of IABO pre-dates the recent large animal studies, and was stimulated by a particularly lethal injury – haemorrhage associated with pelvic fracture. Martinelli et al published a case series of 13 patients who underwent Z3 IABO between 1998 and 2007.[103] Prior to consideration for IABO, trauma patients

were resuscitated with at least three litres of intravenous crystalloid (or at least two litres of crystalloid and one unit of red blood cells), and had a systolic BP of less than 60 mmHg (or less than 90 mmHg after an hour of resuscitation). These patients were considered as imminently dying. In order to bridge to the angiography suite, Z3 IABO was used. Twelve (92%) of the 13 survived to angiography – the mean systolic BP increased to 111 mmHg after balloon inflation. The mean IABO time was 70 (±39) minutes, and six patients had severe hypotension after balloon deflation (resulting in the death of two). The authors do not report any adverse features of prolonged IABO.[103] Six (46%) patients survived to hospital discharge, two of whom were reported to be in haemorrhagic TCA prior to IABO. Although there was no control group, all patients were too unstable to transfer before IABO placement, and it is therefore reasonable to assume that they would have died without Z3 IABO.

Clinical use of Z1 IABO was reported in 2013 by Brenner at el in a case series of four Z1 and two Z3 applications in trauma.[104] Prior to this clinical series, the term 'resuscitative endovascular balloon occlusion of the aorta' (REBOA) had been coined to describe the use of IABO in trauma.[105] As in Martinelli et al's study, the cases reported occurred during routine care of patients who were haemodynamically unstable. In one case the patient, who had sustained a gunshot wound to the pelvis, went into haemorrhagic TCA in the resuscitation room. After inflation of Z1 REBOA the pulse returned, and the systolic BP increased to 100 mmHg. Overall, REBOA resulted in a mean rise in systolic BP of 55 mmHg, and four (67%) patients survived to hospital discharge – the remaining two died of co-existing TBI. This clinical series demonstrates the

application of REBOA, including the successful resuscitation of a patient in TCA. The longest occlusion time was 70 minutes – no sequelae were reported.[104]

These initial clinical reports of successful REBOA in the US included small numbers of carefully selected patients without the use of controls. Retrospective data from the Japanese Trauma Data Bank (2004-2011) was reported by Norii et al in 2015.[106] During this period, 452 trauma patients received REBOA in Japan. The authors used propensity scoring to match each REBOA case with five conventionally managed cases, using age, sex, year of injury, revised trauma score, mechanism of injury, maximum AIS, and receiving hospital. There are no national guidelines for the use of REBOA in Japan; the catheters are usually inserted by emergency physicians as a temporising measure while awaiting the attendance of a trauma surgeon.[106] The crude survival odds in the REBOA group, compared to the propensity score matched control group was 0.3 (95%CI 0.2-0.4). It is tempting to attribute the increased mortality of REBOA patients in this study to factors that were not controlled for during matching, and the authors themselves suggest it was likely that REBOA was used in a 'last-ditch' attempt to resuscitate patients with non-survivable injuries. However, in the absence of a randomised controlled clinical trial this report raises questions about the survival advantage of REBOA.

4.4 Active endovascular resuscitation

REBOA is a passive endovascular process, effective at haemorrhage control, either in the thoracic aorta at Z1 or at the aortic bifurcation at Z3. However, it does not provide flow or perfusion to vital organs, and this theoretically limits its

applicability to manage TCA. Selective Aortic Arch Perfusion (SAAP) is an experimental endovascular technique that describes a Z1 REBOA catheter with a central lumen, through which resuscitation with fluid and drugs can be delivered cephalad to the balloon occlusion, Figure 5.5. The clinical reports have demonstrated that REBOA might be a useful haemorrhage control adjunct in TCA patients.[103,104] However it is likely that these patients are in LOST, in a similar state to the large swine in Morrison et al's most recent study.[101] SAAP, when using oxygenated perfusate, has the potential to manage even the most severe end of the TCA spectrum – those in NOST – by effecting coronary perfusion pressure / flow, and achieving ROSC in the setting of a failing myocardium.[107]

The majority of SAAP studies describe its use in medical cardiac arrest, and have been undertaken by two research groups. The first description was by Manning et al in 1992, and reports the use of SAAP for the management of electrically induced ventricular fibrillation (VF) in 21-36 kg canines.[107] After ten minutes of VF, all animals had a SAAP catheter balloon inflated in Z1. The experimental groups were undertaken sequentially in order to test and adjust subsequent experiments. In group one, six animals then received 60 ml/kg of intra-aortic 0.9% saline over two minutes – this created a mean calculated CPP of 16 mmHg after ten seconds of SAAP, but owing to increasing right atrial pressure, this fell to a mean CPP of 7 mmHg at two minutes. In group two, four animals received 60 ml/kg of intra-aortic oxygenated lactated Ringer's solution containing 2 mg/l epinephrine, which produced a mean calculated CPP of 21 mmHg after 10 seconds of SAAP infusion, and a mean CPP of 46 mmHg after two minutes of SAAP. These animals then underwent closed chest compressions, and were all

successfully defibrillated into a ROSC. In group three, four animals received a SAAP infusion of 20 ml/kg of oxygenated perfluorocarbon, containing 4 mg/l epinephrine, over one minute. After this one minute SAAP infusion, the mean calculated CPP was 58 mmHg, and all animals were successfully defibrillated into a ROSC after closed chest compressions. This study demonstrated the feasibility of active endovascular resuscitation, and its ability to rapidly produce a CPP far in excess of that required for ROSC.[64] It also demonstrated that the addition of epinephrine to the SAAP infusion increases the CPP, and that novel oxygen carrying solutions (a perfluorocarbon) can be used.[107]

Paradis et al subsequently used a canine model of VF to evaluate the use of SAAP with a different novel oxygen carrier.[108] After 20 minutes of untreated VF, seventeen 20-25 kg canines were randomised to either standard advanced life support (closed chest compressions and intravenous epinephrine) or advanced life support with the addition of 450 ml of intra-aortic oxygenated ultrapurified polymerised bovine haemoglobin. During resuscitation, both groups received multiple defibrillation attempts at regular intervals, followed by a period of observation. The SAAP group had a significantly higher mean calculated CPP (62 mmHg) compared to the control group, and a significantly higher ROSC (six out of seven) and one-hour survival (four out of seven). This study therefore corroborates the earlier findings by Manning et al,[107] in showing that SAAP effects a high CPP, and also demonstrates a superior ROSC rate and short-term survival over standard clinical care for medical cardiac arrest.[108]

Paradis and Manning have collectively published three further canine SAAP studies of VF medical cardiac arrest.[109-111] These reports demonstrate that calculated mean CPP may not be the ultimate determinant of ROSC, and that coronary vessel flow during SAAP is likely to be more discriminatory,[109] that SAAP is more efficacious than closed chest compressions for ROSC in medical arrest, [110,111] and that the addition of epinephrine to SAAP infusions increases ROSC.[107,111]

The only report into the use of SAAP in a translational model of haemorrhagic TCA was published by Manning et al in 2001.[112] This study used a model of hepatic injury to achieve TCA (a mean arterial pressure less than 10 mmHg) in small swine (weight range 25-39 kg). Approximately 12 minutes after injury all twelve animals were in TCA (with ongoing cardiac electrical activity, although the heart rates are not reported). Three minutes later swine were randomised to two groups (n=6 per group). Group one received SAAP balloon inflation and 10 ml/kg/minute of oxygenated lactated Ringer's solution, and group two received SAAP balloon inflation and 10 ml/kg/minute of oxygenated HBOC-201. The SAAP infusions were continued until ROSC (defined as a mean arterial pressure of 60 mmHg sustained for one minute). If ROSC was not achieved after three minutes of SAAP, 5 mcg/kg intra-aortic epinephrine was administered every 30 seconds until ROSC (or until 12 minutes had elapsed). Two (33%) of the SAAP with lactated Ringer's group had a ROSC, but none survived an hour, compared to a 100% survival in the six SAAP with HBOC group, in which five of six (83%) went on to survive an hour. This study demonstrates the potential utility of SAAP in successfully resuscitating severe haemorrhagic TCA (with ongoing

cardiac electrical activity, but with a mean arterial pressure lower than 10 mmHg). Furthermore, it suggests that SAAP resuscitation is more efficacious when using an oxygen carrying solution. The additional benefits of SAAP over RT in TCA are that SAAP is less invasive, rapidly increases cerebral blood flow, and effects a high CPP and flow.[112]

As previously explained, RT has been well described in the successful management of penetrating TCA, and can be taught to non-surgeons.[113] However, when considering SAAP as an alternative, the difficulty in accessing the femoral artery in a trauma patient who is in cardiac arrest should not be underestimated. In order to test the ability to place intra-aortic catheters in medical cardiac arrest Manning undertook a two-year clinical protocol in which he successfully placed 22 intra-aortic and right atrium venous catheters pre-hospital – eleven (50%) had a ROSC.[114]

4.5 NCTH management options summary

None of the interventions described for the pre-hospital or early (non-surgical) management of NCTH are well established in clinical practice, and all have potential flaws. The AAJT is the most straightforward intervention, and could be taught to emergency medical service personnel and forward combat medics. It provides a potential solution as a haemorrhage control adjunct in patients with haemorrhage distal to the pelvis – this injury pattern is quite specific to the blast trauma observed during recent conflicts in Iraq and Afghanistan that was frequently associated with pelvic fracture,[115] and may therefore have limited utility. Intra-abdominal foam performed well in animal models of abdominal

haemorrhage, but there are no reports of clinical use, and furthermore this intervention will not control haemorrhage distal to the abdomen. REBOA has demonstrated good efficacy for improving survival in large animal models of NCTH, improving BP and carotid blood flow compared to controls, and is effective in abdominal, pelvic, and junctional groin haemorrhage. There is some evidence from both translational and clinical reports that REBOA can be used to manage haemorrhagic TCA (albeit in the presence of cardiac electrical activity), but there is uncertainty over the survivability of the physiological derangement observed after prolonged balloon occlusion. The ability to use REBOA without radiological imaging means that it could be used in the pre-hospital environment, but the largest evaluation of clinical use has demonstrated an increased mortality compared to matched controls.[106] SAAP involves a higher logistic and training burden compared to REBOA, in that it requires oxygenation of the perfusate (and if used with citrated blood products, the addition of intra-aortic calcium). However, unlike all other potential management options for haemorrhagic TCA secondary to NCTH, SAAP provides active resuscitation by effecting cerebral flow, and a CPP adequate for ROSC. SAAP has the potential to control NCTH whilst actively perfusing the brain and myocardium, and therefore to effectively resuscitate patients at the most severe end of the haemorrhagic TCA spectrum. However, SAAP has not been evaluated in a translational model of severe haemorrhagic TCA against REBOA, or against closed chest compressions. Therefore, a large swine model of severe haemorrhagic TCA was developed in which to address these questions, and is described in the following chapter.

CHAPTER FIVE

MODEL DEVELOPMENT – PART ONE

5.1 Aims and translational model

The overall aim of this research is to address gaps in the current understanding of potential management options for severe haemorrhagic TCA secondary to NCTH. Previous translational research in this field has primarily utilised swine models of haemorrhage.[95-99,101] Swine have many similarities to humans, and importantly in studying NCTH, similar cardiothoracic and abdominal anatomy and physiology.[116,117] Some previous swine studies have described a controlled haemorrhage from either the iliac or femoral artery,[95,97-99] that is not a true representation of clinical NCTH.

However, there have been two published swine models of NCTH (secondary to either uncontrolled splenic or hepatic haemorrhage),[96,101] the latter of which was undertaken in the same animal laboratory as my research.[101] This translational model of laparoscopic hepatic injury has been described in detail, and in the splenectomised swine, resulted in NCTH of 32% of total blood volume, a mean systolic BP of 36.0 (±16.8) mmHg, and a survival of 58% after 60 minutes without intervention.[118] Manning et al's model of NCTH leading to TCA was achieved by an open hepatic injury that resulted in a mean arterial pressure of less than 10 mmHg within 12 minutes.[112]

In order to compare interventions for the management of severe haemorrhagic TCA a large animal model was developed. The initial model was based on the

previously described laparoscopic model of hepatic injury reported by Ross et al,[118] but with a similar end-point to that of Manning et al.[112]

The majority of patients with NCTH die before they reach hospital, and therefore before the opportunity for definitive surgical haemorrhage control. The best available data for median pre-hospital time in clinical NCTH is 61 minutes,[119] and previous translational research has used 60 minutes.[101] The primary outcome for this model will therefore be 60 minute survival post-intervention, as a surrogate for pre-hospital survival.

5.2 Model development ethical review

In accordance with local animal ethical review committee (Institutional Review Board – IRB) approval at the Clinical Research Division of the 59th Medical Wing United States Air Force, a total of 22 large swine (*sus scrofa*) were allocated for model development (protocol number FWH20140020A). The IRB approved this protocol as a non-survival study.

5.3 Aims of model development

The aims of model development were to evaluate the donor blood product, obtain the relevant swine surgical and resuscitation skills (including invasive monitoring, splenectomy, cystostomy, and hepatic surgical repair), adapt the previously described model of laparoscopic hepatic injury to achieve a standardised model of severe haemorrhagic TCA,[118] become proficient with the insertion of intra-aortic balloon catheters, determine an efficacious volume and

rate of SAAP infusion, and to design and test a closed chest compression apparatus for use in large swine.

The following narrative outlines the model at the start of development, based on the laboratory's prior experience with NCTH swine models,[101,118] the laboratory experience of Dr. James Manning,[107,110-112] and discussions with supervisors, including adaptations made to the initial protocol.

5.4 Evaluation of donor blood product

Fresh whole blood (FWB) was collected from a dedicated pool of donors between 24 and 48 hours before experimentation. Each animal had up to three units (450 ml per unit with 10% variance) removed via the external jugular vein, and collected into citrated (CPDA-1) blood donation bags (Teruflex, Terumo Corp. Tokyo, Japan). Each unit of FWB was analysed to confirm haemoglobin concentation. In development, 264 units were used, with a mean haemoglobin of 8.4 (±1.0) g/dl, which is comparable to swine reference values (8.5 (±0.8) g/dl).[120]

Each unit of FWB was separated by centrifuge into red cells and supernatant (plasma). The red cells, stored in blood donation bags containing AS-5 red cell preservative solution, and the plasma, stored in plain donation bags (Teruflex, Terumo Corp. Tokyo, Japan), were collated in pairs in a cold room at 4°C. The CPDA-1 bags contain 63 ml of anticoagulant (citrate phosphate, dextrose, adenine), and the AS-5 contain 100 ml of preservative – increasing the total volume of one FWB unit to 613 ml (range of 568 to 658 ml). On the morning of

development and experimental protocols, paired bags of red cells and plasma were warmed to 38°C in a water bath and transferred to the animal laboratory in a thermal isolation box.

In order to reduce the total number of animals used, the IRB specified that blood donor animals undergo multiple venesections. There was concern that removal of approximately 30% of swine blood volume would result in significant anaemia. The supply and available storage of swine determined that there could be no more than two weeks between venesections. Therefore, during development, four swine (7093, 7094, 7067, 7095) had three units of FWB removed, then following two weeks of rest they had a further three units of FWB removed. The haemoglobin concentration was not significantly different between the first and second venesection – 8.5 (±0.4) g/dl and 8.3 (±1.5) g/dl respectively, paired t-test p=0.6. The protocol was therefore edited to allow a second venesection of individual blood donors, no sooner than two weeks from the first donation. Furthermore, blood samples were obtained from all donor units of FWB for quality control.

The CPDA-1 blood donation bags contain sodium citrate dihydrate to prevent ex-vivo coagulation. Under normal circumstances citrate is metabolised to bicarbonate in the liver, and also forms weakly dissociated salts with divalent cations, particularly calcium – resulting in hypocalaemia.[121] Clinical blood transfusions are administered intravenously, and are therefore diluted by normal systemic blood before supplying the myocardium (an organ that is susceptible to hypocalcacemia). In the clinical setting, this allows calcium to be

administered retrospectively, typically following the transfusion of four or more units of packed red cells. SAAP infusion is delivered directly into the aortic arch, and therefore requires the accurate addition of calcium to the FWB infusion in order to prevent hypocalaemic blood being supplied directly to the myocardium.

An estimation of the required calcium dose to chelate the citrate (and prevent systemic hypocalcaemia) was made from Dr. Manning's prior laboratory experience, and from clinical transfusion protocols. There were no published data on the use of citrated blood product with SAAP. The first five SAAP development animals (D1-7013, D2-7140, D3-7107, D5-7208, D6-7207) received 750mg of intra-aortic calcium chloride per 1000 ml of FWB. Data for ionised calcium concentration immediately before and after the SAAP infusion was available for four animals. Calcium concentration was a mean of 1.29 (±0.03) mmol/l immediately before administration of 800 ml of FWB over one minute, and 0.72 (±0.18) mmol/l after; a mean drop of 0.57 mmol/l (44%). This increased the estimated calcium requirement to 1344 mg of calcium per 1000 ml of FWB. An *in vitro* experiment was undertaken to confirm this.

In the laboratory two bags of donated FWB from the same animal, with a baseline ionised serum calcium of 1.22 mmol/l were separated into 25 ml aliquots. The volume of 10% calcium chloride, equating to 70%, 80%, 90%, 100%, 110% and 120% of the above estimate (1344 mg per 1000 ml of FWB), required for each 25 ml aliquot was calculated. For example, 100% calcium chloride equals 1344mg per 1000ml of FWB, or 0.34 ml of 10% calcium chloride per 25 ml aliquot of FWB. The respective volumes of 10% calcium chloride were

added to five 25 ml aliquots of FWB via a calibrated pipette – a total of 30 samples. The ionised calcium concentration was then measured using a blood gas analyser (ABL800 FLEX, Radiometer, CA, USA). The plotted line intersected the animal's baseline serum ionised calcium (1.22 mmol/l) at 13.88 ml of 10% calcium chloride per 1000 ml of FWB, Figure 5.1.

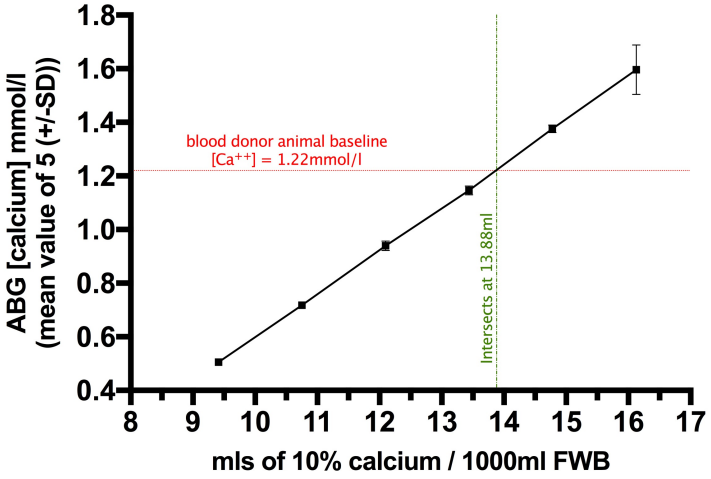

Figure 5.1 – Analysis of the required volume of 10% calcium chloride to return citrated FWB to baseline

Therefore, in future SAAP development protocols, using 800 ml/minute of intra-aortic FWB, calcium chloride was added at a rate of 1110 mg/minute = 11.1 ml/minute of 10% calcium chloride. The 10% variance in the volume of each unit of FWB confers an error to this calculation, and as such in all experimental protocols the volume (by weight) of each unit was checked before use (and discarded if there is more than 10% deviation), and aortic blood samples to confirm serum ionised calcium concentration were obtained during SAAP infusions with FWB. The same calculation was used to determine the required calcium chloride infusion rate for the intravenous FWB infusion.

5.5 Animal preparation

5.5.1 Animal selection

Yorkshire Landrace cross swine (*sus scrofa*, 70-90 kg) were obtained from a single source animal vendor (John Albert, Cibolo, USA, 74-A-1246). To control for sex-linked physiological differences, all animals were male. Swine were quarantined for 72 hours, and fasted for 18 hours before protocols, with free access to water. No adaptions were made to animal preparation.

5.5.2 Animal anaesthesia

Intramuscular ketamine (100 mg + 10 mg/kg), and 2 mg intramuscular buprenorphine were administered for initial sedation and analgesia. Animals were intubated via direct laryngoscopy with a cuffed endotracheal tube. Anaesthesia was maintained on inhaled isoflurane (Abbott, Abbott Park, IL, USA) between 1.5% and 2.5%. Mechanical ventilation tidal volumes were fixed at 7 ml/kg, and minute volume was manipulated by adjustments to ventilation rate. After induction of anaesthesia the fraction of inspired oxygen was maintained at 0.35, until the start of the injury where it was reduced to 0.21 (equivalent to atmospheric air). At the start of the treatment intervention the inspired oxygen fraction was increased to 1.0 in order to emulate clinical management.

During development, detrimental cardiovascular effects of isoflurane were observed. The protocol was therefore amended by titrating the percentage of inhaled anaesthetic against the mean alveolar concentration (MAC) of isoflurane, aiming for a MAC of between 1.1 and 1.3, following agreement with the IRB. In order to produce a more translatable model of haemorrhagic TCA, the IRB were

asked to consider whether the anaesthetic could be switched off at the start of the injury. A compromise was agreed to turn off the anaesthetic at the point of TCA, which was not re-started until ROSC (defined as a systolic BP greater or equal to 50 mmHg) was observed, or the animal showed other signs of awareness. During all protocols, the depth of anaesthesia was continuously monitored using multiple parameters, including MAC of anaesthetic gas, heart rate, jaw tone, muscle tone, palpebral reflexes, and graded pain response).

5.5.3 Physiological monitoring and vascular line placement

When anesthetised, electrocardiography, physiological monitoring, blood sampling, and vascular access lines were inserted percutaneously under ultrasound guidance. For physiological monitoring the following 8.5 Fr catheter introducers (Teleflex, Morrisville, NC, USA) were inserted: 1) right carotid artery - intra-aortic arch blood pressure monitoring via a micromanometer-tipped catheter (Millar Inc. Houston, TX, USA); 2) right external jugular vein (Swan-Ganz thermodilution catheter (Edwards Lifesciences, Irvine, CA, USA); 3) left femoral artery (invasive arterial pressure monitoring). An 8.5 Fr catheter was inserted into the left external jugular vein for intravenous fluid infusion. A 5 Fr catheter was inserted into the left brachial artery for the collection of arterial blood samples. A 14 Fr vascular sheath (Cook Medical Inc. Bloomington, IN, USA) was inserted into the right common femoral artery to allow subsequent insertion of a balloon catheter. A midline cut-down to the left carotid artery was undertaken to place a flow probe (Transonic Systems Inc., Ithaca, NY, USA). A rectal temperature probe was inserted to measure core body temperature, and an oxygen saturation probe was applied to the left ear. Initial baseline physiological

measurements were then obtained. The mean animal weight was 76.7 (±5.1) kg, table 5.1.

Table 5.1 – Baseline physiological measurement of model development animals (n=22)

Baseline variable	Mean (±SD)
Weight (kg)	76.7 (±5.1)
Heart rate (beats/minute)	67.9 (±14.7)
Systolic blood pressure (mmHg)	85.3 (±7.3)
Mean arterial pressure (mmHg)	67.9 (±8.1)
Mean pulmonary artery pressure	18.8 (±4.1)
Cardiac output (l/minute)	4.9 (±1.1)
End-tidal CO_2 (kPa)	5.5 (±0.3)
Carotid artery flow (ml/minute)	351.5 (±77.3)
Central venous pressure (mmHg)	10.6 (±3.5)
Central venous saturation (%)	66.0 (±8.7)
Core temperature (^oC)	36.8 (±0.8)

During development, there were no substantial changes to the above physiological monitoring setup. After D1-7013, the laboratory had the ability to measure tissue oxygenation (StO_2) via near-infrared reflectance spectroscopy (Terumo Corp. Tokyo, Japan). In order to measure tissue oxygenation above the level of a Z1 aortic occlusion balloon an StO_2 cutaneous probe was placed over the right pectoralis muscle. This was tested in the remainder of the development animals, and demonstrated a close relationship with other parameters of perfusion and intervention, with a delay of between one and two minutes, Figure 5.2.

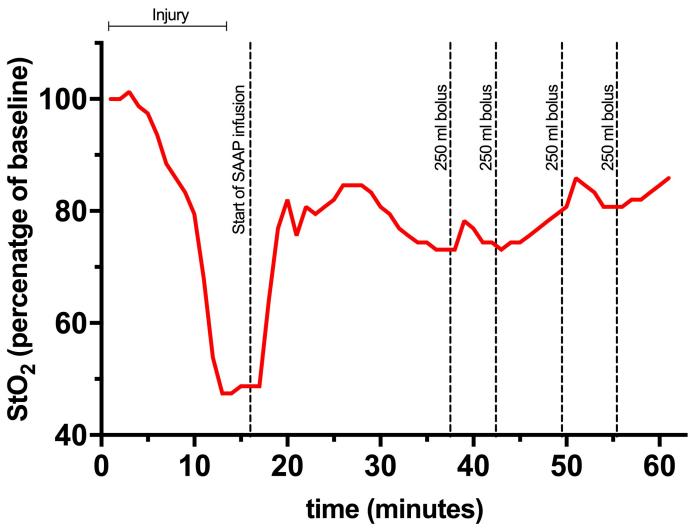

Figure 5.2 – Tissue oxygenation (StO_2) as a percentage of baseline values above the balloon occlusion in D8-7201.

5.5.4 Surgical preparation

Following placement of vascular lines and physiological monitoring, a splenectomy was performed via midline laparotomy. The *sus scrofa* spleen is contractile and theoretically provides an auto-transfusion following injury that may affect the translation of results to clinical practice.[118,122] The left lateral lobe of the liver was marked approximately three to four centimetres from the hilum with electrocautery in order to guide subsequent transection (to effect the NCTH). A direct cystostomy was performed, in order to prevent the physiological effects of a distended urinary bladder during the experiments. The resected spleen was returned to the abdominal cavity in order to restore normal abdominal volume, which could influence the volume of intra-abdominal haemorrhage. To guide the subsequent hepatic injury, four laparoscopic ports were inserted into the anterior abdominal wall. The peritoneum and anterior abdominal wall were then closed with a 1/0 vicryl continuous suture. This was followed by a ten minute stabilisation period prior to the injury phase.

During development, adaptations were made to the placement of laparoscopic ports, in order to better visualise the left lateral lobe of the liver (by retracting liver and bowel), and to better place the endoshears to achieve a 70% transection of the left lateral lobe in under two minutes.

5.5.5 NCTH injury

The model of laparoscopic hepatic injury had been previously developed in the same laboratory.[101,118] This resulted in a mean arterial pressure of 36.0 (±16.8) mmHg.[101] This model, with a longer injury time, was followed in the first four development animals (D1-7013, D2-7140, D3-7107, D4-7131), and produced a mean arterial pressure of 15.8 (±5.3) mmHg and a mean systolic BP of 18.5 (±4.9) mmHg. Dr. Manning's previous swine model demonstrated successful SAAP resuscitation with a mean arterial pressure of less than 10 mmHg.[112] The aim of this research was to evaluate interventions for the management of severe haemorrhagic TCA (NOST), and therefore the aim of this model was to achieve a cardiac arrest systolic BP of less than 10 mmHg, and preferably lower, as dictated by the observed survival of SAAP development animals. The injury time (from the start of the hepatic injury to TCA) was noted to be variable. Therefore, in order to standardise the timings, t0 was moved from the start of the hepatic injury to the point of cardiac arrest. This meant that the intervention started three minutes later (t3), followed by 60 minutes of simulated 'pre-hospital' intervention (ending at t63 – the time of simulated 'hospital arrival').

In order to achieve a NOST, a controlled arterial bleed was added to the protocol. This was started five minutes after the hepatic injury at a rate of 3.0

ml/kg/minute, drawn from the left femoral artery 8.5Fr catheter via a peristaltic pump. In D6-7207 and D7-7193, the volume of controlled arterial bleed was 707 ml and 1,078 ml, resulting in a BP at TCA (t0) of 4/2 mmHg and 3/1 mmHg respectively. Furthermore, this resulted in a relative bradycardia in both D6 and D7 (compared to baseline measurement), indicating a failing myocardium and a NOST. However, owing to collapse of the 8.5 Fr catheter secondary to negative pressure, it was not possible to haemorrhage at the desired rate, and the protocol was edited to draw the blood from the larger right femoral artery vascular sheath. In D8-7201, despite a BP of 0.8/0.0 mmHg at t0, the BP increased spontaneously over the next three minutes (before SAAP intervention) to 14/5 mmHg. A further controlled haemorrhage was therefore added, at a rate of 1.0 ml/kg/minute, between t0 and t2 to ensure the continuation of NOST (the one minute between t2 and t3 was required to unblind the operator (EB) and allow time to setup for the allocated intervention). In this state of haemorrhage there was again difficulty in drawing blood from the peripheral right femoral artery vascular sheath, and for subsequent development protocols the controlled arterial bleed was undertaken via a SAAP catheter sequentially advanced proximally though the vascular sheath to allow the proscribed rate of blood to be drawn. In the subsequent five development animals (D9-7231, D10-7232, D11-7237, D12-7233, D13-7290) the mean systolic BP was 6.4 (±7.2) mmHg at t0, and 0.0 (±0.0) at t3; all animals had a relative bradycardia (compared to baseline values).

The resulting injury model was therefore a laparoscopic hepatic injury (transection of 70% of the left lateral lobe of the liver, in under two minutes),

followed five minutes later by a controlled haemorrhage via a SAAP catheter (advanced via the right femoral artery vascular sheath) of 3 ml/kg/minute until cardiac arrest (defined as a systolic BP less than 10 mmHg = t0). This was followed by two minutes of controlled haemorrhge (via a SAAP catheter) at a rate of 1.0 ml/kg/minute if the systolic BP was greater than 0 mmHg. The operator was unblinded at t2, and the allocated intervention started at t3. Further development animals confirmed that this severe model of haemorrhage was survivable with SAAP intervention.

5.6 Equipment

5.6.1 SAAP infusion circuit

The SAAP circuit consisted of an oxygenator (Quadrox, Maquet IND, Wayne, NJ, USA), connected to a tubing circuit (3/8" ID ECMO circuit, Maquet IND, Wayne, NJ, USA) to allow inflow connection to a 3.0 litre reservoir (Belmont, Billerica, MA, USA), outflow connection to a SAAP catheter, attachment of a Masterflex peristaltic pump (Cole-Parmer, Vernon Hills, IL, USA) to drive the infusion, and a three-way tap to deliver a bolus of 50 ml lactated Ringer's solution into the SAAP catheter. Previous published SAAP experimentation has effectively resuscitated swine from haemorrhagic TCA using an infusion rate of 10 ml/kg/minute.[112] The expected mean animal weight for this research was 80 kg (range of 70-90 kg), and the circuit therefore needed to be capable of delivering at least 800 ml/minute of fluid.

Adaptions were made to this circuit: in D1-7013, D2-7140, D3-7107, and D5-7208, 10% calcium chloride was administered during SAAP via the right carotid

artery 8.5 Fr catheter (the tip of which was demonstrated under fluoroscopy to be at the junction of the common carotid artery and the aortic arch). The aortic BP was measured by a micromanometer-tipped catheter, that was placed through the same 8.5 Fr catheter. During calcium infusion, the BP readings were erratic, and therefore the calcium infusion line was attached to the SAAP circuit for further experiments, Figure 5.3.

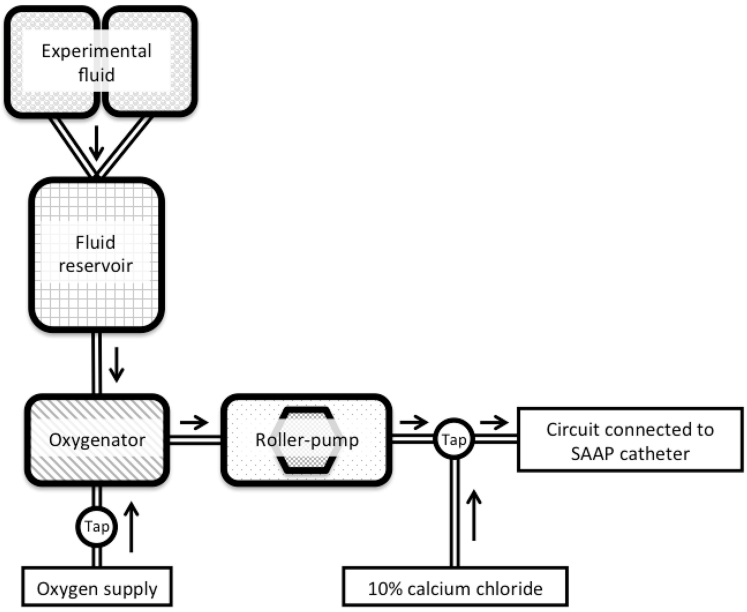

Figure 5.3 – Schematic of the experimental SAAP circuit.

In accordance with the manufacturer's instructions, the oxygenator was supplied with a gas flow of 100% oxygen at 1.0 l/minute. Prior to SAAP infusion, the experimental fluid was circulated through the oxygenator and back into the fluid reservoir for at least ten minutes. Pre-infusion blood gas analysis was undertaken in ten development animals (D8-7201, D10-7232, D11-7237, D12-7233, D16-7316, D17-7317, D18-7330, D19-7333, D20-7361, D21-7359). The

mean partial pressure of oxygen was 79.0 (±7.9) kPa, and the mean partial pressure of carbon dioxide was 2.3 (±0.6) kPa.

The experimental theory of SAAP is that it provides high coronary perfusion pressure and flow. If infused towards an open aortic valve (for example when there is no cardiac activity, or during left ventricular ejection) there is a risk of the SAAP infusion filling the left ventricle in preference to the coronary vessels. Prior unpublished observation by Dr. Manning suggested that a rapid 50 ml intra-aortic flush would be sufficient to close the aortic valve immediately prior to SAAP infusion. This was confirmed under fluoroscopy in D7-7193 and D8-7201, Figure 5.4.

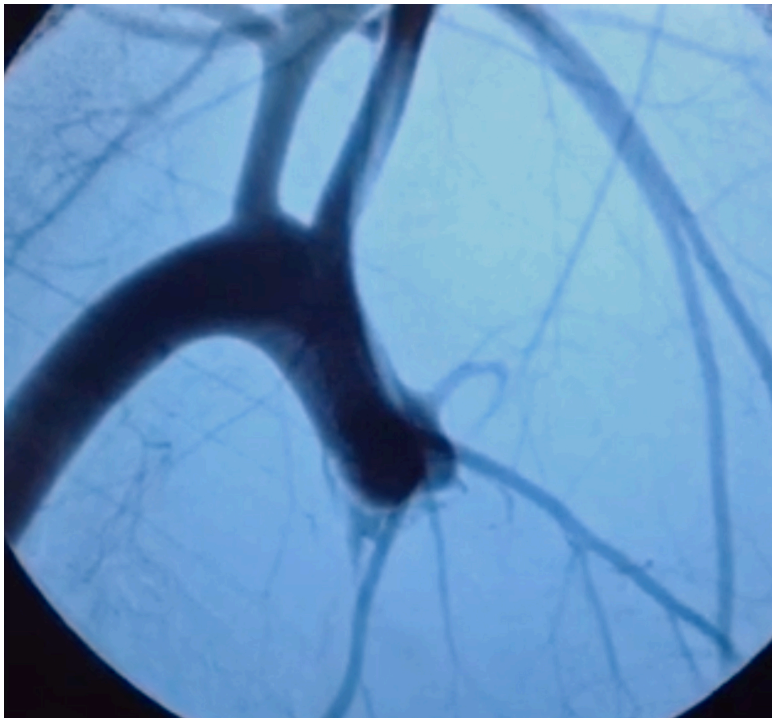

Figure 5.4 – Fluoroscopic image of a swine aortic arch after a 50ml intra-aortic flush of 1:1 lactated Ringer's solution and contrast, demonstrating a closed aortic valve.

5.6.2 SAAP catheter

The SAAP catheter (Vention Medical Inc, Boulder, CO, USA) is an experimental catheter that is 800 mm long with an 11.5 Fr outer diameter. It has a 7.5 Fr central lumen, and a 30 mm (17 ml) balloon near the tip that is inflated via a separate lumen, Figure 5.5.

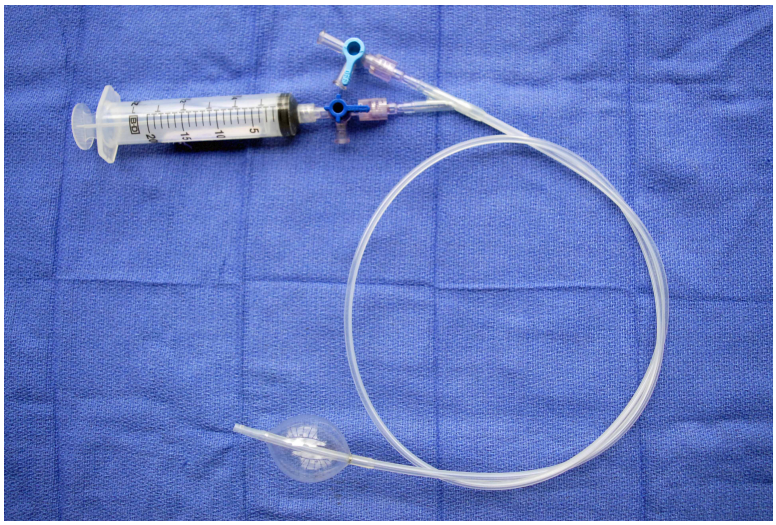

Figure 5.5 – SAAP catheter; the 20 ml syringe is connected the balloon port.

In order to ensure occlusion of Z1 of the aorta, and effectively deliver the SAAP infusion to the aortic arch, the catheter must be placed above the diaphragm. In the first ten SAAP development animals (D1-7013, D2-7140, D3-7107, D5-7208, D6-7207, D7-7193, D8-7201, D9-7231, D10-7232, D11-7237) the catheter was placed under fluoroscopy. After guided placement, the catheter was marked at the point it emerged from the vascular sheath. At the end of each protocol the catheter was measured externally on each swine (in a straight line from the end of the vascular sheath to the sternum), which revealed that correct anatomical

placement could be achieved by inserting the catheter to a depth of 50 mm superior to the inferior edge of the xiphisternum, Figure 5.6.

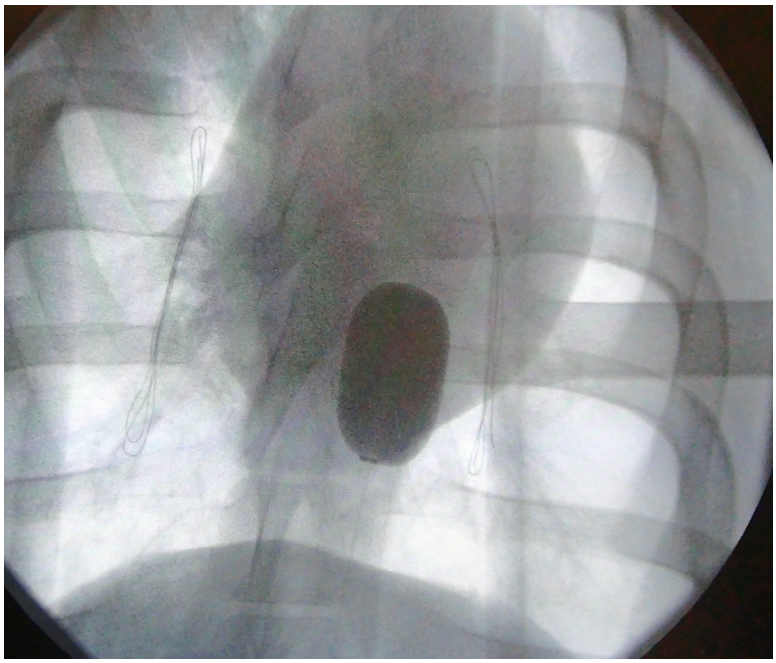

Figure 5.6 – SAAP catheter and balloon inflation in aortic Z1 under fluoroscopy; the balloon was inflated with 17ml of 1:1 lactated Ringer's solution and contrast.

The mean weight and length of these animals was not significantly different to the remainder of the development animals, 77.4 (±5.0) kg, 1579.9 (±47.6) mm, and 76.2 (±5.3) kg, 1619.3 (±63.3) mm, unpaired t-test p=0.58 and p=0.12 respectively. Therefore, in order to save time in further experimental protocols, and mitigate against fluoroscopy failure, the SAAP catheter insertion depth was pre-measured. However, to confirm correct balloon inflation and placement, fluoroscopy was routinely used in all experiments.

The SAAP catheters had been previously used in 25-39 kg swine at an infusion rate of 10 ml/kg/minute – less than 50% of the required infusion volume per

minute for swine in this protocol.[112] There was concern that delivering 800 ml/minute of FWB via the SAAP may cause haemolysis of red blood cells.[123] An *in vitro* study was undertaken to quantify haemolysis at a range of FWB infusions through a SAAP catheter between 200 and 1400 ml/minute; a serum haemoglobin greater than 30 μmol/l was used as the definition of haemolysis.[123] Repeated measurements revealed no significant haemolysis at any infusion rate up to 1400 ml/minute.

5.6.3 SAAP infusion

Prior experimentation with SAAP used an infusion rate of 10 ml/kg/minute for up to two minutes. These experiments used either lactated Ringer's solution or a haemoglobin substitute as the resuscitation fluid.[112] In order to reduce the total FWB requirement from donors, and considering that FWB was likely to be a more effective resuscitation fluid than either lactated Ringer's or haemoglobin substitute, the original IRB approved protocol allowed for one minute of SAAP infusion (800 ml).

Following initial development of animal preparation and the haemorrhage model, five animals (D13-7290, D14-7292, D15-7287, D16-7316, D17-7317) underwent SAAP intervention with 800 ml of FWB over one minute. One animal (D14-7292) had ROSC and survived the 60 minute pre-hospital period (with four further 250 ml boluses of intra-aortic FWB). The other four swine all had a brief period of ROSC within the last ten seconds of the SAAP infusion, and then developed VF that was resistant to further boluses of intra-aortic FWB and DC cardioversion via an external defibrillator. Blood samples taken from the aortic

arch at t4 (the end of the 800 ml SAAP infusion) revealed serum electrolytes (sodium, potassium, and calcium) all to be within the normal range.

The likely cause for the VF was postulated to be secondary to myocardial ischaemia owing to insufficient resuscitation, and therefore the SAAP infusion volume was doubled to 1,600 ml over two minutes, with IRB approval. A further five animals (D18-7330, D19-7333, D20-7361, D21-7359, D22-7360) underwent SAAP intervention with 1,600 ml of FWB over two minutes. One animal (D21-7359) went into VF during the SAAP infusion, but the remaining four all had a sustained ROSC. Furthermore, D19-7333 was in cardiac electrical asystole during the TCA (t0 – t3) and was successfully resuscitated with SAAP. Therefore, the protocol was modified to include 1,600 ml of SAAP infusion over two minutes.

5.6.4 SAAP checklist

In order to ensure compliance with the experimental protocol and reduce the wastage of animals, a challenge-response checklist was developed to be used at key points of the protocol, Appendix A.

5.6.5 Allocation concealment

During development, methods to blind the operator to the experimental allocation were tested. This required a randomised schedule to be produced and communicated to other relevant animal and bench laboratory staff to ensure coordination, and a tent to conceal the preparation of resuscitation fluid.

5.6.6 Closed chest compression table

In order to deliver consistent chest compressions for 60 minutes in large swine, a mechanical compression device was used. Previous swine studies have demonstrated that mechanical compressions outperform manual compressions by effecting a higher CPP.[124] Clinical mechanical compression devices have been developed in recognition that providing consistent, high-quality manual compressions is challenging. Although first described in 1961,[125] they have only been widely adopted in the last ten years.[126] The swine thorax is a different shape to that of the human, and furthermore, unlike a human, swine are not physically stable when placed supine. There was therefore a need to design a table on which to deliver compressions – this required an attachment for the compression device and a lateral support to keep the swine in the supine position, while complying with laboratory infection prevention control measures.

The Model 1008 Life-Stat chest compression device (Michigan Instruments, Grand Rapids, MI, USA) was selected – the Life-Stat is oxygen driven (mitigating failure secondary to loss of electrical power), has a removable base plate (allowing attachment to a bespoke table), has a single side arm (facilitating better access to the swine), and has an adjustable, calibrated compression depth gauge (allowing consistent depth control). A bespoke 'CPR table' was constructed, Figure 5.7.

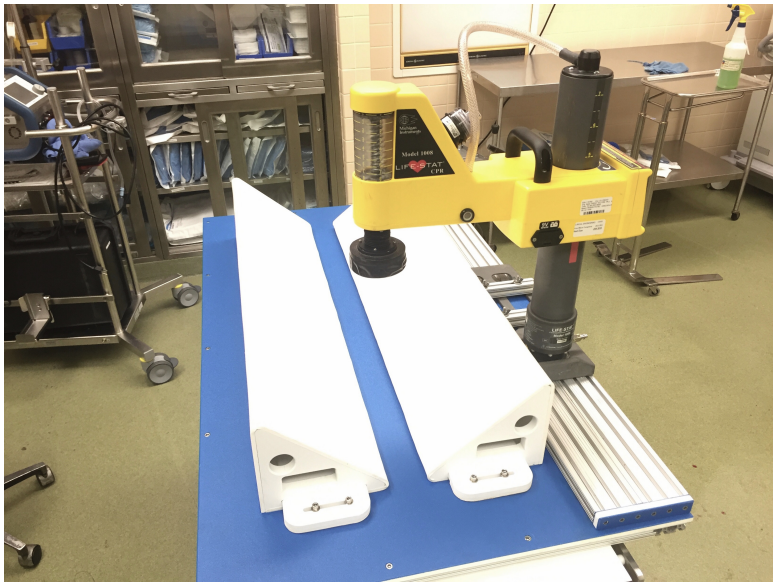

Figure 5.7 – 'CPR table' with Life-Stat mechanical chest compression device attached.

Closed chest compressions were tested in three animals (D4-7131, D19-7333, D20-7361). Chest compressions were started in D4-7131 after NCTH effected a low-output state (BP – 27/20 mmHg, and a carotid artery flow of 30 ml/minute). Simultaneous to the start of chest compressions, 2,452 ml of FWB was administered via the left external jugular vein at a rate of 500 ml/minute (together with a matched calcium infusion).

At the start of the intervention a rapid rise in BP to 58/31 mmHg was observed, followed by a continued rise in systolic BP, and a fall in diastolic BP to 10 mmHg within ten minutes of the start of compressions. At t19 the depth of compression was increased from 4 cm to 5 cm – this increased the systolic BP to 90 mmHg, Figure 5.8.

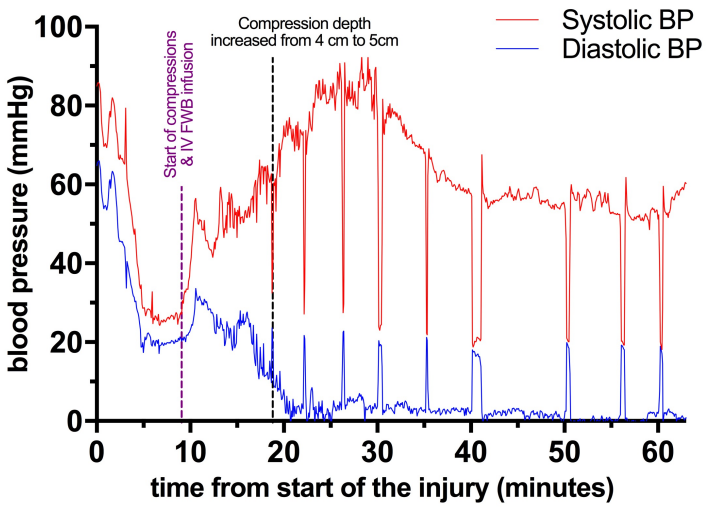

Figure 5.8 – Evaluation of closed chest compressions in D4-7131.

Carotid flow increased to 150 ml/minute at the start of the intervention, and increased to 200 ml/minute when the compression depth was increased to 5 cm. Further brief increases in compression depth greater than 5 cm did not result in a higher systolic BP or carotid flow. The protocol was terminated at t63.

Continuous mechanical ventilation was continued throughout the compressions, without significant impact on the minute volume. Compressions were paused every five to ten minutes in order to assess the underlying cardiac electrical rhythm, Figure 5.8.

Further evaluation of the CPR table was completed on D19-7333 and D20-7361. In D19-7333 compressions were started 22 minutes after a SAAP infusion with FWB had resulted in a BP of 0/0 mmHg and cardiac electrical asystole – the blood pressure increased to 35/10 mmHg with a compression depth of 4 cm. Further 0.5 cm increments in compression depth produced a systolic BP of 52

mmHg and 75 mmHg at 5.0 and 5.5 cm respectively – at 6.0cm the systolic BP dropped to 68 mmHg. In D20-7361 compressions were started 120 minutes after a SAAP with FWB protocol (including surgical control) had resulted in a BP of 30/20 mmHg. Systolic BP increased to a maximum of 110 mmHg with a compression depth of 5.0 cm – higher than a depth of both 4.5 and 5.5cm.

In summary, the CPR table and Life-Stat mechanical compression device performed well. The table (without the Life-Stat fitted) provided a stable platform, and allowed the Life-Stat to be attached in less than 30 seconds – it could therefore be used for all experimental protocols (allowing for allocation concealment during animal preparation in experimental protocols). The Life-Stat was compatible with continuous mechanical ventilation, and produced the highest systolic BP when the depth of compressions was set to between 5.0 and 5.5 cm. It was not possible to read the underlying cardiac electrical activity during compressions. Clinical advanced life support algorithms include a pause in compressions every two minutes to check the underlying rhythm.[41] Therefore, in order to be able to report the underlying cardiac electrical rhythm, and in keeping with clinical practice, compressions were paused every two minutes (for less than ten seconds).

5.7 Summary of model development

Model development demonstrated the feasibility of a swine donor pool, identified the correct amount of calcium in SAAP infusions, adapted the original protocol to cease inhalational anaesthetic during TCA, and informed the correct placement of laparosopic ports to undertake a consistent NCTH injury. It also

allowed a haemorrhage model to be developed, which combined the NCTH with a controlled arterial bleed that continued after the onset of TCA, and resulted in a very severe model of haemorrhagic arrest. The duration and total volume of SAAP infusion was increased from 800 ml over one minute to 1600 ml over two minutes, and appeared to reduce the incidence of VF. A method of allocation concealment was also developed in order to effectively randomise the interventions and blind the operator (a method not previously used in large animal NCTH experiments). Finally, a method to undertake closed chest compressions in large swine was established.

CHAPTER SIX

METHODS – PART ONE

6.1 Part one hypotheses

In a swine model of severe haemorrhagic TCA:

H1. Closed chest compressions with high-volume intravenous FWB will not result in a return of spontaneous circulation, and no animals will survive 60 minutes.

H2. Zone 1 IABO with intravenous FWB will infer a 60 minute survival advantage over closed chest compressions with intravenous FWB.

H3. SAAP with intra-aortic oxygenated FWB will infer a 60-minute survival advantage over SAAP with intra-aortic oxygenated lactated Ringer's solution, REBOA with intravenous FWB, and closed chest compressions with intravenous FWB.

6.2 Animal use ethical review

The experimental protocol was approved by the 59th Medical Wing Institutional Animal Care and Use Committee (IACUC) (protocol number – FWH20140020A). Experiments were performed at the Clinical Research Division, 59th Medical Wing, United States Air Force, Office of the Chief Scientist, in a facility accredited by the American Association for the Accreditation of Laboratory Animal Care. The protocol was conducted in accordance with guidelines established by the

Public Health Service Policy on Humane Care and Use of Laboratory Animals and Office of Laboratory Animal Welfare.

6.3 Donor blood product

6.3.1 Blood donation pool

Whole blood was collected from a dedicated pool of swine the day before each experimental protocol. Previously published data of resuscitation fluid volume requirements in a less severe swine haemorrhage model (but with a similar hepatic injury and animal weight) consisting of 60 minutes of pre-hospital intervention followed by a four hour surgery and critical care phase, indicated that approximately 6,000 ml would be required.[101] This equates to ten units of FWB in volume alone. IRB approval and the capacity of the blood donor pool limited the maximum FWB per protocol to 15 units.

6.3.2 Blood donation procedure

Yorkshire Landrace cross swine (*sus scrofa*, 70-90 kg) were obtained from a single source animal vendor (John Albert, Cibolo, USA, 74-A-1246). Anaesthesia was induced with intramuscular ketamine (10 mg/kg + 100 mg), and maintained on inhaled isoflurane (Abbott Laboratories, Abbott Park, IL, USA) via a snout mask at a MAC of between 1.0 and 1.5. Once anaesthetised, an 8.5 Fr catheter (Teleflex, Morrisville, NC, USA) was placed percutaneously in either the left or right external jugular vein, and blood was drawn as per Section 5.4.

On the morning of experimental protocols, paired bags of red cells and plasma were warmed to 38°C in a water bath and transferred to the animal laboratory in

a thermal isolation box. Pre-infusion samples of reconstituted FWB were undertaken to check haemoglobin, potassium and lactate concentrations, and oxygenation to confirm uniformity.

The intention was to only use FWB that was less than 24 hours since donation. However, in order to provide some flexibility and to reduce wastage, any unused product that had not been removed from cold storage was allocated to the following day's experiment. Therefore, FWB would be a maximum of 48 hours old, with no product over 24 hours old being used for the initial experimental infusions.

6.4 Animal preparation

Yorkshire Landrace cross swine (*sus scrofa*, 70-90 kg) were obtained from a single source animal vendor (John Albert, Cibolo, USA, 74-A-1246). To control for sex-linked physiological difference all animals were male.[127] Swine were quarantined for 72 hours, and fasted for 18 hours before protocols, with free access to water; all protocols began at 0630 hours.

6.4.1 Animal anaesthesia

Intramuscular ketamine (100 mg + 10 mg/kg), and 2 mg intramuscular buprenorphine were administered for initial sedation and analgesia. Animals were intubated via direct laryngoscopy with a cuffed endotracheal tube. Anaesthesia was maintained on inhaled isoflurane (Abbott, Chicago, IL, USA) at a MAC of between 1.1 and 1.3. At the point of TCA (t0) the isoflurane was vapouriser was switched off, and only re-started if there was a ROSC (defined as

a systolic BP greater or equal to 50 mmHg), or the animal showed signs of awareness. Mechanical ventilation tidal volumes were fixed at 7 ml/kg, and minute volume was manipulated by adjustments to ventilation rate to achieve an end-tidal carbon dioxide in the range 5.0 to 5.6 kPa. These ventilator settings were fixed at the time of baseline measurements for the rest of the protocol.

After induction of anaesthesia the fraction of inspired oxygen was maintained at 0.35, until the start of the injury where it was reduced to 0.21 (equivalent to atmospheric air). At the start of the treatment intervention the inspired oxygen fraction was increased to 1.0 in order to emulate clinical management. During all protocols, the depth of anaesthesia was continuously monitored using multiple parameters, including MAC of anaesthetic gas, heart rate, jaw tone, muscle tone, palpebral reflexes, and graded pain response).

6.4.2 Physiological monitoring and vascular line placement

When anesthetised, electrocardiography, physiological monitoring, blood sampling, and vascular access lines were inserted percutaneously under ultrasound guidance, as per Section 5.5.3.

6.4.3 Surgical preparation

As per Section 5.5.4.

6.4.4 NCTH injury and controlled arterial haemorrhage

At the end of the ten minute stabilisation period the abdomen was insufflated via a laparoscopic port to a pressure of 12 mmHg. The left lateral lobe of the liver

was visualised, and approximately 70% excised with 5 mm Metzenbaum endoshears in under two minutes. At the completion of the hepatic injury, the abdomen was rapidly de-sufflated, instruments and ports removed, and the laparoscopic holes approximated with surgical skin staples. Five minutes after the start of the hepatic injury, a controlled haemorrhage was initiated at 3.0 ml/kg/minute via a 7.5 Fr intra-aortic catheter placed via the right femoral artery vascular sheath. At the time of TCA (defined as a systolic BP of less than 10 mmHg, t0), the controlled haemorrhage was continued at a reduced rate of 1.0 ml/kg/minute, for up to two minutes (t0-t2), if the intra-aortic BP was greater than 0 mmHg, Figure 6.1.

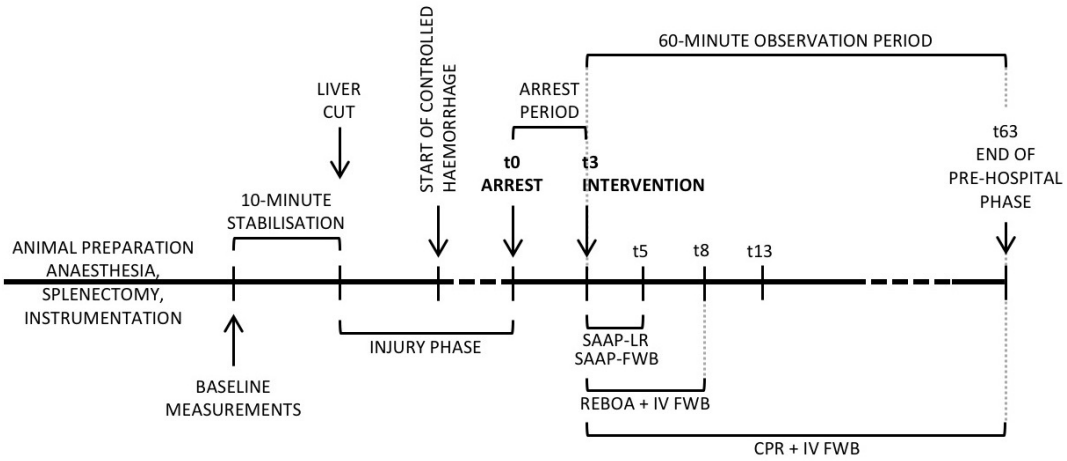

Figure 6.1 – Part One protocol timeline.

6.5 Pre-hospital experimental interventions

Two minutes after the onset of TCA (t2), forty swine were allocated to one of four groups, Table 6.1.

Table 6.1 – Part One experimental groups

Group	Experimental arm	Abbreviation	N
1	Closed chest compressions with IV FWB	CPR	10
2	SAAP with lactated Ringer's solution	SAAP-LR	10
3	SAAP with FWB	SAAP-FWB	10
4	REBOA with IV FWB	REBOA	10

IV – intravenous (external jugular vein), FWB – fresh whole blood, SAAP – selective aortic arch perfusion, REBOA – resuscitative endovascular balloon occlusion of the aorta

6.5.1 Group 1 – Closed chest compressions with IV FWB

At t2, an oxygen-driven Life-Stat mechanical compression device was attached to the experimental operating table, Figure 5.7. The position of the device was adjusted in order that the inferior border of the compression cup sat two fingers breadth (approximately 40 mm) superior to the inferior edge of the swine's xiphisternum. Simultaneously, an infusion line, from a Belmont Rapid Infuser and a syringe driver containing 10% calcium chloride, was connected to the left external jugular vein 8.5 Fr catheter.

At t3, continuous external chest compressions were commenced at a rate of 100 per minute, at a compression decompression ratio of 1:1, and compression depth of 5.0 to 5.5 cm. At t3, four units of FWB (2,452 ml) were infused via the left external jugular vein 8.5 Fr catheter at a rate of 500 ml / minute (over 4.9 minutes). An infusion of 10% calcium chloride (connected to the infusion line via a three-way tap) was administered at a rate of 6.9 ml/minute to prevent hypocalcaemia secondary to citrate chelation, for the duration of the FWB infusion.

Animals were observed between t8 and t63 with no further intervention. From t13 to t63 compressions were paused every two minutes, for less than ten seconds, to confirm the underlying cardiac electrical rhythm and intrinsic systolic BP. Compressions were not re-started if the systolic BP was 50 mmHg or greater, but were re-started if the systolic BP was subsequently less than 50 mmHg.

6.5.2 Group 2 – SAAP-LR

At t2, a SAAP catheter with a 7.5 Fr central lumen, and a 30 mm (17 ml) balloon, was advanced in aortic Z1, and its position confirmed with fluoroscopy. The SAAP catheter central lumen was flushed with LR, and closed distally with a three-way tap.

At t2.75 (two minutes and 45 seconds), the SAAP catheter balloon was inflated, under fluoroscopy, with 17 ml of 1:1 LR and contrast, Figure 5.6.

At t3, 1,600 ml of oxygenated LR was infused into the aortic arch, superior to the balloon, over two minutes, Figure 5.3. Animals with a systolic BP of less than 90 mmHg at t5 received up to seven boluses of 250 ml of oxygenated intra-aortic LR, at a rate of 500 ml/minute, to maintain systolic BP at or above 90 mmHg, until t63, or death. The maximum infusion of LR between t3 and t63 was therefore 3,350 ml.

6.5.3 Group 3 – SAAP-FWB

At t2, a SAAP catheter with a 7.5 Fr central lumen, and a 30 mm (17 ml) balloon, was advanced in aortic Z1, and its position confirmed with fluoroscopy. The SAAP catheter central lumen was flushed with LR, and closed distally with a three-way tap.

At t2.75 (two minutes and 45 seconds), the SAAP catheter balloon was inflated, under fluoroscopy, with 17ml of 1:1 LR and contrast.

At t3, 1,600 ml of oxygenated FWB was infused into the aortic arch, superior to the balloon, over two minutes, together with a two minute infusion of 10% calcium chloride at a rate of 11.1 ml/minute. Animals with a systolic BP of less than 90 mmHg at t5 received up to seven boluses of 250 ml of oxygenated FWB, at a rate of 500 ml/minute, with a simultaneous infusion of 10% calcium chloride at a rate of 6.9 ml/minute to maintain systolic BP at or above 90 mmHg until t63, or death. The maximum infusion of FWB between t3 and t63 was therefore 3,350 ml (5.5 units of FWB).

6.5.4 Group 4 – REBOA

At t2, a SAAP catheter with a 7.5 Fr central lumen, and a 30 mm (17 ml) balloon, was advanced in aortic Z1, and its position confirmed with fluoroscopy. The SAAP catheter central lumen was flushed with LR, and closed distally with a three-way tap.

At t2.75 (two minutes and 45 seconds), the SAAP catheter balloon was inflated, under fluoroscopy, with 17ml of 1:1 LR and contrast.

At t3, four units of FWB (2,452 ml) were infused via the left external jugular vein 8.5 Fr catheter at a rate of 500 ml / minute (over 4.9 minutes). An infusion of 10% calcium chloride (connected to the infusion line via a three-way tap) was administered at a rate of 6.9 ml/minute, for the duration of the FWB infusion.

Animals were observed between t8 and t63, unless they met the definition of death-in-protocol from t13 onwards.

6.6 In-hospital experimental observation

At t63 (the end of the simulated 'pre-hospital' period), animals who had not met death-in-protocol criteria were transitioned into a 30 minute surgery period, followed by a 180 minute critical care period.

6.6.1 Surgery period

At t63, compressions were stopped in the CPR group, and in groups with intra-aortic balloon occlusion (REBOA and SAAP) the balloon was kept inflated. In all groups, fluid resuscitation from t63 onwards was with 250 ml boluses of FWB, together with 6.9 ml of 10% calcium chloride, administered over thirty seconds via the left external jugular vein catheter by a Belmont Rapid Infuser if systolic BP was less than 90 mmHg. Owing to IRB and logistical constraints, a total volume of 5,750 ml of FWB was allocated per animal for this period (equal to 23 250 ml boluses).

In order to mitigate the physiological consequences of opening the peritoneum in NCTH,[128] two 250 ml boluses of FWB were administered prior to surgical intervention.

At t65, in all groups, the abdomen was accessed via a midline laparotomy, and the hepatic injury rapidly controlled by the application of a Doyen's clamp placed across the hilum of the left lateral lobe of the liver, with or without a Satinsky's clamp to control the posterior margin, Figure 6.2.

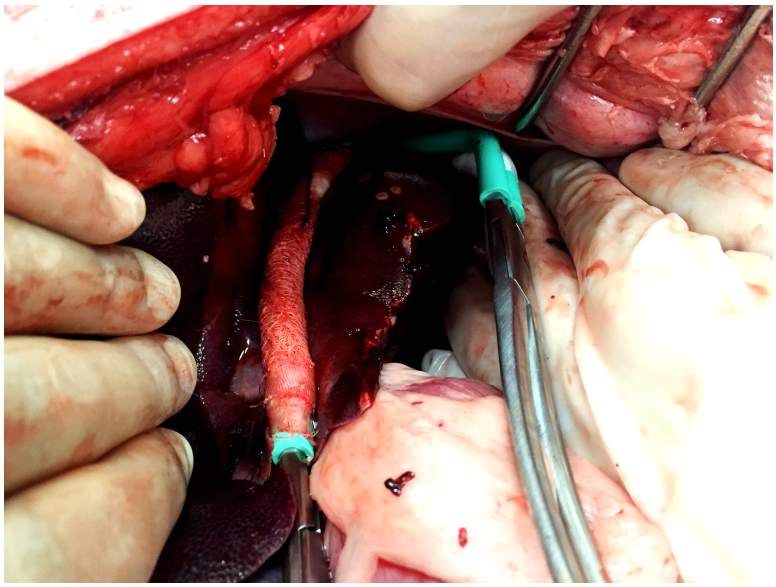

Figure 6.2 – Rapid control of the hepatic injury was achieved with the application of surgical clamps.

Once haemorrhage control was achieved, free blood and clot was removed from the abdomen and weighed for quantification of shed blood volume. The portion of excised liver was also recovered and weighed (in order to later quantify the

hepatic injury during post-experimental procedures – Section 6.7). In all animals with intra-aortic balloon occlusion, the balloon was deflated at a rate of between 0.5 to 2.0 ml/minute, starting at t78 (producing a standardised complete occlusion time of 75 minutes), and completed by t93.

6.6.2 Critical care period

At t93, all animals with a systolic BP greater or equal to 30 mmHg were observed, without further surgical or endovascular intervention, for 180 minutes. Any remaining FWB (5,750 ml, minus the volume infused during the surgery period) was administered if the systolic BP was less than 90 mmHg, using the same methodology as during the surgery period. Once the total available volume of FWB was transfused (5,750 ml), no further fluid was given, and no vasopressors or inotropes were used.

The protocol was terminated at t273 (four and a half hours after the start of the intervention, t3), or sooner if animals met death-in-protocol criteria (Section 6.8.2).

6.7 Post-experimental procedures

At the end of the protocol (t273, or sooner if animals met death-in-protocol criteria), swine were euthanased by a trained laboratory technician with 100 mg/kg of intravenous sodium pentobarbital, in accordance with the American Veterinary Association euthanasia guideline.

Following animal euthanasia, the liver was removed in order to quantify the laparoscopic hepatic injury, as a percentage by weight of the left lateral lobe excised during the injury. In order to mitigate against changes in volume of shed blood secondary to evaporation, the weight of controlled arterial haemorrhage and the blood removed from the abdomen were measured within ten minutes of collection. All suction containers and gauze were pre-weighed, and resulting weights of blood divided by 1.05 to quantify the volume of blood they contained.

6.8 Experimental schedule and definitions

6.8.1 Randomisation schedule

In order to blind the operator during animal preparation and injury, a randomisation schedule was created by one of the laboratory staff (Ms. Jennifer Cox) using a web-based programme (www.randomizer.org). The operator and other key personnel were blinded to the allocation, by transporting resuscitation fluid in a box and with the use of a fabric tent placed over the infusion setup, until one minute before the intervention. The first three experimental groups (closed chest compressions, SAAP-LR, and SAAP-FWB) were included in this schedule. The REBOA group required additional IRB approval, and was undertaken in a subsequent unblinded schedule, which commenced eight days after the completion of the initial experimental groups.

6.8.2 Experimental definitions

TCA (t0) was defined as a systolic BP less than 10 mmHg, together with a cardiac electrical rate lower than the pre-injury baseline. ROSC was defined as the time that the intrinsic systolic BP increased to 50 mmHg or higher following arrest

(with the assumption that this is the threshold at which a central pulse may be palpable). In reference to the secondary outcome, this was observed ten minutes after the start of the intervention in all groups (t13).

In the SAAP and REBOA groups, from t13 onwards (ten minutes after the start of the intervention) death-in-protocol was defined as a systolic BP less than 30 mmHg, despite animals receiving the maximum per protocol permitted resuscitation fluid volume. This cut-off was selected by consensus as non-survivable (without further resuscitation fluid available), and has been used in previous swine translational research.[96] Animals meeting this definition were euthanased, and post-experimental procedures undertaken.

In the CPR group, compressions were continued for the duration of the pre-hospital period (t3-t63), unless ROSC was observed during a pause in compressions. If the systolic BP fell below 50 mmHg following ROSC, compressions were re-started. For the purpose of physiological comparison between groups, observations where possible were made during a pause in compressions (for example at t13 and t63).

6.9 Physiological data acquisition

6.9.1 Data acquisition from the anaesthetic machine

The following variables were automatically recorded at one minute intervals throughout the protocol: cardiac electrical rate, rectal temperature, peripheral oxygen saturation, fraction of inspired oxygen, end-tidal carbon dioxide, MAC of isoflurane, and values from the Swan-Ganz pulmonary artery catheter

(pulmonary artery pressure, central venous pressure, cardiac output, stroke volume, and central venous saturations).

6.9.2 Data acquisition of near-infrared reflectance spectroscopy

Tissue oxygenation data were automatically recorded 11.55 times per minute (as dictated by the manufacturers software) throughout the protocol.

6.9.3 Data acquisition on DAQ board

In order to accurately measure intra-aortic BP, the output from the micromanometer-tipped catheter was recorded at 500 Hz on a DAQ board (DAQpad-6105, National Instruments, Austin, TX, USA). This produced an accurately calibrated BP and cardiac electrical rate. The output from the carotid artery flow probe, and pulmonary artery pressure from the Swan-Ganz catheter were also recorded on the DAQ board at 500 Hz. The data from the DAQ were automatically converted into mean peak values every five seconds.

6.9.4 Manual data recording and time keeping

Manual data sheets were used to record the weight and length of each animal, key timings throughout the protocol, the weights of the spleen, parts of the liver, controlled arterial bleed, NTCH haemorrhage, pertinent observations, protocol deviations, and key physiological variables regularly throughout each protocol (in case of a failure in automated data recording), Appendix B.

Before the start of each experimental protocol the time on all devices (wall clock, anaesthetic machine, and all data recorders) was synchronised to ensure

accuracy. Key time points, including time of arrest (t0), time of the start of the intervention (t3), and the time of the administration of any resuscitation fluid were data logged in an electronic lab book. At t0, the operator (EB) started two identical stopwatches in order that interventions were timed, and double-checked, per protocol.

6.10 Blood sample data acquisition

Blood samples were obtained throughout the experimental protocol, and were analysed with a blood gas analyser (ABL800 FLEX, Radiometer, CA, USA), lab full blood count, clotting (ADVIA 2120i, Siemens Medical Solutions USA Inc., Malvern, PA, USA), lab chemistry (Stanbio Sirrus, Fisher Scientific Co., Pittsburgh, PA, USA) and thromboelastography (TEG 5000, Haemonetics Corp. Braintree, MA, USA).

6.10.1 Arterial blood gas

Immediately following the insertion of the left brachial artery 5 Fr catheter, an arterial blood gas (ABG) sample was obtained, in order to calibrate the Swan-Ganz catheter. Following this, ABG samples were drawn from the brachial arterial line before surgical preparation, at baseline (at the start of the ten-minute stabilisation period), and every ten minutes after the start of the intervention (t13, t23, t33, t43, t53, t63) during the pre-hospital period. An ABG was drawn from the SAAP catheter (in all animals) at the point of arrest (t0) as the severe state of haemorrhage prevented blood being drawn more peripherally from the brachial arterial line. An additional ABG was drawn from the right carotid artery 8.5 Fr catheter at t4 in all SAAP groups.

In the surgery period, an ABG was drawn, via the left brachial arterial line, in all animals every ten minutes (t73, t83, t93). In the critical care period, an ABG was drawn every 30 minutes until protocol completion (t273). The ABG sample included pH, partial pressures of oxygen and carbon dioxide, ionised calcium, potassium, sodium, glucose, lactate, and base deficit.

6.10.2 Full blood count and clotting profile

Blood samples, from the brachial arterial line, were collected into one EDTA specimen tube, and one citrated specimen tube at each time point (before surgical preparation, baseline, t13, t33, t63, t93, t153, t213, and t273). Samples were additionally collected at the point of arrest (t0) via the SAAP catheter.

The EDTA sample was analysed as a full blood count (including haemoglobin, white cell count, and platelet count). The citrated sample was analysed for clotting function (prothrombin time and fibrinogen).

6.10.3 Thromboelastography

At the same time points, and via the same catheters, one citrated specimen tube was drawn to undertake thromboelastography (TEG). The output from TEG included R (the time to initial fibrin formation), K (the time to reach a specific level of clot strength), α (the rate of clot formation), MA (maximum clot amplitude), G (clot elasticity), CI (clotting index), and LY30 (the rate of amplitude reduction from MA to 30 minutes, a measure of fibrinolysis).

6.11 Outcomes

6.11.1 Primary outcome

In order to compare the simulated 'pre-hospital' survival following each experimental intervention, the primary outcome was 60 minute survival post intervention (t63). Survival was defined as an intrinsic systolic BP of 30 mmHg or greater (Section 6.8.2).

6.11.2 Secondary outcomes

In order to compare the physiological effects of each experimental intervention, the first set of secondary outcomes were measured, in all groups, ten minutes after the start of the intervention (t13). In the CPR and REBOA groups, t13 was five minutes after the completion of the intravenous FWB administration, and in the SAAP groups t13 was eight minutes after the completion of the SAAP infusion, Figure 6.1. These time intervals allowed the effect of each intervention to be observed, and to confirm that a ROSC was sustained for more than a few minutes. A t13, the groups were compared with respect to: the proportion of animals with ROSC, systolic BP, carotid artery flow, end-tidal carbon dioxide, and tissue oxygenation. At the same time point, comparison was made of blood samples (pH, the partial pressure of oxygen and carbon dioxide, lactate, base deficit, and ionised calcium).

The second set of secondary outcomes were compared at the end of the pre-hospital period (at t63). This outcome included animals that had not already met death-in-protocol criteria, in order that a comparison could be made between live animals. At t63, live animals were compared with respect to: systolic BP,

carotid artery flow, end-tidal carbon dioxide, and tissue oxygenation. At the same time point, comparison was made of blood sample analyses (haemoglobin, lactate, base deficit, potassium, ionised calcium, prothrombin time, fibrinogen, and TEG).

In addition, there was a descriptive analysis of observations during the intervention in each group.

6.12 Statistical Methods

6.12.1 Sample size calculation

The work by Luna et al in 1988 demonstrated that closed chest compressions reduced the diastolic BP, and theoretically therefore reduced CPP, in a baboon model of less severe haemorrhage.[67] However, the effect of compressions with intravenous FWB in a more severe model of TCA is unknown. There is good translational, and sparse clinical, data that demonstrate REBOA with intravenous FWB can be effective in resuscitation in low-output states,[101,104] but this severe model of haemorrhage resulted in either extremely low or a no output state. Therefore, there is no available survival data of either of these interventions in severe haemorrhagic TCA with which to calculate a sample size for the primary outcome. However, Manning et al's previous swine model of haemorrhagic TCA reports a 60% difference in 60 minute survival in a comparison of SAAP-LR and SAAP with a haemoglobin-based oxygen carrier.[112] Therefore, the sample size for the primary outcome was calculated to demonstrate a 50% difference in survival at 60 minutes, with an alpha of 5%. This calculation produced an experimental group size of n=10.

6.12.2 Statistical analyses

Data are reported as number (percent), and mean (± standard deviation). Proportions are reported as number (percent, 95% confidence interval). The 95% confidence interval was calculated using the Wilcoxon-Brown method. Comparison between the means of two independent groups was analysed with an unpaired t-test with Welch's correction (without assumption of equal standard deviations). Comparison between the means of repeated measurements within the same group was analysed with a paired t-test. Three and four group analyses were by ordinary one-way ANOVA. Analysis of proportions (ROSC and point-survival) was with Fisher's exact test. Survival curves were compared with the log-rank (Mantel-Cox) test. Statistical significance was pre-defined as $p<0.05$.

Percentages and means (± standard deviation) have been calculated in Excel for for Mac (Microsoft, Redmond, WA, USA), version 15.36; standard deviation is reported as standard deviation of the sample. 95% confidence intervals, t-tests, analyses of proportions, and survival curves have been analysed in Prism (GraphPad, La Jolla, CA, USA), version 7.0c.

6.12.3 Protocol deviations

Unintentional protocol deviations were discussed with my primary laboratory supervisor (Dr. Ross), and substitute animals added to the schedule as required, together with IRB review. All protocol deviations were described.

CHAPTER SEVEN

RESULTS – PART ONE

7.1 Overview and exclusions

Experiments were completed between the 4th December 2014 and 16th June 2015. Unintentional protocol deviations occurred in six animals, who were excluded from the final analysis: CPR group (one) - in 7404 the systolic BP between t0 and t3 was greater than 10 mmHg, and the heart rate was 147% of the baseline value. SAAP-LR group (one) – in 7374 the systolic BP between t0 and t3 was greater than 10 mmHg. SAAP-FWB group (two) – in 7421 the SAAP balloon was recognised to be leaking at t18; in 7444, the animal went into VF at t2.25 (pre-intervention). REBOA group (two) – in 7634, the infusion of venous blood was started 30 seconds late, and the infusion was less than 70% of the required volume; in 7729, 250 ml boluses were incorrectly administered after the initial 2,452 ml infusion.

Exclusions were made within 24 hours of each experiment, and additional animals added to the schedule, with IRB agreement. Therefore, a total of 46 swine were used, with 40 included in the data analysis.

7.2 Blood donor pool

A total of 56 swine were used in the blood donor pool, providing 340 units of FWB for experimental protocols. The mean haemoglobin was 8.7 (± 1.3) g/dl. Owing to logistical constraints, 15 swine underwent more than two episodes of

venesection (of up to three units of FWB per session), and two of these underwent four episodes, with an interval of at least 14 days between donations. The mean haemoglobin between venesections was not significantly different (ANOVA, p=0.3), Figure 7.1.

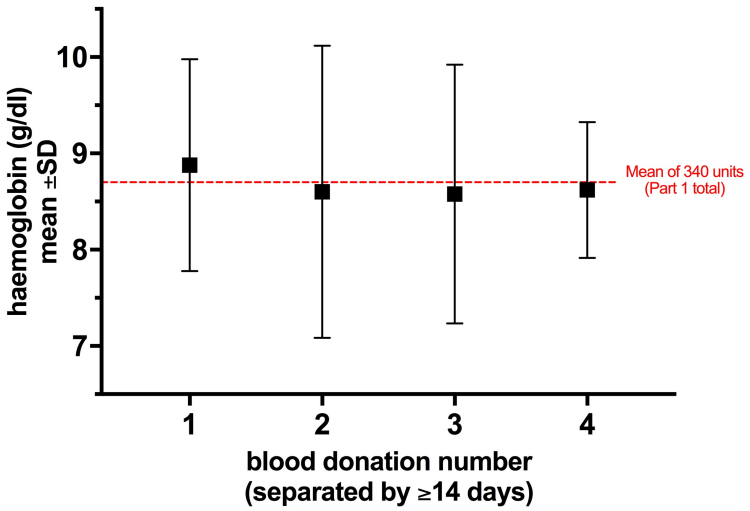

Figure 7.1 – Mean haemoglobin of swine donor pool blood – a comparison of first, second, third, and fourth venesections.

7.3 Animal baseline characteristics

7.3.1 Baseline physiology

All 40 animals were within the required range of 70-90 kg, and the mean weight was 80.1 (±5.7) kg. At baseline, the mean values were: systolic BP 86.8 (±14.8) mmHg, end-tidal carbon dioxide 5.4 (±0.1) kPa, and left carotid artery flow 372.1 (±83.8) ml/minute. There were no statistical differences in baseline physiology between groups, Table 7.1.

7.3.2 Baseline blood sample analyses

At baseline, an ABG, full blood count, and a clotting profile (including TEG) were drawn. The mean arterial partial pressure of oxygen was 15.9 (±3.4) kPa, with an inspired oxygen fraction of 0.21 (PaO_2:FiO_2 = 567.4 (±121.6)). There were no observed differences in blood samples at baseline, Tables 7.2 & 7.3.

Table 7.1 – Baseline physiological characteristics between groups, mean (± standard deviation).

Variable	CPR	SAAP-LR	SAAP-FWB	REBOA	ANOVA
Animal weight (kg)	80.6 (±4.7)	77.6 (±6.2)	79.8 (±6.9)	82.5 (±4.7)	p=0.30
Heart rate (beats/minute)	81.0 (±14.6)	89.9 (±15.7)	86.4 (±12.4)	89.8 (±16.6)	p=0.51
Systolic blood pressure (mmHg)	79.8 (±8.6)	81.7 (±5.7)	88.3 (±9.5)	86.3 (±6.2)	p=0.06
Mean arterial pressure (mmHg)	63.5 (±8.3)	62.8 (±5.3)	68.2 (±6.8)	68.9 (±5.0)	p=0.09
Mean pulmonary artery pressure (mmHg)	23.2 (±3.6)	23.0 (±4.8)	20.6 (±2.6)	23.7 (±2.5)	p=0.22
Cardiac output (l/minute)	4.9 (±0.5)	6.0 (±1.4)	5.8 (±0.8)	5.8 (±1.2)	p=0.12
SaO_2 (%)	98.6 (±1.8)	98.6 (±2.1)	98.8 (±1.5)	98.2 (±1.8)	p=0.90
End-tidal CO_2 (kPa)	5.5 (±0.1)	5.5 (±0.2)	5.3 (±0.2)	5.5 (±0.1)	p=0.07
Left carotid artery flow (ml/minute)	374.5 (±93.3)	360.2 (±90.5)	358.5 (±57.9)	395.1 (±96.0)	p=0.76
Central venous pressure (mmHg)	10.6 (±1.8)	9.4 (±2.7)	9.9 (±1.6)	9.9 (±1.4)	p=0.59
Central venous saturation (%)	65.1 (±8.9)	67.2 (±7.9)	65.3 (±5.1)	66.3 (±5.4)	p=0.90
Core temperature (^oC)	37.1 (±0.7)	37.7 (±0.7)	37.9 (±0.7)	37.4 (±0.7)	p=0.05

Table 7.2 – Baseline ABG, and full blood count analysis between groups, mean (± standard deviation).

Variable	CPR	SAAP-LR	SAAP-FWB	REBOA	ANOVA
Arterial blood gas					
pH	7.48 (±0.03)	7.49 (±0.03)	7.50 (±0.03)	7.47 (±0.03)	p=0.16
pO_2 (kPa)	16.6 (±3.8)	15.8 (±3.8)	16.9 (±2.7)	14.2 (±3.1)	p=0.28
pCO_2 (kPa)	5.4 (±0.4)	5.3 (±0.5)	5.1 (±0.2)	5.4 (±0.3)	p=0.11
Calcium (mmol/l)	1.2 (±0.05)	1.2 (±0.06)	1.2 (±0.04)	1.3 (0.10)	p=0.29
Potassium (mmol/l)	4.3 (±0.4)	4.3 (±0.3)	4.2 (±0.3)	4.2 (±0.3)	p=0.97
Sodium (mmol/l)	140.3 (±3.1)	142.0 (±2.4)	140.8 (±0.8)	140.2 (±2.2)	p=0.29
Glucose (mmol/l)	5.2 (±1.1)	6.3 (±1.5)	5.9 (±1.0)	5.8 (±1.5)	p=0.30
Lactate (mmol/l)	1.5 (±0.4)	1.5 (±0.3)	1.6 (±0.5)	1.7 (±0.4)	p=0.57
Base deficit	-6.1 (±1.9)	-6.7 (±1.4)	-6.1 (±2.1)	-5.8 (±2.0)	p=0.75
Full blood count					
Haemoglobin (g/dl)	11.0 (±1.3)	11.2 (±1.3)	10.9 (±1.2)	11.3 (±1.0)	p=0.91
White cell count ($x10^9$/l)	18.0 (±5.0)	14.3 (±4.2)	15.8 (±4.5)	19.4 (±4.4)	p=0.07
Platelet count ($x10^9$/l)	295.3 (±37.4)	249.5 (±71.4)	279.3 (±90.2)	260.5 (±68.1)	p=0.47

Table 7.3 – Baseline clotting profile, and thromboelastography analysis between groups, mean (± standard deviation).

Variable	CPR	SAAP-LR	SAAP-FWB	REBOA	ANOVA
Clotting profile					
PT (seconds)	13.8 (±0.6)	13.9 (±0.4)	14.2 (±0.4)	13.6 (±0.5)	p=0.09
Fibrinogen (g/dl)	2.0 (±0.4)	2.1 (±0.4)	2.0 (±0.6)	2.1 (±0.4)	p=0.86
Thromboelastograhy					
R (seconds)	4.8 (±1.1)	6.2 (±2.4)	5.2 (±2.2)	6.1 (±1.3)	p=0.26
K (seconds)	1.3 (±0.2)	1.7 (±0.6)	1.7 (±1.6)	1.7 (±0.4)	p=0.72
α angle (degrees)	72.2 (±5.5)	68.1 (±6.4)	68.0 (±11.0)	69.5 (±3.3)	p=0.53
MA (mm)	77.8 (±2.7)	76.8 (±4.8)	74.9 (±6.5)	76.1 (±5.4)	p=0.62
G	17.8 (±2.7)	17.3 (±4.2)	16.7 (±4.8)	16.6 (±3.5)	p=0.88
CI	3.6 (±1.1)	2.1 (±2.6)	2.6 (±2.6)	2.2 (±1.0)	p=0.33
LY-30 (%)	1.6 (±1.2)	1.3 (±0.9)	1.9 (±0.9)	1.6 (±0.8)	p=0.54

7.4 Injury analysis

7.4.1 NCTH and controlled arterial haemorrhage

The hepatic injury was completed in a mean of 105.2 (±17.4) seconds – this was consistent between the CPR and both SAAP groups (p=0.66), but significantly higher compared to the REBOA group (p<0.001). The mean percent excision of the left lateral lobe of the liver was consistent between groups at 69.2 (±9.6) percent by weight, Table 7.4.

The volume of the NCTH was not measured at the time of injury, and was therefore subject to the effects of the intervention over the duration of the pre-hospital period. The mean volume of NCTH was 1,838.2 (±921.7) ml, equating to 22.9 (±11.1) ml/kg, and was not consistent amongst groups. The highest volume of NCTH was observed in the CPR group, followed by the REBOA group, SAAP-LR, and SAAP-FWB, Table 7.4.

The volume of the controlled arterial haemorrhage was a mean of 1,630.8 (±488.5) ml, equating to 20.4 (±6.0) ml/kg, and was consistent between groups. However, when calculated as volume per swine weight, the haemorrhage was significantly lower in the REBOA group compared to the other three groups (p<0.01), Table 7.4.

The time interval from the start of the hepatic injury to the onset of TCA (t0) was a mean of 674.6 (±111.0) seconds (11.2 minutes), and was consistent between groups, Table 7.4.

Table 7.4 – Injury characteristics analysis between groups, mean (± standard deviation).

Variable	CPR	SAAP-LR	SAAP-FWB	REBOA	ANOVA
Animal weight (kg)	80.6 (±4.7)	77.6 (±6.2)	79.8 (±6.9)	82.5 (±4.7)	p=0.30
Spleen weight (g)	641.8 (±111.3)	623.8 (±236.9)	668.9 (±217.7)	611.8 (±180.7)	p=0.92
Liver weight (kg)	1.6 (±0.3)	1.6 (±0.2)	1.9 (±0.3)	1.8 (±0.2)	p=0.07
Injury time (seconds)	665.8 (±95.9)	719.1 (±99.4)	699.8 (±75.7)	613.6 (±146.5)	p=0.16
NCTH					
Hepatic injury (seconds)	107.0 (±20.5)	111.3 (±12.1)	113.0 (±10.0)	89.5 (±16.0)	p<0.01**
Proportion of liver lobe excised (%)	69.4 (±10.2)	70.6 (±11.4)	67.8 (±11.1)	69.0 (±6.1)	p=0.94
Abdominal haemorrhage (ml)	2361.6 (±893.4)	1601.4 (±656.8)	1185.3 (±660.8)	2204.3 (±1012.8)	p<0.01**
Abdominal haemorrhage (ml/kg)	29.4 (±11.1)	20.8 (±8.6)	14.7 (±7.5)	26.6 (±11.9)	p<0.05*
Controlled haemorrhage					
Controlled haemorrhage (ml)	1623.0 (±345.4)	1759.8 (±448.3)	1820.5 (±313.7)	1320.1 (±670.5)	p=0.10
Controlled haemorrhage (ml/kg)	20.2 (±4.2)	22.6 (±5.2)	22.8 (±3.1)	16.2 (±8.3)	p<0.05*
Combined haemorrhage					
Total haemorrhage (ml)	3984.6 (±603.2)	3361.2 (±408.0)	3005.8 (±602.6)	3524.4 (±551.7)	p<0.01**
Total haemorrhage (ml/kg)	49.6 (±8.1)	43.4 (±5.1)	37.5 (±5.8)	42.8 (±7.0)	p<0.01**
Proportion of swine TBV (%)	73.7 (±12.0)	64.5 (±7.5%)	55.8 (±8.6)	63.6 (±10.5)	p<0.01**

7.4.2 Arrest period physiology

At the onset of arrest (t0), a mean of 674.6 (±111.0) seconds after the start of the hepatic injury, all animals had a systolic BP less than 10 mmHg, and a cardiac electrical rate lower than baseline measurement.

In order to compare the three minute arrest period (t0-t3) between groups, the means values for systolic BP, end-tidal carbon dioxide, left carotid artery flow, and tissue oxygenation were calculated per animal during this period, and compared between groups.

This haemorrhage model produced a very severe arrest, with a mean systolic BP of 2.5 (±1.9) mmHg, a mean end-tidal carbon dioxide of 0.9 (±0.2) kPa, a mean left carotid artery flow of 0.8 (±1.0) ml/minute, and a mean StO_2 of 37.6 (±8.1) percent of baseline, Table 7.5.

During the arrest period (t0-t3), the mean cardiac electrical rate was measured over 30 seconds immediately before the start of the intervention (t2.5 to t3), and was a mean of 43.0 (±31.3) beats/minute. There was no statistical difference between the CPR and both SAAP groups (ANOVA, $p=0.72$), but there was a significant difference between these groups and the REBOA group ($p<0.01$), Table 7.5.

Examination of the cardiac electrical rhythm between t2.5 and t3 demonstrated that 12 (30.0%) of animals were in either cardiac electrical asystole or p-wave

asystole – CPR (n=3), SAAP-LR (n=4), SAAP-FWB (n=5). No animals in the REBOA group were in cardiac electrical asystole during the arrest period.

7.4.3 Arrest point blood sample analyses

Blood samples were drawn, via the SAAP catheter, in all groups at the point of TCA (t0). In order to characterise the injury, markers of tissue perfusion (pH, lactate, and base deficit) were examined. No blood could be drawn from CPR10-7521; this group was therefore examined as n=9.

At t0, the pH, lactate, and the base deficit were significantly higher compared to baseline: 7.66 (±0.07) vs 7.49 (±0.03), 2.7 (±0.7) mmol/l vs 1.6 (±0.4) mmol/l, and -5.1 (±1.9) vs -6.2 (±1.8), respectively, all $p<0.001$.

There were no statistical differences in pH, lactate, and base deficit between groups at t0, Table 7.6.

Table 7.5 – Arrest characteristics between groups, mean (± standard deviation).

Variable	CPR	SAAP-LR	SAAP-FWB	REBOA	ANOVA
Arrest period (t0-t3)					
Systolic BP (mmHg)	2.4 (±2.1)	3.2 (±2.0)	2.8 (±1.7)	1.6 (±1.7)	p=0.31
End-tidal CO_2 (kPa)	0.7 (±0.5)	0.9 (±0.5)	0.9 (±0.5)	0.9 (±0.4)	p=0.21
Left carotid artery flow (ml/minute)	0.7 (±0.9)	1.4 (±1.5)	0.5 (±0.5)	0.8 (±1.0)	p=0.13
StO_2 (% of baseline)	36.5 (±5.6)	37.7 (±10.5)	40.7 (±7.4)	37.6 (±8.1)	p=0.54
Between t2.5-t3					
Heart rate (beats/minute)	42.2 (±32.1)	31.5 (±30.4)	35.7 (±35.5)	43.0 (±31.3)	p<0.05*

Table 7.6 – Arrest (t0) blood sample analyses between groups, mean (± standard deviation).

Variable	CPR	SAAP-LR	SAAP-FWB	REBOA	ANOVA
pH	7.62 (±0.08)	7.68 (±0.04)	7.68 (±0.07)	7.63 (±0.06)	p=0.08
Lactate (mmol/l)	2.4 (±0.6)	2.9 (±0.8)	2.9 (±0.9)	2.6 (±0.4)	p=0.38
Base deficit	-4.5 (±1.5)	-5.5 (±1.6)	-5.2 (±2.7)	-5.0 (±1.4)	p=0.68

7.5 Intervention analysis

The intervention started a mean of 184.0 (±2.9) seconds from the onset of arrest (t0), and was consistent between groups, p=0.32.

7.5.1 Analysis of interventional infusions

Three groups (CPR, SAAP-FWB, REBOA) received infusions of FWB. Pre-infusion blood samples of perfusate were obtained to check uniformity. The mean haemoglobin was 7.5 (±0.8) g/dl, and was consistent between the CPR and REBOA groups, p=0.29. However, the FWB infusion for FWB-SAAP had been diluted with lactated Ringer's in the SAAP circuit before sampling, resulting in a mean haemoglobin that was significantly lower than the CPR and REBOA infusions, both $p<0.05$, Table 7.7.

The oxygenation of the SAAP perfusates (SAAP-LR, SAAP-FWB groups) resulted in a mean partial pressure of oxygen of 80.6 (±6.5) kPa, which was consistent between SAAP groups, p=0.11. However, as lactated Ringer's solution does not contain haemoglobin, the oxygen content of this infusion was significantly lower than the SAAP-FWB infusion, 1.8 (±0.1) compared to 11.3 (±0.8) ml of oxygen per 100 ml of infusion, $p<0.001$.

The oxygenation of the SAAP-FWB perfusate effected significant differences in pH as compared to the non-oxygenated CPR and REBOA infusions, Table 7.7.

7.5.2 Volume of interventional infusions

The total FWB volume infused in the CPR and REBOA groups was 2,452 ml per animal, per protocol.

The SAAP-LR group all received an initial 1,600 ml of oxygenated LR via the SAAP catheter over two minutes (t3-t5), and all animals required seven 250 ml boluses of LR via the SAAP catheter during the pre-hospital period (t5-t63), a total mean volume of 3,350 (±0.0) ml (the maximum volume allocated in the pre-hospital period, per protocol).

The SAAP-FWB group all received an initial 1,600 ml of oxygenated FWB via the SAAP catheter over two minutes (t3-t5), and a mean of 650.0 (±625.8) ml in additional 250 ml boluses of FWB via the SAAP catheter during the pre-hospital period (t5-t63), a total mean volume of 2,250 (±625.8) ml.

The total infusion volume was not significantly different between the SAAP-FWB group and the CPR and REBOA groups, $p=0.32$. The total infusion volume in the SAAP-LR group was significantly higher than both the SAAP-FWB group, and the CPR and REBOA groups, all $p<0.001$.

Table 7.7 – Intervention infusions between groups, mean (± standard deviation).

Variable	CPR	SAAP-LR	SAAP-FWB	REBOA	ANOVA
Haemoglobin (g/dl)	7.8 (±0.6)	0.0 (±0.0)	7.0 (±0.6)	7.9 (±0.9)	p<0.001***
pH	7.06 (±0.03)	7.15 (±0.23)	7.50 (±0.13)	7.04 (±0.02)	p<0.001***
pO_2 (kPa)	8.5 (±2.0)	78.3 (±4.4)	82.9 (±7.5)	8.2 (±2.3)	p<0.001***
pCO_2 (kPa)	5.4 (±0.5)	0.0 (±0.0)	1.4 (±0.6)	6.0 (±0.6)	p<0.001***

7.6 Primary outcome

A total of 13 (32.5%) animals survived the pre-hospital period: CPR group - one (10.0%, 95%CI 0.5-40.4), SAAP-LR group - three (30.0%, 95%CI 10.8-60.3), and FWB-SAAP group - nine (90.0%, 95%CI 59.6-99.5). None (0.0%, 95%CI 0.0-27.8) of the animals in the REBOA group survived the pre-hospital period, Figure 7.2.

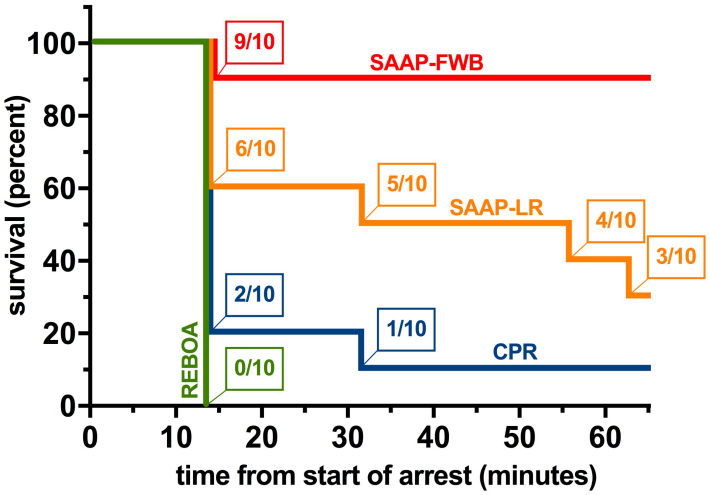

Figure 7.2 – Pre-hospital (60 minute) survival post intervention – a comparison of CPR, SAAP-LR, SAAP-FWB, and REBOA.

Analysis of survival proportions at t63 demonstrated a significant difference between the four groups, $p<0.001$. There was no difference between survival of CPR, SAAP-LR, and REBOA, $p=0.13$. FWB-SAAP demonstrated a significant survival advantage over all other groups, $p<0.01$.

7.7 Secondary outcomes at t13

7.7.1 ROSC

A ROSC was observed in 17 (43.5%) animals at t13 (ten minutes after the intervention): CPR group – two (20%, 95%CI 3.6-51.0), SAAP-LR group – six (60.0%, 95%CI 31.3-83.2), and SAAP-FWB group – nine (90.0%, 95%CI 59.6-99.5). None (0.0%, 95%CI 0.0-27.8) of the animals in the REBOA group had a ROSC at t13, Figure 7.3.

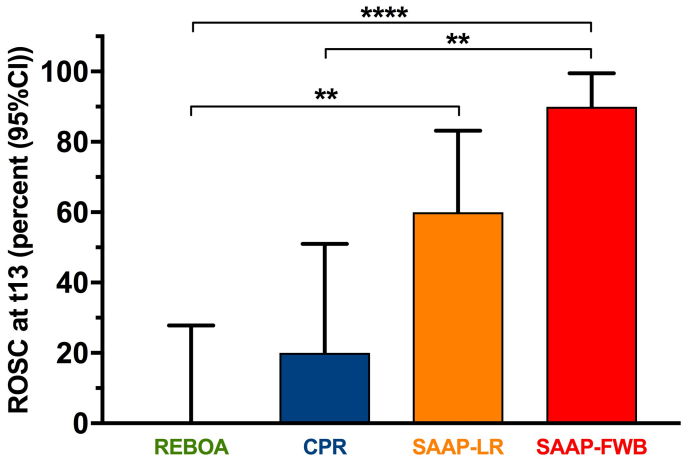

Figure 7.3 – ROSC at t13 – a comparison of CPR, SAAP-LR, SAAP-FWB, and REBOA.

There was no significant difference in the proportion of animals in ROSC at t13 between the CPR and REBOA groups, between the CPR and SAAP-LR groups, and between the SAAP groups, Figure 7.3. However, SAAP-FWB had a significantly higher proportion of animals who achieved ROSC at t13 compared to both the CPR and REBOA groups, $p<0.01$ and $p<0.001$ respectively, and SAAP-LR had a significantly higher proportion of animals who achieved ROSC at t13 compared to REBOA, $p<0.01$. No animals in the CPR and SAAP-LR groups in cardiac

electrical asystole at t3 had a ROSC, compared to four out of five (80.0%) SAAP-FWB animals.

7.7.2 Physiological measurements

The systolic BP, carotid artery flow, end-tidal carbon dioxide, and tissue oxygenation were compared between groups in all animals at t13 (ten minutes after the start of the intervention). In the CPR group, observations were made during the ten second compression pause in order to report the underlying intrinsic values; owing to the latency in change of end-tidal carbon dioxide and tissue oxygenation, these values are affected by the prior compressions.

The highest mean values for all four measurements were observed in the SAAP-FWB group, Figures 7.4a, 7.4b, 7.4c, 7.4d.

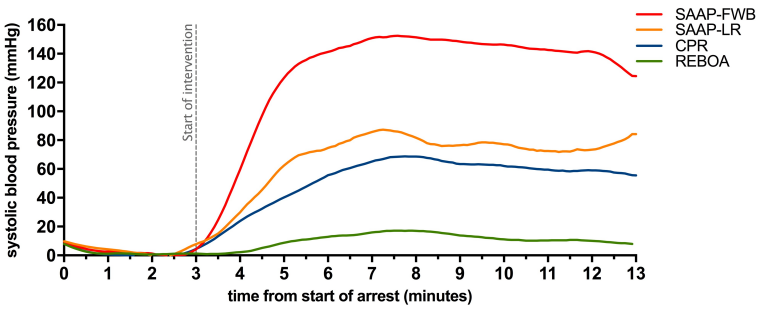

Figure 7.4a – Systolic BP from the time of arrest to t13 – a comparison of CPR, SAAP-LR, SAAP-FWB, and REBOA.

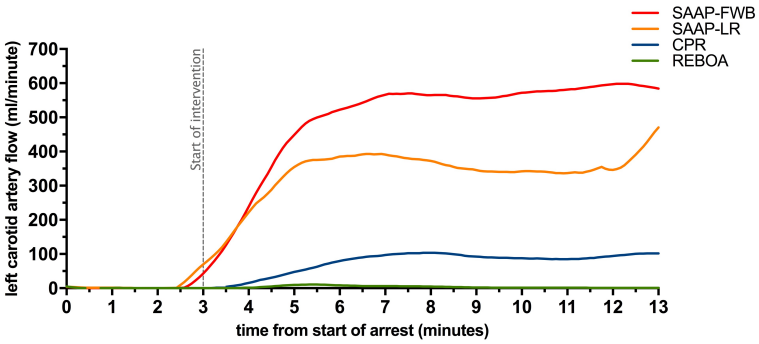

Figure 7.4b – Left carotid artery flow from the time of arrest to t13 – a comparison of CPR, SAAP-LR, SAAP-FWB, and REBOA.

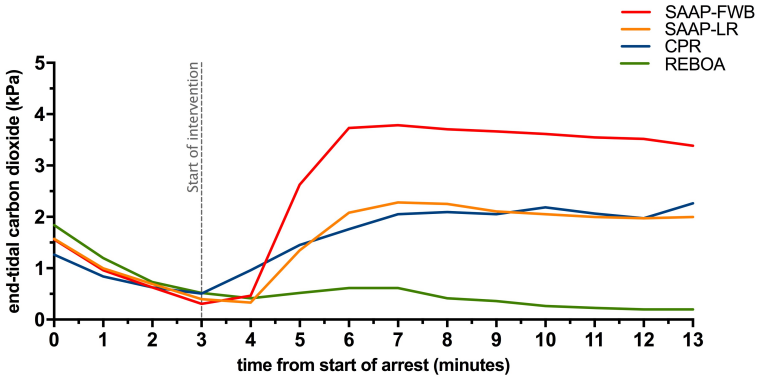

Figure 7.4c – End-tidal carbon dioxide from the time of arrest to t13 – a comparison of CPR, SAAP-LR, SAAP-FWB, and REBOA.

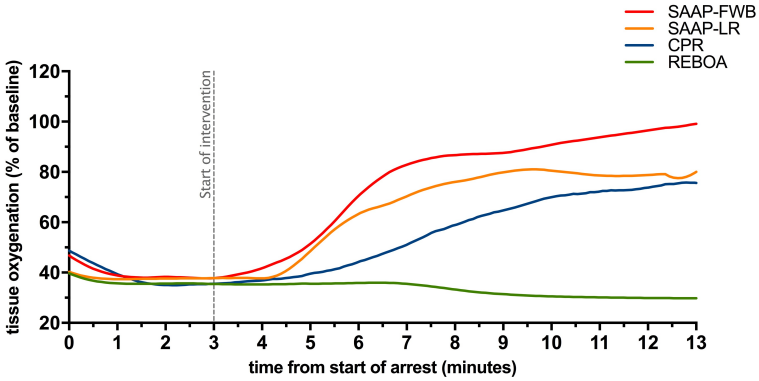

Figure 7.4d – Tissue oxygenation from the time of arrest to t13 – a comparison of CPR, SAAP-LR, SAAP-FWB, and REBOA

The SAAP-FWB group had a significantly higher systolic BP than all other groups, $p<0.01$, a significantly higher left carotid artery flow than the CPR and REBOA groups, $p<0.01$ and $p<0.001$ respectively, and a significantly higher tissue oxygenation compared to the REBOA group, $p<0.001$.

The SAAP-LR group had a significantly higher systolic BP, left carotid artery flow, end-tidal carbon dioxide, and tissue oxygenation than the REBOA group, all $p<0.01$.

The CPR group had a significantly higher end-tidal carbon dioxide and tissue oxygenation, but there was no difference in systolic BP and left carotid artery flow, compared with the REBOA group, $p<0.01$, Table 7.8.

Table 7.8 – Physiological measurements between groups, ten minutes after the start of the intervention (t13), mean (± standard deviation).

Variable	CPR	SAAP-LR	SAAP-FWB	REBOA
Systolic BP (mmHg)	29.0 (±37.8)	67.2 (±48.8)	133.6 (±35.8)	8.1 (±7.5)
Left carotid artery flow (ml/minute)	162.5 (±331.7)	356.4 (±330.4)	589.0 (±296.4)	1.3 (±2.0)
End-tidal carbon dioxide (kPa)	2.3 (±1.7)	2.0 (±1.6)	3.4 (±0.5)	0.2 (±0.2)
StO_2 (% of baseline)	78.6 (±32.8)	79.9 (±34.7)	98.9 (±17.5)	29.8 (±6.5)

7.7.3 Blood sample analyses

At t13, an attempt was made to draw blood samples (an ABG, full blood count) from all animals. Owing to the lack of circulation in one of the SAAP-LR and two of the REBOA group, it was not possible to draw samples from these animals (SAAP-LR5-7439, REBOA6-7750, REBOA10-7778), Table 7.9.

Table 7.9 – Blood sample analyses between groups, ten minutes after the start of the intervention (t13), mean (± standard deviation).

Variable	CPR (n=10)	SAAP-LR (n=9)	SAAP-FWB (n=10)	REBOA (n=8)	ANOVA
Arterial blood gas					
pH	7.34 (±0.16)	7.39 (±0.20)	7.44 (±0.08)	7.56 (±0.23)	p=0.07
pO_2 (kPa)	30.7 (±15.6)	41.1 (±16.4)	49.3 (±11.0)	21.0 (±0.2)	p<0.01**
pCO_2 (kPa)	5.4 (±2.4)	3.2 (±1.9)	3.9 (±0.4)	3.6 (±3.1)	p=0.13
Ionised calcium (mmol/l)	1.5 (±0.3)	1.1 (±0.1)	1.4 (±0.1)	1.1 (±0.2)	p<0.001***
Lactate (mmol/l)	5.5 (±0.9)	13.2 (±6.3)	6.1 (±1.5)	4.9 (±2.1)	p<0.01**
Base deficit	4.3 (±2.7)	2.2 (±2.7)	3.5 (±3.5)	3.6 (±3.2)	p=0.56
Full blood count					
Haemoglobin (g/dl)	8.0 (±1.0)	3.0 (±1.8)	9.2 (±0.6)	7.2 (±0.8)	p<0.001***

The SAAP-FWB group had a significantly higher partial pressure of oxygen than the CPR and REBOA groups, both $p<0.01$, but not compared to the SAAP-LR group, $p=0.22$. However, the SAAP-LR group had a significantly lower haemoglobin than all other groups, all $p<0.001$. Therefore, the calculated oxygen content of the blood sample in the SAAP-LR was significantly lower than that of the SAAP-FWB group, 4.9 (±2.3) compared to 13.5 (±0.9) ml of oxygen per 100 ml of blood respectively, $p<0.001$.

The SAAP-LR group had a lactate significantly higher than all other groups, $p<0.001$. However, there was no significant difference in base deficit between groups. There was no difference in lactate between CPR, SAAP-FWB, and REBOA, Table 7.9.

7.8 Secondary outcomes at t63

7.8.1 Physiological measurements

Per protocol, animals with a systolic BP lower than 30 mmHg were removed from the experiment, starting at t13 (without observations carried forward).

At t63, the physiological measurements in live animals was compared (systolic BP, left carotid artery flow, end-tidal carbon dioxide, tissue oxygenation). The SAAP-FWB group (n=9) had the highest mean systolic BP, left carotid artery flow, and tissue oxygenation compared to the other groups. However, the single CPR animal with an intrinsic systolic BP greater than 30 mmHg at t63 had a higher end-tidal carbon dioxide than both the SAAP groups, Figures 7.5a, 7.5b, 7.5c, 7.5d.

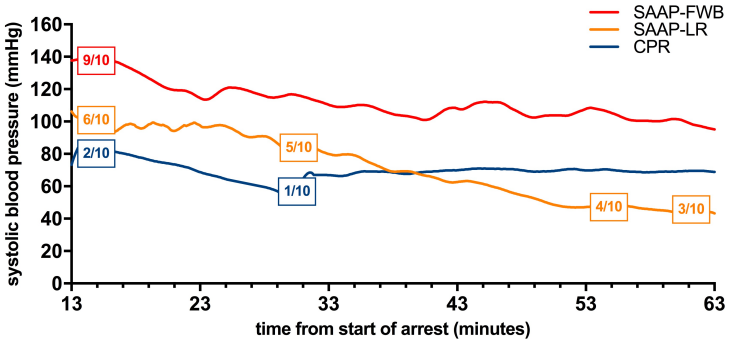

Figure 7.5a – Systolic BP from t13 to t63 – a comparison of CPR, SAAP-LR, and SAAP-FWB.

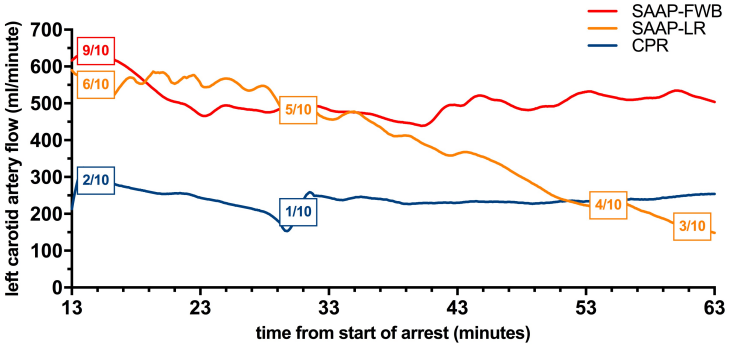

Figure 7.5b – Left carotid artery flow from t13 to t63 – a comparison of CPR, SAAP-LR, and SAAP-FWB.

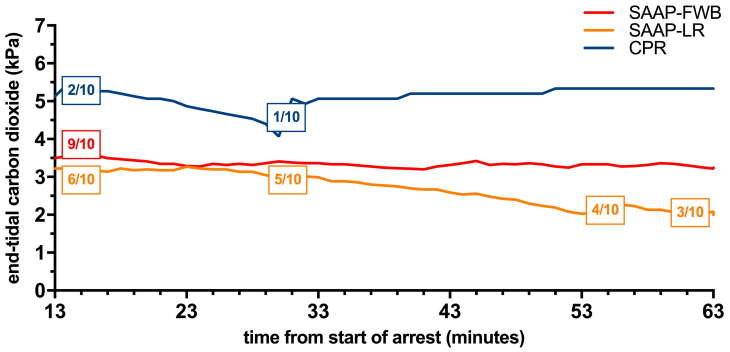

Figure 7.5c – End-tidal carbon dioxide from t13 to t63 – a comparison of CPR, SAAP-LR, and SAAP-FWB.

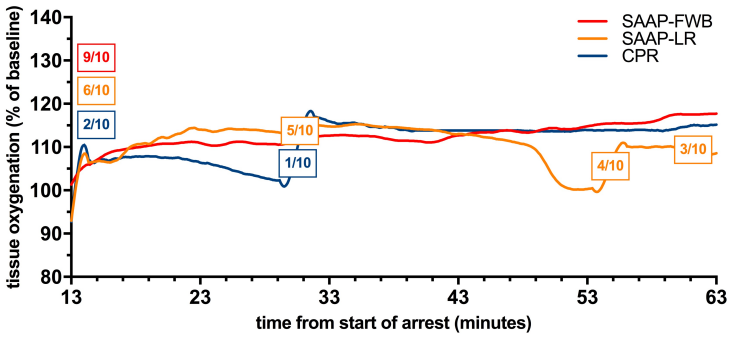

Figure 7.5d – Tissue oxygenation from t13 to t63 – a comparison of CPR, SAAP-LR, and SAAP-FWB.

There was a significant difference between groups in systolic BP, left carotid artery flow, and end-tidal carbon dioxide, but not tissue oxygenation, Table 7.10.

Table 7.10 – Physiological measurements between groups of animals alive at the end of the pre-hospital period (t63), mean values (± standard deviation) in both SAAP groups, single values for the CPR animal.

Variable	CPR	SAAP-LR	SAAP-FWB	ANOVA
N of animals	1	3	9	
Systolic BP (mmHg)	68.5	42.6 (±13.0)	94.7 (±7.3)	p<0.001***
Left carotid artery flow (ml/minute)	253.6	147.0 (±67.2)	500.1 (±87.8)	p<0.001***
End-tidal carbon dioxide (kPa)	5.3	2.0 (±0.0)	3.2 (±0.4)	p<0.001***
StO_2 (% of baseline)	115.5	108.5 (±5.2)	117.7 (±8.3)	p=0.25

The SAAP-FWB group had a significantly higher systolic BP, left carotid artery flow, and end-tidal carbon dioxide compared to the SAAP-LR group, all p<0.001.

7.8.2 Blood sample analyses

At t63, comparison was made of blood samples in live animals, Table 7.11.

Table 7.11 – Blood sample analysis between groups of animals alive at the end of the pre-hospital period (t63), mean values (± standard deviation) in both SAAP groups, single values for the CPR animal.

Variable	CPR	SAAP-LR	SAAP-FWB	ANOVA
N of animals	1	3	9	
Arterial blood gas				
pH	7.36	7.49 (±0.02)	7.35 (±0.05)	p<0.01**
pO_2 (kPa)	41.7	52.7 (±4.5)	57.5 (±4.8)	p<0.05*
pCO_2 (kPa)	5.4	2.8 (±0.3)	3.4 (±0.4)	p<0.001***
Calcium (mmol/l)	1.4	1.2 (±0.1)	1.3 (±0.2)	p=0.50
Potassium (mmol/l)	3.8	4.9 (±0.4)	5.2 (±0.5)	p<0.05*
Sodium (mmol/l)	133.0	143.7 (±2.1)	141.6 (±1.6)	p<0.001***
Glucose (mmol/l)	7.0	3.4 (±2.5)	6.0 (±2.9)	p=0.64
Lactate (mmol/l)	4.9	15.5 (±1.3)	14.2 (±1.3)	p<0.001***
Base deficit	1.9	6.9 (±0.6)	10.6 (±2.1)	p<0.01**
Full blood count				
Haemoglobin (g/dl)	8.8	4.6 (±0.5)	9.2 (±0.9)	p<0.001***
White cell count ($x10^9$/l)	11.4	3.0 (±1.6)	5.7 (±2.4)	p<0.05*
Platelet count ($x10^9$/l)	276.0	158.3 (±52.6)	242.0 (±50.8)	p=0.07
Clotting profile				
PT (seconds)	13.6	15.0 (±0.5)	13.5 (±0.4)	p<0.01**
Fibrinogen (g/dl)	1.9	1.2 (±0.3)	1.6 (±0.1)	p<0.01**

There was no significant difference in the partial pressure of oxygen between the SAAP groups, p=0.16. However, the SAAP-LR group had a significantly lower haemoglobin compared to the SAAP-FWB group, p<0.001, and therefore a significantly lower blood oxygen content – 58.8 (±4.7) compared to 69.8 (±5.7) ml of oxygen per 100 ml of blood, p<0.05.

There was no significant statistical difference in glucose between the SAAP groups, p=0.41. However, two animals in the SAAP-LR group (SAAP-LR3-7407, SAAP-LR10-7596) had low glucose concentrations (1.9 and 2.0 mmol/l respectively).

The number of platelets, prothrombin time, and fibrinogen, were all significantly lower in the SAAP-LR group compared to the SAAP-FWB group, all $p<0.05$. The clotting function was further examined by TEG, Table 7.12.

Table 7.12 – A comparison of thromboelastography values between the SAAP-LR and SAAP-FWB groups at the end of the pre-hospital period (t63), mean (± standard deviation).

Variable	SAAP-LR	SAAP-FWB	t-test
N of animals	3	9	
R (seconds)	5.9 (±3.0)	4.6 (±1.6)	p=0.33
K (seconds)	1.7 (±0.7)	1.6 (±1.2)	p=0.88
α angle (degrees)	66.5 (±9.8)	66.5 (±8.9)	p=1.0
MA (mm)	64.1 (±8.2)	73.5 (±3.9)	p<0.05*
G	9.5 (±3.8)	14.3 (±2.9)	p<0.05*
CI	0.6 (±3.9)	2.7 (±2.3)	p=0.26
LY-30 (%)	1.6 (±1.2)	1.3 (±1.6)	p=0.77

The only significant differences in functional clotting were in clot strength (MA) and clot elasticity (G); both were higher in the SAAP-FWB group. There were no TEG values in the single CPR animal (CPR4-7422) that were more than one standard deviation away from the mean of the SAAP-FWB group. Clotting function in the SAAP-FWB group (n=9) was not statistically different at t63 compared to baseline, Table 7.13.

Table 7.13 – A comparison of thromboelastography values in the SAAP-FWB group (n=9) at baseline and t63, mean (± standard deviation).

Variable	Baseline	t63	t-test
N of animals	9	9	
R (seconds)	5.0 (±2.2)	4.6 (±1.6)	p=0.24
K (seconds)	1.7 (±1.7)	1.6 (±1.2)	p=0.59
α angle (degrees)	68.1 (±11.7)	66.5 (±8.9)	p=0.64
MA (mm)	75.5 (±6.6)	73.5 (±3.9)	p=0.48
G	17.3 (4.6)	14.3 (±2.9)	p=0.13
CI	2.9 (±2.7)	2.7 (±2.3)	p=0.73
LY-30 (%)	1.9 (±1.0)	1.3 (±1.6)	p=0.28

7.9 In hospital observation period

7.9.1 Surgery period

Thirteen animals (CPR group – one, SAAP-LR group – three, SAAP-FWB group – nine) were transitioned into a 30 minute period of surgical control of the hepatic injury. Immediately following surgical control the SAAP balloon was slowly deflated and removed in the SAAP groups. During this period a maximum of 5,750ml of intravenous FWB was allocated per animal, with a transfusion threshold of a systolic BP less than 90 mmHg.

All of the SAAP-LR group required the total volume of 5,750 ml of FWB during this period, compared to 4,750 ml in the CPR animal, and a mean of 3,889 (±1139.6) ml in the SAAP-FWB group. The CPR animal had a systolic BP of 97.0 mmHg at t93.

All SAAP animals demonstrated physiological instability as the SAAP balloon was deflated. Two animals met death-in-protocol criteria during this period, despite having received the maximum volume of 5,750 ml of FWB. SAAP-LR3-7407 died at t81 with the SAAP balloon still partially inflated in Z1, and SAAP-FWB2-7387 died at t90 after the SAAP catheter had been removed. All other animals survived to t93, Figure 7.6.

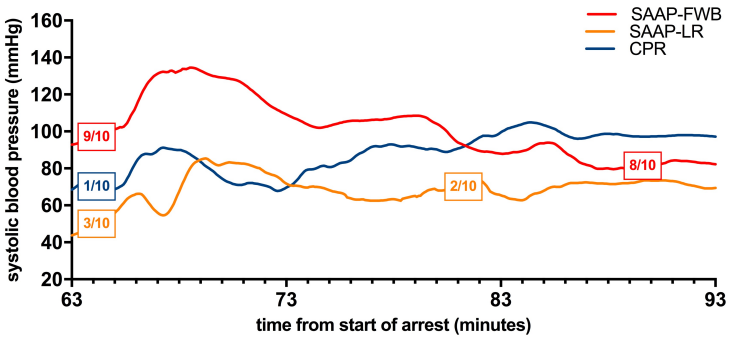

Figure 7.6 – Mean systolic BP from t63 to t93 – a comparison of CPR, SAAP-LR, and SAAP-FWB.

In order to describe the consequences of re-perfusion following balloon deflation, systolic BP, left carotid artery flow, cardiac output, pH, potassium, lactate, and base deficit were compared between t63 and t93 in the eight SAAP-FWB animals alive at t93, Table 7.14.

Table 7.14 – An evaluation of re-perfusion in the SAAP-FWB group (n=8), mean (± standard deviation).

Variable	t63	t93	t-test
Systolic BP (mmHg)	95.4 (±7.4)	81.8 (±13.9)	p<0.05*
Left carotid artery flow (ml/minute)	511.1 (±91.0)	415.1 (±121.6)	p<0.05*
pH	7.35 (±0.05)	7.10 (±0.03)	p<0.001***
Potassium (mmol/l)	5.3 (±0.5)	5.2 (±0.4)	p=0.69
Lactate (mmol/l)	14.0 (±1.2)	14.5 (±1.5)	p=0.38
Base deficit	10.3 (±2.0)	14.9 (±1.7)	p<0.001***

In the SAAP-FWB group (n=8) there was a significant fall in systolic BP and left carotid artery flow after the balloon was deflated, despite these animals receiving a mean volume of 3,656.3 (±963.0) ml of FWB during this period. At t93 these animals had a significant acidaemia, Table 7.14.

7.9.2 Critical care period

The remaining 11 animals alive at t93 transitioned into a 180 minute critical care period. During this period, further boluses of FWB were administered as per the surgery period, up to the combined maximum volume of 5,750 ml.

One animal (SAAP-FWB3-7425) started coughing under anaesthesia, dislodging the hepatic clamp, resulting in further severe haemorrhage and met death-in-protocol criteria at t148. Six further animals in the SAAP-FWB group and one animal in the SAAP-LR group met death-in-protocol criteria during the critical care period, Figure 7.7.

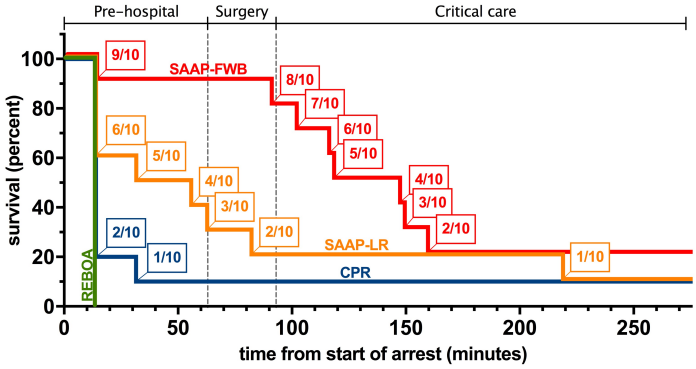

Figure 7.7 – Survival during the whole protocol – a comparison of CPR, SAAP-LR, SAAP-FWB, and REBOA.

There was no statistical difference in whole protocol survival between groups, CPR group – one (10.0%, 95%CI 0.5-40.4), SAAP-LR group – one (10.0%, 95%CI 0.5-40.4), SAAP-FWB group – two (20.0%, 95%CI 3.6-51.0), and REBOA group – none (0.0%, 95%CI 0.0-27.8), p=0.53. This remains non-significant with the assumption that the technical error death in the SAAP-FWB group would have survived to the end of the protocol, p=0.23. However, analysis by time of death

using a Log-rank (Mantel-Cox) test does demonstrate a significant difference between groups, $p<0.01$. Neither of the SAAP-FWB group animals that survived to t273 were in cardiac electrical asystole during the arrest period (t0-t3). There were therefore no survivors to t273 in any group that were in cardiac electrical asystole during the arrest period.

Markers of tissue perfusion (pH, lactate, and base deficit) had all started to trend back towards baseline values by t273. Clotting tests (prothrombin time and fibrinogen) and TEG values were not significantly different to baseline values, with a higher clotting index and less fibrinolysis. However, the potassium in two animals was over 6.5 mmol/l (mean of 6.2 (±1.4), compared to 4.2 (±0.1) at baseline). This was not statistically significant, but may be clinically significant.

7.10 Results of Part One hypotheses

In a swine model of severe haemorrhagic TCA:

H1. *Closed chest compressions with high-volume intravenous FWB will not result in a return of spontaneous circulation, and no animals will survive 60 minutes.*

In the CPR group, two (20.0%, 95%CI 3.6-51.0) animals had a ROSC, and one (10.0%, 95%CI 0.5-40.4) survived to 60 minutes after the start of the intervention.

H2. *REBOA with FWB will infer a 60 minute survival advantage over closed chest compression with FWB.*

None (0.0%, 95%CI 0.0-27.8) of the REBOA group survived to 60 minutes after the intervention, compared to one (10.0%, 95%CI 0.5-40.4) in the CPR group. There was no survival advantage to REBOA compared with CPR, p=1.0.

H3. *SAAP with intra-aortic oxygenated FWB will infer a 60-minute survival advantage over SAAP with intra-aortic oxygenated lactated Ringer's solution, REBOA with FWB, and closed chest compressions with FWB.*

Nine (90.0%, 95%CI 59.6-99.5) of the SAAP-FWB group survived for 60 minutes after the start of the intervention, and was significantly higher than all other groups, p<0.001.

7.11 Further experimental observations

7.11.1 CPR group

In order to quantify the effect of chest compressions during hypovolaemia with and without an intrinsic cardiac output, the CPR group was separated into animals with (n=2) and without (n=8) a ROSC.

In the CPR group without a ROSC, the mean systolic BP increased from 1.9 (±4.8) mmHg at the end of the arrest period, to 61.0 (±18.1) mmHg at the end of the 2,452 ml infusion of FWB. The systolic BP was 77.4% of the baseline measurement at the end of the infusion. The diastolic BP and the left carotid

artery flow at the end of the FWB infusion were 7.6% and 10.1% of baseline measurement respectively, Figures 7.8a & 7.8b.

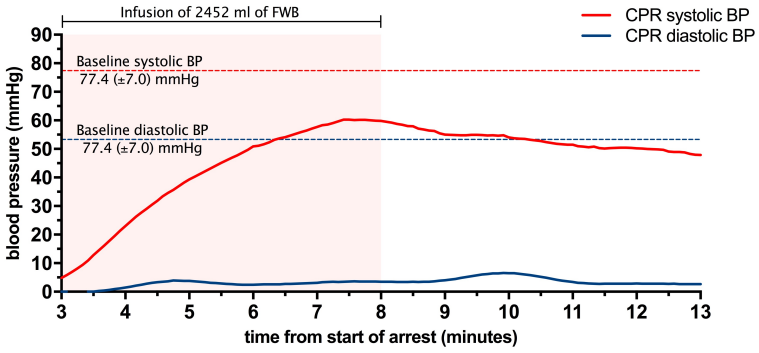

Figure 7.8a – Blood pressure in the CPR group animals without a ROSC (n=8) between t3 and t13.

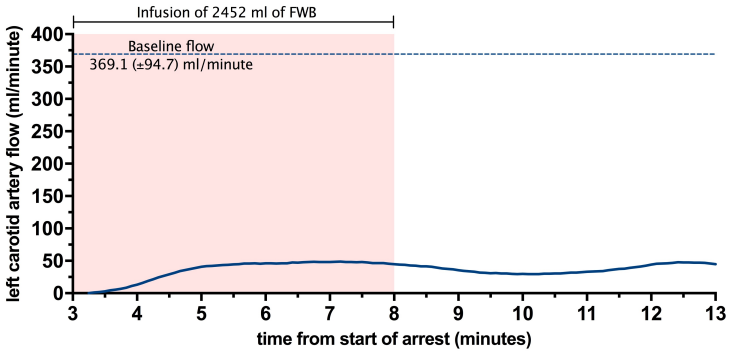

Figure 7.8b – Left carotid artery flow in the CPR group animals without a ROSC (n=8) between t3 and t13.

It is not possible to measure the CPP with the available data, but as coronary filling occurs in diastole (and can be calculated as aortic diastolic pressure minus left ventricular end-diastolic pressure) it can be assumed that the CPP was less than 10 mmHg throughout the period of infusion and compressions in this group of eight animals.

In the CPR group animals with a ROSC, the effect of compressions during infusion of FWB was different to that observed in the other eight animals. Both CPR4-7422 and CPR5-7441 had no intra-aortic pressure fluctuations at the start of the intervention, and during the first two minutes of the intervention both had periods of cardiac electrical asystole. Immediately prior to a return of intrinsic cardiac electrical activity, these two animals had a BP of 55/22 mmHg and 55/15 mmHg respectively. This was followed by a ROSC within the next two minutes in both animals.

7.11.2 SAAP-LR group

In the first ten seconds of SAAP infusion the mean intra-aortic arch pressure was 25.6 (±6.9) mmHg, and the mean left carotid artery flow was 185.4 (±99.2) ml/minute.

Six animals had a ROSC during, or shortly after, the SAAP infusion – the mean time to ROSC was 109.5 (±45.0) seconds. There was no significant difference in the intra-aortic arch pressure and carotid flow between animals with and without a ROSC, $p=0.86$ and $p=0.51$ respectively.

7.11.3 SAAP-FWB group

In the first ten seconds of SAAP infusion the mean intra-aortic arch pressure was 27.0 (±4.8) mmHg, and the mean left carotid artery flow was 139.0 (±46.4) ml/minute. There was no difference in mean intra-aortic pressure and left carotid artery flow between the two different SAAP infusions (FWB and LR), $p=0.61$ and $p=0.20$ respectively. All animals had a ROSC during the SAAP

infusion – the mean time to ROSC was 54.5 (±25.9) seconds, which was significantly faster compared to the ROSC time in the SAAP-LR group, $p<0.01$.

The single SAAP-FWB animal that did not have a ROSC at t13 (SAAP-FWB9-7475), did have a return of cardiac output during the SAAP infusion, with a peak BP of 110/75 mmHg. However, the BP rapidly fell, and the animal met death-in-protocol criteria at t13. Post-mortem examination revealed a percentage-by-weight left lateral lobe of the liver excision that was two standard deviations higher than the mean of the remaining nine animals in this group. The hepatic vascular anatomy was also noted to contain an abnormally wide calibre vein, and in essence was a very proximal, non-survivable, central venous haemorrhage.

Five animals in the SAAP-FWB group died during the critical care period (one animal had a further haemorrhage and died), Figure 7.7. As previously described, there was a significant reduction in pH after SAAP balloon deflation, that in this group of five animals reduced from 7.35 (±0.05) at t63 to 7.10 (±0.03) at t93, $p<0.001$, but no other reported data further explains these deaths. During experiments in this group, it was observed that there was little effect on systolic BP despite a further mean infusion of 2,500.0 (±500.0) ml of FWB, and that the central venous pressure and mean pulmonary artery pressure were high. In order to further describe the physiology during the first 15 minutes of the critical care period in these five animals, systolic BP, central venous pressure, cardiac output, and systemic vascular resistance have been examined, Figure 7.9.

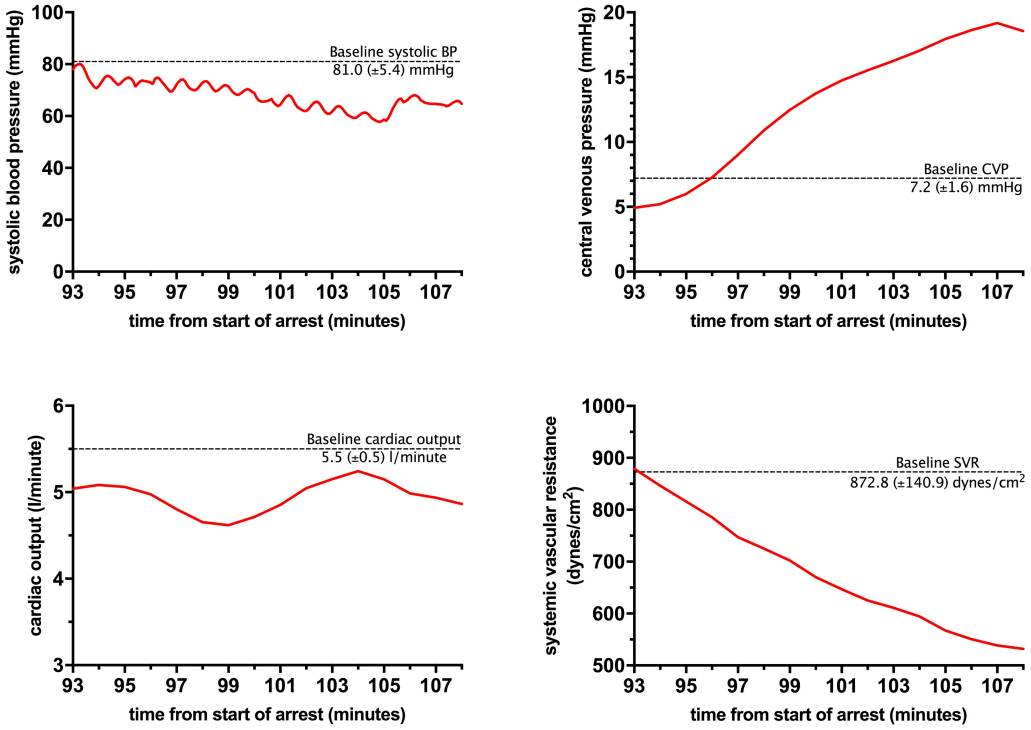

Figure 7.9 – The mean values of systolic BP, central venous pressure, cardiac output, and systemic vascular resistance from t93 to t108, in the five SAAP-FWB animals that died during the critical care period.

There was no significant change in the systolic BP or the cardiac output between t93 and t108, p=0.39 and p=0.78 respectively. However, the central venous pressure significantly increased, p<0.001, and the systemic vascular resistance significantly decreased, p<0.01.

The mean pulmonary artery pressure at t93 was significantly higher than baseline – 42.6 (±2.3) mmHg compared to 19.2 (±1.8) mmHg respectively, p<0.001. The mean pulmonary artery pressure reached a peak at t26, before falling again to a trough at t63 (simulated hospital arrival). There was a further peak at t68; the mean pressure increased from t78, remaining elevated through the first 15 minutes of the critical care period (t108), Figure 7.10.

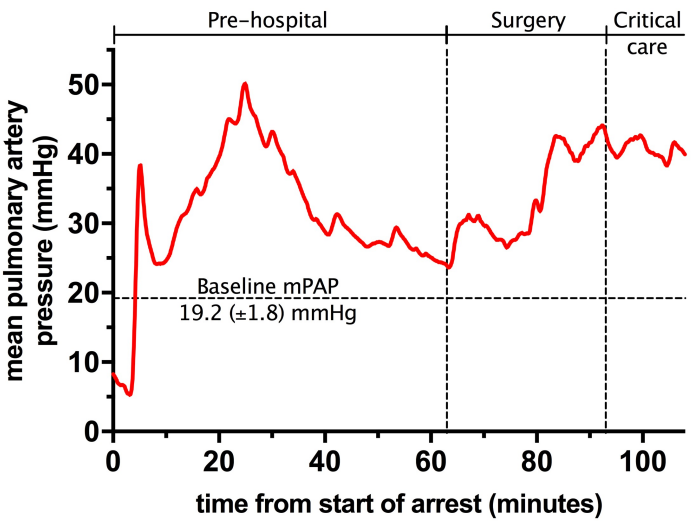

Figure 7.10 – The mean pulmonary artery pressure from the time of arrest (t0) to 15 minutes into the critical care period (t108), in the five SAAP-FWB animals that died during the critical care period.

In order to differentiate the cause of a raised pulmonary artery pressure, the PaO_2:FiO_2 was examined in this subset of the SAAP-FWB group. The baseline PaO_2:FiO_2 was 640.0 (±87.7), and was 366.2 (±53.5) at t13 (ten minutes after the start of the intervention), $p<0.001$. However, there was no significant difference between the PaO_2:FiO_2 at t13 and t63, and t93, $p=0.10$ and $p=0.06$ respectively. There was therefore no evidence of more than a mild impairment of pulmonary gas exchange in these five animals.

7.11.4 REBOA group

An increase in systolic BP was observed during the FWB infusion (t3-t8), despite the mean cardiac electrical rate falling from 43.0 (±31.3) to 29.5 (±15.1), Figure 7.4a. However, when the intra-aortic pressure telemetry was reviewed after each experiment, this increase in systolic BP was not associated with any fluctuations in intra-aortic pressure that would be consistent with an intrinsic

cardiac output, and there was a proportional rise in the diastolic BP. The mean pulmonary artery pressure also increased, and started to do so approximately one minute before the intra-aortic pressure increased, Figure 7.11.

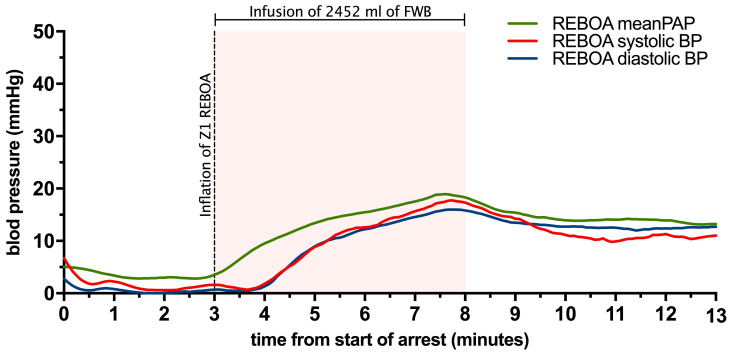

Figure 7.11 – The mean systolic and diastolic BP, and the mean pulmonary artery pressure in the REBOA group between t0 and t13.

There was, therefore, no evidence of resuscitation in the REBOA group, and all animals met death-in-protocol criteria at t13. In order to remove any blood clot from the SAAP catheter, it was flushed with 50 ml of lactated Ringer's after t13; after final blood samples had been obtained, and before the post-mortem laparotomy. An increase in cardiac electrical rate was observed in some of these animals immediately after this small volume flush, Figure 7.12.

Figure 7.12 – The intra-aortic pressure and lead II ECG trace in REBOA1-7655 during a 50 ml intra-aortic flush of lactated Ringer's at approximately t14.

CHAPTER EIGHT

DISCUSSION – PART ONE

8.1 Summary of findings

The aim of these experiments was to evaluate current and potential future management strategies for severe haemorrhagic TCA in a large swine translational model. The injury resulted in severe haemorrhagic TCA, leading to a mean systolic BP of 2.5 (±1.9) mmHg prior to the intervention. SAAP with FWB demonstrated a 60 minute survival advantage over closed chest compressions with IV FWB, REBOA with IV FWB, and SAAP with lactated Ringer's solution, and nine out of ten animals survived to simulated hospital arrival. Furthermore, SAAP with FWB demonstrated its potential to resuscitate cardiac electrical asystole. However, the majority of SAAP-FWB animals died soon after balloon removal, and there was no significant survival advantage at the end of the protocol over the other interventions. This discussion reviews the findings, its translation to clinical practice, and formulates further research questions.

8.2 Blood donor pool, baseline values, and injury model

8.2.1 Blood donor pool

The experimental protocol required a large volume of whole blood – a minimum of 5,750 ml and maximum of 9,100 ml per animal. This is significantly more than the six to eight units used in Morrison's prior large swine REBOA study.[101] Furthermore, all blood in this protocol was less than 48 hours old, and less than 24 hours old if used for the initial experimental infusions in the CPR, SAAP-FWB,

and REBOA groups. This protocol ensured that all blood product met the definition of fresh (less than 72 hours old),[129] and was therefore likely to have significantly more intrinsic clotting function that the donor blood used in Morrison's large swine REBOA experiments that had been stored for up to one week.[101]

This large volume of FWB required dedicated donors, and to my knowledge this is the first description of a dedicated swine donor pool. Model development demonstrated that three units of FWB could be venesected two weeks apart without a significant effect on haemoglobin concentration. Logistical constraints during experimentation led to some donor animals having up to four venesections (all at least two weeks apart), with no significant difference in haemoglobin concentration between donations. However, there was no analysis of clotting function, and it is therefore possible that repeated venesections resulted in impaired intrinsic clotting function of donor blood.

In summary, the dedicated swine blood donor pool was labour intensive, but effective in reducing the number of animals required, and produced large volumes of FWB, with the caveat that the intrinsic clotting function of donated product was unknown.

8.2.2 Baseline measurements

Three of the four groups were randomised with allocation concealment from the operator, whereas the REBOA group was completed, unblinded, at the end of this part of my research. Baseline values were compared between groups to

highlight any differences. Four group one-way ANOVA demonstrated no statistical differences in physiology, or blood samples obtained at baseline between groups, and no clinically significant differences were noted. It can therefore be assumed that there were no substantial differences between these four groups at baseline.

8.2.3 Haemorrhage model

The aim of the haemorrhage model was to adapt previous large swine haemorrhage models to systematically achieve a highly-lethal injury, that was at the extreme of known survivability. The laparoscopic hepatic injury previously described by Ross, et al,[118] and used in a REBOA laboratory experiment by Morrison, et al,[101] achieved a percent by weight excision of just under 70% of the left lateral lobe in these experiments. The mean excision weight was slightly lower than both the previous published reports, but owing to the prominent hepatic vasculature is unlikely to have had a significant effect on the volume of NCTH.

The hepatic injury in Ross et al's study resulted in 40% mortality (in splenectomised swine), and a 100% mortality in Morrison's study, in less than 60 minutes from the start of the injury without intervention.[101,118] There is no definition of death in Ross et al's study, but in Morrison's it is defined as cardiac electrical asystole. The hepatic injury alone therefore is highly lethal, but does not systematically effect a very severe arrest with little or no cardiac output in a consistent time interval. Previous evaluation by Manning has demonstrated that SAAP can resuscitate swine from a mean arterial pressure of less than 10

mmHg,[112] and in model development the hepatic injury alone resulted in blood pressures higher than this. Therefore, a controlled arterial haemorrhage was added to the protocol, starting five minutes after the hepatic injury.

The mean resulting volume of NCTH in this model was 1,838.2 (±921.7) ml, which is significantly lower than the NCTH volumes reported by Morrison et al, $p<0.001$.[101] However, the total haemorrhage (NCTH and controlled haemorrhage) was significantly higher than Morrison's NCTH volume, $p<0.05$. It is therefore likely that although the controlled haemorhage was required to effect a severe TCA in a consistent time frame, it reduced the rate of haemorrhage from the hepatic injury. The volume of NCTH in both Morrison's and these experiments are approximations. The NCTH cannot be reliably measured during the injury, and is collected retrospectively. The volume of blood in the abdomen may therefore be affected by the resuscitative interventions. For example, it might be expected that aortic balloon occlusion would reduce the total volume of NCTH by reducing hepatic arterial blood flow, whereas closed chest compressions may increase the total volume of NCTH through mechanical clot breakdown. In addition to this, animals received different volumes of different resuscitation fluids (FWB or lactated Ringer's solution). The largest volume of NCTH in these experiments was observed in the CPR group, and the smallest volume observed in the SAAP-FWB group.

The assumption that the intervention would affect the nature of the NCTH was further corroborated by the appearance of the haemoperitoneum in these 40 animals. Those in the SAAP-LR group had a large volume of blood-stained fluid

and sparse clot, whereas those in the REBOA and SAAP-FWB group had large well-formed clots, and the CPR group had large volumes of liquid blood. This was not quantified, but was consistently observed, and is in keeping with the physical properties of each intervention.

Previous publications of similar models of laparoscopic hepatic injury have not reported the time taken to complete the injury, only that it was completed in less than two minutes.[101,118] In my research, the injury was also completed within two minutes, per protocol, but was significantly faster in the REBOA group compared to the other groups. The REBOA group was added at the end of the protocol, and the time difference is likely to be due to an improvement of technical skill, having already completed more than 30 laparoscopic hepatic injuries. This difference does not appear to have had an effect on the model, as there was no significant difference between groups of the time from the start of the injury to the onset of TCA.

The resultant hybrid model of haemorrhagic cardiac arrest is unique in both the method used, and also the very severe physiological state achieved. At the onset of TCA, all animals had a systolic BP of less than 10 mmHg, and a cardiac electrical rate lower than baseline. The clinical definition of TCA is by necessity simple, and most commonly uses the lack of a palpable central pulse as a threshold.[41] This definition provides a binary output – dichotimising patients into those in TCA, and those not. However, haemorrhage is a spectrum of disease, and in haemorrhagic TCA there is a concept of patients in a low-output state in trauma (LOST) and those in a no-output state in trauma (NOST).[46] In

LOST there is no palpable pulse, but it is likely that there is some degree of cardiac output (and organised cardiac electrical activity) – these patients are salvageable with early and aggressive haemorrhage control and rapid high-volume fluid resuscitation. At the other end of the spectrum there is a no-output state in trauma (NOST), where there is no cardiac output, and either a brady-agonal or asystolic cardiac rhythm – haemorrhage control and resuscitation with fluid filling alone is unlikely to be successful in this group, but prior to this work there is no good evidence to support this.

The LOST and NOST concepts also encourage a simplified, binary output to the spectrum of haemorrhage. In addition to a fall in blood pressure, haemorrhage also leads to tachycardia, followed by an inappropriate bradycardia as the blood supply to the myocardium falls.[130] The ability to transduce beat-to-beat intra-aortic pressure in the laboratory gives a much higher fidelity of the state of haemorrhage, and cardiac output, compared to clinical practice. Therefore, this protocol included an observation (the cardiac electrical rate) that could be translated to clinical practice, and describe the point on the spectrum of haemorrhage of this model.

During the three minute arrest period, the mean systolic BP was 2.5 (±1.9) mmHg, with a mean left carotid artery flow of less than 1.0 ml/minute. The severity of this injury is therefore not comparable to any previously described large animal model. The cardiac electrical rate was measured over 30 seconds at the end of the arrest period, a mean of 43.0 (±31.3) beats/minute. The animals were therefore manifesting signs of myocardial depression as an inappropriate

bradycardia. Not all of the animals were in NOST, but 12 (30.0%) were, and were either in asystole or p-wave asystole. Clinically, asystole secondary to haemorrhage is regarded as almost universally non-survivable, with only a single case report of successful resuscitation with confirmed cardiac standstill.[131]

Markers of perfusion (pH, lactate, base deficit) were all altered at the time of arrest compared to baseline. The lactate and base deficit were both elevated, as would be expected during haemorrhage, and previously demonstrated in swine models.[96,101] However, the pH was also elevated compared to baseline, which was unexpected. This may be explained by the fact that the animals were mechanically ventilated throughout the injury, and were therefore unable to adapt their ventilation in response to reduced carbon dioxide production secondary to hypovolaemia and reduced tissue perfusion. Carbon dioxide and the formation of carbonic acid in the blood has a significant effect on pH, and the low levels of end-tidal carbon dioxide (less than 1.0 kPa) in these animals at arrest was the likely cause of the increase in pH observed during the arrest period.

Measuring tissue oxygenation by near infra-red reflectance spectroscopy during the injury was of limited value. At baseline, despite comparable partial pressures of oxygen and other markers of perfusion (systolic BP, left carotid artery flow, lactate, and base deficit) there was considerable variation in the tissue oxygenation. There was consistency between groups in the percentage fall in tissue oxygenation at the point of arrest, and the changes in tissue oxygen observed during each intervention were consistent with the physiological state

of the animals (measured by systolic BP, carotid artery flow, and end-tidal carbon dioxide). However, this was only calculable when the baseline tissue oxygenation for the individual animal was known. In the clinical trauma setting, this is unworkable as patients do not have tissue oxygenation monitoring in place prior to haemorrhage. Instead, other physiological markers can be more readily used, for example heart rate and blood pressure.

In summary, this hybrid haemorrhage model consistently produced a very severe injury that resulted in 30% of animals being in cardiac electrical asystole. It is the most severe haemorrhagic injury described in the literature, and is therefore not comparable to previous models used to evaluate REBOA. The model achieved the aim of a systematic highly-lethal injury, that was at the extreme of swine survivability.

8.3 Arrest period and intervention

8.3.1 Arrest period

As per the protocol, the intervention was started after a three minute arrest period, a mean time of 184.0 (±2.9) seconds. The few seconds of delay was caused by ensuring the intervention was ready, and was particularly difficult to achieve in the technically-demanding SAAP-FWB group. This arrest period was not used in previous large animal REBOA and SAAP experiments.[101,112] However, in model development, even after the hybrid haemorrhage model resulted in a systolic BP less than 10 mmHg, a rebound increase in BP was observed. This was controlled by ongoing haemorrhage at a reduced rate. During the arrest period the systolic BP and left carotid artery flow continued to fall, and a number of

animals went into cardiac electrical asystole. The inclusion of the arrest period therefore further increased the injury severity, and reduced the chance of spontaneous recovery.

8.3.2 Intervention infusions

The haemoglobin concentration of the FWB infusion was not significantly different between the CPR and REBOA groups. However, the SAAP-FWB infusion had a significantly lower haemoglobin concentration that the two other blood groups. The SAAP circuit required a small, unmeasured, volume of lactated Ringer's solution to prepare the circuit and remove gas. It is therefore likely that the lower haemoglobin was due to dilution with LR. The difference in mean haemoglobin was less than 1.0 g/dl, and would not be considered clinically significant, particularly as there are other aspects of the interventions, for example intravenous versus intra-aortic infusion, that are likely to have a considerably greater effect on outcome.

There was a significant difference in the pH of infusions between the CPR and REBOA group, and the SAAP-FWB group. The mean pH of the non-oxygenated FWB was less than 7.1, and can be partly explained by the acidity of the anticoagulants and preservatives (CPDA-1, AS-5) in the blood donation bags.[132,133] Glycolysis in red blood cells continues *ex vivo*, despite the bags being in cold storage, producing lactate, that further reduces pH. However, the mean pH of the oxygenated FWB infusion, in SAAP-FWB, was 7.5.

The concentration of haemoglobin in the SAAP-FWB infusion, together with oxygenation in the circuit, produced a mean calculated oxygen content of 11.3 ml of oxygen per 100 ml of infusion. Therefore, even though the SAAP-LR infusion had a comparable partial pressure of oxygen to the SAAP-FWB infusion, the oxygen content was significantly lower.

8.4. Primary outcome

Clinical data have demonstrated that the majority of deaths secondary to NCTH occur pre-hospital,[37] and that the median pre-hospital time in NCTH is 61 minutes.[119] These experiments were therefore designed to compare 60 minute survival in potential interventions for TCA secondary to NCTH. It was powered to detect a 50% difference in survival, with an alpha of 5%.

These experiments have demonstrated that SAAP with FWB infers a significant 60 minute survival advantage over SAAP with LR, REBOA with FWB, and chest compressions with FWB in severe haemorrhagic TCA in large swine. This is the first time that these interventions have been compared in a translational model, and is a novel finding.

The only previous published report of the use of SAAP in a large animal model of haemorrhage compared SAAP-LR and SAAP with a haemoglobin based oxygen carrier (HBOC).[112] This model used a less severe haemorrhagic injury (an arrest point of a mean arterial pressure less than 10 mmHg) compared to these experiments, and demonstrated an 83.3% 60 minute survival in the n=6 SAAP-HBOC group.[112] This is a comparable 60 minute survival in the SAAP-FWB group

of this research (83.3% versus 90.0%). However, in Manning et al's experiments none of the six SAAP-LR swine survived for 60 minutes after the intervention, compared to three (30.0%) in these experiments. This difference in difficult to explain – Manning used a similar membrane oxygenator, identical resuscitation fluid, and the same rate of infusion (ml/kg) as these SAAP-LR experiments. Manning does not report the total LR volume per kg infused, but according to the protocol it is likely to be a significantly higher volume than these experiments, and Manning administered intra-aortic epinephrine if initial SAAP-LR infusion was unsuccessful.[112] The inclusion of the SAAP-LR arm in this research was used as a control group, and the outcome was unexpected – this is further discussed below (Section 8.8).

The outcome in the REBOA group (0% survival at 60 minutes) was also unexpected. REBOA has not been previously evaluated in a translational model of severe haemorrhagic TCA, but has been shown to be an effective intervention in groups of swine with a mean systolic BP between 28 and 48 mmHg.[101] It was therefore expected that a proportion of REBOA group animals in these experiments would be successfully resuscitated with this intervention. REBOA provides haemorrhage control and increases afterload, but relies on a functioning myocardium to pump intravenously administered resuscitation fluid. In these experiments, the injury was so severe that it resulted in a failing myocardium, that was unable to respond even with REBOA and 2,452 ml of FWB. The REBOA group therefore acted as a direct comparator for the CPR group, that received the same volume of intravenous FWB together with external chest compressions, but without aortic balloon occlusion. The expectation was that

none of the CPR group would survive for 60 minutes, owing to the previously reported detrimental effect on CPP due to compressions in hypovolaemia,[67] together with the lack of haemorrhage control, further aggravated by mechanical clot disruption caused by compressions. However, one CPR animal did survive for 60 minutes, and was in cardiac electrical asystole before ROSC. The REBOA and CPR groups are further discussed in their relevant sections (Section 8.9 and 8.10 respectively).

8.5 Secondary outcomes

8.5.1 ROSC

A return of spontaneous circulation was defined as a systolic BP of at least 50 mmHg ten minutes after the start of the intervention (t13). This ensured that the effect of each intervention was sustained, and also allowed sufficient time for the effect of the intervention to be observed.

ROSC at t13 was observed in nine out of ten (90.0%) of the SAAP-FWB group, but the remaining animal had a ROSC during the initial SAAP infusion (but died by t13), and at post-mortem was found to have a particularly severe hepatic injury exacerbated by atypical vascular anatomy. Even if this one animal had had a ROSC at t13 there would still have not been a statistical difference with the ROSC rate in the SAAP-LR group (60.0%). Comparison of the ROSC rate at t13 between SAAP-FWB and other groups only demonstrated a statistical difference compared to the CPR and REBOA groups. However, the time to ROSC was significantly faster in the SAAP-FWB group, compared to the SAAP-LR group (a mean of 54.5 seconds compared to 109.5 seconds respectively). There were no

differences between the volume of infusion, the infusion partial pressure of oxygen, intra-aortic pressure, and left carotid artery flow achieved between SAAP-FWB and SAAP-LR. However, owing to the haemoglobin content in the FWB, the oxygen content of the infusion was significantly more than in the SAAP-LR infusion.

A further relevant difference between the infusions is the concentration of electrolytes (potassium and calcium) and pH. LR contains 4.0 mmol/l of potassium, and 1.5 mmol/l of calcium, and analysis of the infusions in the SAAP-LR group demonstrated a mean potassium of 3.7 (±0.1) mmol/l and a mean ionised calcium of 0.9 (±0.0). Therefore the LR infusion contained a near normal concentration of potassium, but a low level of calcium compared to swine reference values.[120] The low pH of the infusion will have increased the ratio of ionised calcium to total calcium, but evidently some calcium was still bound to lactate in this infusion. The low pH of the SAAP-LR infusion (a mean of 7.15) may have impaired myocardial performance,[134] and together with the low calcium concentration and oxygen content of the SAAP-LR infusion may explain the longer time to ROSC in these animals compared to the SAAP-FWB group.

Manning's previous SAAP-LR swine experiments used a method of continuous LR infusion at 10 ml/kg/minute until ROSC was observed, for up to 15 minutes.[112] In these experiments, when ROSC was observed in the SAAP-LR group it was close to the end of the two minute initial infusion, and all animals required the maximum per protocol additional LR volume of 1,750 ml via SAAP.

It is therefore possible that a longer initial SAAP infusion in the SAAP-LR group may increase the ROSC rate observed.

None of the REBOA group had a ROSC at any time; the injury was too severe to be responsive to aortic occlusion and high-volume FWB. However, two (20.0%) of the CPR group achieved ROSC at t13, and both had been in cardiac electrical asystole during the initial intervention (although not during the arrest period). As previously described, in this model of injury the REBOA group unintentionally acted as a control for the CPR group. Although in this sample size there is no statistical difference in ROSC between the two groups, it is possible that the addition of compressions had a positive effect on myocardial perfusion.

8.5.2 Physiological measurements at t13

The systolic BP, left carotid artery flow, end-tidal carbon dioxide, and tissue oxygenation measured at t13 are not independent of the proportion of animals in ROSC at this time point, but instead allow comparison of whole group physiological characteristics.

The SAAP-FWB group had the highest proportion of animals in ROSC at t13, and had the highest mean values in all four measurements. The mean systolic BP in the SAAP-FWB group was significantly higher than all other groups, but the left carotid artery flow was only significantly higher than the CPR and REBOA groups. The lack of statistical difference in left carotid artery flow between SAAP-FWB and SAAP-LR might be explained by the wide variance (including animals without a ROSC, and those with a ROSC at t13) of values in a small

sample. However, when left carotid artery flow was compared in these groups in only animals with a ROSC, there was no statistical difference (SAAP-FWB – 648.6 (±80.9) ml/minute, SAAP-LR – 575.5 (±92.9) ml/minute). The same was not true for systolic BP between SAAP-FWB and SAAP-LR in animals with a ROSC at t13 (SAAP-FWB – 142.0 (±25.2) mmHg, SAAP-LR – 103.3 (±18.0) mmHg, $p<0.01$. The relationship between carotid flow and intra-aortic was therefore not linear.

The low values of these four variables in the REBOA group was expected, as none of them had a ROSC. However, only two (20.0%) of the CPR group had a ROSC at t13, but the mean end-tidal carbon dioxide was not significantly different to either of the SAAP groups. There is latency in the rate of change of end-tidal carbon dioxide, and compressions were only paused for ten seconds before measurements were recorded, and this relatively high end-tidal carbon dioxide is therefore likely in part to be secondary to ongoing tissue perfusion effected by flow due to compressions. This is further discussed in the CPR section (Section 8.10).

8.5.3 Blood sample analyses at t13

In the same way as the physiological measurements at t13, the blood sample results have been compared in all animals between groups. However, owing to the lack of circulation in three animals, it was not possible to draw samples from one SAAP-LR group animal and two REBOA group animals.

The most marked differences between groups were that the SAAP-LR group had a significantly lower haemoglobin than the other groups (a mean value of 3.0

g/dl), and a very high lactate concentration (a mean of 13.2 mmol/l). Both of these differences can be explained by the nature of LR, that has a lactate concentration of 28 mmol/l and contains no haemoglobin. In normal physiology, lactate is metabolised in the liver producing bicarbonate and glucose, and excess lactate is excreted by the kidneys. However, in the SAAP-LR group there was no arterial flow to either the liver or the kidneys, resulting in a raised lactate concentration.

The partial pressure of oxygen in the SAAP-LR group was not significantly different to that of the SAAP-FWB group. However, the lower concentration of haemoglobin in the SAAP-LR group resulted in a significantly lower oxygen content compared to the SAAP-FWB group. This lower oxygen content would have a detrimental effect on oxygen delivery to tissues, and although not statistically different, the mean StO_2 (as a percentage of baseline) was lower in the SAAP-LR group compared to the SAAP-FWB group at t13.

8.5.4 Physiological measurements at t63

These values were reported for animals still alive at t63, and is arguably a more appropriate comparison than values at t13. In translation, these physiological values are those that might be expected in patients at their time of hospital arrival.

The SAAP-FWB group (n=9) had a significantly higher systolic BP, left carotid artery flow, and tissue oxygenation compared to the CPR (n=1) and SAAP-LR

(n=3) groups. This means that irrespective of survival, SAAP-FWB confers a better physiological state following TCA at the time of simulated hospital arrival.

The highest end-tidal carbon dioxide was observed in the single CPR group survivor (5.3 kPa). Once a stable minute volume via mechanical ventilation had been established at baseline, alterations were not made to the ventilation strategy. However, in the presence of intra-aortic balloon occlusion (both SAAP groups), less than half of the swine body is being perfused, and therefore the amount of carbon dioxide produced is reduced. This may be the explanation for the significantly higher end-tidal carbon dioxide in the CPR animal.

8.5.5 Blood sample analyses at t63

The high end-tidal carbon dioxide recorded in the CPR animal alive at t63 was corroborated with a significantly higher arterial partial pressure of carbon dioxide at t63 compared to the SAAP groups. The CPR animal also had a significantly lower lactate concentration and base deficit than the two SAAP groups at t63. This comparison provides some insight into the metabolic burden caused by Z1 aortic occlusion for 60 minutes.

The SAAP-LR group had a clinically (but not statistically significantly) lower glucose concentration at t63 compared to other groups; one SAAP-LR animal had a glucose of 1.9 mmol/l. In comparison, the SAAP-FWB and CPR groups both received FWB that had been preserved with CPDA-1 (which contains glucose), and the CPR animal had a vascularised liver capable of gluconeogenesis. The single CPR animal alive at t63 had the highest glucose of any of these thirteen

animals at this time point. Hypoglycaemia is well documented to have detrimental effects on the myocardium,[135] and may have affected the survival to t63 in the SAAP-LR group.

Further effects of LR resuscitation were observed in the clotting function of blood samples at t63. The SAAP-LR group had a significantly higher prothrombin time, and lower fibrinogen concentration than the SAAP-FWB group and the CPR animal. Comparison in functional clotting, using TEG, between SAAP-LR and SAAP-FWB animals at t63 demonstrated significant differences between maximum clot amplitude and clot elasticity. Despite the severe haemorrhagic TCA the TEG values in the SAAP-FWB group at t63 were all within one standard deviation of the mean of baseline values; the clot index was 2.6 at baseline, and 2.7 at t63. The benefits of FWB resuscitation in clinical haemorrhagic shock have not been well described, but in a retrospective study of 100 military patients who received FWB, Spinella et al demonstrated a significantly higher 30-day survival compared to a matched cohort of 254 who did not receive FWB.[136] This research supports the theory that FWB retains high levels of intrinsic clotting ability.

8.6 In-hospital observation period

Thirteen animals survived and were transitioned into a period of haemorrhage control, and intra-aortic balloon deflation and removal where relevant. The single CPR group animal had a systolic BP of 68.5 mmHg at t63, and required 4,750 ml of intravenous FWB during the 30 minute surgery period to maintain a

systolic BP of at least 90 mmHg, and both lactate and base deficit started to improve before t93.

All animals in the SAAP-LR (n=3) and SAAP-FWB (n=9) groups demonstrated physiological instability on deflation of the SAAP balloon. All of the SAAP-LR animals required the maximum volume of intravenous FWB (5,750 ml) in order to deflate the balloon, and one animal in this group died with the balloon still partially inflated. The SAAP-FWB group required a mean FWB volume of 3,889 ml to deflate the balloon, and one animal died shortly after the catheter was removed at t90. In the comparison of physiological variables, and markers of perfusion between t63 and t93 in the SAA-FWB group, there was a significant fall in the systolic BP, left carotid artery flow, and pH – despite receiving a mean of almost four litres of FWB. The resulting mean pH (7.10) in this group is likely to have had a detrimental effect on myocardial performance.[137]

8.7 SAAP-FWB group

The most unexpected finding in this study was the death of five animals in the SAAP-FWB group during the critical care period, after successful haemorrhage control and SAAP balloon catheter removal. In the first 15 minutes of the critical care period, there was no significant change in systolic BP or cardiac output, despite these animals receiving a further mean volume of 2,500 ml of FWB. Examination of the systemic vascular resistance and central venous pressure demonstrated that the SVR fell, and the CVP increased. This was accompanied by a high mean pulmonary artery pressure, that did not significantly change during this 15 minutes and was not associated with more than a mild impairment of

pulmonary gas exchange. These findings are consistent with acute right ventricular and left ventricular systolic dysfunction, together with systemic vasodilation (in the absence of significant pulmonary disease).

This cardiac failure is likely to have occurred secondary to a combination of the severe haemorrhagic arrest, the increased afterload of 75 minutes of proximal aortic balloon occlusion, and acidaemia.[137] There are no comparable studies that are as severe a model as this, but previous REBOA translational reports have examined the physiological tolerance of IABO. In 2012, Markov et al compared 30 and 90 minutes of balloon occlusion in large swine.[98] There were no animal deaths associated with deflation of the IABO, despite a peak in lactate to over 11 mmol/l in the 90 minute occlusion group. Furthermore, there was no statistical difference in serum troponin between the 90 minute IABO group and a no-balloon control group, and no histological evidence of myocardial ischaemia.[98] This is in contrast to a large swine model of IABO reported by Avaro et al, where there was a high proportion of animal deaths following deflation of IABO following 60 minutes of occlusion.[96] This model had a less severe haemorrahge model than Markov et al (an arrest point of 60 mmHg, compared to 33 (±8) mmHg), but histopathology demonstrated significant evidence of myocardial ischaemic injury compared to the 40 minute IABO group. However, Avaro et al concluded that the cause of the high number of deaths after balloon deflation was due to the high serum potassium observed.[96] Further data on cardiac function in a translational model has been reported by Morrison et al.[101] This study has already been described, and used a similar hepatic injury to this research, achieving a mean systolic BP in the 60 minute IABO group of 31 (±14)

mmHg. There was a significant rise in cardiac troponin between baseline and the end of the study, and the lowest pH was recorded after IABO deflation.[101] Furthermore, one animal (of seven alive at this time point) died after IABO deflation that was reported as "cardiogenic shock refractory to inotropic support" with intravenous norepinephrine.[101]

Cardiac histopathology and serum troponin were not collected in my experiments, so it is not possible to confirm the cause of these deaths following IABO deflation, but it is likely that there was significant myocardial failure, caused by a combination of the initial haemorrhage, prolonged increased afterload, and the acidaemia observed following reperfusion.

The initial response to resuscitation with SAAP-FWB in these large swine experiments is very positive. However, after IABO deflation, there was no statistical difference in point survival between SAAP-FWB, SAAP-LR, CPR and REBOA at the end of the protocol. Further large animal work could investigate the effects of occlusion and reperfusion on cardiac function, and investigate methods to mitigate this injury.

8.8 SAAP-LR group

Six (60.0%) of the SAAP-LR group animals had a ROSC, and three survived the simulated pre-hospital period. In order to confirm the appropriateness of the death-in-protocol criteria, the SAAP-LR animal that 'died' at t62 received a 500 ml bolus of intravenous FWB at t63 without an increase in systolic BP.

As previously explained there is only one other study on the use of SAAP-LR – Manning et al reported a ROSC rate of two out of six animals (33.3%), with a mean time to ROSC of 10.0 (±6.8) minutes.[112] Both the rate of ROSC, and the mean time to achieve ROSC are quite different to that observed in this research. Manning used a less severe model of haemorrhage, but an identical infusion rate per body weight to the animals in these experiments. One reason for these differences may be that Manning's definition of ROSC is a mean arterial pressure of greater than 60 mmHg, compared to a systolic BP of at least 50 mmHg used in these experiments. Manning et al used a similar oxygenator for the SAAP-LR infusion, but the partial pressure of oxygen in the infusion is not reported, nor is carotid artery flow. It is therefore possible that the increased rate of ROSC is due to higher oxygen content of the LR infusion, together with a definition of ROSC that uses a lower systolic BP.

Manning et al reported no survivors to 60 minutes after the intervention, using a similar death-in-protocol definition – a mean arterial pressure of less than 20 mmHg, compared to a systolic BP of less than 30 mmHg. However, three (30.0%) SAAP-LR animals survived in this research, and although the total volume per kg of LR is likely to be higher in Manning's study per protocol, it is not reported. It is therefore not clear why this difference in the efficacy of SAAP-LR was observed, and warrants further examination. The logistical burden of pre-hospital blood product is considerable, and the ability to resuscitate patients in NOST, at least initially, with lactated Ringer's is an attractive one. The mean time to ROSC in the SAAP-LR group was 110 seconds, only ten seconds before the end of the initial SAAP infusion – all animals then required the maximum

further volume of LR. Future large swine translational research should therefore evaluate whether a longer initial period of SAAP with LR improves 60 minute survival.

8.9 REBOA group

The severity of haemorrhage achieved in this model was too severe for IABO and high-volume intravenous FWB to be an efficacious intervention. The most interesting observation made in the REBOA group was after all animals had met death-in-protocol criteria – in a number of animals an increase in cardiac electrical rate was observed after a 50 ml intra-aortic flush of lactated Ringer's. This effect was observed even though there had been no evidence of cardiac output for at least 13 minutes.

The reason this observation has potential utility relates to the complexities of REBOA and SAAP. In clinical practice, insertion of a REBOA catheter is a relatively rapid process, that has been successfully undertaken pre-hospital.[138] However, SAAP requires oxygenated resuscitation fluid in an infusion circuit, and if used with a citrated blood product the careful titration of calcium chloride, and will inevitably take longer to commence than REBOA. This, together with the fact that there are no defined clinical criteria that determine which patients can be resuscitated with REBOA, and which need active endovascular intervention, means that a clinical paradigm might start with REBOA and intravenous filling, and then progress to SAAP if there is no ROSC. This paradigm could be tested in a future translational large swine model.

8.10 CPR group

The CPR group was included as a control for REBOA and SAAP interventions, and also to describe the effect of closed chest compressions in haemorrhagic arrest. As previously described, at the start of this project the only published data on compressions in hypovolaemia was a study of three baboons by Luna et al in 1989.[67] However, in 2016 Jeffcoach et al published an evaluation of closed chest compressions in hypovolaemic canines.[68] The investigators effected a controlled arterial haemorrhage in 17-25 kg dogs until a palpable central pulse was lost – blood pressure was not reported, but the haemorrhage was approximately 45 ml/kg. The dogs were then allocated to one of three groups for a 20 minute resuscitation period: chest compressions alone (with a pulse check every two minutes), fluid alone (20 ml/kg of lactated Ringer's solution repeated after five minutes, followed by 20 ml/kg of whole blood five minutes later, again repeated five minutes later – a total of 80 ml/kg of resuscitation fluid), and a combination group of CPR and fluid (as per the above). Four out of six (67%) dogs in the CPR group survived 20 minutes, compared to all (100%) of the other two groups. There was no evaluation of CPP, or central BP, and Jeffcoach concludes that compressions may not be of benefit in hypovolaemia.[68] The utility of this study is limited – it is both logical and previously understood that restoring circulating volume in a model of controlled haemorrhage with ongoing cardiac output is effective in resuscitation.[67] Previous case reports have also highlighted that patients in a low-output state in trauma can be resuscitated with fluid alone.[45] The real unknowns in this area of medicine are whether compressions are of benefit together with fluid resuscitation in NOST, the effect of compressions on

CPP in severe haemorrhagic arrest, and whether compressions are detrimental to intrinsic haemostasis in NCTH.

The first and last of these questions can be informed by these experiments. The key findings were that closed chest compressions and high-volume intravenous FWB are not an effective intervention in a swine model of severe haemorrhagic TCA, secondary to a hybrid of NCTH and a controlled arterial bleed – two (20.0%) animals had a ROSC, and only one (10.0%) survived for 60 minutes. However, this was a higher ROSC rate and survival to the REBOA group, albeit this did not reach significance, although this may be due to a type 2 error in a small sample.

The REBOA group was not intended to act as a control group for the CPR group, but the animals received the same volume and type of resuscitation fluid, with the only differences being that one group received closed chest compressions, and the other IABO. The observation that both CPR group animals that had a ROSC did so after periods of cardiac electrical asystole is important in the consideration of why compressions may have improved observed survival over the REBOA group. Although Luna et al demonstrated that compressions reduced the diastolic BP in hypovolaemia (and therefore likely reduced the CPP),[67] compressions in the presence of no cardiac output and no coronary artery flow, may have produced some flow in these animals. However, further data are required to make this assertion.

Both animals had received over 1,000 ml of FWB (12.5 ml/kg) when in cardiac electrical asystole, and had intra-aortic BPs of 55/22 and 55/15 mmHg during compressions prior to a return of cardiac electrical activity. It would be reasonable to assume that in the absence of compressions the intra-aortic BP would be 0/0 mmHg. However, in the REBOA group, without cardiac output, the mean BP was a maximum of 19.0/16.4 mmHg at the end of the intravenous infusion of 2,452 ml of FWB. In the absence of cardiac output, the mechanism by which the intravenous infusion increased the intra-aortic pressure is unclear. It is possible that increasing the intra-thoracic volume by 2,452 ml effected a global increase in intra-thoracic pressure, that exerted external pressure on the aorta. However, the increase in mean pulmonary artery pressure in the REBOA group during the 2,452 ml infusion of FWB suggests that the high-volume infusion alone prompted flow of blood through the low-resistance pulmonary circulation, and into the left side of the heart. This is further corroborated by the time difference between the increases in pressure between the pulmonary artery and the aorta observed in the REBOA group.

The exact mechanism by which compressions effect the flow of blood is not completely understood, but is likely to be a combination of the thoracic pump model (where changes in intra-thoracic pressure effect flow) and the cardiac pump (where compressing the heart effects flow). Either way, in the two CPR group animals that had a ROSC, there was sufficient blood volume and pressure to produce an intra-aortic diastolic pressure of at least 15 mmHg during compressions. The previous study by Luna et al, investigated the effect on diastolic pressure during compressions with a low pre-load. The limited data

from my study suggests that with an increased pre-load (by high-volume intravenous filling) it may be possible to achieve a diastolic BP that is commensurate with ROSC with compressions. It also suggests that this intervention may be more likely to result in a ROSC following severe haemorrhagic TCA than REBOA with high-volume FWB, but further data are needed.

The most notable difference between the CPR group animals with a ROSC, and those without (n=8) is that the mean diastolic pressure did not increase above 5 mmHg in those without a ROSC. It is not possible to calculate the CPP, but it is likely that it was lower than 5 mmHg in the animals without a ROSC. The cause of the difference between these two subsets is not clear. However, it is clear that while compressions with FWB infusion increased the mean systolic BP to over 70% of baseline, the left carotid artery flow only increased to 10% of baseline. Therefore, in this setting, compressions were ineffective at producing adequate carotid artery flow.

Chest compressions have recently been de-emphasised in the management of haemorrhagic TCA.[41,45,46] The reasons for this are the recognition that in LOST there are other interventions that take precedence, for example high-volume intravenous filling, and that compressions in LOST may reduce coronary perfusion. However, in the clinical setting of NOST (potentially differentiated from LOST by an inappropriate bradycardia, a brady-agonal, or asystolic cardiac electrical rate) intravenous filling alone is unlikely to produce a ROSC if there is no flow (as seen in the REBOA group). In the single case report of successful

short-term resuscitation from cardiac electrical asystole with confirmed cardiac standstill secondary to haemorrage, compressions (external and internal) were used together with high-volume intravenous filling.[131] The findings in the two CPR group animals with a ROSC suggest that clinically, it might be appropriate to augment high-volume intravenous filling with compressions in NOST.

The effect of compressions on the NCTH can only be estimated by comparison with the REBOA group. The volume of NCTH was slightly higher in the CPR group, compared to the REBOA group, 29.4 (±11.1) ml/kg and 26.6 (±11.9) ml/kg respectively, although this was not statistically significant, p=0.60. Therefore, despite the observation that the CPR group had a large volume of liquid haemoperitoneum, there was no evidence that compressions had a negative effect on haemostasis in this model. This injury, albeit severe, uncontrolled, and mixed arterial and venous, has limited utility in translation of the effect of compressions on clinical NCTH, and it is possible that more widespread NCTH would be adversely affected by compressions.

8.11 Clinical translation

This model of injury was designed as a translational study, in which to compare four different interventions for the management of severe haemorrhagic TCA. It is the first study to include animals in NOST secondary to haemorrhage, and replicates a clinical situation that is widely reported as almost universally fatal.

It would be appropriate to suggest that REBOA in a very severe haemorrhagic arrest is not an effective intervention. The challenges in defining NOST in the

clinical setting have been discussed, but it might be differentiated from LOST by evaluation of cardiac electrical rate. The clinical use of chest compressions is widespread, and this research suggests that they are frequently ineffective in achieving a ROSC, even with high-volume FWB infusion. However, in the setting of NOST, and once intravenous filling has started it might be appropriate to consider the use of chest compressions in order to effect some intra-thoracic flow. The combination of aortic balloon occlusion, intravenous filling, and compressions has not been studied, but might present a potential clinical solution.

However, these two interventions are inferior to SAAP in the setting of NOST. It would be appropriate to suggest that SAAP with oxygenated blood product is an effective short-term intervention in NOST patients. However, the rate of death on balloon deflation questions the clinical utility of this experimental intervention, that may be dependent on occlusion time. It is possible that currently available in-hospital endovascular intervention that can support a failing myocardium, for example extra-corporeal life support, may provide a clinical solution to the physiological burden effected by SAAP intervention in my research.

8.12 Conclusion – Part One

A dedicated swine blood donor pool can provide large volumes of FWB over time without a significant drop in haemoglobin concentration, and may reduce the total number of animals required. This hybrid model of NCTH and controlled arterial haemorrhage resulted in a highly-lethal injury, with 30% of large swine

in a no-output state at the point of arrest, in a short time period. SAAP with FWB conferred a superior short-term survival over SAAP with LR, REBOA with IV FWB, and CPR with IV FWB in this swine model of severe haemorrhagic TCA. SAAP can achieve ROSC even from cardiac electrical asystole, but the physiological burden of this intervention resulted in no significant difference between interventions at the end of the study period. There is very limited evidence that CPR with high-volume FWB infusion might be effective in cardiac electrical asystole. Further evaluation of SAAP is required before considering clinical implementation.

PART TWO

CHAPTER NINE

MITIGATING THE EFFECTS OF BALLOON OCCLUSION

9.1 The physiological effects of IABO

The swine experiments described in Part One demonstrated that SAAP-FWB was an effective short-term intervention for severe haemorrhagic TCA. However, the majority of animals died within an hour of balloon deflation, despite further large volumes of intravenous FWB. The physiological measurements made during the initial period of reperfusion suggest that the cause of these deaths was acute right ventricular failure and left ventricular systolic dysfunction, without significant impairment of pulmonary gas exchange, in the presence of significant acidaemia and a falling systemic vascular resistance.

Cardiovascular collapse on deflation of IABO has been described clinically,[103] and in large animal models.[95] There are multiple physiological and metabolic effects that may cause this, including a drop in cardiac afterload, and the release of metabolites into the circulation on reperfusion.[96,98,101] Additionally, Avaro et al demonstrated histological evidence of myocardial ischaemia, and Morrison et al demonstrated biochemical evidence of myocardial ischaemia following IABO deflation in large animal models.[96,101] It is however not clear from these studies if myocardial injury occurs secondary to the initial haemorrhagic injury, the increased afterload caused by the IABO, by the effect of washout of toxic metabolites, or a combination of these factors.

It is both logical, and has been demonstrated in large animal models, that longer occlusion times are associated with increased evidence of myocardial ischaemia, increased serum lactate, and reduced survival.[96,98] The longest reported duration for clinical Z1 IABO is 70 minutes; in this case there were no reported sequalae of balloon deflation.[104] A more recent analysis of 96 REBOA cases from six countries included 21 patients who had over 60 minutes of IABO. The authors do not specifically comment on the effects of balloon deflation, but do report that multiple organ failure was observed in 35% of the reported cases.[139] Retrospective observational data from the Japanese Society of Diagnostic and Interventional Radiology in Emergency, Critical Care and Trauma (DIRECT) has reported an association between occlusion time and survival. Between 2011-2015, 142 patients received REBOA for refractory haemorrhagic shock (a median pre-REBOA systolic BP of 60 mmHg). There was a significant difference in total occlusion time between survivors and non-survivors at 24 hours (34 minutes versus 78 minutes, $p<0.001$).[140] However, it is possible that patients requiring a longer occlusion time had more a more severe injury, potentially confounding this observation.

As demonstrated by White et al, IABO results in less physiological disturbance than open aortic cross clamping in large swine.[95] However, the clinical technique of manually compressing the thoracic aorta to control NCTH is over 40 years old,[128] and as such provides some insight into the physiological and metabolic effects of proximal aortic occlusion and subsequent reperfusion that is relevant to IABO. Furthermore, in recent history, prior to the advent of endovascular

aortic aneurysm repair,[141] open surgical repair with proximal aortic control also provides some data.

9.2 Myocardial dysfunction

In 1912, Anrep demonstrated that proximal aortic occlusion in a canine model resulted in acute dilation of the left ventricle.[142] It has also been shown that the dilation persists for approximately 30 minutes, and that an increase in cardiac contractility effects a return to normal left ventricular pressure.[143] However, during the initial period of aortic occlusion, the dilation of the left ventricle in another canine model led to under-perfusion of the subendocardial layers of the left ventricle.[144] This phenomenon has been characterised in a clinical study, showing that Z1 aortic occlusion led to left ventricle wall motion abnormalities in four (33%) of twelve adult patients.[145] This has not been examined in the setting of IABO, but is likely to be an important factor in the cause of myocardial ischaemia observed in large animal IABO studies.[96,101]

9.3 Ischaemia and reperfusion injury

Hypoxia and ischaemia distal to aortic occlusion leads to vasodilation, that is partly responsible for the hypotension observed on reperfusion. The acidaemia and vasodilation is further compounded by an increased production of carbon dioxide in tissues that are perfused after removal of an aortic clamp.[146] However, the physiological and biochemical effects of aortic occlusion are more complex than this simple explanation, and the collective disease processes are termed ischaemia and reperfusion injury.

Ischaemia and reperfusion injury is a well recognised complication of aortic cross clamping, and has previously been described as 'aortic declamping injury'.[147] Translational and clinical research have demonstrated multiple mechanisms for this disease process, including the release of pro-inflammatory mediators (IL-6, IL-8, and TNF-α), an increase in neutrophil activation and leucocyte count, the release of thromboxane, elevated levels of hypoxanthine, and complement activation (C3a, C5a). IL-6 and TNF-α are both associated with the development of the acute respiratory distress syndrome,[148] that is compounded by the accumulation of thromboxane and leucocytes in the lungs,[149,150] and an increase in pulmonary vascular permeability caused by an increase in C3a and C5a complement.[151] Furthermore, complement may independently cause myocardial depression, and vasodilation.[152] During ischaemia, hypoxanthine is metabolized from ATP, and causes cell damage through the production of reactive oxygen species,[153,154] and may also cause myocardial depression.[155] During aortic clamping, the up-regulation of the renin-angiotensin system is a protective renal mechanism, leading to increased renal vascular resistance.[156,157] However, this reduction in renal perfusion continues after the aortic clamp is removed, and may be partly responsible for the high prevalence of acute tubular necrosis observed following aortic occlusion.[158]

The effects of aortic occlusion are complex, but potentially result in dysfunction of several organs, including the cardiovascular, pulmonary, and renal systems.

9.4 Strategies to mitigate the effects of IABO

Due to the nature of the clinical indication for IABO (the control of exsanguinating NCTH), there are no published guidelines on the maximum occlusion time, so instead IABO is used until haemorrhage control can otherwise be achieved. One strategy to mitigate the effects of IABO deflation is to minimise the occlusion time – this depends on the efficiency of the trauma system, but several studies have examined the feasibility of intermittent occlusion.

9.4.1 Intermittent IABO

Morrison et al compared two groups of eight large swine, that received either continuous IABO for 60 minutes, or intermittent IABO – where the balloon was deflated for one minute, 20 and 40 minutes into the 60 minute occlusion period.[101] There was no statistical difference in the survival between groups, and one animal in each group died after IABO deflation. However, there was a trend towards lower serum troponin in the intermittent occlusion group compared to the continuous occlusion group – 1.8 (±1.5), and 2.9 (±1.5) ng/ml respectively. Both of these findings are limited by the small sample size, potentially producing a type 2 error. It would be logical to assume that intermittent deflation of an IABO would result in further haemorrhage, and a requirement for more resuscitation fluid compared to continuous REBOA. In Morrison et al's swine study there was no statistical significance in NCTH volume or resuscitation fluid volume between intermittent and continuous REBOA.[101] The reason for this is likely to be that the period of deflation was very short, and as the authors state, longer periods of deflation were not tolerated. There are no other large animal reports of intermittent IABO.

Despite no good translational evidence of benefit, there have been clinical reports of the use of intermittent IABO. In a case series previously described, Sadeghi et al report the use of 'non-continuous' IABO in 46 (48%) of 96 IABO patients.[139] The authors use the term non-continuous IABO for a variety of inflation methods, including partial occlusion. There was no mortality difference between groups, and the only reported statistical difference was a lower systolic BP after IABO in the non-continuous group compared to the continuous IABO group. The difference in BP after IABO may be an important factor in the myocardial injury associated with IABO, but more evidence is needed. Furthermore, as demonstrated in Morrison et al's swine model, the opportunity to use intermittent IABO is dependent on the physiological state incurred when the balloon is temporarily deflated.[101]

9.4.2 Partial IABO

By avoiding rapid changes in BP, cardiac afterload and left ventricle dilation, and allowing some distal perfusion, partial occlusion of the aorta provides a potential superior solution to complete IABO. Russo et al compared partial IABO to complete IABO in a large swine model of controlled haemorrhage.[159] In the partial inflation group (n=5), the balloon was inflated to effect a 50% proximal to distal aortic pressure gradient, that was continuously monitored and adjusted. In the complete IABO group (n=5) the balloon was inflated to maintain complete occlusion. Both groups of animals were observed for 90 minutes of IABO, and for 15 minutes after balloon deflation. One (20%) of the complete IABO group died immediately after balloon deflation, compared to none of the partial IABO group. Partial IABO resulted in a significantly higher mean pH, lower serum lactate, and

lower histological evidence of small bowel and renal injury compared to the complete IABO group.[159] These differences in metabolic burden are likely to reduce the detrimental effects of reperfusion, and may therefore also increase the potential duration of occlusion. In Russo et al's model of controlled haemorrhage, the rebound BP on complete IABO produced a mean arterial pressure of 222 mmHg in one animal. As stated by the authors, this supranormal BP and cardiac afterload is likely to cause myocardial injury, but the magnitude of this has not been studied. The major limitation of this animal study is that it uses a controlled haemorrhage, as such there is no assessment of the potential for partial IABO in the setting of NCTH. Its practical use therefore is likely to be limited to in-hospital use, when there is little constraint on available resuscitation fluid. The previously described Japanese Society DIRECT observational report also included an analysis of patients who underwent partial occlusion. They observed a significantly better haemodynamic response in patients who received partial (n=78) compared to complete occlusion (n=37), and a longer total occlusion time.[140] However, there was no difference in 24-hour or 30-day survival between the two groups. This finding is complicated by the fact that all patients initially received complete occlusion, and if they became haemodynamically unstable during partial occlusion they were once again fully-occluded. Therefore, the patients self-selected for either partial or complete occlusion based on their response to REBOA (and likely their physiological state), and further conclusions cannot be made.

Owing to the concerns about the detrimental effects of reperfusion following complete IABO, a clinical guideline has been published: Johnson et al state that

partial IABO can be considered when "proximal hemodynamics suggests resuscitation reserves are adequate to support titrated distal perfusion".[160] This logic may be flawed – BP and carotid flow typically increase to supranormal levels after complete IABO, even in cases of severe hypovolaemia, that may give the impression of adequate resuscitation reserves.[95,98,101] This also highlights an inherent difficulty when selecting the correct patients for IABO in the first instance. There is no clinical test or physiological variable that determines whether a patient with NCTH will maintain a cardiac output, or die without the use of IABO. However, as discussed, IABO is not without considerable, and currently poorly understood, risk. The same argument is not as strong for the use of active endovascular intervention (for example SAAP) in patients already in a NOST, as their clinical condition is almost universally fatal. Therefore, in this partial IABO clinical guideline, the provider is asked to judge whether deflation of the balloon is feasible, despite the likely increased BP and carotid flow observed with complete IABO. The risks of balloon deflation in the wrong circumstances are self-evident: compromising clot integrity, and allowing further haemorrhage.

This situation is further complicated in the pre-hospital or emergency setting, where measuring invasive BPs above and below the IABO would be challenging, and with the knowledge that there is not a linear relationship between the degree of occlusion and the balloon volume.[161] The benefits of partial versus complete IABO in reducing myocardial ischaemia are only theoretical, but are likely to be compromised by the initial use of complete occlusion in Johnson et al's guideline and the Japanese Society's DIRECT report. Further research is

required to determine both the correct patient group, and the optimal risk benefit ratio of partial IABO.

9.4.3 Acidaemia

No large animal IABO studies specifically describe the change in blood pH on IABO deflation. The results described in Part One show that pH decreased after IABO deflation from a mean of 7.35 (±0.05) to 7.10 (±0.03) in the five animals in the SAAP-FWB group that subsequently died in the critical care period. This is more severe than the lowest mean pH of 7.17 (±0.10) reported by Morrison et al in their swine study of IABO after balloon deflation, but likely represents the same ischaemia and reperfusion phenomenon.[101]

Several other animal studies have reported the presence of hyperkalaemia after IABO deflation.[96,98,162] Potassium is released into the serum by cells damaged by ischaemia, and is an important factor in reperfusion injury. Morrison et al report the use of intravenous insulin and dextrose to manage hyperkalaemia.[101] This is a widely accepted clinical technique that works through promoting the translocation of sodium potassium ATPase, but maximum effect takes between 45 and 60 minutes and there is a risk of precipitating hypoglycaemia,[163,164] itself detrimental to myocardial performance.

However, serum potassium concentration is modulated by a number of factors, including pH.[165] Although pH was not reported in these three animal studies, it is possible that hyperkalaemia was in part due to acidaemia associated with reperfusion. Acidaemia has multiple detrimental effects on the cardiovascular

system – impairing myocardial function (by inhibiting calcium ion influx to the cardiac myocytes) and causing vasodilation.[134,166] Historically, bicarbonate has been used as a buffer, but this has been shown to paradoxically lower intracellular pH secondary to the production of carbon dioxide.[166] Carbon dioxide concentration can be rapidly controlled by ventilation. Increasing minute volume will result in a reduction of plasma carbon dioxide concentration, and a respiratory alkalosis. The increased mortality and morbidity associated with high tidal volumes is well documented,[167] but a potential strategy could include increasing the rate of ventilation with a low tidal volume. This may have limited effect, particularly in the setting of pulmonary dysfunction secondary to reperfusion injury, but could be safely started before IABO deflation.

9.4.4 Blood purification

Previous large swine models of haemorrhage have demonstrated increased serum IL-6, IL-8, and TNF-α following balloon occlusion, that was dependent on total occlusion time[101,168] The authors correlated this finding with the trend of increased norepinephrine requirements to maintain BP in 30 and 60 minute IABO compared to 90 minute.[168] Furthermore TNF-α is strongly associated with pulmonary injury following aortic occlusion.[148] Theoretically, reducing the concentration of pro-inflammatory mediators in IABO may mitigate some of the negative effects of aortic occlusion. This has not been examined in the setting of aortic occlusion, but extra-corporeal blood purification, with the aim of filtering pro-inflammatory mediators, has been used experimentally in the management of sepsis for over a decade.[169]

There are two techniques currently under development (haemofiltration and haemadsorption).[169] High-volume haemofiltration (HVHF) has demonstrated improved haemodynamics and survival in the management of septic shock and in survivors of cardiac arrest.[170,171] HVHF involves haemofiltration of between 50 and 120 ml/kg/hour, and is thought to have two actions in the reduction of circulating pro-inflammatory mediators: convection across the membrane, and adsorption by the membrane. Despite the documented haemodynamic benefit of HVHF in pro-inflammatory states, the pathophysiological effects are not completely understood.[172] Haemadsorption uses a range of extra-corporeal compounds (polymyxin B, biopolymers, synthetic resins) that adsorb pro-inflammatory mediators (cytokines and or endotoxins).[169] Cytosorb (Cytosorbents Medical Inc., Monmouth Junction, New Jersey, USA), is the only extra-corporeal cytokine adsorber approved for use in Europe. This product utilises an extra-corporeal blood volume of 150 ml, allows flow rates of between 100 and 700 ml/minute, and data from an international registry of 198 patients in sepsis or post cardiac surgery has demonstrated its safety and potential to improve survival.[169]

Both of these techniques are experimental, although both have been used clinically despite limited evidence of benefit. Additionally, both techniques are easily integrated into other extra-corporeal interventions (for example extra-corporeal membrane oxygenation), and therefore warrant further investigation into their use following aortic occlusion.

9.4.5 Pharmacological adjuncts

Two pharmacological adjuncts, hydrogen sulphide (H2S) and angiotensin converting enzyme inhibitors (ACE-i), have demonstrated potential to mitigate some of the adverse effects of aortic occlusion.

H2S minimises the activation of the systemic inflammatory cascade associated with ischaemia and reperfusion injury.[173] In addition, Causey et al used a swine model of controlled haemorrhage to demonstrate that administration of H2S during 50 minutes of Z1 aortic occlusion led to significantly lower mean arterial pressures compared to a control group, and that the animals who received H2S required less resuscitation (defined as total fluid and inotrope requirements) following reperfusion.[174] The authors suggest that H2S may be beneficial in aortic occlusion both by reducing cardiac stress during occlusion and by reducing the activation of pro-inflammatory mediators during reperfusion.

The renin-angiotensin system controls BP and tissue perfusion.[175] Both renin and angiotensin levels are increased during aortic occlusion above the renal arteries, and may contribute to the hypertension observed during aortic occlusion.[156,157] Hong et al used a rat model to demonstrate that pre-treatment with enalapril, an ACE-i, resulted in significantly less hypertension during aortic occlusion than controls.[176]

The value of either of these interventions in the setting of IABO is unknown, but the limitation of the ability to pre-treat trauma patients before aortic occlusion

may limit the applicability of ACE-i. The current data on H2S warrants further investigation into its potential to mitigate some effects of IABO.

Morrison et al used boluses of intravenous norepinephrine during a critical care period in their swine REBOA experiments.[101] The specific indication was not explained, but reported as "refractory to volume repletion". The authors report one animal death that was thought to be secondary to cardiogenic shock, refractory to norepinephrine, and although not fully described, has similarities to the five SAAP-FWB animal deaths in my experiments. The use of vasopressors in the early management of haemorrhagic shock is controversial. Despite large animal translation data that demonstrates some benefit of vasopressors, specifically vasopressin over placebo,[177] a retrospective analysis of 921 blunt trauma patients demonstrated an 80% higher risk of mortality in those administered vasopressors (including vasopressin and norepinephrine) within 12 hours of injury compared to those who did not. Conversely, early aggressive intravenous volume loading was independently associated with a 40% reduction in mortality.[178] It is likely that norepinephrine was used, at least in part, in Morrison et al's study to keep animals alive to the end of the protocol in the absence of a limitless supply of blood product.

9.5 Summary

From the available large animal and clinical data, it is clear that IABO has a significant physiological effect during and after occlusion. Increased afterload and left ventricular dilation are the likely causes of myocardial ischaemia, and the release of potentially toxic chemicals (potassium, carbon dioxide, and

inflammatory mediators) into the systemic circulation on balloon deflation are the likely cause of myocardial dysfunction, pulmonary injury, and vasodilation. There are numerous potential strategies to mitigate these effects; pharmacological adjuncts (H2S and ACE-i), partial or intermittent IABO, blood purification, aggressive mechanical ventilation and vasopressors. However, none of these strategies have either a proven benefit in reducing organ dysfunction in the setting of IABO, or target every negative effect of IABO. Extra-corporeal life support is an endovascular intervention that provides both exogenous oxygenation and vascular flow, and as such has the potential to mitigate the known negative effects of IABO.

CHAPTER TEN

EXTRA-CORPOREAL LIFE SUPPORT

10.1 ECLS introduction

Extra-corporeal life support (ECLS) provides mechanical cardiopulmonary support via an external circuit that includes a membrane oxygenator and pump. Its primary use in clinical practice has historically been in the management of severe pulmonary dysfunction in the neonate, but more recently this has evolved into the use of ECLS in providing both pulmonary and cardiac support in patients of all ages. The experimental intervention of SAAP, described in previous chapters, has some similarities with ECLS, and indeed may be considered a type of limited duration extra-corporeal life support. The adverse effects of IABO primarily result in pulmonary and cardiovascular dysfunction that may be managed with ECLS. It is therefore a logical extension to SAAP intervention, but its efficacy in the setting of mitigation of IABO has not been previously examined. This chapter describes the historical development of ECLS, the main components and considerations of use, the literature pertaining to translational models of ECLS in trauma, and the recent clinical evidence.

10.2 The historical development of ECLS

The invention of the 'heart-lung' machine, and its first successful clinical use in 1953 to effect total cardiopulmonary bypass, revolutionised cardiac surgery.[179] However, the direct method of blood oxygenation damaged red blood cells, limiting the duration of therapy to a maximum of one hour.[180] In the 1960s, the

development of silicone with its properties of gas permeability enabled future innovators to produce a membrane oxygenator that mitigated the earlier issues with blood cell damage. In 1971, this work culminated in the successful use of a heart-lung machine for severe ARDS secondary to trauma for 75 hours.[181] The results of the first randomised clinical trial of extra-corporeal membrane oxygenation (ECMO) were published in 1979.[182] A total of 90 adult patients at nine hospitals with severe respiratory failure were randomised to either conventional mechanical ventilation or ECMO. Four patients in each group survived, leading the authors to conclude that ECMO can provide artificial oxygenation, but does not increase survival.[182] These findings slowed the progression of ECMO development in adults, but in 1982 Bartlett et al reported a 56% survival following the use of ECMO in 45 neonates with presumed lethal respiratory failure.[183] This was followed in 1988 by a larger clinical study, demonstrating survival in 81% of 751 neonates with severe respiratory failure, who received ECMO in 18 different hospitals.[184] This culminated in two randomised clinical trials that demonstrated the effectiveness of ECMO in the management of severe respiratory failure of the neonate.[185,186] ECMO is therefore currently a well-established intervention in neonates with severe pulmonary dysfunction.

Despite several promising case reports of the use of ECMO in adults with severe pulmonary dysfunction, the 1979 clinical trial stalled further development. Retrospective scrutiny of this early trial suggests that a number of factors, including over-heparinisation and mechanical ventilation alongside ECLS, resulted in the finding of no survival advantage.[187] Further developments in

critical care (for example the recognition of ventilator-associated lung injury),[188] and technological advances in ECMO (for example the use of non-microporous polymethylpentene oxygenator membranes),[189] led to two clinical trials that demonstrated the benefit of ECMO in adults.[190,191] As a result, ECLS is now a recognised intervention for cardiopulmonary support in patients of all ages, with an ever-increasing list of indications, including refractory cardiogenic shock, medical cardiac arrest, massive pulmonary embolism, accidental hypothermia, drug overdose, and septic shock.[192]

10.3 The concept of ECLS

10.3.1 Overview

The main components of ECLS are a membrane oxygenator (to diffuse carbon dioxide out of, and oxygen in to the blood), a pump (to effect flow), tubing (to make an extra-corporeal circuit), and cannulae (to draw blood out, and infuse blood into the patient).[187] There are two main ECLS configurations – venovenous (VV), in which blood is drawn from and returned to the venous system, and venoarterial (VA), in which blood is drawn from the venous system and returned to the arterial system. VV provides pulmonary support, by providing exogenous gas exchange, without cardiac support. VA provides both pulmonary and cardiac support, by providing exogenous blood gas exchange and by working in parallel to the heart – pumping blood from the central venous circulation into the aorta.[193] In mitigating the effects of IABO (pulmonary and cardiovascular dysfunction), VA-ECLS is likely to be a more effective intervention than VV-ECLS, and this approach is further described.

Differing terminology is used with reference to VA-ECLS, and it is often described as VA-ECMO. As the intervention provides cardiac support and exogenous oxygenation, the term ECLS is used throughout this thesis to indicate venoarterial extra-corporeal life support / membrane oxygenation, but it should be noted that the terms are interchangeable.

10.3.2 ECLS cannulae

There are two cannulae placement options in ECLS. Cannulation can be central – the venous cannula is inserted into the right atrium, and the arterial cannula is placed in the proximal ascending aorta. This approach requires a thoracotomy, and is therefore usually only used following open cardiothoracic surgery.[192] The other cannulation option is peripheral – typically, the femoral vessels are accessed through either a percutaneous or open incision, and then, using the Seldinger technique, the venous cannula is advanced to the vena cava at the level of the right atrium, and the arterial cannula is advanced to the level of the aortic birfurcation.[193]

The size of cannulae is an important consideration. The resistance to blood flow is directly proportional to the length of the cannula, and inversely proportional to the fourth power of the radius – the internal diameter therefore has the greatest impact on flow.[194] In adult patients a suitable arterial cannula is between 17 Fr and 19 Fr (an internal diameter of 5.7 mm to 6.3 mm), and a suitable venous cannula is between 21 Fr and 23 Fr (7.0 mm to 7.7 mm).[195] The length of the cannulae is dictated by the distance from the insertion site to the level of the right atrium for the venous cannula, and the distance to the aortic

bifurcation for the arterial cannula – the venous cannula is therefore longer, and they are typically 550 mm and 150 mm in length respectively.[195]

10.3.3 ECLS gas exchange

The membrane oxygenator acts as the patient's artificial lung, and is therefore required to oxygenate the blood and remove carbon dioxide. Membrane materials and structure have evolved over time, and modern non-microporous polymethlypentene membranes have been demonstrated to provide more efficient gas exchange and less blood cell damage than silicone membranes.[189] Polymethylpentene is a hydrophobic thermoplastic polymer, that in ECLS oxygenators provides a hollow fiber, plasma tight membrane with a high surface area and low resistance to flow.[189]

Oxygenation in the ECLS circuit is controlled by altering the fraction of oxygen in the gas supply, and oxygen delivery is a combination of this and the flow rate in the circuit (a higher flow rate will oxygenate more blood per minute). The removal of carbon dioxide is controlled by the countercurrent sweep gas – this is typically checked and adjusted via outflow arterial blood gas analysis.[192]

10.3.4 ECLS pump

ECLS machines use two types of pump – roller and centrifugal. Centrifugal pumps have been shown to produce less red cell haemolysis than roller pumps, and are smaller.[196] However, the risk of air embolism is theoretically higher with the use of centrifugal pumps, as they create a high negative pressure on the venous side of the circuit.[187] The use of centrifugal pumps therefore requires

constant observation, in order to clamp the circuit if air is entrained to avoid arterial gas embolism.

10.3.5 ECLS anticoagulation

Blood contact with ECLS circuit components is known to cause a combination of activation, destruction, and dysfunction of platelets and consumption of clotting factors, resulting in the majority of patients being thrombocytopenic and requiring transfusion during therapy, as well as forming clot.[197,198] In addition to this, anti-coagulation is required in order to reduce the risk of clotting within the circuit (causing obstruction and preventing gas exchange at the membrane) and to reduce the risk of emboli. Anti-coagulation is achieved with unfractionated intravenous heparin.

The optimum dosing of heparin is unknown, but the extra-corporeal life support organization (ELSO) recommends a loading dose of 50 to 100 units/kg, followed by a continuous infusion guided by a point-of-care activated clotting time (ACT) of 1.5 times the upper limit of normal (between 200 and 300 seconds).[199] This has traditionally limited the use of ECLS in trauma patients, specifically those with intracranial haemorrhage. However, a recent systematic review has demonstrated that bleeding related mortality is less than previously estimated, and since 1995 is lower than 15%.[198] Some of the studies included used heparin-bonded ECLS circuits, and lower target ACTs, and the future aspiration is that advancements in ECLS components can further reduce platelet activation and the requirement for systemic anti-coagulation.[198]

10.4 Animal studies of ECLS in trauma

There are no published reports of animal studies examining the use of ECLS in the mitigation of the negative effects of aortic occlusion. The majority of non-neonatal ECLS animal studies are designed to test novel non-heparinised circuits. The most extreme of these, reported by Nishinaka et al, maintained ECLS for 151 days in a large goat using a novel anti-thrombogenic circuit coating.[200]

Larsson et al used a swine model to evaluate the haemodynamic effects of ECLS in major venous haemorrhage.[201] Ten 35-42 kg swine were cannulated for ECLS, and high pulmonary pressures were achieved by increasing the positive end-expiratory pressure and administration of intravenous dextran. ECLS significantly reduced the central venous pressure and significantly increased the mean arterial pressure, therefore suggesting that ECLS may be an effective intervention for major venous haemorrhage.[201]

Tisherman et al have used ECLS in a canine model to instigate deep hypothermic circulatory arrest, followed by resuscitation, after inducing haemorrhagic shock through a controlled haemorrhage.[202] This study demonstrated the early translational work, that is now known as emergency preservation and resuscitation (EPR),[203] and may potentially form part of a clinical paradigm of care that starts with IABO and SAAP, and culminates with EPR.[204]

There are no other translational studies that are relevant to the use of ECLS in the management of traumatic haemorrhage.

10.5 Clinical studies of ECLS in trauma

The first description of the use of ECLS following trauma was the 1971 case report by Hill et al – ECLS was maintained for 72 hours.[181] However, this patient was six days post-trauma and had undergone definitive surgical control. The use of VV-ECLS for the management of severe pulmonary dysfunction secondary to chest injury remains controversial, owing to concerns about anti-coagulation, but there is some evidence of survival benefit.[198,205]

The use of ECLS in the early management of trauma has only recently been reported. In 2010, Arlt et al reported the use of ECLS in three trauma patients who all had cardiopulmonary failure secondary to haemorrhage, despite receiving massive transfusion.[206] ECLS was commenced prior to surgical control in all patients, and cannulae were placed percutaneously via the femoral vessels. Systemic heparinisation was delayed until surgical control had been achieved and haemorrhagic shock resolved – there were no thromboembolic events or clots observed in the circuit. Two (67%) of the patients survived to hospital discharge without neurological deficit.[206]

In 2013, Bonacchi et al described the use of ECLS early in the management of trauma in 14 patients at a single hospital in Italy. The in-hospital ECLS team evaluated 30 patients (8% of the total major trauma patients) in whom it was considered that no other conventional therapy would be successful. Twelve were excluded owing to "massive intractable bleeding", prolonged hypoxia, and age over 75 years old.[207] Of the remaining 18 patients, 16 (89%) had ECLS commenced in the emergency department or intensive care unit, and two (11%)

had ECLS commenced in the operating theatre – 14 patients had VA-ECLS, 4 had VV-ECLS. The primary indication for VA-ECLS was cardiopulmonary failure secondary to haemorrhagic shock; all cannulae were inserted percutaneously via the femoral vessels. Heparin was omitted in all cases until surgical control had been achieved, and commenced after a mean time on ECLS of 16.7 hours. Two (14%) VA-ECLS patients survived, and six (43%) underwent organ harvest for donation. In four patients, VA-ECLS was unsuccessful owing to the inability to maintain adequate circuit flow.[207] This small clinical study has demonstrated the feasibility to start ECLS in the acute management of cardiopulmonary failure secondary to haemorrhage, and suggests it may improve survival.

In 2016, Huh et al reported a cohort of five hospital patients with suspected cardiac and major vessel injury; those with confirmed intracranial haemorrhage were not included.[208] All patients had an in-hospital TCA, and ECLS (with systemic heparinisation) was commenced ten minutes after the start of chest compressions. All patients then underwent definitive surgical control in an operating theatre. All five patients survived to hospital discharge. This report demonstrates the potential use of ECLS in the acute management of trauma. The lack of a control group and the small sample limit the validity of the findings, but the authors state that the injuries were only repairable on ECLS.[208]

These reports demonstrate that ECLS is being used clinically as a management option in a few specialised centres. The small samples and lack of control groups mean that the effectiveness of this intervention is uncertain. However, the

increasing interest and technical skill in endovascular resuscitation is likely to produce further clinical reports and clinical trials.

10.6 ECLS summary

ECLS has been developed over the last 40 years, and is now established in the management of severe pulmonary dysfunction and cardiogenic shock. It requires careful patient selection, and additional clinical skills in endovascular resuscitation, that are similar to those required for IABO and SAAP. The ability of ECLS to support patients in severe sepsis and those with cardiopulmonary failure secondary to haemorrhage make it an attractive option for further investigation to mitigate the effects of IABO observed in Part One of this thesis.

CHAPTER ELEVEN

MODEL DEVELOPMENT – PART TWO

11.1 Aims of model development

Part One demonstrated that SAAP with FWB was capable of resuscitating large swine in haemorrhage-induced cardiac electrical asystole, that ROSC resulting from SAAP with lactated Ringer's solution occurred near the end of a two minute infusion, and that further cardiac support was required after balloon deflation. Therefore, the aims of model development in Part Two were: 1) To evaluate the potential to effect a more severe injury model, with the goal of achieving systematic cardiac electrical asystole during the arrest period, 2) to examine the effect of a longer initial SAAP infusion on ROSC, and 3) to establish the use of peripheral ECLS in this model.

11.2 Model development ethical review

In accordance with local animal ethics committee (Institutional Review Board – IRB) approval at the Clinical Research Division of the 59th Medical Wing, United States Air Force, a total of five large swine (*sus scrofa*) were allocated for model development (protocol number FWH20150068A). The IRB approved this protocol as a non-survival study. The lower number of development animals compared to Part One (n=22) was in recognition of the similarity of the protocols. Model development was completed between 13th July 2015 and the 29th July 2015.

11.3 Swine donor pool

A swine donor pool was utilised using the same methodology as Part One (Section 6.3). As per Part One, during SAAP-FWB infusions at 800 ml/minute, calcium chloride was added at a rate of 1110 mg/minute = 11.1 ml/minute of 10%. The 10% variance in the volume of each unit of FWB confers an error to this calculation, and as such in all experimental protocols the volume (by weight) of each unit was checked before use (and discarded if there is more than 10% deviation), and aortic blood samples to confirm serum ionised calcium concentration were obtained during SAAP infusions with FWB. The same calculation was used to determine the required calcium chloride infusion rate for the intravenous FWB infusion.

11.4 Animal preparation

11.4.1 Animal selection

No adaptions were made to animal preparation from Part One (Section 6.4).

11.4.2 Animal anaesthesia

No adaptations were made to animal anaesthesia from Part One (Section 6.4.1).

11.4.3 Physiological monitoring and vascular line placement

When anesthetised, electrocardiography, physiological monitoring, blood sampling, and vascular access lines were inserted percutaneously under ultrasound guidance as per Part One (Section 5.5.3), with the omission of the tissue oxygenation sensor.

Initial baseline physiological measurements were then obtained. The mean animal weight was 77.0 kg, Table 11.1.

Table 11.1 – Baseline physiological measurements of model development animals (n=5), mean (± standard deviation).

Baseline variable	Mean (±SD)
Weight (kg)	77.0 (±3.9)
Heart rate (beats/minute)	79.0 (±15.8)
Systolic blood pressure (mmHg)	95.6 (±10.3)
Mean arterial pressure (mmHg)	74.6 (±11.5)
Mean pulmonary artery pressure (mmHg)	21.8 (±3.1)
Cardiac output (l/minute)	5.0 (±0.9)
End-tidal CO_2 (kPa)	5.4 (±0.2)
Carotid artery flow (ml/minute)	355.6 (±95.9)
Central venous pressure (mmHg)	9.4 (±0.5)
Central venous saturation (%)	69.4 (±9.2)
Core temperature (^oC)	37.1 (±0.3)

During Part One, the left femoral artery 8.5 Fr catheter was not used either for the controlled haemorrhage, or for BP monitoring. Therefore, this catheter was omitted in Part Two, however, an 8.5 Fr catheter was inserted in the left femoral vein, in order to allow exchange for the venous ECLS cannula later in the protocol. The arterial ECLS cannula would be exchanged for the 14 Fr vascular sheath already placed in the right femoral artery for the SAAP catheter when required.

11.4.4 Surgical preparation

No adaptations were made to surgical preparation from Part One (Section 5.5.4).

11.5 NCTH injury and controlled arterial haemorrhage

The model of laparoscopic hepatic injury has been previously developed in this laboratory.[101,118] This resulted in a mean arterial pressure of 36.0 (±16.8) mmHg.[101] Through Part One model development a more severe model of haemorrhage was developed that was a hybrid of the previously described laparoscopic hepatic injury and a controlled arterial haemorrhage, that started five minutes after the hepatic injury at 3.0 ml/kg/minute, and was reduced to 1.0 ml/kg/minute at the point of arrest (systolic BP less than 10 mmHg) for a further two minutes. This novel model of haemorrhage resulted in a very severe arrest, with a mean systolic BP of 2.5 (±1.9) mmHg, a mean cardiac electrical rate of 43.0 (±31.3) beats/minute. Furthermore 12 of 40 (30.0%) of animals were either in cardiac electrical asystole or p-wave asystole during the arrest period. Part One demonstrated the ability of SAAP-FWB to resuscitate swine from asystole, which is a novel finding, and one aim of Part Two model development was to evaluate the possibility of the injury resulting in asystole in all animals.

There was a need to further increase the severity of the haemorrhage model. The hybrid model was effective in Part One, and the protocol was well known to the laboratory team. Therefore, the method of haemorrhage was not changed; the initial rate of controlled haemorrhage was 3.0 ml/kg/minute, reduced to 1.0 ml/kg/minute when the intra-aortic systolic BP was <10 mmHg. However, the arrest point (t0) was moved to the time that animals had no intra-aortic pressure fluctuations, with the expectation that cardiac electrical asystole would occur during the three minute arrest period (t0-t3). As per Part One, a controlled haemorrhage of 1.0 ml/kg/minute could be used between t0 and t2 if there was

a spontaneous recovery. The cut-off point for this arrest model (no intra-aortic pressure fluctuations) is potentially subjective. The intra-aortic pressure output (measured by a micromanometer-tipped catheter), was displayed live on a large screen in the laboratory, and consensus from three of the research team (EB, JR, JC) was required to define the point of 'no pressure fluctuations'.

This method was undertaken in the first development animal (2D1-7815). The injury resulted in no intra-aortic pressure fluctuations, and very small, wide ECG complexes after 589 seconds (9 minutes, 49 seconds). This was maintained for 50 seconds without further haemorrhage before the animal went into VF (at t0.83). Owing to the high rates of VF observed in Part One model development, defibrillation pads had already been attached to this animal, and despite a SAAP infusion and multiple defibrillations, the animal remained in VF, and was euthanased. VF is atypical in human haemorrhagic TCA,[45,52] and to date in swine experiments has been resistant to treatment with SAAP and defibrillation. Therefore, in future protocols, animals who are in VF during the arrest period will be excluded (as per Part One methodology).

The method was repeated in the second development animal (2D2-7775). This resulted initially in an ischaemic ECG (ST segment depression, and deep inverted T waves) followed by no intra-aortic pressure fluctuations and a brady-agonal cardiac electrical rate of 28 per minute at t0. Following the 180 second arrest period, a SAAP-FWB infusion successfully resuscitated the animal. It is possible that further prolonging the arrest period would have resulted in cardiac

electrical asystole, but it was also possible that this would increase the risk of VF, and therefore no further changes were made to the model.

In the third development animal (2D3-7779), the injury method was repeated. This resulted in an ischaemic ECG, followed by no intra-aortic pressure fluctuations, and then cardiac electrical asystole during the arrest period (t1.0). SAAP-FWB infusion restored organised cardiac electrical activity, but after 176 seconds of SAAP (at t5.9) the animal went into VF that was resistant to further SAAP infusion and defibrillation. Asystole was also achieved in 2D4-7813, but after six beats of sinus rhythm during SAAP infusion, the animal went into VF that was again resistant to further SAAP infusion and defibrillation.

The new injury model was systematically resulting in animals with no intra-aortic pressure fluctuations, and a high proportion of asystole before the start of the intervention. Therefore, in the final development animal, the equipment (particularly the calcium infusion) was double-checked, and the same injury model was repeated. In 2D5-7820, this resulted in three minutes of no intra-aortic pressure fluctuations, and an ischaemic ECG, but not asystole. The FWB-SAAP infusion effected a ROSC without VF.

In summary, the new injury model systematically achieved an arrest period with no intra-aortic pressure fluctuations. However, one animal went into VF during the arrest period, and two went into VF that was resistant to further SAAP infusion and defibrillation. The cause of the VF was unknown, and if it continued

into the Phase Two experiments it may adversely affect the ability test the hypotheses as planned.

11.6 Equipment

The CPR table was used in Part One for all initial experimental protocols in order to allow blinding of the intervention, Figure 5.7. However, the animal stability it afforded over the standard operating table and sand bags, meant that it was also used for the add-on REBOA group. The CPR table (without the mechanical CPR device attached) was also used for Part Two.

Part Two included an evaluation of ECLS after SAAP balloon deflation, and the model development of this equipment, including ECMO cannulae, is described later (Section 11.9). The SAAP infusion circuit functioned satisfactorily throughout Part One, and was not altered for use in Part Two.

11.7 Intervention

11.7.1 SAAP intervention

The SAAP intervention with both FWB and LR was developed in Part One (Section 5.6.3). The rate of infusion (10 ml/kg/minute) had been originally taken from Manning et al's previous work with SAAP in swine,[112] and was efficacious in the first set of experiments. The SAAP infusion in Part One was for two minutes regardless of ROSC, and effected an initial ROSC in all SAAP-FWB animals, but only six (60.0%) of the SAAP-LR group. In the six SAAP-LR animals with a ROSC, the mean time to ROSC was only ten seconds prior to the cessation of the infusion. Therefore, in order to confirm that SAAP-LR ROSC and 60 minute

survival could not be increased by a longer initial SAAP infusion, the methodological plan in Part Two was to increase the duration of the SAAP infusion to five minutes.

However, the effects of ongoing SAAP infusion, after a ROSC, were observed in Part One in the SAAP-FWB group. The mean time to initial ROSC was after 54.5 seconds of SAAP. The continuation of SAAP in this group, after ROSC, in the nine animals who survived to t13, resulted in very high systolic BPs (a range of 119.1 to 250.4 mmHg). The effect of these high systolic BPs is unknown, but may have contributed towards further haemorrhage (from surgical wounds, and via collateral circulation), and potentially caused myocardial injury by dilating the left ventricle. In translation, if these interventions are being undertaken pre-hospital, there is also the concern about unnecessary use of limited resources, particularly with respect to blood products.

Manning et al have previously described the use of a longer initial SAAP infusion, that was stopped when the animal had a ROSC.[112] This methodology was therefore planned for Part Two experiments – an initial SAAP infusion at 10 ml/kg/minute, stopped if there was an intrinsic systolic BP of 90 mmHg or greater, and up to a maximum duration of five minutes (a maximum SAAP volume of 4,000 ml). In keeping with the methodology of Part One, animals with a ROSC at t13 could receive up to a further seven 250 ml boluses of resuscitation fluid.

The development of this new SAAP technique was only tested in two animals. As previously explained, one animal had VF during the arrest period, and two further animals had VF during the SAAP infusion. In the remaining two animals (2D2-7775, 2D5-7820) the SAAP-FWB infusion successfully resuscitated the animals from the new arrest model. In 2D2-7775, SAAP-FWB infusion effected a ROSC, and after 101 seconds of SAAP the intrinsic systolic BP was greater than 90 mmHg, Figure 11.1.

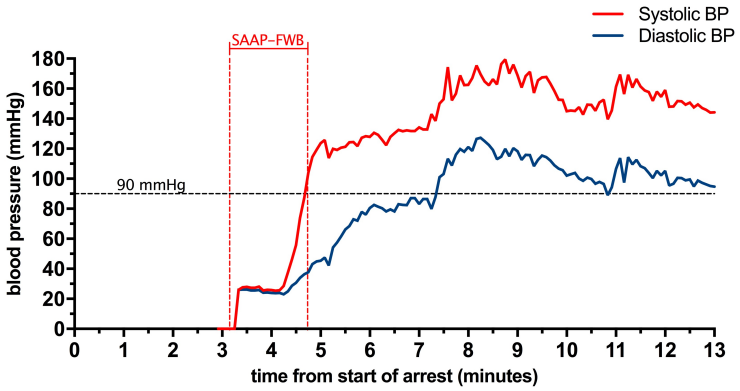

Figure 11.1 – Initial SAAP-FWB infusion in 2D2-7775.

There was a brief run of spontaneously resolving ventricular tachycardia on cessation of the SAAP infusion, and in order to ensure that enough SAAP fluid had been administered the protocol was edited to stop the SAAP infusion after two consecutive systolic BP readings (five seconds apart) were greater than 90 mmHg. This animal required two 250 ml boluses of SAAP-FWB to maintain a systolic BP of at least 90 mmHg until t63.

In 2D5-7820, two consecutive systolic BP readings over 90 mmHg were achieved after 90 seconds of SAAP-FWB infusion. This animal had multiple episodes of

spontaneously resolving ventricular tachycardia, and also required two 250 ml boluses of SAAP-FWB to maintain a systolic BP over 90 mmHg to t63. An intra-aortic ABG sample was obtained in 2D5-7820 at t5 that demonstrated an ionised calcium of 1.11 mmol/l, compared to 1.19 mmol/l at t0, confirming the appropriate volume of SAAP calcium chloride infusion.

11.8 Surgery Period

In Part One, surgical control of the hepatic injury was rapidly achieved with the use of a Doyen's and Satinsky's clamps. However, one SAAP-FWB animal was lost to further analysis after coughing under anaesthesia and dislodging the clamp, sustaining further hepatic haemorrhage. In order to mitigate against this a set of bespoke, smaller, hepatic clamps were fabricated, Figure 11.2.

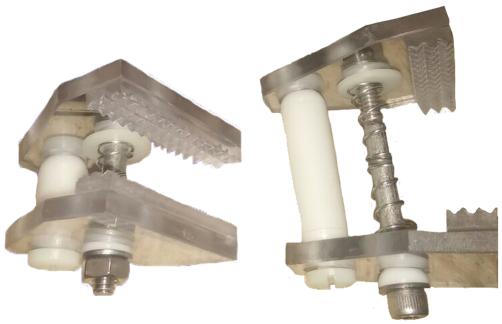

Figure 11.2 – Bespoke hepatic clamps fabricated for Part Two model development.

In Part Two, the methodological plan was for the SAAP-FWB animals to transition into the surgery period, in order to observe the effects of ECLS through a 180 minute critical care period. As in Part One, these animals received a 500 ml bolus of intravenous FWB before the abdomen was opened. After

haemorrhage control, and removal of NCTH blood and clots, the SAAP catheter was deflated at a rate of between 0.5 and 2.0 ml/minute, starting at t78 (75 minutes after the balloon inflation), completed by t93.

Simultaneouly, the ECLS machine was primed and checked, and ECLS cannulae were prepared for the start of the 180 minute critical care period.

11.9 ECLS equipment

Part Two experiments required the use of ECLS in the SAAP-FWB group after deflation of the SAAP balloon. The necessary features for the ECLS machine were that it was commercially available (to translate to clinical practice), and relatively simple to use (as there were only five development animals in which to test and adjust).

A pre-hospital medical team, Samu de Paris in France, has been using a portable ECLS machine (Cardiohelp, Maquet, Rastatt, Germany) for a number of years for pre-hospital clinical ECLS in medical cardiac arrest patients.[209] The Cardiohelp is a fully integrated, portable ECLS system including a non-microporous polymethlypentene membrane oxygenator, pump, and multiple flow and pressure sensors with automatic bubble-sensing. The complete unit is small and lightweight – measuring 455 x 255 x 427 mm (HxWxD), and weighing 11.5 kg,[210] Figure 11.3.

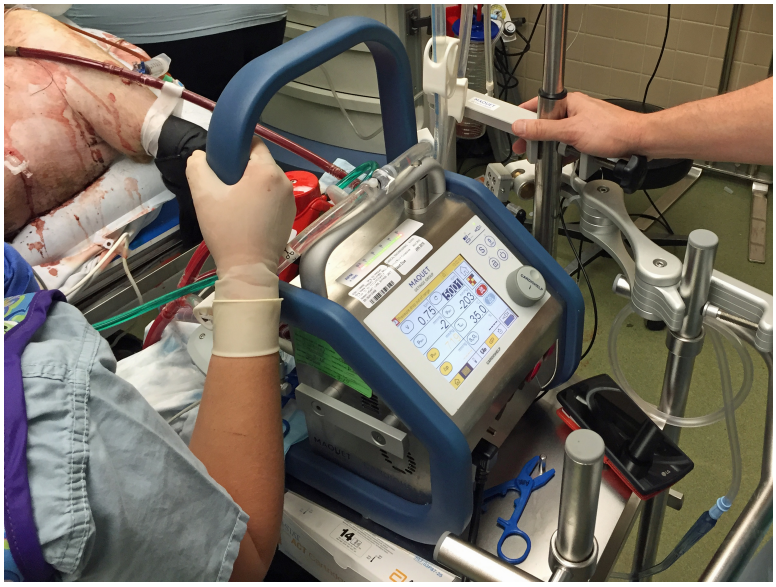

Figure 11.3 – The Maquet Cardiohelp in use in the large animal laboratory.

The Cardiohelp uses a polymethlypentene oxygenator membrane, that has been shown to provide more efficient gas exchange and less blood cell damage than silicone membranes.[189] The Cardiohelp also uses a centrifugal pump, that has been demonstrated to produce less red cell haemolysis than roller pumps, and is smaller.[196] The maximum flow rate is 5,000 ml/minute using the HLS Advanced 5.0 oxygenator, which is adequate for providing ≥50% exogenous support of cardiac output in 80 kg swine.

The Maquet HLS ECLS cannulae (Maquet, Rastatt, Germany) were used for the arterial (15 Fr, 150 mm length) and venous (23 Fr, 550 mm length) catheters. The arterial cannula allows a flow of up to 4,000 ml/minute at a pressure drop of 200 mmHg, and the venous cannula a flow of up to 7,000 ml/minute at a pressure drop of 120 mmHg.[211]

11.10 ECLS anti-coagulation

The available ECLS components were not heparin-bonded, and in order to reduce laboratory costs, the circuit and oxygenator had to be used for multiple experimental animals. Therefore, systemic heparinisation was achieved with a bolus of 100 units/kg of intravenous heparin at the start of ECLS.[199] Further administration would be guided by point of care ACT with an i-STAT analyser (Abbott Laboratories, Abbott Park, IL, USA), aiming for an ACT twice as long as swine baseline values (approximately 200 seconds).

11.11 Critical care period

Two development animals were alive at t63, and were transitioned into the 30 minute surgery period.

11.11.1 2D2-7775

2D2-7775 received the maximum allowance of 5,750 ml of intravenous FWB during the surgery period, and demonstrated significant cardiovascular instability as the balloon was deflated. At t88, the SAAP catheter was removed and the animal was in TCA.

The right femoral artery 14 Fr vascular sheath was exchanged for a 15 Fr arterial ECLS cannula, and a 23 Fr venous cannula was inserted via the left femoral vein and advanced into the proximal inferior vena cava while the ECLS circuit was primed with LR, and a bolus of 8,000 units of heparin was administered. ECLS was started at t90.5 at a flow rate of 2,800 ml/minute, which resulted in a ROSC three minutes later, Figure 11.4.

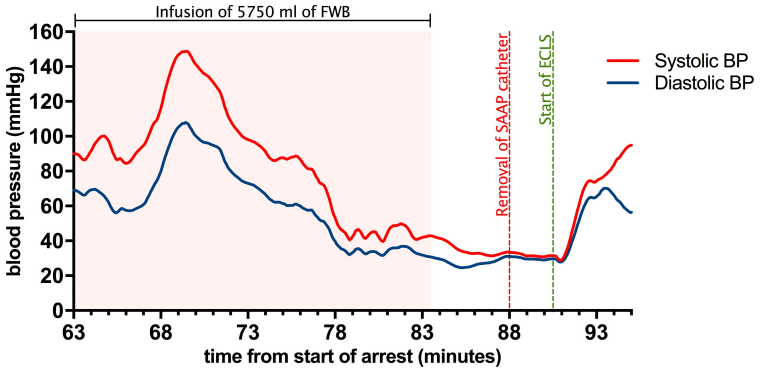

Figure 11.4 – Blood pressure in 2D2-7775 during the surgery period, removal of the SAAP catheter, and the start of ECLS.

The animal survived the three hour critical care period, which allowed some adjustments in ECLS flow to be tested. At t110 the ECLS flow was increased to 3.2 l/minute, and at t118 it was increased to 5.0 l/minute; neither adjustment improved the systolic BP or the left carotid artery flow. At t128, after ten minutes at a flow of 5.0 l/minute, venous cannula 'chatter' (excessive vibration secondary to very low venous pressure) was observed with pressures of -300 mmHg, the ECLS flow was therefore turned down to 1.0 l/minute, which effected a fall in both systolic BP and left carotid artery flow. This effect has been described in the clinical series by Bonacchi et al,[207] and in order to maintain enough circulating volume to run ECLS at a flow of at least 50% of estimated cardiac output, a bolus of 1,000 ml of intravenous LR was given. This enabled ECLS flow to be increased to 3.9 l/minute, and restored the systolic BP and carotid flow. Three further 1,000 ml boluses of LR were administered during the critical care period when low venous cannula pressures (< -150 mmHg) caused chatter, and necessitated a brief reduction in ECLS flow rate. ECLS flow was

maintained between 2.8 and 3.9 l/minute until t265, were it was reduced to 2.0 l/minute until the end of the protocol (t273).

Blood samples were obtained from the left brachial artery catheter throughout the protocol. Despite the administration of 4,000 ml of LR during the critical care period, the haemoglobin increased from 7.1 g/dl at t93 to 8.6 g/dl at t273, and the TEG clot index increased from 2.6 to 3.2. The lowest pH (6.89), and highest lactate (16.0 mmol/l) and base deficit (17.8) were observed at t93 and t123 respectively. At t273, the pH was 7.25, the lactate was 9.1, and the base deficit was 7.6 – all had improved. This indicated improved tissue perfusion, despite the systolic BP being lower than 90 mmHg throughout the critical care period.

There was no observed benefit of increasing ECLS flow from 2.8 to 3.2 l/minute, or from 3.2 l/minute to 5.0 l/minute. Therefore, in future protocols ECLS flow was maintained at 2.8 l/minute, equal to 50% of the mean cardiac output at baseline in the 40 Part One animals.

A single bolus of 100 units/kg of intravenous heparin was administered at the start of ECLS. After the protocol, there were multiple small clots in the oxygenator – highlighting the need for further anticoagulation.

11.11.2 2D5-7820

2D5-7820 did not display overt cardiovascular instability on deflation of the balloon, and only required 1,500 ml FWB to maintain a systolic BP of 90 mmHg

between t63 and t93. Technical difficulties with inserting the ECLS venous cannula and setting up the ECLS circuit meant that ECLS was not started until t134, during which period the animal required a further 4,250 ml (the maximum FWB allowance) to keep the systolic BP over 90 mmHg.

ECLS flow was started at 2.8 l/minute, and maintained throughout the critical care period, requiring six 1,000 ml boluses of LR. The haemoglobin fell from 9.9 g/dl at t93 to 7.0 at t273. However, the pH, lactate and base deficit all improved throughout the critical care period – the lowest pH (7.11) was observed at t93, and increased to 7.39 at t273. In order to improve the venous flow, and potentially reduce the requirement for large volumes of lactated Ringer's solution during ECLS, a larger venous cannula was used for subsequent experimental protocols (25 Fr).

An initial bolus of 100 units/kg of heparin was given five minutes before the start of ECLS flow; the ACT just before starting ECLS was 118 seconds (below the target of 200 seconds), so a second bolus of 100 units/kg was given, which resulted in an ACT of 388 seconds at t123. The ACT was 258 seconds at t153 (258), and an on-going infusion of 60 units/kg/hour resulted in an ACT at the end of the protocol of 218 seconds, with no evidence of either clotting in the circuit, or bleeding from cannulae insertion points, or at post-mortem in the abdomen. Therefore, in future protocols, a single bolus of 100 units/kg of heparin was given at t78 (when the hepatic injury had been controlled), followed by an infusion of 60 units/kg/hour (starting at t93, the start of ECLS), with ACT measurements obtained every 60 minutes from t123 onwards.

The level of mixing of ECLS blood and the swine's intrinsic cardiac output blood is unknown. Therefore, in order to check the partial pressure of oxygen and carbon dioxide, and markers of tissue perfusion (pH, lactate, base deficit) in both circulations, an arterial blood gas was obtained every 30 minutes during the critical care phase from the ECLS outflow and from the left brachial artery.

11.12 Summary of Part Two model development

A limited number of animals and laboratory days were allocated to Part Two development, and the ability to test and adjust ECLS was further restricted by three animals not surviving to receive ECLS. However, a new model of injury that resulted in three minutes of no intra-aortic pressure fluctuations has been described, and the feasibility of setting-up ECLS in large swine in this laboratory has been demonstrated. High-rates of VF in Part Two may impair the ability to test the planned hypotheses, and the large volumes of LR required to maintain ECLS flow in the critical care period may cause anaemia, and affect valid comparison with the SAAP-FWB Part One historical controls.

CHAPTER TWELVE

METHODS - PART TWO

12.1 Part Two hypotheses

In a swine model of severe haemorrhagic TCA, without intra-aortic pressure fluctuations:

H4. SAAP with FWB will not result in a significantly higher proportion of animals with a ROSC ten minutes after the start of the intervention, compared to SAAP with LR.

H5. A significantly smaller volume of resuscitation fluid will be required to effect ROSC ten minutes after the start of the intervention in animals that receive SAAP with FWB, compared to SAAP with LR.

H6. A larger volume of initial SAAP infusion (up to 4,000 ml) with LR will result in a significantly higher proportion of animals with a systolic BP equal to or greater than 50 mmHg ten minutes after the start of the intervention, compared to Part One historical controls (that received 1,600 ml of SAAP with LR), despite a more severe injury.

H7. SAAP with FWB will infer a significant 60 minute survival advantage over SAAP with LR.

H8. ECLS during a critical care period will infer a significant three-hour survival advantage after IABO removal, compared to Part One historical controls (without ECLS).

12.2 Animal use ethical review

The experimental protocol was approved by the 59th Medical Wing Institutional Animal Care and Use Committee (IACUC) (protocol number – FWH20150068A). Experiments were performed at the Clinical Research Division, 59th Medical Wing, United States Air Force, Office of the Chief Scientist, in a facility accredited by the American Association for the Accreditation of Laboratory Animal Care. The protocol was conducted in accordance with guidelines established by the Public Health Service Policy on Humane Care and Use of Laboratory Animals and Office of Laboratory Animal Welfare.

12.3 Swine donor blood

12.3.1 Blood donation pool

Whole blood was collected from a dedicated pool of swine the day before each experimental protocol. In Part One of this thesis, up to 15 units per animal were used (in the SAAP-FWB group), with an initial fixed SAAP volume of 1,600 ml. The Part Two SAAP infusion is variable, up to a maximum volume of 4,000 ml, with the same additional pre-hospital fluid allowance of 1,750 ml (seven boluses of 250 ml). Only the SAAP-FWB (SAAP-FWB2) group will be transitioned into the surgery and critical care period. Therefore, the Part Two SAAP-LR (SAAP-LR2) group require no FWB, and the SAAP-FWB2 group will require 11,500 ml of

FWB per protocol. IRB approval was given for 19 units of FWB per SAAP-FWB2 experiment.

12.3.2 Blood donation procedure

No adaptions were made to the blood donation procedure from Part One (Section 6.3.2).

12.4 Animal preparation

No adaptions were made to animal preparation from Part One (Section 6.4).

12.4.1 Animal anaesthesia

No adaptions were made to animal anesthesia from Part One (Section 6.4.1).

12.4.2 Physiological monitoring and vascular line placement

No adaptions were made to physiological monitoring and vascular line placement from Part One (Section 5.5.3), with the exceptions of the omission of the left femoral artery 8.5Fr catheter and the addition of a left femoral vein 8.5Fr catheter to allow subsequent insertion of an ECLS venous cannula.

12.4.3 Surgical preparation

Surgical preparation was identical to Part One, and can be found at Section 6.4.3. This included a splenectomy, marking of the left lateral lobe of the liver with electrocautery, a direct cystostomy, and insertion of four laparoscopic ports. The spleen was returned to the abdominal cavity, and the abdomen was then closed.

Baseline physiological measurements and blood samples were then obtained, followed by a ten minute stabilisation period.

12.4.4 NCTH injury and controlled arterial haemorrhage

The only adaptation to the Part One injury (Section 6.4.4) was that at the time of TCA (defined as no intra-aortic pressure fluctuations, t0), the controlled haemorrhage was continued at 1.0 ml/kg/minute between t0 and t2 if there was a spontaneous return of intra-aortic pressure fluctuations.

12.5 Pre-hospital experimental interventions

Two minutes after the onset of TCA (t2), sixteen swine were allocated to one of two groups, Table 12.1.

Table 12.1 – Part Two experimental groups.

Group	Part Two experimental arm	Abbreviation	N
2-1	SAAP with LR	SAAP-LR2	8
2-2	SAAP with FWB	SAAP-FWB2	8

12.5.1 Group 2-1 – SAAP-LR2

At t2, a SAAP catheter with a 7.5 Fr central lumen, and a 30 mm (17 ml) balloon, was advanced in aortic Z1, and its position confirmed with fluoroscopy. The SAAP catheter central lumen was flushed with LR, and closed distally with a three-way tap.

At t2.75 (two minutes and 45 seconds), the SAAP catheter balloon was inflated, under fluoroscopy, with 17ml of 1:1 LR and contrast.

At t3, oxygenated LR was infused into the aortic arch at a rate of 800 ml/minute, using the SAAP circuit, Figure 5.3. SAAP infusion was continued until either the animal had a ROSC, and two consecutive intra-aortic systolic BP readings above 90 mmHg, or until the maximum volume of 4,000 ml of LR had been administered (t8). Animals with a systolic BP of 30 mmHg or greater at t13, were administered up to seven 250 ml boluses of SAAP-LR if the systolic BP was less than 90 mmHg between t13 and t63. The maximum infusion of LR in the SAAP-LR2 group was therefore 5,750 ml.

At t63, or sooner if death-in-protocol criteria were met (Section 12.8.2), animals were euthanased and post-experimental procedures were undertaken.

12.5.2 Group 2-2 – SAAP-FWB2

At t2, a SAAP catheter with a 7.5 Fr central lumen, and a 30 mm (17 ml) balloon, was advanced in aortic Z1, and its position confirmed with fluoroscopy. The SAAP catheter central lumen was flushed with LR, and closed distally with a three-way tap.

At t2.75 (two minutes and 45 seconds), the SAAP catheter balloon was inflated, under fluoroscopy, with 17ml of 1:1 LR and contrast.

At t3, oxygenated FWB was infused into the aortic arch at a rate of 800 ml/minute, together with an infusion of 10% calcium chloride at a rate of 11.1 ml/minute using the SAAP circuit. SAAP infusion was continued until either the animal had a ROSC, and two consecutive intra-aortic systolic BP readings above 90 mmHg, or until the maximum volume of 4,000 ml of FWB had been administered (t8). Animals with a systolic BP of 30 mmHg or greater at t13, were administered up to seven 250 ml boluses of SAAP-FWB, together with an infusion of 3.5 ml of 10% calcium chloride, over 30 seconds, if the systolic BP was less than 90 mmHg between t13 and t63. The maximum infusion of FWB in the SAAP-FWB2 group was therefore 5,750 ml.

12.6 In-hospital period

The SAAP-LR2 group protocol was completed at t63, but the SAAP-FWB2 group who were alive at t63 were transitioned into a 30 minute surgery period, followed by a 180 minute critical care period.

12.6.1 Surgery period (SAAP-FWB2 group)

At t63, the SAAP catheter balloon was kept inflated, and 500 ml of intravenous FWB was administered, together with 6.9 ml of 10% calcium chloride, over one minute by a Belmont Rapid Infuser before the abdomen was opened. Further intravenous boluses of 250 ml of FWB, and 3.5 ml of 10% calcium chloride were administered to maintain systolic BP over 90 mmHg. Owing to IRB and logistical constraints, a total volume of 5,750 ml of FWB was allocated per animal for the in-hospital period (equal to 23 x 250 ml boluses).

At t64, the abdomen was opened via midline laparotomy, and the hepatic injury was rapidly controlled with the use of bespoke plastic clamps, Figure 11.2. Once haemorrhage control had been achieved, NCTH blood was removed from the abdomen and weighed for quantification of shed blood volume. The portion of excised liver was recovered and weighed. Fifteen minutes into the surgery period, at t78, the SAAP catheter balloon was deflated in a controlled manner (between 0.5 and 2.0 ml/minute), and removed from the animal at t93. Simultaneously, the abdomen was packed with gauze, and closed with surgical staples. In preparation for ECLS in the critical care period, once haemorrhage control had been achieved, at t78, a bolus of 8,000 units of heparin (approximately 100 units/kg) was administered via the left internal jugular vein 8.5 Fr catheter.

12.6.2 Critical care period (SAAP-FWB2 group)

At t93, all animals with a systolic BP greater than or equal to 30 mmHg were transitioned into a 180 minute critical care period. During the surgery period, an ECLS circuit (Cardiohelp, Maquet, Rastatt, Germany) was primed with warmed lactated Ringer's solution. At t93, a heparin infusion, of 60 units/kg/hour was commenced to provide anti-coagulation during ECLS. At t93, a 17 Fr arterial ECLS cannula was inserted over a wire into the right femoral artery to a depth of 150 mm, and a 25 Fr venous ECLS cannula (Maquet, Rastatt, Germany) was inserted over a wire into the left femoral vein to a depth of 550 mm.

ECLS was started, at a flow rate of 2.8 l/minute (approximately 50% of baseline cardiac output) as soon as the cannulae were placed and the circuit was ready, between t93 and t98.

During the critical care period, the remaining FWB volume (from the maximum 5,750 ml allocated in the in-hospital period) was administered in 250 ml boluses (with 3.5 ml of 10% calcium chloride) over 30 seconds via the left internal jugular vein catheter if systolic BP was less than 90 mmHg. If the venous pressure in the ECLS circuit was lower than -150 mmHg, a bolus of 500 ml of warmed LR was administered via the left internal jugular vein catheter over one minute, in order to maintain ECLS flow and prevent venous chatter.

The protocol was terminated at t273 (four and a half hours after the start of the intervention, t3), or sooner if animals met death-in-protocol criteria (Section 12.8.2).

12.7 Post-experimental procedures

At the end of the protocol - t63 in the SAAP-LR2 group, t273 in the SAAP-FWB2 group, or sooner if animals met death-in-protocol criteria, swine were euthanased by a trained laboratory technician with 100 mg/kg of intravenous sodium pentobarbital, in accordance with the American Veterinary Association euthanasia guideline.

Following animal euthanasia, the liver was removed in order to quantify the laparoscopic hepatic injury, as a percentage by weight of the left lateral lobe excised during the injury.

In order to mitigate against changes in volume of shed blood secondary to evaporation, the weight of controlled arterial haemorrhage and the blood removed from the abdomen were measured within ten minutes of collection. All suction containers and gauze were pre-weighed, and resulting weights of blood divided by 1.05 to quantify the volume of blood they contained.

In the SAAP-FWB2 group, sections of the heart (apical – left and right ventricle, and inferior septum), and lungs (apex and base from both lungs) were obtained for histological analysis.

12.8 Experimental schedule and definitions

12.8.1 Randomisation schedule

As per Section 6.8.1.

12.8.2 Experimental definitions

TCA (t0) was defined as the absence of intra-aortic pressure fluctuations, and a cardiac electrical rate lower than the pre-injury baseline rate.

ROSC was defined as an intrinsic systolic BP of 50 mmHg or greater, ten minutes after the start of the intervention (t13).

In all groups, from t13 onwards (ten minutes after the start of the intervention), death-in-protocol was defined as a systolic BP less than 30 mmHg, despite animals having received the maximum per protocol permitted volume of resuscitation fluid. This cut-off was selected by consensus as non-survivable (without further resuscitation fluid available), and has been used in previous swine translational research.[96] Animals meeting this definition were euthanased, and post-experimental procedures undertaken.

12.9 Physiological data acquisition

12.9.1 Data acquisition from the anaesthetic machine

The following variables were automatically recorded at one minute intervals throughout the protocol: cardiac electrical rate, rectal temperature, peripheral oxygen saturation, fraction of inspired oxygen, end-tidal carbon dioxide, MAC of isoflurane, and values from the Swan-Ganz pulmonary artery catheter (pulmonary artery pressure, central venous pressure, cardiac output, stroke volume, and central venous saturations).

12.9.2 Data acquisition on DAQ board

In order to accurately measure intra-aortic BP, the output from the micromanometer-tipped catheter was recorded at 500 Hz on a DAQ board, this produced an accurate BP and heart rate. The output from the carotid artery flow probe, and pulmonary artery pressure from the Swan-Ganz catheter were also recorded on the DAQ board at 500 Hz.

12.9.3 Manual data recording and time keeping

As per Section 6.9.4; the manual data recording sheet is at Appendix C.

12.10 Blood sample data acquisition

As per Section 6.10.

12.10.1 Arterial blood gas

Immediately following the insertion of the left brachial artery 5 Fr catheter, an ABG sample was obtained, in order to calibrate the Swan-Ganz catheter. Following this, ABG samples were drawn from the brachial arterial catheter at baseline (at the start of the ten-minute stabilisation period), and every ten minutes after the start of the intervention (t13, t23, t33, t43, t53, t63) during the pre-hospital period. An ABG was drawn from the SAAP catheter (in all animals) when the intra-aortic BP fell below 10 mmHg – in Part Two model development it was demonstrated that samples could not be reliably obtained at the onset of TCA (t0 – no intra-aortic pressure fluctuations), as there was not enough blood in the thoracic aorta. An additional ABG was drawn from the right carotid artery 8.5 Fr catheter at t5.

In the surgery period, an ABG was drawn, via the left brachial arterial line every ten minutes (t73, t83, t93). In the critical care period, an ABG was drawn from both the ECLS outflow, and from the left brachial artery every 30 minutes until protocol completion (t273). The ABG sample included: pH, partial pressures of oxygen and carbon dioxide, ionised calcium, potassium, sodium, glucose, lactate, and base deficit.

12.10.2 Activated clotting time

In order to quantify systemic anticoagulation during ECLS, a point-of-care ACT was obtained at baseline, at t123 (30 minutes after the start of ECLS), at t183, and at t243 using an i-STAT. This method of quantifying anticoagulation is widely used in clinical ECLS management as it produces a rapid result, requires a small volume of blood, can be undertaken by non-laboratory personnel, and provides a global picture of clotting function.[212]

12.10.3 Full blood count and clotting profile

Blood samples, from the brachial arterial line, were collected into one EDTA specimen tube, and one citrated specimen tube at each time point (before surgical preparation, baseline, t13, t33, t63, t93, t153, t213, and t273). Samples were additionally collected at the point of arrest (t0) via the SAAP catheter.

The EDTA sample was analysed for full blood count (including haemoglobin, white cell count, and platelet count). The citrated sample was analysed for clotting function (prothrombin time and fibrinogen).

An additional SST2 sample was drawn at baseline, t63, t93, and t153 in all animals, but only analysed in the SAAP-FWB2 group. This sample was used to measure cardiac troponin-I, and pro-inflammatory mediators (HMGB-1, IL-1B, IL-2, IL-6, IL-8, TNF-α).

12.10.4 Thromboelastography

At the same time points, and via the same catheters, another citrated specimen tube was drawn to undertake TEG. The output from TEG included R (the time to initial fibrin formation), K (the time to reach a specific level of clot strength), α (the rate of clot formation), MA (maximum clot amplitude), G (clot elasticity), CI (clot index), and LY-30 (the rate of amplitude reduction from MA to 30 minutes, a measure of fibrinolysis).

12.11 Outcomes

12.11.1 Primary outcome

In order to compare the simulated pre-hospital survival between groups, the primary outcome was 60 minute survival post intervention. Survival was defined as an intrinsic BP of 30 mmHg or greater at t63 – 60 minutes after the start of the intervention.

12.11.2 Secondary outcomes

In order to compare the ability of the SAAP resuscitation fluid to effect ROSC, both the proportion of animals with a ROSC ten minutes after the start of the intervention (t13), and the volume of resuscitation fluid administered to those with a ROSC were compared between the SAAP-LR2 and the SAAP-FWB2 groups.

In order to quantify the effects of SAAP resuscitation with LR and FWB, systolic BP, left carotid artery flow, and end-tidal carbon dioxide were compared between groups in all animals at t13. The partial pressures of oxygen and carbon dioxide, haemoglobin, markers of perfusion (pH, lactate, base deficit), and

clotting function (platelet count, prothrombin time, fibrinogen, and TEG values) were compared between groups in animals at t13. These analyses were repeated in animals with a ROSC at t13, in order to compare the physiological state in live animals at this time point.

In order to quantify the effects of SAAP resuscitation with LR and FWB at simulated hospital arrival, systolic BP, left carotid artery flow, and end-tidal carbon dioxide were compared between groups in animals alive at t63. The partial pressures of oxygen and carbon dioxide, haemoglobin, markers of perfusion (pH, lactate, base deficit), and clotting function (platelet count, prothrombin time, fibrinogen, and TEG values) were also compared between groups in animals alive at t63.

12.12 Pro-inflammatory mediators and cardiac troponin I

In order to quantify the changes in pro-inflammatory mediators and cardiac troponin I throughout the protocol in the SAAP-FWB2 group samples were analysed between baseline and simulated hospital arrival, after balloon deflation, and after 60 minutes of ECLS. In addition, the correlation of these pro-inflammatory mediators and troponin at t153 with animal pulmonary function (PaO_2:FiO_2) will be described.

12.13 Comparison of the SAAP-FWB2 group with historical controls

In order to quantify the effects of ECLS in the critical care period in the SAAP-FWB2 group, comparisons were made with the seven Part One animals in the SAAP-FWB group who died in the critical care period after balloon deflation

(excluding the one animal who died secondary to technical error). The primary comparison was survival to the end of the protocol (t273).

12.14 Statistical Methods

12.14.1 Sample size calculation

The sample size was based on the results from Part One, and estimated to provide a similar number of SAAP-FWB2 group animals to make a comparison with the critical care period of the SAAP-FWB group. In Part One there was a 60.0% difference in 60 minute survival between the SAAP-FWB group (90.0%), and the SAAP-LR (30.0%) group. Assuming a similar survival percentage in Part Two experiments, a sample size of eight animals per group would result in a significant difference, using an alpha of 0.05. In reference to providing a similar number of animals in the SAAP-FWB2 group to compare with the five animals from Part One, an initial group of eight and a survival of 80.0% to t93 would result in six animals in the SAAP-FWB2 group for ECLS.

12.14.2 Statistical analyses

Data are reported as number (percent), and mean (± standard deviation). Proportions are reported as number (percent, 95% confidence interval). The 95% confidence interval was calculated using the Wilcoxon-Brown method. Comparison between the means of two independent groups was analysed with an unpaired t-test with Welch's correction (without assumption of equal standard deviations). Comparison between the means of repeated measurements within the same group was analysed with a paired t-test. Three group analyses were by ordinary one-way ANOVA. Analysis of proportions

(survival and ROSC) was with Fisher's exact test. Correlation was determined with the Pearson correlation coefficient. Statistical significance was pre-defined as $p<0.05$.

Percentages and means (± standard deviation) have been calculated in Excel for for Mac (Microsoft, Redmond, WA, USA), version 15.36; standard deviation is reported as standard deviation of the sample. 95% confidence intervals, t-tests, analyses of proportions, correlation, and survival curves have been analysed in Prism (GraphPad, La Jolla, CA, USA), version 7.0c.

12.14.3 Protocol deviations

Unintentional protocol deviations were discussed with my primary laboratory supervisor (Dr. Ross), and substitute animals added to the schedule as required, together with IRB review. All protocol deviations will be described.

CHAPTER THIRTEEN

RESULTS – PART TWO

13.1 Overview and exclusions

Experiments were completed between 30th July 2015 and 30th September 2015. There were no unintentional protocol deviations during the setup and injury phases in either group. However, five animals in the SAAP-FWB2 group went into VF during the first minute of the SAAP infusion (7955, 7956, 7964, 8001, 8011). The cause of the VF is unknown and during model development was resistant to treatment.

The Part Two hypotheses cannot be answered with animals in VF. These animals were excluded from further intervention, and additional animals added to the schedule with IRB review. Therefore, a total of 21 animals were used for Part Two, with 16 included in the data analysis. A description of the five excluded animals is presented at the end of Part Two results (Section 13.16).

13.2 Blood donor pool

A total of 38 swine were used in the blood donor pool, providing 208 units of FWB for experimental protocols. The mean haemoglobin was 9.7 (±1.0) g/dl. All episodes of venesection were at least two weeks apart, and three animals (7910, 7911, 7862) had three venesections; there was no significant difference in mean haemoglobin between the first, second, and third venesections (ANOVA, p=0.1).

13.3 Animal baseline characteristics

13.3.1 Baseline physiology

All 16 animals were within the required range of 70-90 kg, with a mean weight of 74.6 (±3.9) kg. At baseline, the mean values were: systolic BP 96.6 (±9.1) mmHg, end-tidal carbon dioxide 5.5 (±0.3) kPa, and left carotid artery flow 367.0 (±146.8) ml/minute. There were no statistical differences in baseline physiology between groups, Table 13.1.

13.3.2 Baseline blood sample analyses

At baseline, an ABG, and a clotting profile (including TEG) were drawn. The mean arterial partial pressure of oxygen was 14.8 (±4.2) kPa, with an inspired fraction of oxygen of 0.21 (PaO_2:FiO_2 = 528.6 (±148.3) mmHg). There were no observed differences in blood samples at baseline between groups, Table 13.2.

Table 13.1 – Baseline physiological characteristics between groups, mean (± standard deviation).

Variable	SAAP-LR2	SAAP-FWB2	t-test
Animal weight (kg)	75.5 (±4.1)	73.6 (±3.7)	p=0.35
Heart rate (beats/minute)	95.0 (±18.7)	97.4 (±20.4)	p=0.81
Systolic blood pressure (mmHg)	96.1 (±7.7)	97.1 (±11.0)	p=0.84
Mean arterial pressure (mmHg)	80.0 (±5.1)	79.5 (±10.2)	p=0.90
Mean pulmonary artery pressure (mmHg)	24.6 (±2.1)	24.8 (±4.5)	p=0.92
Cardiac output (l/minute)	6.0 (±1.1)	6.0 (±1.2)	p=0.98
SaO_2 (%)	98.6 (±1.1)	99.3 (±1.2)	p=0.28
End-tidal CO_2 (kPa)	5.6 (±0.3)	5.5 (±0.3)	p=0.49
Left carotid artery flow (ml/minute)	352.6 (±126.2)	381.4 (±172.6)	p=0.71
Central venous pressure (mmHg)	8.5 (±1.8)	9.8 (±2.3)	p=0.25
Central venous saturation (%)	69.9 (±8.3)	72.9 (±6.7)	p=0.44
Core temperature (^oC)	37.2 (±0.6)	37.0 (±0.7)	p=0.48

Table 13.2 – Baseline blood sample analysis between groups, mean (± standard deviation).

Variable	SAAP-LR2	SAAP-FWB2	t-test
Arterial blood gas			
pH	7.48 (±0.03)	7.48 (±0.3)	p=0.86
pO_2 (kPa)	14.4 (±5.3)	15.2 (±2.9)	p=0.70
pCO_2 (kPa)	5.3 (±0.3)	5.6 (±0.4)	p=0.13
Ionised calcium (mmol/l)	1.3 (±0.1)	1.3 (±0.0)	p=0.26
Potassium (mmol/l)	4.1 (±0.2)	4.3 (±0.3)	p=0.27
Sodium (mmol/l)	137.8 (±1.3)	138.9 (±1.2)	p=0.10
Glucose (mmol/l)	5.6 (±0.5)	6.0 (±1.2)	p=0.40
Lactate (mmol/l)	1.7 (±0.5)	1.7 (±0.9)	p=0.87
Base deficit	-5.7 (±1.2)	-6.6 (±1.5)	p=0.21
Full blood count			
Haemoglobin (g/dl)	11.2 (±0.7)	10.8 (±0.7)	p=0.22
White cell count ($x10^9$/l)	17.9 (±5.5)	15.7 (±5.1)	p=0.44
Platelet count ($x10^9$/l)	322.9 (±77.7)	340.8 (±96.6)	p=0.69
Clotting profile			
PT (seconds)	13.3 (±0.5)	13.4 (±0.4)	p=0.69
Fibrinogen (g/l)	2.0 (±0.4)	2.3 (±0.6)	p=0.28
Thromboelastography			
R (seconds)	5.1 (±1.1)	5.6 (±2.1)	p=0.57
K (seconds)	1.3 (±0.4)	1.4 (±0.5)	p=0.47
α angle (degrees)	72.9 (±4.6)	70.4 (±7.5)	p=0.44
MA (mm)	74.7 (±5.9)	77.7 (±4.4)	p=0.28
G	15.5 (±5.3)	18.2 (±4.6)	p=0.31
CI	3.0 (±1.6)	2.9 (±2.4)	p=0.87
LY-30 (%)	1.9 (±1.3)	2.0 (±1.2)	p=0.98

13.4 Injury analysis

13.4.1 NCTH and controlled arterial haemorrhage

The hepatic injury was completed in a mean of 101.5 (±10.0) seconds, and was not significantly different between groups. The mean percent excision of the left lateral lobe of the liver by weight was 66.6 (±7.1) %, and was also not significantly different between groups, Table 13.3.

Table 13.3 – Injury characteristics between groups, mean (± standard deviation).

Variable	SAAP-LR2	SAAP-FWB2	t-test
Animal weight (kg)	75.5 (±4.1)	73.6 (±3.7)	p=0.35
Spleen weight (g)	517.3 (±139.2)	519.0 (±129.1)	p=0.98
Liver weight (kg)	1456.3 (±158.6)	1636.9 (±314.2)	p=0.18
Injury time (seconds)	782.1 (±146.2)	776.7 (±149.2)	p=0.94
NCTH			
Hepatic injury (seconds)	98.5 (±9.7)	104.5 (±9.9)	p=0.24
Proportion of liver lobe excised (%)	65.5 (±8.5)	67.7 (±5.8)	p=0.55
Abdominal haemorrhage (ml)	2475.1 (±1217.5)	1212.0 (±678.4)	p<0.05*
Abdominal haemorrhage (ml/kg)	32.4 (±15.4)	16.7 (±9.5)	p<0.05*
Controlled haemorrhage			
Controlled haemorrhage (ml)	1412.5 (±515.3)	1497.8 (±548.1)	p=0.75
Controlled haemorrhage (ml/kg)	19.0 (±7.4)	20.8 (±7.5)	p=0.64
Combined haemorrhage			
Total haemorrhage (ml)	3887.6 (±1163.9)	2709.7 (±422.4)	p<0.05*
Total haemorrhage (ml/kg)	51.4 (±14.7)	37.5 (±7.3)	p<0.05*
Proportion of swine TBV (%)	76.4 (±21.9)	55.7 (±10.9)	p<0.05*

The volume of NCTH, measured after the intervention, was a mean of 1,843.5 (±1,154.1) ml, and was a significantly higher volume in the SAAP-LR2 group. The mean volume of controlled arterial haemorrhage was 1,455.1 (±515.8) ml, equivalent to 19.9 (±7.2) ml/kg, and was not statistically different between groups, Table 13.3.

The mean time interval between the start of the hepatic injury to the onset of TCA (t0 – no intra-aortic pressure fluctuations) was 779.4 (±142.7) seconds, and was not statistically different between groups, Table 13.3.

13.4.2 Arrest period physiology

At the onset of TCA (t0), a mean of 779.4 (±142.7) seconds after the start of the hepatic injury, none of the animals had intra-aortic pressure fluctuations, and all had a cardiac electrical rate lower than baseline measurement.

This haemorrhage model produced a very severe arrest, after an injury period that was a mean of 104.8 seconds longer than Part One, $p<0.05$. Owing to the protocol definition of TCA in Part Two, the mean systolic BP and left carotid artery flow were both zero. The mean end-tidal carbon dioxide at t0 was 0.7 (±0.2) kPa, and was not significantly different between groups – SAAP-LR2 0.6 (±0.3) kPa, SAAP-FWB2 0.7 (±0.1) kPa, $p=0.24$.

During the arrest period (t0-t3), the mean cardiac electrical rate was measured over 30 seconds immediately before the start of the intervention (t2.5 to t3), and was a mean of 60.2 (±24.7) beats/minute. There was no significant difference in cardiac electrical rate between groups – SAAP-LR2 63.8 (±24.7) beats/minute, SAAP-FWB2 56.6 (±28.6) beats/minute, $p=0.58$.

Evaluation of lead II rhythm strips (t2.5 to t3) revealed that no animals in either group had a normal ECG – seven (43.8%) had ST segment elevation or

depression, and or T wave inversion, four (25.0%) had pauses for over five seconds, four (25.0%) had complete (third degree) heart block, and one (6.3%) was in cardiac electrical asystole (SAAP-FWB2-2-7916).

13.4.3 Arrest point blood sample analyses

An ABG was drawn via the SAAP catheter, from the thoracic aorta, in all animals when the intra-aortic systolic BP fell below 10 mmHg - the same time point as Part One. In order to characterise the injury, markers of tissue perfusion (pH, lactate, base deficit) were examined.

The pH, lactate, and base deficit were all significantly higher compared to baseline measurement: 7.64 (±0.07) vs 7.48 (±0.03), 2.8 (±1.3) mmol/l vs 1.7 (±0.7) mmol/l, and -5.1 (±1.4) vs -6.2 (±1.4), respectively, all $p<0.01$. There was no statistical difference in pH, lactate, or base deficit between groups, Table 13.4.

Table 13.4 – Blood sample analysis at systolic BP <10 mmHg, mean (± standard deviation).

Variable	SAAP-LR2	SAAP-FWB2	t-test
pH	7.62 (±0.07)	7.65 (±0.08)	p=0.48
Lactate (mmol/l)	2.4 (±0.6)	3.2 (±1.7)	p=0.22
Base deficit	-4.8 (±1.3)	-5.4 (±1.6)	p=0.45

13.5 Intervention analysis

SAAP was started a mean of 188.6 (±6.4) seconds from the onset of TCA (t0), and was not significantly different between groups, p=0.50.

The SAAP infusions were different by design, either oxygenated FWB (SAAP-FWB2 group) or oxygenated LR (SAAP-LR2 group). The FWB infusion had a mean haemoglobin of 7.1 (±0.6) g/dl, a partial pressure of oxygen of 82.7 (±13.0) kPa, a partial pressure of carbon dioxide of 2.9 (±1.5) kPa, and a pH of 7.46 (±0.15). The LR infusion contained no haemoglobin, had a partial pressure of oxygen of 76.4 (±10.9) kPa, a partial pressure of carbon dioxide of 0.3 (±0.4) kPa, and a pH of 7.12 (±0.12). As in Part One, there was no statistical difference between the partial pressure of oxygen in the FWB and LR infusions, $p=0.31$. However, as LR contains no haemoglobin, the oxygen content was significantly lower compared to the FWB infusion, 1.8 (±0.3) and 11.5 (±0.9) ml of oxygen per 100 ml of infusion respectively, $p<0.001$.

SAAP infusions were terminated either when the intra-aortic systolic BP was over 90 mmHg, or when the total maximum volume (4,000 ml) had been administered – these results answer one of the hypotheses, and are reported in Section 13.7.2.

13.6 Primary outcome

A total of seven (43.8%) animals survived the pre-hospital period: SAAP-LR2 group – one (12.5%, 95%CI 0.6-47.1), SAAP-FWB2 group – six (75.0%, 95%CI 40.9-95.6), $p<0.05$, Figure 13.1.

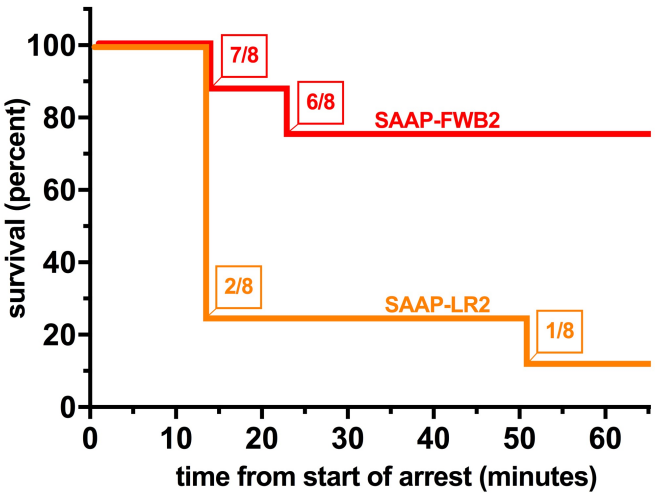

Figure 13.1 – Pre-hospital (60 minute) survival post intervention, a comparison of SAAP-LR2 and SAAP-FWB2.

13.7 Secondary outcomes

13.7.1 ROSC

ROSC, defined as a systolic BP of 50 mmHg or greater, was observed in nine (56.3%) animals at t13 (ten minutes after the start of the intervention): SAAP-LR2 group – two (25.0%, 95%CI 4.4-59.1), SAAP-FWB2 group – seven (87.50%, 95%CI 52.9-99.4), $p<0.05$, Figure 13.2.

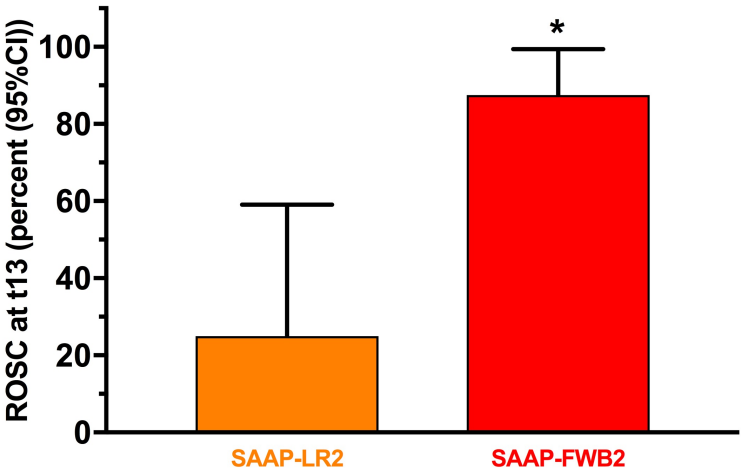

Figure 13.2 – ROSC at t13, ten minutes after the start of the intervention, a comparison of SAAP-LR2 and SAAP-FWB2.

13.7.2 The volume of SAAP infusion

The SAAP infusion was stopped if animals had an intrinsic systolic BP of 90 mmHg or greater, or when the maximum volume (4,000 ml) had been administered. Seven (87.5%) of the SAAP-LR2 group received 4,000 ml of SAAP infusion, compared to two (25.0%) of the SAAP-FWB2 group. The time (and therefore volume) to achieve a systolic BP greater than 90 mmHg was compared in those with a ROSC (systolic BP ≥50 mmHg) at t13.

There was a trend of shorter SAAP infusion times and lower SAAP volumes (in animals with a ROSC) in the SAAP-FWB2 group (n=7), compared to the SAAP-LR2 group (n=2) – 1,742.9 (±1034.5) ml of FWB in 130.7 (±77.6) seconds, and 2,580.0 (±2,008.2) ml of LR in 193.5 (±150.6) seconds respectively, but this did not reach significance, p=0.66.

13.7.3 Animals without a ROSC

Seven (43.8%) animals in Part Two did not have a ROSC at t13 – six in the SAAP-LR2 group, and one in the SAAP-FWB2 group.

In the SAAP-LR2 group, three animals without a ROSC (SAAP-LR2-1-7873, SAAP-LR2-2-7875, SAAP-LR2-7-7986) had no evidence of a return of intrinsic cardiac output during SAAP infusion. The other three animals (SAAP-LR2-4-7977, SAAP-LR2-6-7972, SAAP-LR2-8-8002) did have a return of intrinsic cardiac output during SAAP. 7977 had a maximum BP of 25.8/18.3 mmHg after 158 seconds of SAAP, 7972 had a maximum BP of 74.0/25.9 mmHg after 156 seconds of SAAP, and 8002 had a maximum BP of 67.1/24.2 mmHg after 244 seconds of SAAP. However, as per protocol, all three animals received ongoing SAAP infusion, and none had evidence of intrinsic cardiac output at the end of the infusion, and all met death-in-protocol criteria at t13.

In the SAAP-FWB2 group, one animal without a ROSC (SAAP-FWB2-2-7916) had a return of intrinsic cardiac output during SAAP, and had a maximum BP of 81.4/47.6 mmHg after 90 seconds of SAAP. This animal continued to receive SAAP infusion with FWB, did not have an intrinsic cardiac output at the end of the SAAP infusion, and met death-in-protocol criteria at t13.

13.7.4 Physiology and blood samples at t13 – all animals

The systolic BP, left carotid artery flow, and end-tidal carbon dioxide were compared between groups, in all animals at t13 (ten minutes after the start of

the intervention). The highest mean values were observed in the SAAP-FWB2 group, Figures 13.3a, 13.3b, 13.3c.

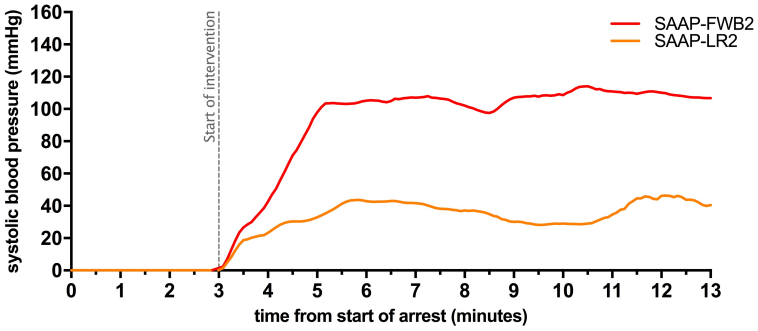

Figure 13.3a – Mean systolic BP from the time of arrest to t13 – a comparison of SAAP-LR2 and SAAP-FWB2.

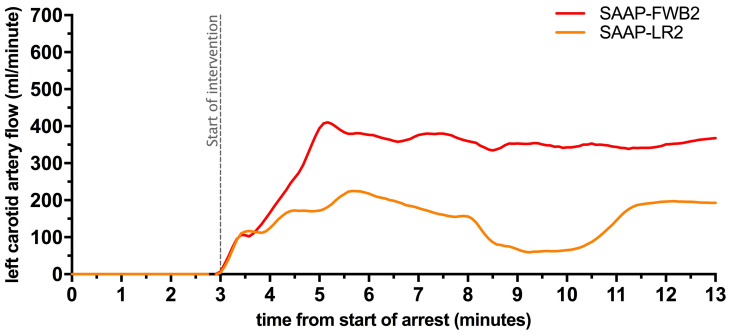

Figure 13.3b – Mean left carotid artery flow from the time of arrest to t13 – a comparison of SAAP-LR2 and SAAP-FWB2.

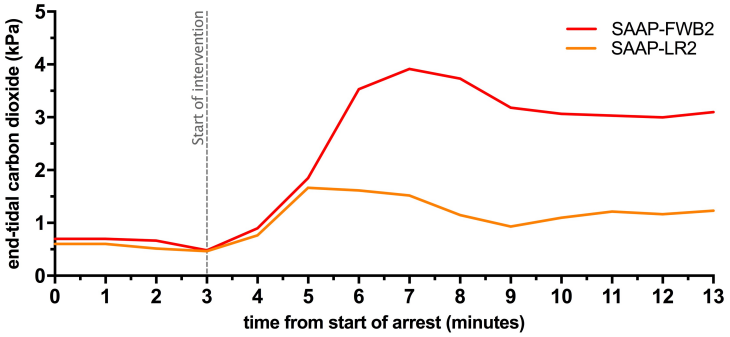

Figure 13.3c – Mean end-tidal carbon dioxide from the time of arrest to t13 – a comparison of SAAP-LR2 and SAAP-FWB2.

The SAAP-FWB2 group had a significantly higher systolic BP and end-tidal carbon dioxide at t13 compared to the SAAP-LR2 group, but there was no significant difference in the left carotid artery flow, Table 13.5.

Table 13.5 – A comparison of systolic BP, left carotid artery flow, and end-tidal carbon dioxide between SAAP-LR2 and SAAP-FWB2 at t13, mean (± standard deviation).

Variable	SAAP-LR2	SAAP-FWB2	t-test
Systolic BP (mmHg)	40.6 (±57.3)	106.7 (±52.4)	p<0.05*
Left carotid artery flow (ml/minute)	168.5 (±358.3)	368.8 (±224.7)	p=0.22
End-tidal carbon dioxide (kPa)	1.2 (±1.8)	3.1 (±1.4)	p<0.05*

At t13, there was an attempt to draw blood samples (an ABG, full blood count, and clotting profile (including TEG)). Owing to the lack of circulation in one SAAP-FWB2 animal (SAAP-FWB2-2-7916) no samples were obtained from this animal. Additionally, the citrated blood samples in one SAAP-LR2 animal (SAAP-LR2-8-8002) were clotted and were not included. These variables have therefore been analysed as n=7.

There was no significant difference in pH at t13 between the two groups, but the SAAP-FWB2 group had significantly higher partial pressures of oxygen and carbon dioxide, a significantly lower lactate, and a significantly higher ionised calcium concentration, Table 13.6.

Table 13.6 – A comparison of blood samples between SAAP-LR2 and SAAP-FWB2 at t13, mean (± standard deviation).

Variable	SAAP-LR2	SAAP-FWB2	t-test
Arterial blood gas			
pH	7.48 (±0.15)	7.39 (±0.10)	p=0.18
pO_2 (kPa)	25.7 (±19.9)	52.1 (±20.6)	p<0.05*
pCO_2 (kPa)	3.5 (±1.4)	4.9 (±0.8)	p<0.05*
Ionised calcium (mmol/l)	1.1 (±0.1)	1.3 (±0.1)	p<0.01**
Lactate (mmol/l)	12.3 (±2.2)	6.0 (±0.9)	p<0.001***
Base deficit	5.3 (±3.4)	3.0 (±2.7)	p=0.17
Full blood count			
Haemoglobin (g/dl)	3.6 (±1.5)	8.5 (±0.8)	p<0.001***
Platelet count ($x10^9$/l)	156.9 (±55.8)	260.1 (±41.7)	p<0.01**
Clotting profile			
PT (seconds)	23.8 (±15.2)	13.5 (±0.4)	p=0.16
Fibrinogen (g/l)	0.6 (±0.3)	1.7 (±0.3)	p<0.001***
Thromboelastography			
R (seconds)	3.7 (±1.2)	5.6 (±3.9)	p=0.26
K (seconds)	3.4 (±4.7)	1.2 (±0.4)	p=0.27
α angle (degrees)	62.1 (±15.6)	72.8 (±5.1)	p=0.13
MA (mm)	52.6 (±15.5)	70.7 (±6.4)	p<0.05*
G	6.3 (±2.9)	12.7 (±3.4)	p<0.01**
CI	-0.4 (±5.1)	2.3 (±3.3)	p=0.29
LY-30 (%)	0.8 (±1.1)	1.3 (±1.7)	p=0.47
Pulmonary gas exchange			
PaO_2:FiO_2	193.1 (±149.2)	390.9 (±154.5)	p<0.05*

The SAAP-FWB2 group had a significantly higher haemoglobin, platelet count, and fibrinogen concentration than the SAAP-LR2 group. There was a trend of a lower prothrombin time in the SAAP-FWB2 group, but this did not reach significance. In comparison of TEG values, there was a trend of higher clot function in the SAAP-FWB2 group, but the maximum amplitude and clot elasticity were the only values that reached significance, Table 13.6.

13.7.5 Physiology and blood samples – live animals

In order to present the physiological state of animals alive at t13 (compared to the earlier assessment of all animals in both groups), further comparison was

undertaken between the seven animals in the SAAP-FWB2 group and the two animals in the SAAP-LR2 group that were alive at this time point. The SAAP-LR2 group (n=2) had a higher mean systolic BP, left carotid artery flow, and end-tidal carbon dioxide compared to the SAAP-FWB2 group (n=7) at t13, but these did not reach significance (p=0.93, p=0.60, p=0.76 respectively), Figures 13.4a, 13.4b, 13.4c.

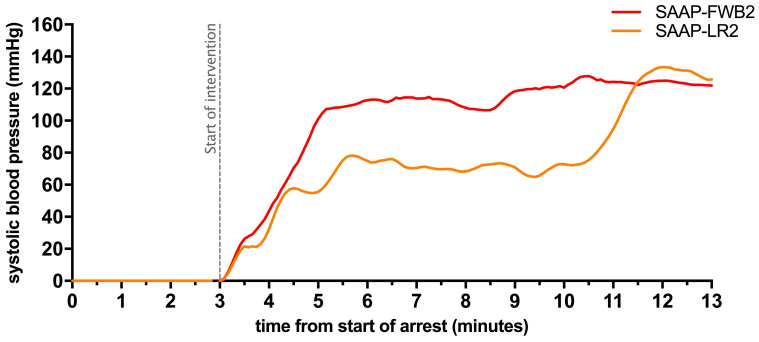

Figure 13.4a – Mean systolic BP from the time of arrest to t13 – a comparison of live animals, SAAP-LR2 (n=2) and SAAP-FWB2 (n=7).

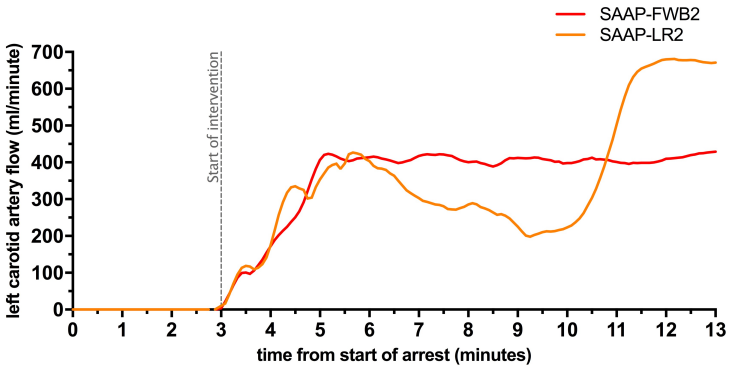

Figure 13.4b – Mean left carotid artery flow from the time of arrest to t13 – a comparison of live animals, SAAP-LR2 (n=2) and SAAP-FWB2 (n=7).

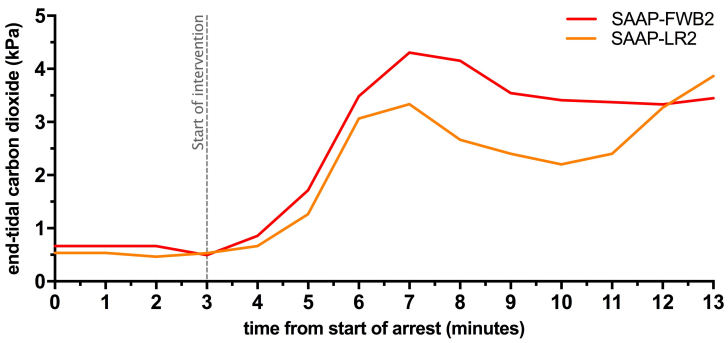

Figure 13.4c – Mean end-tidal carbon dioxide from the time of arrest to t13 – a comparison of live animals, SAAP-LR2 (n=2) and SAAP-FWB2 (n=7).

The SAAP-LR2 group (n=2) were significantly more alkalaemic than the SAAP-FWB2 group (n=7) – 7.49 (±0.01) and 7.39 (±0.01) respectively, $p<0.05$, but there was no significant difference in partial pressures of oxygen and carbon dioxide, base deficit, lactate, or calcium. The SAAP-LR2 group (n=2) had a significantly lower haemoglobin concentration compared to the SAAP-FWB2 group – 3.2 (±0.7) g/dl and 8.5 (±0.8) g/dl respectively, $p<0.05$, and a lower fibrinogen concentration – 0.7 (±0.1) g/l and 1.7 (±0.3) respectively, $p<0.001$, but there was no statistical difference in platelet count, prothrombin time, or any TEG values.

Although there was no statistical difference in the partial pressure of oxygen at t13 in alive animals, as the haemoglobin concentration was significantly lower in the SAAP-LR2 group, there was a significant difference in the calculated oxygen content. At t13, the mean calculated oxygen content in the SAAP-LR2 group was 5.6 (±1.4) ml of oxygen per 100 ml of blood, compared to 12.6 (±1.4) ml of oxygen per 100 ml of blood in the SAAP-FWB group, $p<0.05$.

In summary, although there was a significantly lower proportion of the SAAP-LR2 group animals alive at t13, compared to the SAAP-FWB2 group, there was not a significant difference in physiological variables (systolic BP, left carotid artery flow, end-tidal carbon dioxide), markers of tissue perfusion (lactate, base deficit), or clotting function (thromboelastography) between animals alive at t13.

13.7.6 Physiology and blood samples at 63

Seven (43.8%) animals survived to t63 (simulated hospital arrival), six (75.0%) in the SAAP-FWB2 group, and one (12.5%) in the SAAP-LR2 group, preventing statistical analysis by t-test between groups. However, the systolic BP and the end-tidal carbon dioxide in the single SAAP-LR2 animal were both lower than two standard deviations from the mean of the six SAAP-FWB2 group animals, Table 13.7.

Table 13.7 – A comparison of physiological measurements, and blood samples in SAAP-LR2 and SAAP-FWB2 at t63, mean only for SAAP-LR2, mean (± standard deviation) for SAAP-FWB2.

Variable	SAAP-LR2	SAAP-FWB2	>2 SD
N of animals	1	6	
Physiological measurements			
Systolic BP (mmHg)	65.4	99.4 (±12.1)	*
Left carotid artery flow (ml/minute)	454.5	453.0 (±126.9)	
End-tidal carbon dioxide (kPa)	2.5	4.2 (±0.6)	*
Arterial blood gas			
pH	7.53	7.29 (±0.04)	*
pO_2 (kPa)	26.3	65.6 (±4.0)	*
pCO_2 (kPa)	2.8	4.5 (±0.4)	*
Ionised calcium (mmol/l)	1.2	1.3 (±0.1)	
Lactate (mmol/l)	16.0	13.3 (±0.9)	*
Base deficit	4.5	9.8 (±1.2)	*
Full blood count			
Haemoglobin (g/dl)	3.6	9.0 (±0.6)	*
Platelets ($x10^9$/l)	141.0	248.3 (±24.6)	*
Clotting profile			
PT (seconds)	18.5	13.6 (±0.8)	*
Fibrinogen (g/l)	0.6	1.8 (±0.4)	*
Thromboelastography			
R (seconds)	3.8	4.7 (±2.1)	
K (seconds)	1.6	1.2 (±0.4)	
α angle (degrees)	67.7	73.2 (±5.3)	
MA (mm)	56.7	71.1 (±6.3)	*
G	6.5	13.0 (±3.7)	*
CI	1.2	3.0 (±2.6)	
LY-30 (%)	0.0	1.9 (±2.4)	

The pH and lactate in the SAAP-LR2 animal at t63 were more than two standard deviations higher than the mean of the SAAP-FWB2 group. The partial pressures of oxygen and carbon dioxide, the base deficit, haemoglobin, platelet count, fibrinogen, were more than two standard deviations lower than the mean of the SAAP-FWB2 group. The only TEG differences observed were the maximum clot amplitude and clot elasticity in the SAAP-LR2 animal - both were more than two standard deviations lower than the mean of the SAAP-FWB2 group, Table 12.7.

At t63, the SAAP-LR2 group reached the end of the protocol, and post-experimental procedures were undertaken.

13.8 Results of Part Two hypotheses (H4, H5, H6, H7)

In a swine model of severe haemorrhagic TCA, without intra-aortic pressure fluctuations:

H4. *SAAP with FWB will not result in a significantly higher proportion of animals with a ROSC ten minutes after the start of the intervention, compared to SAAP with LR.*

Seven (87.5%, 95%CI 52.9-99.4) animals in the SAAP-FWB2 group had a ROSC ten minutes after the start of the intervention (t13), compared to two (25.0%, 95%CI 4.4-59.1) in the SAAP-LR2 group, $p<0.05$. There was therefore a significantly higher proportion of SAAP-FWB animals with a ROSC compared to SAAP-LR in the Part Two injury model.

H5. *A significantly smaller volume of resuscitation fluid will be required to effect ROSC ten minutes after the start of the intervention in animals that receive SAAP with FWB, compared to SAAP with LR.*

Including only animals that had a ROSC at t13, there was a trend of shorter SAAP infusion times and lower SAAP volumes in the SAAP-FWB2 group (n=7), compared to the SAAP-LR2 group (n=2) – 1,742.9 (±1,034.5) ml of FWB in 130.7

(±77.6) seconds, and 2,580.0 (±2,008.2) ml of LR in 193.5 (±150.6) seconds respectively, but this did not reach statistical significance, p=0.66.

H6. *A larger volume of initial SAAP infusion (up to 4,000 ml) with LR will result in a significantly higher proportion of animals with a systolic BP equal to or greater than 50 mmHg ten minutes after the start of the intervention, compared to Part One historical controls (that received 1,600 ml of SAAP with LR), despite a more severe injury.*

In Part One, the SAAP-LR group (n=10) all received 1,600 ml of LR over two minutes, and six (60.0%, 95%CI 31.3-83.2) had a systolic BP equal to or greater than 50 mmHg at t13. In Part Two, with a more severe injury, the SAAP-LR2 group received up to 4,000 ml of LR over five minutes. Seven (97.5%) animals in the SAAP-LR2 group received 4,000 ml, and one received 1,160 ml, a mean volume of 3,053.3 (±1,639.7) ml. Two (25.0%, 95%CI 4.4-59.1) animals in the SAAP-LR2 group had a systolic BP equal to or greater than 50 mmHg at t13. Therefore, there was a lower proportion of animals with a systolic BP equal to or greater than 50 mmHg at t13 in the SAAP-LR2 group compared with the SAAP-LR group from Part One, although this did not reach significance, p=0.19.

H7. *SAAP with FWB will infer a significant 60 minute survival advantage over SAAP with LR.*

In Part Two, including a haemorrhagic injury that resulted in no intra-aortic pressure fluctuations for three minutes, six (75.0%, 95%CI 40.9-95.6) animals in

the SAAP-FWB2 group, and one (12.5%, 95%CI 0.6-47.1) animal in the SAAP-LR2 group survived to 60 minutes after the start of the intervention, $p<0.05$. SAAP with FWB in this model of injury did therefore infer a significant survival advantage over SAAP with LR.

13.9 Surgery period – SAAP-FWB2 group

Six (75.0%) animals in the SAAP-FWB2 group survived to t63, and were transitioned into the surgery period. As per Part One methodology, the SAAP balloon was kept inflated in situ, and 500 ml of intravenous FWB was administered via the left internal jugular vein 8.5 Fr catheter over one minute, together with 10% calcium chloride. The maximum FWB resuscitation fluid allocated per animal was identical to Part One – a total of 5,750 ml from t63 onwards, with an infusion trigger of a systolic BP less than 90 mmHg.

At t64, the abdomen was opened via midline laparotomy, and rapid control of the hepatic injury was achieved in all animals. At t78 (after 75 minutes of IABO), the SAAP catheter was deflated in a controlled manner – the catheter was removed from the right femoral artery sheath at t93.

All six animals survived the surgery period, and received a mean intravenous FWB volume of 3,791.7 (±1,133.8) ml. All animals displayed cardiovascular instability on deflation and removal of the SAAP catheter, Figures 13.5a, 13.5b.

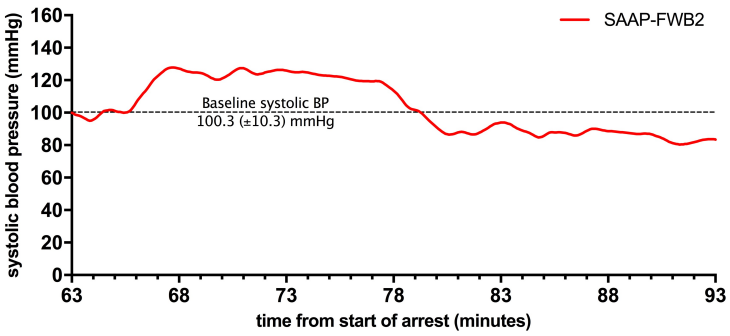

Figure 13.5a – Mean systolic BP in SAAP-FWB2 animals (n=6) during the surgery period (t63 to t93).

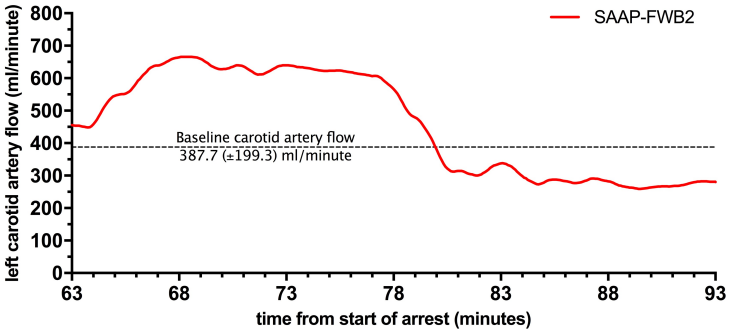

Figure 13.5b – Mean left carotid artery flow in SAAP-FWB2 animals (n=6) during the surgery period (t63 to t93).

The cardiovascular instability observed was associated with a significant decrease in pH, the partial pressure of oxygen, and PaO_2:FiO_2, and a significant increase in end-tidal carbon dioxide, the partial pressure of carbon dioxide, and base deficit, Table 13.8.

Table 13.8 – A comparison of physiological variables and blood samples in SAAP-FWB2 animals (n=6) between t63 and t93, mean (± standard deviation).

Variable	t63	t93	t-test
Physiological measurements			
Systolic BP (mmHg)	99.4 (±12.1)	82.5 (±10.1)	p=0.09
Left carotid artery flow (ml/minute)	453.0 (±126.9)	278.4 (±60.3)	p=0.07
End-tidal carbon dioxide (kPa)	4.2 (±0.6)	7.4 (±0.4)	p<0.001***
Arterial blood gas			
pH	7.29 (±0.04)	7.01 (±0.03)	p<0.001***
pO_2 (kPa)	65.6 (±4.0)	47.9 (±17.1)	p<0.05*
pCO_2 (kPa)	4.5 (±0.4)	8.0 (±0.4)	p<0.001***
Potassium (mmol/l)	5.2 (±0.5)	5.0 (±0.4)	p=0.07
Lactate (mmol/l)	13.3 (±0.9)	13.6 (±1.5)	p=0.67
Base deficit	9.8 (±1.2)	14.6 (±1.0)	p<0.01**
PaO_2:FiO_2	492.2 (±29.9)	359.5 (±128.1)	p<0.05*

13.10 Critical care period – SAAP-FWB2 group

Six animals in the SAAP-FWB2 group survived to t93, and were transitioned into the critical care period.

13.10.1 ECLS setup

At t93, arterial and venous ECLS cannulae were inserted over a wire into the right femoral artery and left femoral vein respectively. In one animal (SAAP-FWB2-3-7919), it was not possible to advance the venous ECLS cannula via the left femoral vein, and therefore the cannula was placed in the right femoral vein – effecting a delay in starting ECLS flow. In SAAP-FWB2-4-7915, the wire used to insert the arterial ECLS cannula resulted in a vascular injury, and the animal died at t110. This animal was not replaced, reducing the group to n=5.

13.10.2 ECLS maintenance

Once the ECLS circuit had been primed, flow was increased from zero to 2.8 l/minute over two minutes. The mean time to start ECLS flow, after removal of the SAAP catheter (t93) was 149.3 (±217.6) seconds. The group received a mean additional volume of FWB of 2,350 (±675.5) ml using the trigger of a systolic BP of less than 90 mmHg, and a mean volume of LR of 6,900 (±1,474.8) ml to maintain the venous ECLS pressure greater than -150 mmHg between t93 and t273.

All five animals in the SAAP-FWB2 group that received ECLS survived to the end of the protocol (t273). The SAAP-FWB2 group therefore had a total study survival of five out of eight (62.5%, 95%CI 30.6-86.3), including one animal that died after an unsuccessful attempt to place the ECLS cannulae, Figure 13.6.

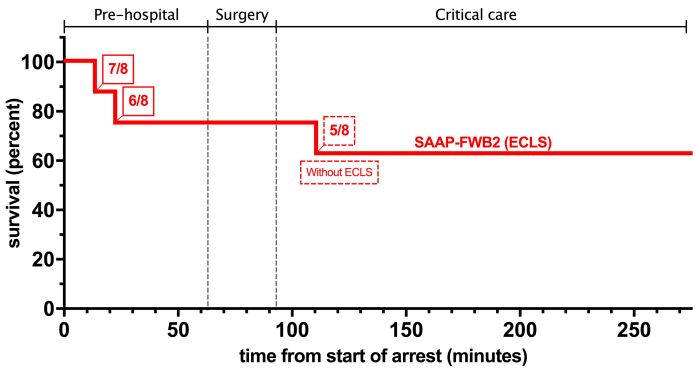

Figure 13.6 – Total protocol survival in the SAAP-FWB2 group, including one animal that died without ECLS at t110.

The five animals in the SAAP-FWB2 group that survived to the end of the protocol had a mean systolic BP of 78.4 (±11.0) mmHg, and a mean left carotid artery flow of 328.8 (±96.8) ml/minute at t273, Figures 13.7a, 13.7b.

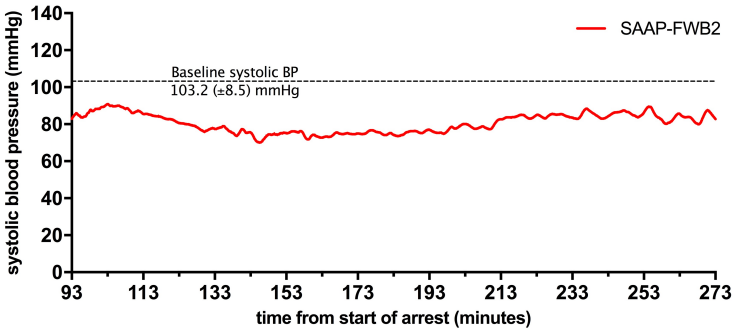

Figure 13.7a – Mean systolic BP in SAAP-FWB2 animals (n=5) during the critical care period (t93 to t273).

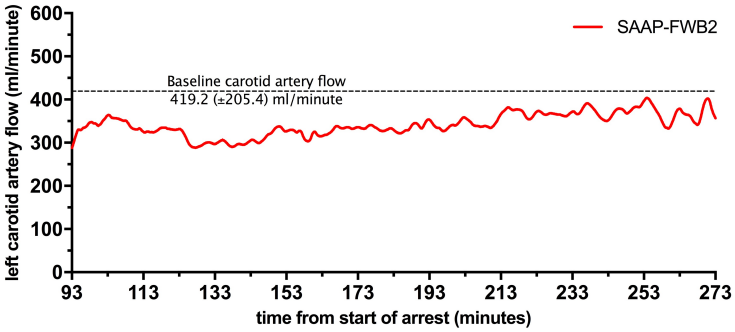

Figure 13.7b – Mean left carotid artery flow in SAAP-FWB2 animals (n=5) during the critical care period (t93 to t273).

During three hours of ECLS, the mean haemoglobin did not significantly change – 9.9 (±0.8) g/dl at t93, and 9.6 (±1.1) g/dl at t273, p=0.71. There was a trend of decreasing lactate, although this did not reach statistical significance, but there was a significant increase in pH – 7.01 (±0.03) to 7.30 (±0.06), p<0.001, and a

significant fall in base deficit – 14.9 (±0.9) to 8.5 (±1.6), p<0.01, Figures 13.8a, 13.8b, 13.8c, 13.8d.

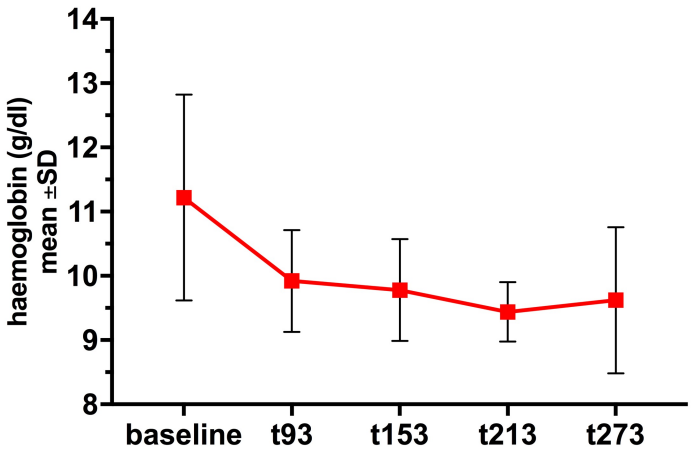

Figure 13.8a – Mean (± standard deviation) haemoglobin concentration in SAAP-FWB2 animals (n=5) at baseline and during the critical care period (t93 to t273).

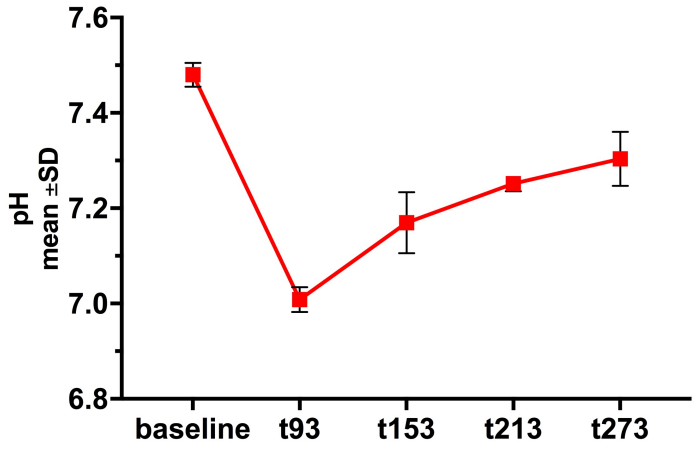

Figure 13.8b – Mean (± standard deviation) pH in SAAP-FWB2 animals (n=5) at baseline and during the critical care period (t93 to t273).

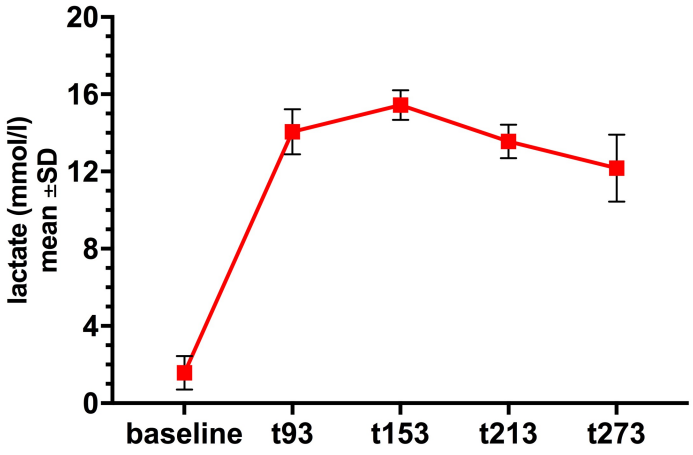

Figure 13.8c – Mean (± standard deviation) lactate concentration in SAAP-FWB2 animals (n=5) at baseline and during the critical care period (t93 to t273).

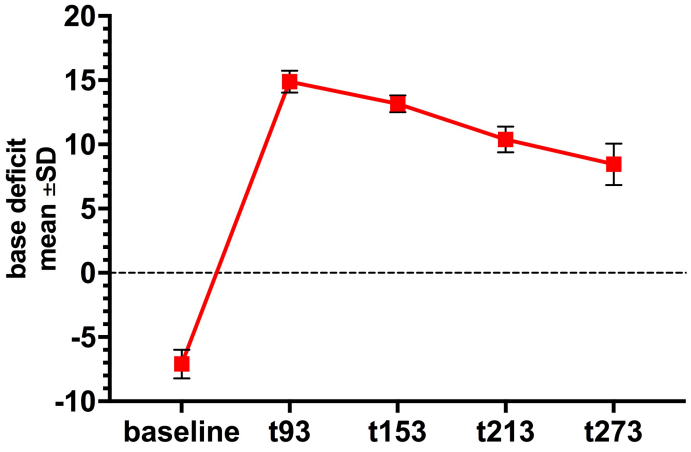

Figure 13.8d – Mean (± standard deviation) base deficit in SAAP-FWB2 animals (n=5) at baseline and during the critical care period (t93 to t273).

13.10.3 ELCS anticoagulation

All animals received a bolus of heparin at t78, followed by a heparin infusion of 60 units/hour between t93 and t273 (per protocol). ACT was a mean of 98.8 (±13.0) seconds at baseline, increasing to 161.8 (±16.5) seconds at t123. The

mean ACT between t123 and t243 was 169.6 (±23.5) seconds (a ratio to baseline of 1.7 (±0.4)), with a trend of increasing ACT throughout ECLS. This was associated with a significant increase in the prothrombin time between t63 and t273, from 13.4 (±0.3) to 16.2 (±1.4) seconds, $p<0.05$. In assessment of clotting function by TEG there was a trend towards reduced clot maximum amplitude and clot index between t63 and t273, but no TEG values reached statistical significance.

However, there was a significant fall in platelet count and fibrinogen concentration during ECLS – 236.2 (±22.9) to 180.2 (±33.7) $x10^9$/l, $p<0.01$, and 1.7 (±0.3) to 1.2 (±0.3) g/l, $p<0.05$ respectively. There was no observed bleeding from line insertion sites during the protocol, and observation of the ECLS circuit throughout experiments and during post-experimental maintenance did not reveal any visible clots.

At post-mortem, after t273, all surgical packing material was removed from the abdominal cavity, which revealed a small amount of blood-stained serous fluid in all animals, estimated to be less than 100 ml.

13.10.4 ECLS gas exchange

ABG samples were obtained from both the ECLS circuit outflow, and the left brachial artery every 30 minutes from t123 onwards, which demonstrated a differential hypoxaemia, Figure 13.9.

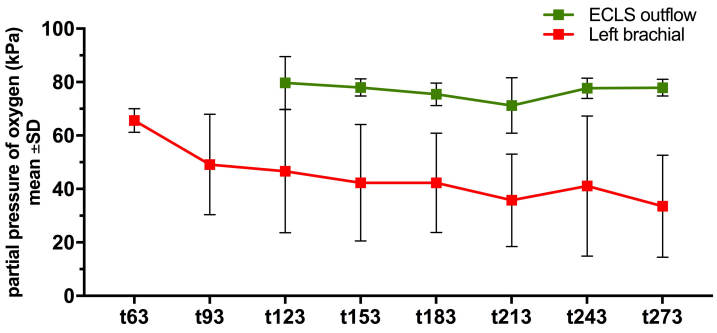

Figure 13.9 – The partial pressures of oxygen, mean (± standard deviation) from the ECLS outflow and the left brachial artery, from t63 to t273.

After the rise in the partial pressure of carbon dioxide at t93 after SAAP catheter removal, the partial pressure of carbon dioxide reduced throughout ECLS, Figure 13.10.

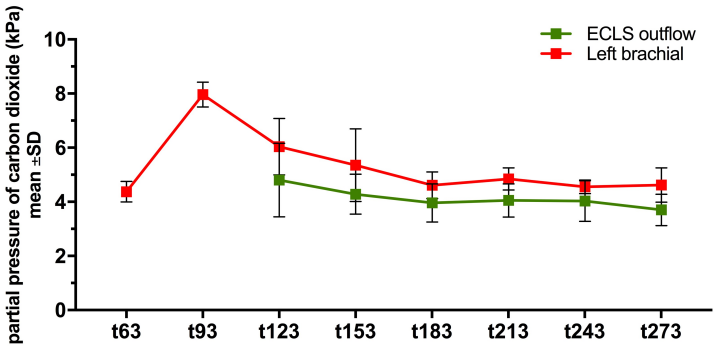

Figure 13.10 – The partial pressures of carbon dioxide, mean (± standard deviation) from the ECLS outflow and the left brachial artery, from t63 to t273.

13.11 Comparison of baseline and end of the protocol

In order to quantify the effects of the injury, followed by SAAP resuscitation with FWB, then three hours of ECLS, physiological variables and blood samples were compared in the SAAP-FWB2 group of n=5 at baseline and at t273. In summary,

animals at the end of the protocol had a significantly lower systolic BP, and higher mean pulmonary artery pressure, but carotid artery flow, central venous saturations, and core temperature were not significantly different. The pH at t273 was significantly lower than baseline, with biochemical evidence of hypoperfusion. Serum potassium concentration was significantly higher at t273 compared to baseline, and serum sodium concentration was significantly lower. There was evidence of impaired pulmonary gas exchange, with a significantly lower PaO_2:FiO_2 at t273 compared to baseline – furthermore, there was not a significant difference in the partial pressure of oxygen despite animals receiving a fraction of inspired oxygen of 0.21 at baseline, and 1.0 at t273. Animals at t273 had a significantly prolonged prothrombin time, reduced maximum clot amplitude and clot elasticity compared to baseline, Table 13.9.

Table 13.9 – A comparison of physiological variables and blood samples in SAAP-FWB2 animals alive at t273 (n=5) between baseline and t273, mean (± standard deviation).

Variable	Baseline	t273	t-test
Physiological measurements			
Heart rate (beats/minute)	100.2 (±18.3)	106.8 (±14.7)	p=0.26
Systolic BP (mmHg)	103.2 (±8.5)	81.8 (±10.6)	p<0.05*
Mean arterial pressure (mmHg)	86.0 (±5.8)	63.0 (±12.6)	p<0.05*
Mean pulmonary artery pressure (mmHg)	26.2 (±5.2)	43.2 (±6.2)	p<0.01**
Left carotid artery flow (ml/minute)	419.2 (±205.4)	326.4 (±99.4)	p=0.33
Central venous saturation (%)	74.6 (±8.0)	84.4 (±2.4)	p=0.07
Core temperature (^{o}C)	36.9 (±0.7)	35.8 (±1.6)	p=0.07
Arterial blood gas			
pH	7.48 (±0.02)	7.30 (±0.06)	p<0.01**
pO_2 (kPa)	15.4 (±1.6)	33.5 (±19.1)	p=0.10
pCO_2 (kPa)	5.6 (±0.3)	4.6 (±0.6)	p=0.09
Ionised calcium (mmol/l)	1.3 (±0.0)	1.3 (±0.2)	p=0.98
Potassium (mmol/l)	4.1 (±0.1)	6.7 (±1.3)	p<0.05*
Sodium (mmol/l)	139.2 (±1.5)	135.2 (±2.2)	p<0.05*
Lactate (mmol/l)	1.6 (±0.9)	12.2 (±1.7)	p<0.001***
Base deficit	-7.2 (±1.0)	8.5 (±1.6)	p<0.001***
PaO_2:FiO_2	548.6 (±57.3)	251.3 (±143.1)	p<0.05*
Full blood count			
Haemoglobin (g/dl)	10.9 (±0.9)	9.6 (±1.1)	p=0.08
White cell count (x10^9/l)	15.3 (±4.1)	4.7 (±3.2)	p<0.05*
Platelets (x10^9/l)	335.8 (±90.4)	180.2 (±33.7)	p<0.05*
Clotting profile			
PT (seconds)	13.5 (±0.5)	16.2 (±1.4)	p<0.05*
Fibrinogen (g/l)	2.4 (±0.8)	1.2 (±0.3)	p=0.06
Thromboelastography			
R (seconds)	5.7 (±0.9)	4.1 (±0.3)	p<0.05*
K (seconds)	1.4 (±0.1)	1.3 (±0.2)	p=0.46
α angle (degrees)	69.4 (±6.3)	71.3 (±2.5)	p=0.56
MA (mm)	77.1 (±4.7)	65.9 (±3.3)	p<0.05*
G	17.6 (±4.8)	9.8 (±1.5)	p<0.05*
CI	2.7 (±1.3)	2.5 (±0.9)	p=0.88
LY-30 (%)	1.8 (±1.2)	0.4 (±0.7)	p<0.01**

13.12 Cardiopulmonary dysfunction

13.12.1 Cardiac dysfunction

The physiological effects of balloon deflation followed by three hours of ECLS have already been described. The serum concentration of cardiac troponin I was

measured at baseline, t63, t93, and t153, in SAAP-FWB2 animals alive at t273. The concentration of troponin increased from 0.01 (±0.01) to 0.67 (±0.44) ng/ml between baseline and t63, $p<0.05$, and was significantly higher than baseline at t93 and t153, both $p<0.05$, Figure 13.11.

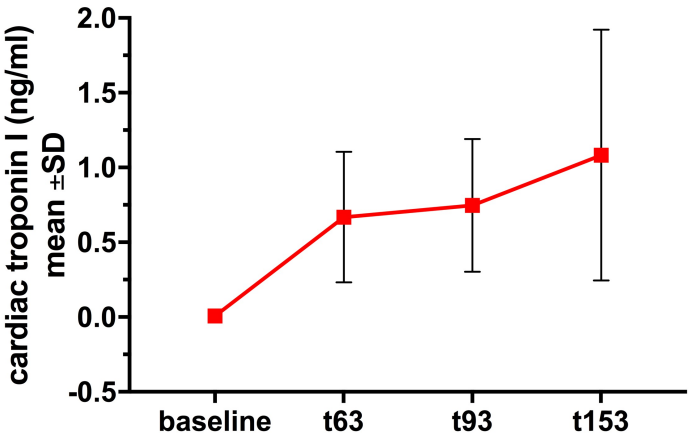

Figure 13.11 – Serum cardiac troponin I in SAAP-FWB2 animals (n=5) from baseline to t153 – mean (± standard deviation).

Histological examination of the left and right ventricles and inter-ventricular septum demonstrated no abnormal findings in two animals (SAAP-FWB2-1-7868, SAAP-FWB2-3-7919); these animals had relatively low serum troponin concentrations at t153 – 0.53 and 0.49 ng/ml respectively. Two animals had a serum troponin of 2.0 ng/ml at t153 (SAAP-FWB2-5-7995, SAAP-FWB2-7-8031); both had acute subendocardial haemorrhages in the left ventricle and septum, and interstitial perivascular inflammation in all samples. The animal (SAAP-FWB2-8-8008) with the lowest serum troponin (0.40) in this group of five

animals had no evidence of subendocardial haemorrhage, but did have interstitial perivascular inflammation in all samples.

13.12.2 Pulmonary dysfunction

As previously described, there was a significant decrease in the PaO_2:FiO_2 between baseline and t273, and between t63 and t93. In order to demonstrate the change in pulmonary gas exchange, the PaO_2:FiO_2 has been graphically presented throughout the protocol, Figure 13.12.

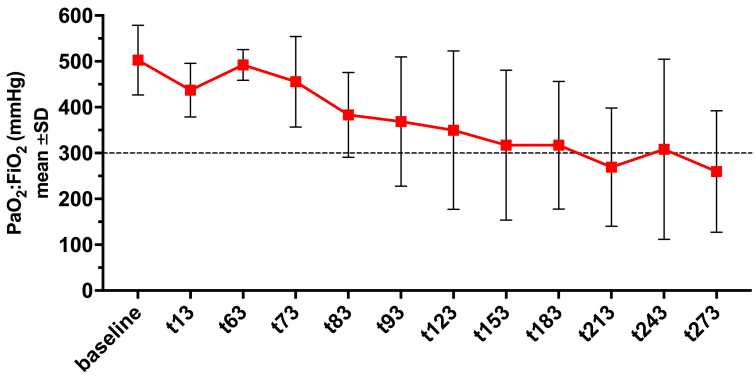

Figure 13.12 – PaO_2:FiO_2 in SAAP-FWB2 animals (n=5) from baseline to the end of the protocol (t273), mean (± standard deviation).

There was no significant change in pulmonary function between baseline and t63, and a trend towards reducing PaO_2:FiO_2 from t73 onwards.

At t273, three (60.0%, 95%CI 23.1-92.9) animals met the definition of the acute respiratory distress syndrome; one mild (<300 mmHg), one moderate (<200 mmHg), and one severe (<100 mmHg).

The serum levels of pro-inflammatory mediators (TNF-α, IL-1B, IL2, IL6, IL8) were measured at baseline, t63, t93, and t153. Between baseline and t63, there was a significant rise in IL-6, $p<0.01$, and a trend towards increased concentration in TNF-α, IL-1B, and IL8, but these did not reach significance; the concentration of IL-2 did not change.

There were significant increases at t153, compared to baseline, in serum concentrations of TNF-α, IL-1B, and IL-6.

There were significant correlations between serum concentrations of all analysed pro-inflammatory mediators at t153, and the PaO_2:FiO_2 at the end of the protocol (t273), Figure 13.13.

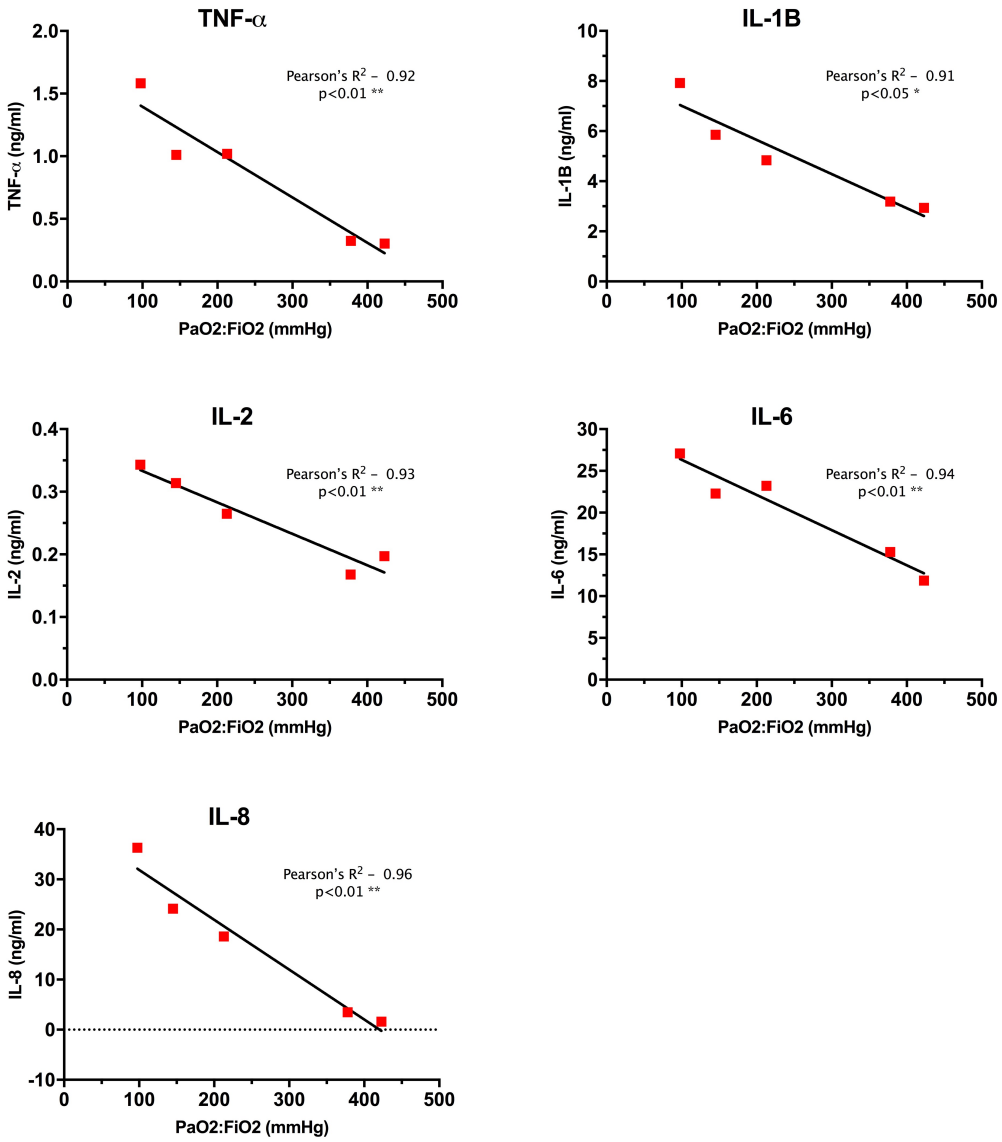

Figure 13.13 – Correlation of the serum concentration of pro-inflammatory mediators at t153, and PaO_2:FiO_2 at the end of the protocol (t273) in n=5 SAAP-FWB2 animals.

Histological examination of the apex and base of the left lung in the n=5 SAAP-FWB2 animals that survived to the end of the protocol revealed that two had mild focal atelectasis and one had moderate oedema at the lung apex. All five animals had moderate, diffuse oedema at the base, and two had acute moderate

alveolar haemorrhage. However, these findings did not differentiate animals with respect to calculated pulmonary function (PaO_2:FiO_2).

13.13 Comparison with Part One historical controls

In order to describe the effect of ECLS in Part Two on the mitigation of aortic balloon occlusion, comparisons have been made between the n=5 SAAP-FWB2 animals that received ECLS, and the Part One animals in the SAAP-FWB group who were alive at t93 (excluding the one animal that died after a technical error – SAAP-FWB3-7425), n=7.

At t93, the two groups had comparable physiology – the only difference in the examined variables was that the SAAP-FWB2 group had a significantly lower pH and higher partial pressure of carbon dioxide compared to the SAAP-FWB group, Table 13.10.

Table 13.10 – A comparison of physiological variables and blood samples at t93 in the Part One SAAP-FWB group (n=7), and the Part Two SAAP-FWB2 group (n=5), mean (± standard deviation).

Variable	SAAP-FWB	SAAP-FWB2	t-test
N of animals	7	5	
Physiological measurements			
Heart rate (beats/minute)	107.4 (±6.4)	125.2 (±28.8)	p=0.24
Systolic BP (mmHg)	80.9 (±14.7)	84.7 (±9.5)	p=0.59
Mean arterial pressure (mmHg)	56.3 (±8.4)	60.8 (±5.7)	p=0.29
Mean pulmonary artery pressure (mmHg)	41.6 (±2.4)	43.4 (±8.1)	p=0.65
Left carotid artery flow (ml/minute)	393.1 (±112.8)	276.6 (±69.4)	p=0.06
Central venous pressure (mmHg)	14.1 (±4.0)	13.4 (±2.5)	p=0.70
Arterial blood gas			
pH	7.10 (±0.03)	7.01 (±0.03)	p<0.001***
pO_2 (kPa)	56.4 (±7.4)	49.1 (±18.8)	p=0.46
pCO_2 (kPa)	5.9 (±1.0)	8.0 (±0.5)	p<0.001***
Ionised calcium (mmol/l)	1.2 (±0.0)	1.4 (±0.3)	p=0.38
Potassium (mmol/l)	5.2 (±0.4)	5.0 (±0.4)	p=0.34
Lactate (mmol/l)	14.6 (±1.6)	14.1 (±1.2)	p=0.50
Base deficit	14.9 (±1.8)	14.9 (±0.9)	p=0.94
PaO_2:FiO_2 (mmHg)	422.7 (±55.2)	368.6 (±141.0)	p=0.45
Full blood count			
Haemoglobin (g/dl)	9.9 (±0.6)	9.9 (±0.8)	p=0.89
Platelets ($x10^9$/l)	220.0 (±40.5)	236.2 (±22.9)	p=0.40
Volume of FWB resuscitation			
Initial SAAP infusion (ml)	1600.0 (±0.0)	1,301.3 (±295.4)	p=0.09
Pre-hospital boluses (ml)	607.1 (±556.3)	700.0 (±958.5)	p=0.85
Surgery period (ml)	3,228.6 (±1058.2)	3,400.0 (±675.5)	p=0.74
Total resuscitation (ml)	5,435.7 (±1009.0)	5,401.3 (±1070.9)	p=0.96

Two (28.6%, 95%CI 5.1-64.1) of the SAAP-FWB group (n=7) survived to the end of the protocol (t273), compared to five (100.0%, 95%CI 56.6-100.0) of the SAAP-FWB2 group (n=5), p<0.05, Figure 13.14.

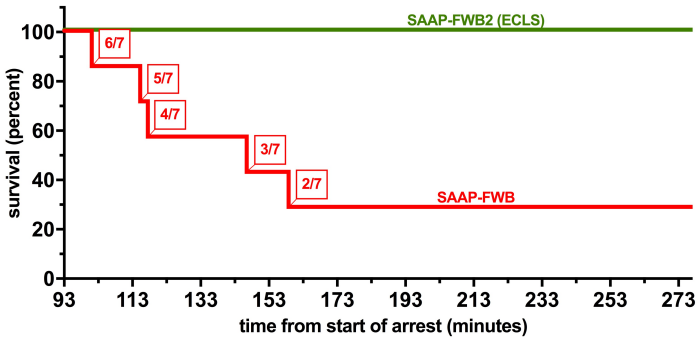

Figure 13.14 – Survival from t93 to t273 in Part One animals (SAAP-FWB, n=7), and Part Two animals (SAAP-FWB2, n=5) that received ECLS.

The SAAP-FWB group received a mean FWB volume of 2,035.7 (±1,025.0) ml after t93, compared to 2,350.0 (±675.5) ml in the SAAP-FWB2 group, p=0.57. However, the SAAP-FWB2 group received an additional mean LR volume of 6,900.0 (±1,474.8) ml in order to maintain the ECLS flow. The SAAP-FWB2 group therefore received a significantly higher volume of resuscitation fluid after t93 compared to the SAAP-FWB group, 9,250.0 (±1,211.9) ml and 2,035.7 (±1,025.0) ml respectively, p<0.001.

13.14 Result of Part Two hypothesis (H8)

In a swine model of severe haemorrhagic TCA, without intra-aortic pressure fluctuations:

H8. *ECLS during a critical care period will infer a significant three-hour survival advantage after IABO removal, compared to Part One historical controls (without ECLS).*

Two (28.6%, 95%CI 5.1-64.1) of the Part One SAAP-FWB group (n=7) survived to the end of the protocol (t273), compared to five (100.0%, 95%CI 56.6-100.0) of the Part Two SAAP-FWB2 group (n=5). There was a significant difference in survival at t273, $p<0.05$.

ECLS did therefore infer a three-hour survival advantage over historical controls. However, the SAAP-FWB2 group required a mean LR volume of 6,900.0 (±1,474.8) ml in order to maintain the ECLS flow, and the effect of this volume of resuscitation alone is unknown.

13.15 Analysis of VF exclusions

Five animals in the SAAP-FWB2 group were excluded from protocol and replaced owing to a cardiac rhythm of VF during the SAAP infusion.

13.15.1 Prior observations of VF

VF was originally observed in Part One model development, with four (80.0%) out of five animals observed to have a few beats of normal sinus rhythm followed by VF after the start of SAAP. These initial five animals received 800 ml of FWB over one minute of SAAP with FWB, and after increasing the infusion to 1,600 ml over two minutes, VF was only observed in one (20.0%) animal out of five. During Part One experiments, one animal was excluded owing to VF during the haemorrhage phase, but no animals went into VF during the SAAP infusion.

In Part Two model development, VF occurred in the haemorrhage phase in one animal, and in a further two during SAAP infusion.

13.15.2 Analysis of VF in Part Two exclusions

VF was observed during the SAAP infusion in five (38.5%) out of thirteen animals randomised to receive SAAP-FWB in Part Two – 7955, 7956, 7964, 8001, 8011. In these animals, the cardiac rhythm changed to VF after a mean of 44.4 (±21.7) seconds = 592.0 (±289.9) ml of SAAP infusion with FWB.

In order to explore associations with VF during SAAP, comparisons were made between those with VF (VF group, n=5), and those without (NoVF group, n=8).

13.15.2.1 Baseline physiology and blood samples

There was a trend of a higher baseline heart rate in the VF compared to the NoVF group, but this did not reach statistical significance. There were no differences in baseline physiological variables examined between groups, Table 13.11.

There were also no statistical differences between groups in baseline blood sample analysis, or PaO_2:FiO_2, Table 13.11.

Table 13.11 – A comparison of baseline physiological measurements, and blood samples between animals with VF (VF) and those without VF (NoVF) during FWB SAAP infusion, mean (± standard deviation).

Variable	VF	NoVF	t-test
N of animals	5	8	
Physiological measurements			
Heart rate (beats/minute)	129.3 (±30.8)	97.4 (±20.4)	p=0.08
Systolic blood pressure (mmHg)	98.0 (±7.8)	97.1 (±11.0)	p=0.88
Diastolic blood pressure (mmHg)	72.8 (±5.8)	70.5 (±11.0)	p=0.62
Cardiac output (l/minute)	6.2 (±1.0)	6.0 (±1.2)	p=0.74
SaO_2 (%)	98.9 (±1.3)	99.3 (±1.2)	p=0.55
Left carotid artery flow (ml/minute)	389.6 (±139.6)	381.4 (±172.6)	p=0.93
Central venous pressure (mmHg)	9.0 (±1.6)	9.8 (±2.3)	p=0.50
Central venous saturation (%)	78.2 (±8.1)	72.9 (±6.7)	p=0.26
Core temperature (^oC)	37.1 (±0.1)	37.0 (±0.7)	p=0.73
Arterial blood gas			
pH	7.49 (±0.02)	7.48 (±0.03)	p=0.38
pO_2 (kPa)	13.3 (±2.6)	15.2 (±2.9)	p=0.25
pCO_2 (kPa)	5.4 (±0.2)	5.6 (±0.4)	p=0.35
Ionised calcium (mmol/l)	1.3 (±0.1)	1.3 (±0.0)	p=0.80
Potassium (mmol/l)	4.0 (±0.2)	4.3 (±0.3)	p=0.12
Sodium (mmol/l)	140.0 (±3.3)	138.9 (±1.2)	p=0.50
Lactate (mmol/l)	2.2 (±0.7)	1.7 (±0.9)	p=0.29
Base deficit	-7.0 (±1.5)	-6.6 (±1.5)	p=0.70
Full blood count			
Haemoglobin (g/dl)	11.7 (±1.1)	10.8 (±0.7)	p=0.17
White cell count ($x10^9$/l)	16.3 (±3.4)	15.7 (±5.1)	p=0.83
Platelets ($x10^9$/l)	265.2 (±30.8)	340.8 (±96.6)	p=0.07
Pulmonary gas exchange			
PaO_2:FiO_2	476.3 (±93.3)	543.9 (±104.0)	p=0.25

13.15.2.2 SAAP infusion characteristics

In the first ten seconds of SAAP infusion, there was no statistical difference in intra-aortic pressure or in left carotid artery flow between the VF and NoVF groups, Table 13.12.

The SAAP infusion was analysed by ABG immediately before administration, and there was no statistical difference between groups in the partial pressure of oxygen, haemoglobin concentration, or calculated oxygen content. However, in

the VF group, the SAAP infusion had a significantly higher pH, lower partial pressure of carbon dioxide, and lower potassium concentration compared to the NoVF group, Table 13.12.

Table 13.12 – A comparison of FWB SAAP infusions between animals with VF (VF) and those without VF (NoVF) during FWB SAAP infusion, mean (± standard deviation).

Variable	VF	NoVF	t-test
N of animals	5	8	
First ten seconds of SAAP			
Intra-aortic pressure (mmHg)	27.2 (±3.1)	25.5 (±3.6)	p=0.39
Left carotid artery flow (ml/minute)	151.0 (±35.8)	131.6 (±30.6)	p=0.35
SAAP infusion			
pH	7.64 (±0.17)	7.42 (±0.15)	p<0.05*
pO_2 (kPa)	91.1 (±4.5)	77.3 (±17.5)	p=0.07
pCO_2 (kPa)	1.4 (±0.6)	2.9 (±1.5)	p<0.05*
Potassium (mmol/l)	3.9 (±0.5)	5.0 (±0.8)	p<0.05*
Haemoglobin (g/dl)	7.4 (±0.4)	7.1 (±0.6)	p=0.36
Oxygen content (ml/100 ml)	12.1 (±0.6)	11.4 (±1.0)	p=0.14

13.15.2.3 Aortic arch blood samples at t5

An ABG was obtained in all animals from the aortic arch at t5 – approximately two minutes after the start of the SAAP infusion. At this time point, all of the animals in the VF group were in VF and were still receiving a SAAP infusion, and four (50.0%) of the VF group were still receiving a SAAP infusion.

The VF group had a significantly higher serum potassium concentration, and a significantly lower partial pressure of carbon dioxide compared to the NoVF group, but there was no difference between the mean ionised calcium concentrations, Table 13.13.

Table 13.13 – A comparison of arterial blood gas samples obtained at t5 between animals with VF (VF) and those without VF (NoVF) during FWB SAAP infusion, mean (± standard deviation).

Variable	VF	NoVF	t-test
N of animals	5	8	
Arterial blood gas			
pH	7.36 (±0.14)	7.28 (±0.08)	p=0.28
pO_2 (kPa)	51.8 (±25.4)	22.1 (±21.1)	p=0.06
pCO_2 (kPa)	3.7 (±2.0)	6.4 (±1.0)	p<0.05*
Ionised calcium (mmol/l)	1.3 (±0.3)	1.4 (±0.2)	p=0.56
Potassium (mmol/l)	3.4 (±0.2)	4.5 (±0.7)	p<0.01*

13.15.2.4 ECG characteristics

Lead II of the printed electrocardiogram (ECG) was analysed at t3 (immediately before the SAAP infusion) and at t3.5 (approximately 30 seconds into the SAAP infusion) for cardiac electrical rate, QRS duration, QTc duration (using Framingham's formula, QTc = QT+0.154(1-RR)),[213] and for ST segment deviation. In the thirteen animals, there was a significant increase in the mean QTc interval between t3 and t3.5 – 355.5 (±172.6) milliseconds and 591.6 (±222.4) milliseconds respectively, p<0.001, but no difference in the other measured ECG characteristics. However, there was no difference in the mean QTc interval at either t3 or t3.5 between the VF and NoVF groups, Table 13.14.

Table 13.14 – A comparison of lead II ECG characteristics at t3 and t3.5 in the VF and NoVF groups, mean (± standard deviation).

Variable	VF	NoVF	t-test
N of animals	5	8	
t3 ECG characteristics			
Heart rate (beats/minute)	78.7 (±10.2)	58.7 (±28.8)	p=0.11
QRS interval (milliseconds)	112.0 (±52.2)	71.5 (±39.8)	p=0.18
QTc interval (milliseconds)	387.1 (±171.0)	335.8 (±182.3)	p=0.62
ST segment deviation (mm)	1.8 (±2.7)	1.7 (±1.3)	p=0.95
t3.5 ECG characteristics			
Heart rate (beats/minute)	79.2 (±19.7)	66.2 (±13.2)	p=0.24
QRS interval (milliseconds)	94.4 (±39.5)	79.5 (±16.8)	p=0.46
QTc interval (milliseconds)	547.4 (±270.4)	619.2 (±202.0)	p=0.63
ST segment deviation (mm)	2.4 (±2.1)	1.9 (±2.0)	p=0.67

13.15.3 Summary of VF analysis

In summary, the only observed differences between the VF and NoVF groups was that the SAAP infusion had a significantly higher pH, lower partial pressure of carbon dioxide, and lower concentration of potassium in the VF group compared to the NoVF group.

CHAPTER FOURTEEN

DISCUSSION – PART TWO

14.1 Summary of findings

The aims of Part Two were to evaluate SAAP in a more severe injury model than Part One, to evaluate the effect of a longer SAAP infusion with LR on ROSC rates, and to evaluate the use of ECLS to mitigate the effects of IABO using Part One SAAP FWB animals as a historical comparison group. The five animals excluded owing to VF also provided an opportunity to investigate the associations with VF during SAAP infusion.

The injury model resulted in a very severe arrest, with a mean systolic BP of 0.0 (±0.0) mmHg, and no intra-aortic pressure fluctuations. However, only one animal was in cardiac electrical asystole during the arrest period. In this model, SAAP with FWB inferred a significant 60 minute survival advantage over SAAP with LR, and a larger volume (up to 4,000 ml) of LR did not improve survival compared to Part One controls. ECLS was demonstrated to be a feasible intervention in this large swine model, and demonstrated a significant three hour survival advantage over non-ECLS Part One control animals. The only observed differences between Part Two animals with VF during SAAP and those without VF was a higher pH, lower partial pressure of carbon dioxide, and lower potassium concentration in the SAAP infusion. This discussion reviews the data, its relevance to and possible translation into clinical practice, and formulates further research questions.

14.2 Blood donor pool, baseline values, and injury model

14.2.1 Blood donor pool

Part Two required a smaller total volume of FWB compared to Part One, and no animals in the SAAP-FWB2 group required the maximum volume of 9,100 ml allocated. As in Part One, some animals had more than two episodes of venesection, and there was no difference in mean haemoglobin between donations. However, the intrinsic clotting function of donated product was not tested. No protocols were delayed or cancelled owing to the lack of FWB supply, and with some caveats described in Part One (Section 8.2.1), the swine donor pool was an effective means of obtaining large volumes of FWB whilst reducing the total number of animals used.

14.2.2 Baseline values

Animals were randomly allocated to one of two SAAP infusions, and there were no statistically or clinically significant differences between groups.

14.2.3 Injury model

The initial aim of the injury model was to further develop the method used in Part One in order to achieve cardiac electrical asystole. Model development demonstrated that asystole did not readily occur, even after three minutes of no intra-aortic pressure fluctuations. Therefore, in order to standardise the onset time of TCA (t0) in a more severe model than Part One, the time that intra-aortic pressure fluctuations were lost was used. This was achieved by live-displaying the intra-aortic pressure trace on a large monitor in the laboratory, which was estimated to be accurate to at least 0.1 mmHg at the maximum scale. This was

an effective way in defining the point of TCA in the laboratory, and there were no disagreements amongst the research team during experiments.

There was no significant difference in the mean percent excision of the left lateral lobe of the liver – 66.6 (±7.1) % in Part Two and 69.2 (±9.6) % in Part One. The time interval from the start of the hepatic injury to t0 was significantly longer in the Part Two model compared to Part One, and all physiological variables compared at this time point (systolic BP, left carotid artery flow, end-tidal carbon dioxide) were significantly lower in the Part Two injury model.

There was no difference in the volume of controlled haemorrhage between the two groups in Part Two. However, there was a significant difference in the volume of abdominal haemorrhage – 1,212.0 (±678.4) ml in the SAAP-FWB2 group, and 2,475.1 (±1,217.5) ml in the SAAP-LR2 group. Observation in Part One suggested that the resuscitation fluid administered had an effect on the haemoperitoneum, but no significant difference in volume was found. Using the volume of haemoperitoneum to define the initial hepatic injury is flawed, and in this set of experiments, where some of the SAAP-LR2 group received 4,000 ml of initial SAAP resuscitation, there appears to be an effect on the volume of intraperitoneal blood. There are no reports in the published literature of how to accurately record contemporaneous uncontrolled abdominal haemorrhage with a closed abdomen. This could be attempted with surgical drains inserted around the point of haemorrhage, but this may in turn have an effect on the intrinsic clotting ability (at laparotomy large clots were observed around the hepatic injury in all animals). Manning et al used an open-abdomen method in their

swine NCTH, but this reduces the possible translation into clinical practice, and likely affected intrinsic clot formation around the liver.[112] The volume of abdominal haemorrhage recovered at laparotomy, and following 60 minutes of resuscitation, is therefore unlikely to be a valid method of defining this injury.

The time interval from the start of the hepatic injury to TCA in Part Two was a mean of 779.4 (±142.7) seconds, and was statistically longer than Part One, by a mean of 104.8 seconds. This is not a surprising finding – the injury methodology was the same in both sets of experiments, but the definition of TCA in Part Two was of a more severe injury. However, it was not possible to draw blood samples at the time of arrest owing to a very low central blood volume.

Previous large animal models of haemorrhage have utilised a less severe injury end-point than this research and makes comparison difficult. Neither Ross et al's or Morrison et al's models of laparascopic hepatic injury resulted in a no-output state, and resulted in 60 minute mortality of 40% and 100% respectively.[101,118] In comparison, the haemorrhage model described in Part Two systematically resulted in a no intra-aortic pressure fluctuations that would logically be expected to result in a 100% mortality within a few minutes without intervention, dependent on the definition of death used.

As previously explained, using the volume of haemoperitoneum to describe injury severity is flawed when animals receive resuscitation fluid during the pre-hospital period. A study by Ross et al using a similar laparoscopic hepatic injury to this model, but without a controlled haemorrhage, and without the

administration of resuscitation fluid, likely provides the best estimate of abdominal haemorrhage – 1,257 (±206) ml in 58.4 (±5.6) kg swine.[118] This equates to 22 (±4) ml/kg of haemoperitoneum, and is comparable to the Part Two SAAP-FWB2 group – 16.7 (±9.5) ml/kg, and lower than the SAAP-LR2 group – 32.4 (±15.4) ml/kg, that received a significantly higher mean volume of resuscitation fluid in the pre-hospital period. Morrison et al used the same model of laparoscopic hepatic injury in swine with a mean weight of 74.4 (±2.1) kg, that received a mean volume of 569 (±493) ml of hydroxyethyl starch (Hextend) in the pre-hospital period with continuous balloon occlusion.[101] The mean haemoperitoneal volume in Morrison's report is 3,285 (±813) ml, and is higher than both Part Two groups in this research, SAAP-FWB2 – 1,212.0 (±678.4) ml, SAAP-LR2 – 2,475.1 (±1,217.5) ml, despite animals in Part Two receiving a higher volume of resuscitation fluid than Morrison's animals. This suggests that the addition of the controlled haemorrhage in my model may have reduced the volume of abdominal haemorrhage.

In summary, in animal models of abdominal haemorrhage with the administration of resuscitation fluid, the volume of haemoperitoneum does not appear to be an accurate method of defining the extent of the injury, and the addition of a controlled haemorrhage appears to reduce the volume of haemoperitoneal blood. This finding has little relevance to animal studies in which the injury end-points are measured physiologically (for example systolic BP), and of no relevance to clinical practice.

The Part Two injury model is novel, and produced a more severe TCA compared to Part One. However, the difference in physiological variables measured at the time of arrest (systolic BP, left carotid artery flow, end-tidal carbon dioxide, and lack of intra-aortic pressure fluctuations) between the two models are either not measured clinically, or are unlikely to have clinical relevance.

14.3 Arrest period and intervention

The time from the point of arrest to the start of the intervention was a mean of 188.6 (±6.4) seconds. This interval was not different to Part One (184.0 (±2.9) seconds), and demonstrates that even with greater technical experience of SAAP, there were still delays in starting the infusion. The three minute arrest period has not been used in previous large animal models of balloon occlusion, but as described in Part One, is aimed at reducing the chance of a spontaneous recovery.

In model development, it was not possible to draw blood samples at t0 in the Part Two model as there was insufficient volume of blood in the aorta. Therefore, markers of tissue perfusion were drawn at the same time point as Part One – when the intra-aortic BP fell below 10 mmHg. The pH, lactate, and base deficit were all significantly different to baseline values, and were comparable to Part One measurements. The lactate and base deficit were both elevated – as would be expected during haemorrhage and reduced tissue perfusion.[214] However, the pH was also raised, and as explained in the discussion to Part One may be due to the continuation of mechanical ventilation during haemorrhage in the context of reduced carbon dioxide production in the animal.

All animals had a cardiac electrical rate that was lower than their baseline measurement at t0, per protocol. The mean cardiac electrical rate was measured at the end of the arrest period, between t2.5 and t3, and was a mean of 60.2 (±24.7) beats/minute – higher than that observed in Part One animals – a mean of 43.0 (±31.3) beats/minute. Part of this difference is due to only one animal in Part Two being in cardiac electrical asystole at this time point, compared to 12 animals in the Part One group, despite other measurements indicating a more severe injury. Observation of Part One animals suggested that there was not a linear fall in heart rate during haemorrhage, but that cardiac electrical activity abruptly ceased following an inappropriate bradycardia. Asystole in haemorrhage results from ischaemia of the sinoatrial node, and it would therefore be expected that a higher proportion of asystole would have been observed in Part Two animals, that all had no intra-aortic pressure fluctuations. There are no other large animal studies that include animals in asystole secondary to haemorrhage, and no studies that investigate the factors affecting the onset of asystole secondary to haemorrhage. The cause and timing of asystole secondary to haemorrhage is therefore an area that requires further research.

14.4 Primary outcome

The primary outcome was survival at 60 minutes after the start of the intervention, defined as a systolic BP of 30 mmHg or greater. Part One and previous translational experiments of NCTH have used this time interval to represent pre-hospital time,[101] and clinical data also show that the median pre-hospital time in NCTH is approximately 60 minutes.[119]

In this more severe model of haemorrhagic TCA, SAAP with FWB inferred a significant 60 minute survival advantage over SAAP with LR. The difference in percentage survival between the two interventions was similar to Part One results, but survival proportions at t63 were lower following both interventions in the Part Two model. Survival following SAAP-FWB treatment was 90.0% in Part One and 75.0% in Part Two, and following SAAP-LR treatment was 30.0% in Part One, and 12.5% in Part Two. This might be explained by the more severe injury model used in Part Two experiments.

The only prior published report on the use of SAAP-LR by Manning et al in 2001 describes a zero percent 60 minute survival, despite a less severe definition of haemorrhagic TCA.[112] Manning's definition of death-in-protocol is not fully explained, but is described as "no signs of spontaneous circulation" 15 minutes after the start of the SAAP intervention. It would be reasonable to assume that this means the animals had no recordable cardiac output, and is therefore a similar death-in-protocol definition. At this time point, Manning et al's animals would have received 150 ml/kg of LR and 0.12 mg/kg of intra-aortic epinephrine.[112] This volume of LR is approximately three times the upper limit of the initial SAAP infusion in my Part Two experiments (4,000 ml, or 50 ml/kg).

A different method of hepatic injury was used by Manning et al that included injury to all four lobes of the liver, compared to a single lobe in my experiments. Furthermore, in order to quantify the rate of haemorrhage, Manning suctioned blood from the swine's' abdomens,[112] that as previously described may have reduced the formation of hepatic clot. It is therefore likely that in Manning's

model there was an increased rate of ongoing hepatic haemorrhage during SAAP intervention (SAAP only controls aortic blood flow, and the injury is mixed arterial and venous). However, the volume of haemoperitoneum during SAAP is not reported by Manning et al, and is not accurately recorded in my experiments; ruling out the possibility of testing this theory.

The physiological severity of the haemorrhage model in Part Two is more severe than any previous swine translational studies, but has provided further evidence that SAAP with FWB has the potential to resuscitate severe haemorrhagic TCA, and is superior to SAAP with LR in large swine.

14.5 Secondary Outcomes

14.5.1 ROSC

The definition of ROSC, a systolic BP of 50 mmHg or greater ten minutes after the start of the intervention, was the same as Part One methodology. This definition ensured that the effect of each intervention was sustained, and also allowed sufficient time for the effect of the intervention to be observed.

ROSC was observed in nine (56.3%) of sixteen animals: SAAP-LR2 group – two (25.0%), SAAP-FWB2 group – seven (87.5%), $p<0.05$. The proportion of SAAP-LR2 animals with a ROSC was lower than that observed in Part One LR animals (60.0%), and might be explained by the more severe injury model. This occurred despite SAAP-LR2 animals receiving a greater volume of SAAP infusion (a maximum of 4,000 ml, compared to a fixed volume of 1,600 ml in Part One).

In the SAAP-LR2 animals without a ROSC (n=6), three (50.0%) had a return of cardiac output during the SAAP infusion, one had a maximum systolic BP of 25 mmHg, and two had a maximum systolic BP of approximately 70 mmHg. However, per protocol, these animals continued to receive further SAAP infusion with LR, and all had no recordable intra-aortic pressure fluctuations at the end of the 4,000 ml SAAP infusion. The cause of this is not clear from the data available, and this phenomenon is not described by Manning et al in their study of SAAP with LR.[112] Once the heart has an intrinsic output, it is logical to deduce that ongoing SAAP infusion at 10 ml/kg/minute will increase cardiac afterload, in turn increasing the workload of the myocardium. At the same time, ongoing SAAP infusion with LR will further dilute the animal's blood, reducing the haemoglobin concentration (and therefore oxygen delivery to the myocardium), and reducing the calcium concentration (and therefore reducing myocardial contractility). The one SAAP-FWB2 group animal that did not have a ROSC at t13, also had a return of cardiac output during SAAP infusion, and had a maximum intrinsic systolic BP of 81 mmHg. However, with ongoing SAAP infusion with FWB, the BP fell, and there were no intra-aortic pressure fluctuations at the end of the SAAP infusion. This finding supports the theory that it is the increased afterload associated with SAAP infusion that is causing this phenomenon, rather than a product of the LR itself, but further data are needed. Potential theoretical solutions to this include reducing the rate of SAAP infusion, or converting to central venous fluid administration, once an intrinsic cardiac output is observed, or using partial aortic occlusion – either initially, or once an intrinsic output is detected.

One aim of the Part Two experiments was to determine if a longer period, and therefore a greater total volume, of SAAP LR infusion would increase the proportion of animals with a ROSC compared to the Part One SAAP-LR animals. The ability to answer this question was compromised by using a more severe injury model in Part Two animals, but from the evidence available, it appears that a longer period of SAAP with LR is not associated with a higher proportion of animals with a ROSC.

The two SAAP-LR2 group animals with a ROSC, SAAP-LR2-3-7888 and SAAP-LR2-5-7973 received 4,000 ml and 1,160 ml of LR respectively. 7888 had a systolic BP of 48 mmHg at the end of the 4,000 ml of SAAP (t8.3), but with no further fluid administration spontaneously recovered, and had a systolic BP of 167 mmHg at t13. The cause of this spontaneous recovery is unknown, but may be secondary to the reduction in cardiac afterload on cessation of the SAAP infusion. 7973 had a rapid response to SAAP with LR, but required the total maximum boluses after t13 (7 x 250 ml of LR), and met death-in-protocol before simulated hospital arrival (t63).

Therefore, there were limited data in which to compare the volume of SAAP infusion required to effect a ROSC in FWB compared to LR, but there was a trend of lower SAAP volumes in the SAAP-FWB2 group compared to the SAAP-LR2 group. It is possible that with a larger sample size this difference would be significant. However, the clinical relevance of this finding in comparison to the significant difference in 60 minute survival is questionable.

14.5.2 Physiology and blood samples at t13 in all animals

An initial comparison was made at t13 between all animals, regardless of their ROSC status, of mean systolic BP, left carotid artery flow, and end-tidal carbon dioxide. The SAAP-FWB2 group (n=8) had a significantly higher systolic BP and end-tidal carbon dioxide compared to the SAAP-LR2 group (n=8), but there was no statistical difference in mean left carotid artery flow between groups. These values are intrinsically linked to another secondary outcome (ROSC), and are therefore not discussed further.

An initial comparison was also made in blood samples at t13 in all animals between groups. The SAAP-LR2 group (n=8) had significantly reduced gas exchange, haemoglobin concentration, platelet count, fibrinogen concentration, maximum clot amplitude, and clot elasticity compared to the SAAP-FWB2 group (n=8). As in Part One, the SAAP-LR2 group had a significantly higher lactate concentration, likely secondary to the high lactate concentration in LR, compared to the SAAP-FWB2 group. However, like the physiological measurements, these values are intrinsically linked to another secondary outcome (ROSC), and are therefore not discussed further.

14.5.3 Physiology and blood samples at t13 in live animals

These variables were also compared in animals with a ROSC at t13, SAAP-FWB2 – seven, and SAAP-LR2 – two. There was no statistical difference between the systolic BP, left carotid artery flow, and end-tidal carbon dioxide between the groups of animals with a ROSC at t13, and the mean values were slightly higher in the SAAP-LR2 group.

This demonstrates that although there was a significant difference in ROSC between SAAP with FWB and LR, the animals in the LR2 group alive at this time point had comparable physiology to animals that had received SAAP with FWB. These data were not reported by Manning et al in the only published study of SAAP with LR, and not reported in Part One of this research. However, retrospective review of the six SAAP-LR animals in Part One reveals that in animals with a ROSC at t13, the mean systolic BP, left carotid artery flow, and end-tidal carbon dioxide were all comparable to the SAAP-FWB animals that also had a ROSC at t13.

The SAAP-LR2 group with a ROSC (n=2) were significantly more alkalaemic than the SAAP-FWB2 group (n=7) at t13, and had a mean pH that was comparable to baseline – 7.49 (±0.01) and 7.48 (±0.03) respectively. This difference in pH is likely to have been caused by a lower mean partial pressure of carbon dioxide in the SAAP-LR2 group, but neither arterial partial pressures of oxygen nor carbon dioxide were statistically significantly different between groups. The only other differences in the SAAP-LR2 group compared to the SAAP-FWB2 group at t13 were a lower haemoglobin and fibrinogen concentration (as expected after LR resuscitation), and a lower calculated oxygen content (a product of the lower haemoglobin concentration). There were no differences in platelet count or prothrombin time, or in TEG values between groups of live animals at t13.

Data from Part One and Part Two of this research demonstrate that SAAP with FWB is superior to SAAP with LR. However, the availability of pre-hospital blood products is limited, with a small number of specialist pre-hospital services

carrying blood, and the imminent start of a clinical trial that has potential to paradoxically result in fewer services delivering blood pre-hospital.[215] The availability of non-blood resuscitation fluid, and the logistical and administrative constraints of blood products, makes resuscitation with non-blood fluids a highly attractive management strategy. Prior unpublished research by Dr. James Manning has effectively used FWB diluted 1:1 with LR to resuscitate large swine, which would potentially reduce the volume of blood products required for a pre-hospital SAAP intervention, but would also reduce the oxygen carriage and have a detrimental effect on clotting, and still requires pre-hospital blood.

A potential solution is to use a novel oxygen carrying fluid, for example an HBOC or a perfluorocarbon emulsion. Manning et al have described the use of both of these experimental oxygen carrying fluids in large animal models,[111,112] and demonstrated that SAAP resuscitation with HBOC-201 inferred a significant 60 minute survival advantage compared to SAAP with LR. Manning et al used a model of uncontrolled hepatic haemorrhage, but they do not report the clotting function after SAAP resuscitation with HBOC, which is likely to be deranged. These experimental fluids are not currently in widespread clinical practice, but could, if administered together with lyophilised plasma (that has been used pre-hospital),[216] potentially provide a blood-product free pre-hospital SAAP solution.

14.5.4 Physiology and blood samples at t63

This analysis only included live animals at t63, and included seven animals – six (75.0%) of the SAAP-FWB2 group, and one (12.5%) of the SAAP-LR2 group. The results were similar to those observed in Part One animals; the SAAP-FWB2

group had a mean systolic BP, left carotid artery flow, and end-tidal carbon dioxide that were comparable to baseline values. However, the single SAAP-LR2 animal had a systolic BP and end-tidal carbon dioxide that were more than two standard deviations lower than the mean of the SAAP-FWB2 group. The unexpected finding was that the SAAP-LR2 animal had a left carotid artery flow that was comparable with the SAAP-FWB2 group at t63 - 454.5 ml/minute and 453.0 (±126.9) ml/minute respectively. This animal, SAAP-LR2-3-7888, had a haemoglobin concentration at t63 of 3.6 g/dl, more than two standard deviations lower than the mean of the SAAP-FWB2 group. The circulating blood was therefore of a lower viscosity in the SAAP-LR2 animal,[217] which may explain the relatively high left carotid artery flow observed in this animal.

The SAAP-LR2 animal alive at t63 was administered a further 1,750 ml (7 x 250 ml) of LR, after the initial SAAP infusion of 4,000 ml, during the pre-hospital period. This resulted in a platelet count, fibrinogen concentration, and maximum clot amplitude that were two standard deviations lower than the SAAP-FWB2 group, and a prothrombin time that was more than two standard deviations higher than the SAAP-FWB2 group.

Therefore, although at ten minutes after the start of the SAAP intervention, the SAAP-LR2 and SAAP-FWB2 groups had comparable physiology with no evidence of clotting dysfunction, at t63 the single surviving LR group animal had significant derangement of measured physiological and blood samples analyses. It is unclear whether the initial LR infusion, or the infusion after t13, was responsible for the differences in physiology and clotting function at t63, but it is

tempting to suggest that in a logistically-constrained environment initial SAAP resuscitation with LR (or a novel oxygen carrier with or without lyophilised plasma) may be a successful clinical intervention. Further data are needed on this strategy, which will become more realistic if novel oxygen carrying resuscitation fluids are adopted into widespread clinical use.

14.6 Surgery period – SAAP-FWB2 group

Six (75.0%) of the SAAP-FWB2 group survived to t63 and underwent surgical control of haemorrhage. The novel hepatic clamps were effective at rapidly controlling the anterior section of the hepatic injury, but owing to a bulky hilum several animals required a Satinsky's clamp to be placed over the posterior portion of the left lateral lobe of the liver. As per Part One, all animals in Part Two demonstrated cardiovascular instability on balloon deflation, characterised by a significantly lower pH, and a significantly higher partial pressure of carbon dioxide, and base deficit at t93 compared to t63, requiring an additional mean FWB volume of 3,791.7 (±1,133.8) ml.

14.7 Critical care period – SAAP-FWB2 group

14.7.1 ECLS setup

The technical challenges of placing large ECLS cannulae were realised in the failure to cannulate the femoral vein of one animal, necessitating a contralateral cannulation, and iatrogenic vascular injury in another animal that resulted in the failure to commence ECLS and animal death at t110. Despite a delay in one experiment, the interval from removal of the SAAP catheter to starting ECLS flow in these five animals was a mean of less than three minutes. This was achieved

with a team of two – one to place the cannulae (EB), and one to prime and circulate the ECLS circuit (Miss Jennifer Cox), and demonstrates the relative ease of setup using the Maquet Cardiohelp system in the laboratory setting, that could translate to clinical use. Indeed, a five-person medical team in Paris have been using this system for years in the pre-hospital management of medical cardiac arrest.[209]

14.7.2 Maintaining ECLS flow

All animals (n=5) started on ECLS survived to the end of the three hour critical care period. None of these animals required the maximum allocated volume of post-t63 FWB (5,750 ml) during the surgery period, and were administered an additional mean volume of 2,350 (±675.5) ml of FWB during ECLS. During Part Two model development it was evident that animals would require large volumes of LR during to maintain ECLS flow of 2.8 l/minute, with a concern that haemodilution would result. Despite animals receiving a mean LR volume of 6,900 (±1,474.8) ml over three hours there was no significant fall in mean haemoglobin concentration. Post-mortem visual inspection of the abdominal cavity did not demonstrate large amounts of fluid, and it is likely therefore that a proportion of the infused LR accumulated in the extravascular space leading to tissue oedema. This has the potential to result in pulmonary oedema, and may be partly responsible for the fall in PaO_2:FiO_2 throughout ECLS.

14.7.3 The effect of ECLS

There are no previous large animal studies of the use of ECLS after balloon occlusion, but Part One demonstrated a high mortality after balloon deflation

that was associated with acidaemia, myocardial failure, and pulmonary dysfunction. The most comparable large animal study, by Morrison et al, used a less severe haemorrhage model followed by 60 minutes of balloon occlusion and a three hour critical care period.[101] This resulted in a mean pH of 7.17 (±0.00) after balloon deflation, compared to 7.01 (±0.03) in this study, and Morrison's swine required a mean volume of 1922 (±1745) ml of LR during the three hour critical care period, but also 4.7 (±12.3) mg of norepinephrine and an undisclosed volume of whole blood. Aside from survival, the most striking effect of ECLS in this study was the increase in pH observed – 7.30 (±0.06) after three hours – higher than the mean pH reported in Morrison et al's swine at the same time point – 7.22 (±1.45). It would be reasonable to suggest therefore that short-term ECLS has a beneficial effect on pH after balloon deflation. Furthermore, one animal in Morrison et al's study died during the critical care period of "cardiogenic shock", that may have been amenable to ECLS.[101]

The lowest pH recorded (at t93) coincided with the highest arterial partial pressure of carbon dioxide recorded, and it is likely that correction in concentration of carbon dioxide had a substantial effect on the increase in pH throughout ECLS. The exogenous removal of carbon dioxide from venous blood is a key function of ECLS – allowing the reduction of ventilator settings and further lung injury.[187] However, in these experiments, the exogenous removal of carbon dioxide appears to have had the added benefit of increasing pH, and potentially improving cardiovascular function.[134,166]

There was evidence of incomplete mixing of intrinsic cardiac output and ECLS flow, shown by the differential hypoxaemia observed during ECLS. During ECLS, the animals were ventilated at a fixed tidal volume of 7 ml/kg, and a ventilation rate that was set at baseline to produce an end-tidal carbon dioxide of between 5.0 and 5.6 kPa, with an inspired oxygen fraction of 1.0. In order to reduce further pulmonary injury, it would have been advantageous to reduce both the minute volume and the fraction of inspired oxygen during ECLS, but the mean partial pressure of oxygen in the brachial artery sample at t273 was only 33.5 (±19.1) kPa. There are two potential solutions to this situation; one is to override the intrinsic cardiac output with a higher ECLS flow (likely requiring larger volumes of LR administration to maintain flow, and risking anaemia), or inserting a venous return catheter to undertake venous-arterial-venous ECLS. This technique has been described in the clinical setting, using an additional 17 Fr cannula in the subclavian vein,[218] and may be a useful technique in the use of ECLS in the management of post-IABO cardiovascular dysfunction.

14.7.4 ECLS anticoagulation

There were limited data from Part Two development from which to reliably calculate the dose of heparin required to inhibit clot formation in the ECLS circuit. The dose of the initial heparin bolus, and ongoing infusion was derived from the ELSO guidelines,[199] and resulted in a mean ACT of 1.7 (±0.4) times the group's mean baseline measurement. Most importantly, there were no incidences of visible clot formation within the ECLS circuit, and no evidence of bleeding from surgical incisions or line insertion sites, so this seemed to be an efficacious method. These data have relevance for future large swine

translational models, but are of limited utility in clinical practice with the advent of heparin-bonded ECLS circuits.[206,207]

The use of heparin in this model was associated with a significant increase in prothrombin time during ECLS, but this did not result in a significant reduction in clotting function measured by TEG. Blood contact with ECLS components is known to cause a combination of activation, destruction, and dysfunction of platelets and consume clotting factors.[197,198] During ECLS there was a significant fall in platelet count and fibrinogen concentration that may be explained by the interaction of blood with components of the ECLS circuit. This effect was mitigated in part by the use of FWB during the protocol, including during ECLS, but future large animal translational models should consider the administration of targeted blood components during prolonged periods of ECLS.

14.7.5 Cardiac dysfunction

The cardiac dysfunction observed following 75 minutes of IABO and subsequent reperfusion have already been reported and discussed. Cardiac troponin I is both very specific and sensitive for myocardial cell damage,[219] and was therefore measured in this study to provide data on the timing of myocardial ischaemia. Troponin was measured at baseline, at t63 (after 60 minutes of IABO), at t93 (after IABO deflation), and at t153 (60 minutes into the critical care period). The greatest increase in mean troponin concentration was observed between baseline and t63, with no further significant increase on balloon deflation, or after 60 minutes of ECLS. Therefore, the greatest myocardial injury occurred between baseline and t63, with no evidence of further significant injury on

balloon deflation, or during the first 60 minutes of ECLS. However, from these data, it is not possible to differentiate whether the myocardial ischaemia occurred during the haemorrhagic injury, during IABO, or a combination of both.

Two previous large animal IABO studies have reported cardiac troponin concentrations, and demonstrated a significant rise in troponin between baseline and the end of the protocol,[101] with a trend of increasing troponin concentration with duration of occlusion, from 30 minutes to 90 minutes, measured eight hours after intervention.[168] There was no measurement of baseline troponin in the second study, and owing to a single sample taken eight hours after intervention it is unclear at what time point this myocardial injury occurred. However, together with my data, it supports the theory that at least a portion of the myocardial injury is caused by the proximal aortic occlusion.

Histological analysis of myocardial samples revealed subendocardial haemorrhages in the left ventricle and septum of the two animals with the highest serum troponin concentrations at t153. Subendocardial haemorrhages are caused by myocardial cell necrosis secondary to a number of aetiologies, including direct trauma, ischaemia, and catecholamine surge.[220] One previous large animal IABO study has described myocardial histology, and demonstrated an increased incidence of cardiac injury following 60 minutes of IABO, compared to 40 minutes.[96] This gives more weight to the argument that proximal IABO causes myocardial injury, but as with the troponin data it is unclear what proportion of this injury is due to the initial haemorrhagic injury and what proportion is due to IABO.

These findings are in keeping with previous studies of proximal aortic occlusion with an aortic clamp, that have demonstrated acute left ventricle dilation, subendocardial ischaemia, and left ventricle wall motion abnormalities.[142,144,145] A potential solution to reduce the myocardial injury associated with IABO is therefore to use partial occlusion without prior complete occlusion, but further research is needed in this area.

14.7.6 Pulmonary dysfunction and pro-inflammatory mediators

There was no significant difference in PaO_2:FiO_2 between baseline and t63, but there was a significant decrease in PaO_2:FiO_2 between t63 and t93. This decrease in pulmonary function coincided with IABO deflation, and it would be reasonable to suggest that re-perfusion was the cause of the observed pulmonary dysfunction.

A proportion of the observed pulmonary dysfunction may have been secondary to cardiac failure as previously described, leading to pulmonary oedema and impaired gas exchange. However, previous large animal IABO studies have reported an increase in serum concentrations of TNF-α and IL-6, that are both associated with pulmonary injury.[101,168] In this study there was no significant rise in TNF-α at t93 compared to baseline, but there was a significant rise at t153, and a significant rise in IL-6 at t63 and at t93. IL-6 is released in response to increased levels of TNF-α and IL-1B, and has been shown to correlate with ARDS severity.[148] No previous large animal IABO studies have reported serum concentrations of IL-1B, but in these animals there was a significant rise in IL-1B at t93 compared to baseline.

There was a correlation between the serum concentration of all measured pro-inflammatory mediators (TNF-α, IL-1B, IL-2, IL-6, IL-8) at t153, and the PaO_2:FiO_2 at the end of the study. This is likely to represent a general systemic inflammatory response syndrome, caused by a combination of the initial haemorrhagic injury, balloon occlusion, and reperfusion. However, there was considerable variation observed in levels of pro-inflammatory mediators between individual animals, indicating that there is not a consistent inflammatory response in swine to this intervention.

Pulmonary function continued to fall after t93 to the end of the protocol, and at t273 three (60.0%) animals met the definition of ARDS.[221] Whilst the exact mechanism of the pulmonary dysfunction has not been explained, it is clear that after IABO following haemorrhagic TCA there is a release of pro-inflammatory mediators associated with a reduction in pulmonary gas exchange that may require exogenous membrane oxygenation. In clinical translation, providers should be prepared to provide lung-protective ventilation and potentially ECLS.

14.8 Comparison with Part One historical controls

In order to describe the effect of ECLS in large swine after balloon deflation, the seven Part One animals in the SAAP-FWB group that did not receive ECLS were compared with the five Part Two animals in the SAAP-FWB2 group that did receive ECLS. The more severe initial injury model in Part Two resulted in a significantly lower pH and higher partial pressure of carbon dioxide after IABO deflation (t93) compared to Part One animals. However, there were no other significant differences in physiology or blood sample analyses.

Despite the lower pH and higher partial pressure of carbon dioxide in the SAAP-FWB2 group compared to the SAAP-FWB group, all animals survived the three hour period of ECLS. ECLS was therefore a successful intervention in improving survival after balloon deflation. However, without a longer period of observation, including weaning from ECLS, it is unclear if this would translate to increased long-term survival.

As previously discussed, the effect of ECLS included eliminating carbon dioxide, with an associated increase in pH, which may have been a key factor in the difference in survival observed. The validity of the comparison was compromised by the requirement to administer large volumes of LR during ECLS to maintain flow, and it is possible that more animals in the SAAP-FWB group would have survived after balloon deflation with the same volume of LR infusion. However, data from Part One demonstrated that the five animals in the SAAP-FWB group who died after balloon deflation did so after receiving a mean FWB volume of 2,500.0 (±500.0) ml with a significant rise in central venous pressure – indicating a failure of the cardiac pump. From the data available, it is therefore reasonable to suggest that even with large volumes of LR these animals would have still died without ECLS.

14.9 Analysis of VF exclusions

14.9.1 VF throughout experiments

VF was observed in a total of 14 animals throughout model development and experiments in Parts One and Two; two during the haemorrhagic injury, and 12 during the SAAP infusion with FWB. There is therefore some evidence that VF

occurs more readily during haemorrhage in swine compared to humans, in whom VF during haemorrhage is a rare arrhythmia.[45,52] This observation is supported by a study that demonstrated that the VF electrical threshold of swine myocardium is significantly lower than that of humans.[222] The difference is likely to be in the anatomy of the Purkinje fibres that in humans are in the subendocardium, compared to swine where they extend through the ventricular wall to the subepicardium.[223]

Personal communication has revealed that VF is a relatively common occurrence during swine haemorrhage studies, and other research groups have pre-dosed animals with amiodarone to reduce the incidence of VF, but this has not been reported in the literature.

14.9.2 VF during SAAP infusion in Part Two

There were no significant differences in baseline physiology and blood sample analyses between the five animals with VF and the eight animals without VF during SAAP infusion with FWB in Part Two. There was a trend of a higher baseline heart rate in the animals who had VF during the SAAP infusion, but this did not reach significance in this small sample. Whilst this may have represented a group of animals with an underlying illness, there were no differences in white cell counts or core temperatures between groups.

There was also no difference between the mean intra-aortic pressure and mean left carotid artery flow during the first ten seconds of SAAP infusion between groups. However, in pre-infusion samples of SAAP FWB, the animals that went

into VF during SAAP had a significantly higher pH, lower partial pressure of carbon dioxide, and lower potassium concentration compared to those without VF. The duration of re-circulation of FWB around the SAAP ex vivo SAAP circuit prior to administration was not standardised, and was dependent on the ease of initial circuit setup and the duration of the injury period. An explanation for the observed differences in the SAAP infusion is that in the VF group there was a longer period of *ex vivo* circuit circulation, resulting in a lower partial pressure of carbon dioxide. This theory is supported by a higher partial pressure of oxygen in the SAAP infusion in the VF group, compared to the NoVF group. The lower partial pressure of carbon dioxide in the SAAP infusion would have increased the pH, which in turn would have reduced the potassium concentration.[165]

Potassium is known to have an important role in myocardial electrical stability, and hypokalaemia has been demonstrated in animal models to reduce the VF threshold.[224] This effect has been demonstrated in a clinical setting where a serum potassium concentration lower than 3.5 mmol/l is associated with an increased incidence of ventricular arrhythmias in acute coronary syndromes (and hence ischaemia).[225] However, the mean potassium concentration in the VF group SAAP infusion was 3.9 (±0.05) mmol/l, and although significantly lower than the VF group SAAP infusion, is not considerably lower than swine reference values – 4.4 (±0.4) mmol/l,[120] or the mean baseline measurement in the SAAP-FWB2 group – 4.3 (±0.3) mmol/l.

An ABG was obtained two minutes after the start of the SAAP infusion in all animals (t5). The utility of this sample to identify potential causes of VF is

affected by the fact that all animals in the VF group were in VF at this time point and all were still receiving SAAP, compared to the NoVF group in which only four (50.0%) animals were still receiving SAAP. However, the VF group had a significantly lower serum potassium concentration, and a significantly lower partial pressure of carbon dioxide compared to the NoVF group at t5. This is likely to reflect a combination of the differences observed in SAAP infusions between groups, but also the return of intrinsic cardiac activity in 50.0% of the NoVF group, due to washout of potassium and carbon dioxide in the ischemic venous blood, and these are therefore of questionable use in identifying potential causes of VF during SAAP.

Calcium is another electrolyte that is important to myocardial electrical stability.[224,226] In model development, the required volume of 10% calcium chloride solution per 1,000 ml of citrated FWB infused to maintain calcium homeostasis was calculated, and the calcium was infused simultaneously to FWB through the SAAP catheter using a syringe driver. This method resulted in none of the ten animals in the SAAP-FWB group in Part One going into VF during SAAP, and was therefore considered effective. Blood samples obtained at t5 from the aortic arch demonstrated no differences in ionised calcium concentration between the VF and NoVF groups – 1.3 (±0.3) and 1.4 (±0.2) mmol/l respectively. However, as previously explained these samples were taken after the onset of VF, and do not describe the ionised calcium concentration in blood perfusing the coronary vessels at the onset of VF. Evaluation of lead II of the ECG at t3 (prior to SAAP) and t3.5 (during the first 30 seconds of SAAP), revealed that there was an increase in the mean QTc interval

in both groups between t3 and t3.5, but no statistical difference between groups at t3.5. The QTc interval can be prolonged by a number of aetiologies, including ischaemia, hypokalaemia, and hypocalcaemia.[227] The exact cause for QTc prolongation in these animals cannot be determined from the data available, but significantly increased myocardial ischaemia is unlikely in the setting of active coronary perfusion by SAAP, as is hypokalaemia as the SAAP infusion contains potassium. Hypocalcaemia is therefore likely to have had the greatest effect on prolongation of the QTc interval, and questions the effectiveness of delivery of calcium via SAAP in the first 30 seconds of the infusion.

The calcium syringe driver was competing with the pressure of flow in the SAAP circuit, and although total volumes of both calcium and FWB infused were confirmed to be accurate after two minutes of infusion in Part One, the volumes infused during the first 30 seconds are unknown. Further data are needed to confirm the volume of calcium infused using this setup in the first 30 seconds of SAAP infusion.

Subsequent to Part Two experiments, Manning et al undertook an evaluation in large swine of the effects of citrated FWB SAAP infusion without concomitant calcium infusion – all animals went into VF during SAAP.[226] This confirms the association of hypocalcaemia and VF during SAAP, but did not provide further data on the serum calcium concentration in the aortic arch during initial SAAP. In the same set of large swine SAAP experiments, Manning et al also compared three other infusions: FWB with calcium, FWB diluted by LR with calcium, and FWB diluted by 0.9% sodium chloride with calcium. The FWB was diluted to a

haemoglobin concentration of 4.0 g/dl. In the FWB with calcium group, two (50.0%) of animals had VF during SAAP infusion, but both were successfully defibrillated – personal correspondence reveals that this was achieved with trans-oesophageal defibrillation (one defibrillation pad on the anterior wall of the thorax, and the other wrapped around a gastroscope in the oesephageous at the level of the heart). Multiple attempts at transthoracic defibrillation were unsuccessful in Part One model development, possibly related to the differences in thoracic impedance between swine and humans.[228]

No VF was observed during SAAP infusion in either of Manning's groups that received diluted FWB with calcium.[226] The cause for this is unknown and potentially includes a number of variables, for example the viscosity of the infusion and the oxygen content in low-haemoglobin infusions. However, comparing the VF and NoVF groups in Part Two there were no differences in haemoglobin concentration (as a measure of viscosity) or oxygen content, providing no further evidence for this theory. Throughout my research I have not observed VF during SAAP in any animals that received LR. Evaluation of the SAAP-LR infusions in Part One demonstrated a mean ionised calcium concentration of 0.9 (±0.0) mmol/l. This concentration is lower than swine baseline values, but is higher than the reported intra-aortic calcium concentration in Manning's animals who received FWB without calcium,[226] and reinforces the requirement to obtain further data on the calcium concentration of blood in the aortic arch during the first 30 seconds of SAAP infusion with FWB. It also strengthens the argument for the use of novel oxygen carrying fluids, that do not require concomitant administration of calcium, for SAAP.[112,229]

14.9.3 Summary of observed VF

There are limited data available from which to ascertain the cause of VF during SAAP infusion with FWB. However, possible explanations are that swine have a lower VF threshold, and that the method for delivering calcium during SAAP results in hypocalcaemia during the first 30 seconds of SAAP infusion with citrated FWB. Prior to clinical translation further data are needed on the concentration of ionised calcium in the aortic arch during the initial SAAP infusion.

14.10 Clinical translation

These further experiments were designed as a translational study, in which to compare SAAP in a more severe model of haemorrhagic TCA, and to evaluate the use of ECLS in improving survival after IABO deflation. It is the first study to describe a model of haemorrhagic injury of this severity, and also the first to describe the use of ECLS in mitigating the premature animal death observed in Part One after balloon deflation.

It would be appropriate to suggest that SAAP with FWB could be an effective clinical management strategy in severe haemorrhagic TCA, but that providers should expect cardiovascular dysfunction after prolonged IABO. There is limited evidence that ECLS is an effective intervention for improving short-term survival after IABO removal, so more data are required to determine whether this effect extends beyond the three hour critical care period and whether animals in a translational study could be weaned from ECLS.

The high proportion of VF observed in Part Two during SAAP infusion is concerning, but this may be a product of swine susceptibility to VF during haemorrhage, and with ongoing SAAP infusion, defibrillation (using a defibrillator that is designed for use in humans) is likely to be an effective rescue strategy. Further data are needed on the concentration of ionised calcium achieved with the current infusion system, but this could be mitigated with the development of novel oxygen carriers – a technological advance that would also reduce the logistic burden of large volumes of pre-hospital blood product that potentially limits the clinical use of SAAP.

14.11 Conclusion – Part Two

The hybrid model of NCTH and controlled arterial haemorrhage resulted in a highly-lethal injury that was more severe than that described in Part One. SAAP with FWB conferred a superior short-term compared to SAAP with LR, even with a SAAP infusion of up to 4,000 ml. Further data are needed on the effect of aortic balloon occlusion on cardiac and pulmonary dysfunction. ECLS is a feasible intervention in large swine, and inferred a significant three hour survival advantage after balloon deflation compared to historical controls. The cause of VF observed during SAAP with FWB in swine is unknown, but hypocalcaemia during initial SAAP infusion may be an important factor.

PART THREE

CHAPTER FIFTEEN

A TRANSLATIONAL PARADIGM

15.1 Passive endovascular intervention

15.1.1 The clinical experience of endovascular intervention

Throughout this research it has become evident that even in the laboratory setting, the technical skill, equipment required, and the time to complete the endovascular intervention increases from REBOA to SAAP, and from SAAP to ECLS. This effect is likely to be amplified in the clinical setting, particularly in the logistically-constrained pre-hospital environment. Internationally, a single physician-paramedic team delivers pre-hospital Z3 REBOA for haemorrhagic shock,[138] and a single physician-led medical team delivers pre-hospital ECLS in medical cardiac arrest.[209] More recently there has also been a report of military use of REBOA by a far forward special operations surgical team;[230] a medical capability that sits between the pre-hospital and the traditional hospital setting. This is in contrast to the fact that in 2016 at least 90 US hospitals undertook ECLS for medical cardiac arrest, and 36 did so in their emergency departments.[231] There have only been three reported case series, at different institutions, on the early use of ECLS in traumatic haemorrhage,[206-208] but the overall experience in endovascular intervention in the emergency setting is rapidly increasing.[232,233]

15.1.2 Differentiating patients for REBOA

The decision to undertake REBOA is complicated. On one hand, earlier haemorrhage control is logically associated with better outcomes, and although this has not been demonstrated in NCTH, it has been in severe limb haemorrhage.[234] Data from the Japan Trauma Data Bank reported an increased mortality associated with the use of REBOA in NCTH, and the authors suggest that REBOA was used as a "last ditch" attempt at resuscitation.[106] This is likely to represent poor patient selection, or not using REBOA early enough.

However, there is no single test that reliably differentiates NCTH patients into those who will exsanguinate before the opportunity for definitive haemorrhage control without REBOA, and those who will survive to the operating theatre without REBOA.[235,236] In clinical standard operating procedures, the decision to use REBOA can be based on a systolic BP cut-off, [235,237] but more recently its use has been described after a transient or failed response to resuscitation.[238] This decision is therefore based on a combination of a physiological variable (BP) that may have limited specificity in haemorrhagic shock,[239,240] and recognition-primed decision making that requires clinicians to have substantial experience of shocked trauma patients as well as REBOA. However, there are a growing number of providers placing REBOA who have limited experience in endovascular resuscitation and minimal training,[232] and the frequency of eligible patients in the ten busiest trauma hospitals in England and Wales is estimated to be one patient every 46 days.[35] Therefore, there is considerable difficulty in selecting patients who would most benefit from REBOA, particularly outside the small number of specialist centres.

15.1.3 The morbidity of REBOA

Part One and Part Two have described some of the risks associated with IABO; iatrogenic vascular injury, cardiac dysfunction, pulmonary dysfunction, and cardiovascular instability after balloon deflation. There are limited clinical data that describe the morbidity of REBOA, the recognition of which is compounded by the poor physiological state of the patients prior to IABO. Davidson et al have reviewed the current understanding of the risks of REBOA, and separated them according to the steps of placement – arterial access, balloon positioning, balloon inflation, management during occlusion, balloon deflation, and removal of the vascular sheath.[232] This review includes a list of potential complications and ways to potentially mitigate the risks, but does not describe their prevalence. The case series by Brenner et al describes a number of patients who required limb fasciotomy or limb amputation following REBOA, and one patient who died of bowel necrosis secondary to prolonged Z1 aortic occlusion.[238]

Accurately recording the morbidity of REBOA is complicated by the emergency setting and high mortality of patients in whom REBOA is indicated – further data are therefore needed to both reduce the morbidity, and better inform decision makers in their time-pressured risk benefit analyses.

In terms of patient selection, the team at the R Cowley Adams Shock Trauma Center in Baltimore advocate the exclusion of patients with cardiac tamponade (with the use of bedside ultrasound), and those with thoracic haemorrhage (by review of the chest radiograph, and bilateral thoracostomies) from REBOA intervention.[238] This methodology is likely to identify patients that would benefit

more from RT, and would potentially be harmed by REBOA. This case series includes 50 patients who underwent REBOA for TCA – 32% with non-thoracic penetrating injury, and 68% from blunt injury (all those with penetrating thoracic injury in this institution receive RT, and were not included in this case series).[238] This group of patients, who were transient or non-responders to conventional resuscitation and did not have an indication for RT, likely represent those with the greatest benefit to risk ratio for the use of REBOA. 29 of 50 (58%) of patients in TCA had a ROSC with REBOA, 20 patients survived to receive surgery, and five (10%) survived to 30 days. All of the survivors had REBOA initiated in the operating theatre, and although not described it is likely that all of these patients were in LOST prior to REBOA, in a similar way to the single survivor from haemorrhagic TCA in Brenner et al's earlier case series.[104] This theory is reinforced by the reported mean increase in systolic BP after REBOA inflation in these 50 TCA patients – from 15.1 (±30.2) mmHg to 71.0 (±74.2) mmHg.[238]

The standard deviation of the mean systolic BP after REBOA inflation suggests that some patients did not respond to REBOA. It is therefore tempting to suggest that conversion from passive endovascular intervention (REBOA) to active endovascular intervention (SAAP) may have increased the survival rate of patients in Brenner's series that did not respond to REBOA, and who were likely therefore to have been in NOST.

15.1.4 REBOA - speed of intervention

The first and most time consuming step in initiating REBOA is vascular access.[232] The challenge of obtaining rapid vascular access is potentially affected by the complex resuscitation environment, vasospasm secondary to haemorrhagic shock, groin obstruction by a pelvic binder, and distorted anatomy secondary to the injury itself.[232,241] The skill of achieving vascular access should not be underestimated, particularly in TCA when other simultaneous interventions, for example chest compressions, may further increase the difficulty of arterial access. For these reasons, many providers advocate the early placement of a small calibre arterial line (for example a 5 Fr micropuncture) under ultrasound guidance whilst assessing the risk benefit ratio of aortic occlusion.[232] This arterial access can be used for invasive BP monitoring and for arterial blood samples, and can be 'up-sized' using a Seldinger technique for REBOA as required. The difficulty in obtaining percutaneous arterial access is illustrated in Brenner et al's most recent REBOA case series, in which eight patients in TCA underwent surgical cut-down after a failed attempt at percutaneous access.[238] This case series additionally provides the best estimate on the time taken to access the common femoral artery – a mean of 141 (±105) seconds in severe haemorrhage, and 300 (±201) seconds in TCA, $p<0.001$. In comparison, there was no significant difference in the subsequent time to aortic occlusion, with a total procedure time in the TCA group of 537 (±257) seconds (approximately nine minutes).[238]

In summary, there is no accurate method to determine in which patients the benefit of REBOA outweighs the risks of no intervention, and the morbidity of

the procedure has not been well described. Patients in TCA from NCTH, without an indication for RT, are the group in which the risk benefit analysis is most in favour of balloon occlusion. It is likely that later haemorrhage control is associated with an increased mortality,[106] and in a recent clinical series only 10% of patients who received REBOA in TCA survived to 30 days.[238] It is therefore logical to suggest that the risk benefit ratio of balloon occlusion improves as the patient continues to deteriorate along the spectrum of haemorrhage, from hypotension, to peri-arrest, to LOST, and finally to NOST. However, the effectiveness of REBOA in TCA is questionable; it is likely that in a proportion of patients (those in LOST) REBOA may be an effective intervention, but Part One of this thesis demonstrated that in a translational model REBOA is not effective in NOST. As previously described, there are no clinically validated methods to differentiate LOST from NOST, but SAAP is effective in translational models at resuscitating NOST. It would therefore be logical to suggest that in TCA secondary to NCTH a strategy of escalating endovascular intervention, starting with REBOA and proceeding to SAAP as required, may be an effective clinical paradigm.

15.2 Active endovascular intervention

15.2.1 Rescue-SAAP

SAAP is an experimental intervention, and as such there are no data on the time from initiating the procedure to starting the SAAP infusion, or on potential complications in the clinical setting. A distinct benefit in considering REBOA as a precursor to SAAP is that a SAAP catheter could be used, as in these animal models, as a REBOA catheter in the first instance, whilst the SAAP perfusion

circuit is prepared, and the response to REBOA is reviewed. In clinical practice, there has been a move towards inserting REBOA via smaller vascular sheaths, from 12 Fr, that would accommodate the current iteration of the experimental SAAP catheter, to 7Fr that would not.[238,242] However, it would be technically feasible to rapidly exchange the vascular sheath for a larger one using a Seldinger technique.

The time to initiate SAAP, after the initial placement of a REBOA catheter, is dependent on the setup of the infusion circuit. From my laboratory experience I estimate that with prior preparation and training this would take between three and five minutes, and could be accomplished with a team of three people.

SAAP has two additional potential risks over REBOA. Irrespective of the resuscitation fluid, there is a risk of introducing an arterial gas embolism, that could result in cerebral or myocardial injury. The use of citrated blood product requires the co-administration of calcium in the SAAP infusion, with the inherent risk of precipitating a ventricular arrhythmia.[226]

It would therefore be theoretically feasible to initiate SAAP within five minutes if the initial intervention with REBOA had not resulted in a ROSC. This experimental technique is termed 'Rescue-SAAP' (R-SAAP).

15.2.2 SAAP-ECLS

R-SAAP would require a large volume of resuscitation fluid, and at an infusion rate of 10 ml/kg/minute could administer 100% of total blood volume within

seven minutes. Continuation of R-SAAP for more than five minutes would both be logistically challenging, and would risk fluid overload. However, there may be patients who require ongoing cardiopulmonary support after a TCA.

Part Two described the use of ECLS to mitigate the effects of IABO deflation. A similar extracorporeal technique could be used to continue SAAP resuscitation after the circulating volume had been restored, by removing blood from the central venous circulation, passing it though an ECLS circuit, and returning the oxygenated blood via the SAAP catheter. This experimental technique is termed 'SAAP-ECLS'. This technique has not been previously described, and there are therefore no data available on setup time, which would involve inserting a venous ECLS cannulae and setting up an ECLS circuit. Data from ECLS setup in Part Two demonstrated that the mean time to ECLS flow after removal of the SAAP catheter was 149.3 seconds. In clinical practice this would realistically take longer, and placement of the venous ECLS cannula alone is likely to take a similar time to insertion of REBOA in TCA (a mean of 300 seconds in Brenner's study).[238]

15.3 A translational paradigm of escalating endovascular intervention

Haemorrhagic TCA is a spectrum of disease, where at the least severe end the patient may meet the definition of TCA (no palpable central pulse), but still have a cardiac output – described as a low output state in trauma (LOST).[46] REBOA, and simultaneous intravenous fluid resuscitation, may be an effective intervention in LOST TCA, but there is no reliable way to differentiate which patients will respond to this intervention. In those who do not respond, active

endovascular resuscitation with R-SAAP may be effective, although this would take longer to initiate. In those patients who require ongoing endovascular resuscitation after R-SAAP, SAAP-ECLS is the logical next step that may improve survival.

In order to test this translational paradigm of escalating endovascular intervention, a large animal model of severe haemorrhagic TCA secondary to NCTH and a controlled arterial bleed was used to test the interventions sequentially during a simulated 60 minute pre-hospital period.

CHAPTER SIXTEEN

MODEL DEVELOPMENT - PART THREE

16.1 Aims of model development

The aims of model development in Part Three were to develop the novel technique of converting REBOA to R-SAAP, and R-SAAP to SAAP-ECLS, and to undertake analysis of the calcium concentration in the first minute of SAAP infusion with citrated FWB.

16.2 Model development ethical review

Part Three experiments were undertaken under the same local animal ethics committee review as Part Two (FWH20150068A). Five animals were allocated to Part Three model development.

16.3 Swine donor pool

A swine donor pool was utilised using the same methodology as Parts One and Two (Section 6.3).

16.4 Animal preparation

Animal selection, animal anaesthesia, and initial physiological monitoring setup was the same as Part One, with the exception that near-infrared reflectance spectroscopy was not used (Section 6.4).

In 3D1-7817 and 3D2-7812, the left femoral vein 8.5 Fr catheter was placed as part of the initial animal preparation. Prior to SAAP-ECLS, this catheter was exchanged over a wire with a 21 Fr venous ECLS cannula (PVL2155, Maquet, Rastatt, Germany). However, this was technically challenging and resulted in significant blood loss. Therefore, from 3D3-7863 onwards, a 24 Fr Check-flo vascular sheath (Cook Medical LLC, Bloomington, IN, USA) was inserted to approximately 100 mm during initial animal preparation. This 250 mm vascular sheath was fully inserted immediately prior to introducing the 21 Fr venous ECLS cannula, and starting ECLS flow, as required during the protocol.

16.4.1 Surgical preparation

There were no adaptations made in surgical preparation from Parts One or Two during Part Three model development (Section 5.5.4). Baseline measurements were then obtained prior to the intervention, Table 16.1.

Table 16.1 – Baseline physiological measurements of model development animals (n=5), mean (± standard deviation).

Baseline variable	Mean (±SD)
Weight (kg)	75.6 (±4.7)
Heart rate (beats/minute)	84.6 (±9.1)
Systolic blood pressure (mmHg)	89.2 (±17.8)
Mean arterial pressure (mmHg)	71.0 (±15.5)
Mean pulmonary artery pressure (mmHg)	22.6 (±4.4)
Cardiac output (l/minute)	4.8 (±0.8)
End-tidal CO_2 (kPa)	5.7 (±0.5)
Carotid artery flow (ml/minute)	407.8 (±108.5)
Central venous pressure (mmHg)	10.2 (±1.8)
Central venous saturation (%)	70.6 (±9.6)
Core temperature (^{o}C)	36.6 (±0.8)

16.5 NCTH injury and controlled arterial haemorrhage

The model of laparoscopic hepatic injury has been previously developed in this laboratory,[101,118] and described in Parts One and Two of my research (Sections 5.5.5 & 11.5).

Specifically, the Part One model of NCTH injury and controlled arterial haemorrhage was used, in order to theoretically reduce the risk of VF during haemorrhage, and also to allow comparisons with the Part One REBOA group.

In summary, approximately 70% of the left lateral lobe of the liver was excised laparoscopically with Metzenbaum endoshears in under two minutes, followed five minutes later by a 3 ml/kg/minute controlled arterial haemorrhage from a SAAP catheter placed via the right femoral artery 14 Fr vascular sheath. The point of TCA was defined as an intra-aortic systolic BP of less than 10 mmHg for two consecutive heart beats. At this point, a t0 blood sample was drawn, and the haemorrhage rate was reduced to 1.0 ml/kg/minute if the intra-aortic systolic BP was greater than 0 mmHg, for a maximum of two minutes.

During Part Three model development, this produced an injury state at t0 that was similar to Part One, with no incidences of VF during haemorrhage. Therefore, no adaptions were made to the haemorrhage model from Part One methodology (Section 6.4.4).

16.6 REBOA intervention

Using an identical methodology to the Part One REBOA group, at t2, a SAAP catheter was advanced into Z1 of the aorta, and it position confirmed with fluoroscopy. At t2.75, the SAAP catheter balloon was inflated, and at t3 four units of FWB (2,452 ml) were infused via the left external jugular vein 8.5 Fr catheter at a rate of 500 ml/minute (over 4.9 minutes, approximately t3 to t8). All five Part Three development animals underwent this intervention, and none had a return of intrinsic cardiac output. No adaptations were made from Part One methodology (Section 6.5.4).

16.7 Rescue-SAAP intervention

During the 4.9 minute REBOA period, a SAAP FWB infusion was prepared using the same methodology as Parts One and Two. At t8, none of the five development animals had a ROSC (defined as an intra-aortic systolic BP of 50 mmHg or greater), and all received the R-SAAP intervention. At t8, all animals received a 50 ml rapid flush of intra-aortic LR immediately followed by oxygenated FWB at 800 ml/minute, together with 11.1 ml/minute of 10% calcium chloride. This infusion was continued until the animal had a return of intrinsic cardiac output and a systolic BP greater than 90 mmHg, or for a maximum duration of five minutes (total maximum FWB infusion of 4,000 ml).

One animal (3D4-7865) had a ROSC during R-SAAP. Three others (3D2-7812, 3D3-7863, 3D5-7866) went into VF during the REBOA intervention that was resistant to multiple trans-thoracic defibrillations and 4,000 ml of R-SAAP. The

remaining animal (3D1-7817) had a return of intrinsic cardiac function with R-SAAP, but its maximum systolic BP was 67.1 mmHg.

Previous large animal research with SAAP by Manning et al has used intra-aortic epinephrine, which appears to increase the ROSC rate after initial failed SAAP intervention.[112,226] In order to increase the likelihood of a ROSC with R-SAAP, experimental protocols included a 1 mg dose of intra-aortic epinephrine if the intrinsic systolic BP was below 50 mmHg after two minutes of R-SAAP.

Animals converting into the cardiac rhythm of VF during haemorrhage and during interventions has been an issue throughout this research project, and will not allow the hypotheses to be tested. Therefore, animals that converted into VF at any point during the Part Three protocol will be excluded.

16.8 SAAP-ECLS intervention

Three animals were not commenced on SAAP-ECLS flow owing to clot in the inferior vena cava – large clots were drained via the ECLS venous cannula. The one animal that was started on SAAP-ECLS was not in VF, and was not observed to have clot in the inferior vena cava. Therefore, it is possible that this was due to stasis, which may not occur in animals that are not in VF. However, in order to mitigate against this, and to preserve the limited number of ECLS circuits and oxygenators available for this project, systemic heparinisation was planned for the experimental protocols. This was initiated in all animals by administration of 1,000 units of heparin in 100 ml of LR, via the left femoral vein 24 Fr vascular sheath three minutes after the start of the R-SAAP infusion in animals with a

systolic BP lower than 50 mmHg that were therefore likely to require SAAP-ECLS.

The one animal that received SAAP-ECLS until t63 required 4,000 ml of intravenous LR to maintain ECLS flow at 800 ml/minute with a venous pressure greater than -150 mmHg. Using the same methodology as Part Two ECLS in the critical care period, 500 ml boluses of warmed LR were administered via the left external jugular vein 8.5 Fr catheter if the venous ECLS pressure was lower than -150 mmHg (Section 12.6.2).

16.9 *In vitro* analysis of SAAP calcium administration

In reference to the concerns discussed in Part Two about the serum ionised calcium concentration in the aortic arch during the first 30 seconds of SAAP infusion, a bench laboratory experiment was undertaken using the experimental SAAP circuit and catheter.

16.9.1 Adaptions to the calcium infusion setup

In the first instance, the calcium infusion line was shortened and an unnecessary three-way tap was removed to minimise dead space. One bag of warmed, citrated FWB, from an animal in the donor pool with a baseline ionised calcium of 1.22 mmol/l, was circulated and oxygenated in the experimental SAAP circuit, Figure 5.3.

In the first two runs, the calcium infusion was set up as described in Parts One and Two methodology, and approximately 10 ml of SAAP infusion was collected

in plain collection pots at ten, 20, 30, 40, 50, and 60 seconds from the start of the infusion (Group 1). In the latter two runs, the calcium infusion system was 'pre-pressurised' by running the infusion pump against a closed three-way tap for ten seconds (Group 2). Samples were then obtained at the same time intervals as the first group. The samples were immediately analysed using an ABG analyser.

On the day of the *in vitro* experiments, the calcium infusion was erroneously run at 8.25 ml/minute, rather than the 11.1 ml/minute rate that was used in previous model development and experimental protocols. This resulted in SAAP infusion ionised calcium concentrations lower than the animal baseline. The data have therefore been multiplied by 1.35 (8.25 ml/minute x 1.35 = 11.1 ml/minute) in order to provide an estimate of expected values at 11.1 ml/minute.

In Group 1, the ionised calcium concentration was lower than the detectable range at ten seconds, and was not within 10% of the baseline value until 30 seconds into the SAAP infusion. In Group 2, the ionised calcium concentration was not within 10% of the baseline value until 20 seconds into the SAAP infusion, Figure 16.1.

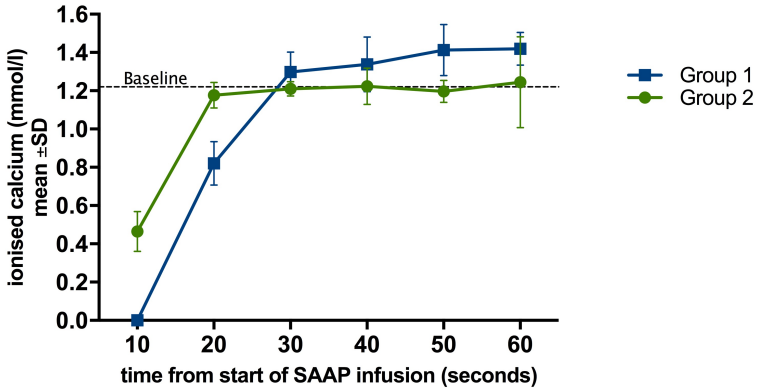

Figure 16.1 – *In vitro* SAAP infusion ionised calcium concentration at ten second intervals during the first 60 seconds of SAAP, mean (± standard deviation) of two samples per group; Group 1 – unpressurised system, Group 2 – pressurised system. Data have been multiplied by 1.35 to correct for a reduced rate of calcium infusion.

Pressurising the calcium infusion prior to administration (Group 2) increased the ionised calcium concentration of SAAP blood infusion in the first 30 seconds. In Group 1, the mean ionised calcium concentration after 30 seconds of infusion was higher than the baseline of the donor blood. This is likely to represent capacitance in the calcium infusion setup. The syringe driver used for calcium administration was an MRI Compatible Syringe Pump (Harvard Apparatus, Holliston, MA, USA), which is accurate to ± 0.35%, and can drive against over 800 N.[243] However, a pressure infusion line was not used, and therefore the delay in administering calcium in the first 20 seconds of SAAP may be as a result of capacitance in the infusion line. During Part Three experimental protocols, *in vivo* samples of intra-aortic arch ionised calcium will be obtained, and a pressure infusion line will be used once available.

16.10 Aims of the Part Three experimental protocol

The aim of this study was to demonstrate the efficacy of a translational paradigm of escalating endovascular intervention for the management of haemorrhagic TCA in large swine.

CHAPTER SEVENTEEN

METHODS - PART THREE

17.1 Part Three hypotheses

In a swine model of severe haemorrhagic TCA:

H9. Intervention with REBOA and intravenous FWB will not result in a return of spontaneous circulation in any animal.

H10. R-SAAP (up to 4,000 ml of intra-aortic oxygenated FWB over five minutes), followed by SAAP-ECLS (venoarterial circulation and oxygenation of blood at 800 ml/minute) as required will result in 100% of animals surviving for 60 minutes after unsuccessful REBOA intervention.

H11. Escalating endovascular intervention, from REBOA to R-SAAP to SAAP-ECLS as required will infer a significant 60 minute survival advantage over Part One historical controls that received REBOA and FWB only.

17.2 Animal use ethical review

The experimental protocol was approved by the 59th Medical Wing Institutional Animal Care and Use Committee (IACUC) (protocol number – FWH20150068A). Experiments were performed at the Clinical Research Division, 59th Medical Wing, United States Air Force, Office of the Chief Scientist, in a facility accredited by the American Association for the Accreditation of Laboratory Animal Care.

The protocol was conducted in accordance with guidelines established by the Public Health Service Policy on Humane Care and Use of Laboratory Animals and Office of Laboratory Animal Welfare.

17.3 Swine donor blood

Whole blood was collected from a dedicated pool of swine the day before each experimental protocol, using the same methodology as Part One (Section 6.3). Part Three includes one group of animals that sequentially moved through a series of escalating endovascular interventions, dependent on their response to each intervention. The maximum FWB per animal was 2,452 ml during the REBOA intervention and 4,000 ml during the R-SAAP intervention, a total of 6,452 ml – equating to 11 units of FWB per protocol. IRB approval was obtained for up to 11 units of FWB per Part Three experiment.

17.4 Animal preparation

No adaptions were made to animal preparation from Part One (Section 6.4).

17.4.1 Animal anaesthesia

No adaptions were made to animal anesthesia from Part One (Section 6.4.1). During all protocols, the depth of anaesthesia was continuously monitored by a trained laboratory technician using multiple parameters, including MAC of anaesthetic gas, heart rate, jaw tone, muscle tone, palpebral reflexes, and graded pain response.

17.4.2 Physiological monitoring and vascular line placement

When anaesthetised, electrocardiography, physiological monitoring, blood sampling, and vascular access lines were inserted percutaneously under ultrasound guidance. No adaptations were made to physiological monitoring and vascular line placement from Part One (Section 5.5.3), with the exception that the left femoral artery 8.5 Fr catheter was omitted, and a 24 Fr vascular sheath (Cook Medical Inc. Bloomington, IN, USA) was inserted approximately 100 mm into the left femoral vein to allow subsequent insertion of a 21 Fr ECLS venous cannula.

17.4.3 Surgical preparation

Surgical preparation was identical to Part One, and can be found at Section 6.4.3. This included a splenectomy, marking of the left lateral lobe of the liver with electrocautery, a direct cystostomy, and insertion of four laparoscopic ports. The spleen was returned to the abdominal cavity, and the abdomen was then closed. Baseline physiological measurements and blood samples were then obtained, followed by a ten minute stabilisation period.

17.5 NCTH injury and controlled arterial haemorrhage

At the end of the ten minute stabilisation period, the abdomen was insufflated via a laparoscopic port to a pressure of 12 mmHg. The left lateral lobe of the liver was visualised, and approximately 70% excised with 5 mm Metzenbaum endoshears in under two minutes. At the completion of the hepatic injury, the abdomen was rapidly desufflated, instruments and ports removed, and the laparoscopic holes approximated with surgical skin staples. Five minutes after

the start of the hepatic injury, a controlled haemorrhage was initiated at 3 ml/kg/minute via a 7.5 Fr intra-aortic (SAAP) catheter placed via the right femoral artery vascular sheath. At the time of TCA (defined as a systolic BP of less than 10 mmHg, t0), the controlled haemorrhage was continued at a reduced rate of 1.0 ml/kg/minute, for up to two minutes (t0-t2), if the intra-aortic BP was greater than 0 mmHg (Part One injury model), Figure 17.1.

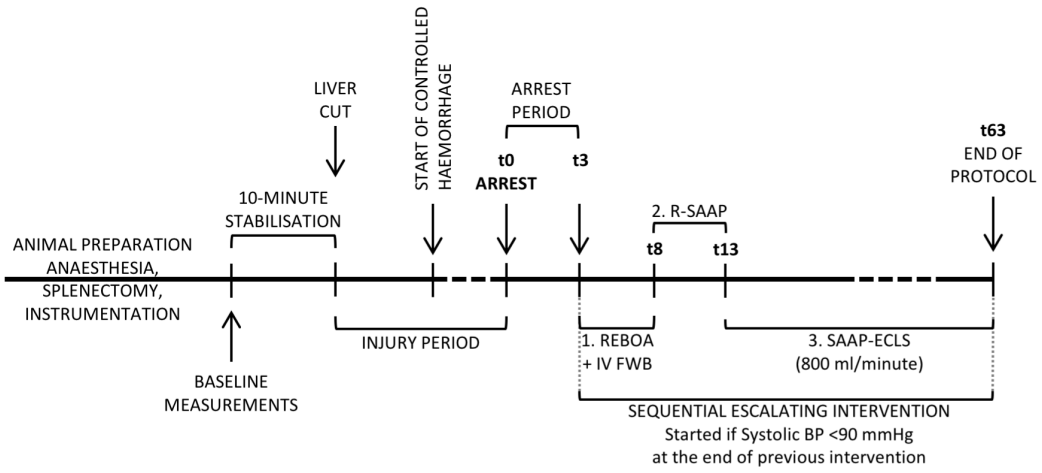

Figure 17.1 – Part Three protocol timeline. All animals received Z1 REBOA + 2,452 ml of intravenous FWB over five minutes (t3-t8). Animals with a systolic BP less than 90 mmHg at t8 received R-SAAP (800 ml/minute of intra-aortic oxygenated FWB, for a maximum of five minutes). Animals with a systolic BP less than 90 mmHg at t13 received SAAP-ECLS at 800 ml/minute until t63.

17.6 Experimental interventions

There was one experimental group in Part Three (n=12) – at t3, all animals received REBOA, those with a systolic BP lower than 90 mmHg at t8 received R-SAAP, and those with a systolic BP lower than 90 mmHg at t13 received SAAP-ECLS, Figure 17.1.

17.6.1 REBOA

At t2, a SAAP catheter with a 7.5 Fr central lumen, and a 30 mm (17 ml) balloon, was advanced in aortic Z1, and its position confirmed with fluoroscopy, Figure 5.6. The SAAP catheter central lumen was flushed with LR, and closed distally with a three-way tap.

At t2.75 (two minutes and 45 seconds), the SAAP catheter balloon was inflated, under fluoroscopy, with 17ml of 1:1 LR and contrast.

At t3, four units of FWB (2,452 ml) was infused via the left external jugular vein 8.5 Fr catheter at a rate of 500 ml/minute (over 4.9 minutes). An infusion of 10% calcium chloride (connected to the infusion line via a three-way tap) was administered at a rate of 6.9 ml/minute, for the duration of the FWB infusion.

Animals with a systolic BP of 90 mmHg or greater at t8 were observed, with no further intervention, until t63 or until they meet death-in-protocol criteria (Section 17.8). Animals with a systolic BP lower than 90 mmHg were transitioned to R-SAAP.

17.6.2 R-SAAP

At t8, eligible animals received R-SAAP: 800 ml/minute of intra-aortic oxygenated FWB, together with 10% calcium chloride at a rate of 11.1 ml/minute. The infusion was terminated if the animal had a systolic BP of 90 mmHg or greater, and re-started if the systolic BP fell below 90 mmHg between

t8 and t13. The maximum duration of R-SAAP was five minutes (t8-t13), a maximum volume of 4,000 ml of FWB.

After two minutes of R-SAAP infusion, animals with a systolic BP lower than 50 mmHg were administered 1 mg of intra-aortic epinephrine.

After three minutes of R-SAAP infusion, animals with a systolic BP lower than 50 mmHg were administered 1,000 units of heparin in 100 ml of 0.9% saline via the left femoral vein 24 Fr vascular sheath.

Animals with a systolic BP of 90 mmHg or greater at t13 were observed, with no further intervention, until t63 or until they meet death-in-protocol criteria. Animals with a systolic BP lower than 90 mmHg were transitioned to SAAP-ECLS.

17.6.3 SAAP-ECLS

At t13 (five minutes after the start of the R-SAAP infusion), in animals with a systolic BP lower than 90 mmHg the 24 Fr left femoral vascular sheath was fully inserted (to a depth of 250 mm), and a 21 Fr venous ECLS cannula (PVL2155, Maquet, Rastatt, Germany) was inserted. The SAAP catheter was attached to the ECLS arterial outflow, and the 21 Fr venous ECLS cannula was attached to the venous inflow. ECLS flow was then commenced at 800 ml/minute (approximately 10 ml/kg/minute).

During SAAP-ECLS, if the venous ECLS pressure was lower than -150 mmHg a 500 ml bolus of warmed LR was administered via the left internal jugular vein 8.5 Fr catheter. All animals commenced on SAAP-ECLS continued this therapy until t63 (the end of the protocol).

17.7 Post-experimental procedures

At the end of the protocol (t63, or sooner if animals met death-in-protocol criteria), swine were euthanised by a trained laboratory technician with intravenous administration of 100 mg/kg of sodium pentobarbital, in accordance with the American Veterinary Association euthanasia guideline.

Following animal euthanasia, the liver was removed in order to quantify the laparoscopic hepatic injury, as a percentage by weight of the left lateral lobe excised during the injury.

In order to mitigate against changes in volume of shed blood secondary to evaporation, the weight of controlled arterial haemorrhage and the blood removed from the abdomen were measured within ten minutes of collection. All suction containers and gauze were pre-weighed, and resulting weights of blood divided by 1.05 to quantify the volume of blood they contained.

17.8 Experimental definitions

Traumatic cardiac arrest (t0) was defined as a systolic BP less than 10 mmHg, together with a heart rate lower than the pre-injury baseline, sustained for three minutes.

ROSC, in reference to hypothesis H8, was defined as a systolic BP of 50 mmHg or greater, with the assumption that this is the threshold at which a central pulse may be palpable.

Death-in-protocol was defined as a systolic BP lower than 30 mmHg, despite animals receiving all experimental interventions, and this therefore could not occur until after SAAP-ECLS had been commenced (after t13). Therefore, survival was defined as a systolic BP of 30 mmHg or greater, 60 minutes after the start of the REBOA intervention (t63).

17.9 Physiological data acquisition

17.9.1 Data acquisition from the anaesthetic machine

The following variables were automatically recorded at one minute intervals throughout the protocol: cardiac electrical rate, rectal temperature, peripheral oxygen saturation, fraction of inspired oxygen, end-tidal carbon dioxide, MAC of isoflurane, and values from the Swan-Ganz pulmonary artery catheter (pulmonary artery pressure, central venous pressure, cardiac output, stroke volume, and central venous saturations).

17.9.2 Data acquisition on DAQ board

In order to accurately measure intra-aortic BP, the output from the micromanometer-tipped catheter was recorded at 500 Hz on a DAQ board, which produced an accurately calibrated BP and cardiac electrical rate. The output from the carotid artery flow probe, and pulmonary artery pressure from

the Swan-Ganz catheter were also recorded on the DAQ board at 500 Hz. The data from the DAQ was automatically converted into mean peak values every five seconds.

17.9.3 Manual data recording and time keeping

As per Section 6.9.4; the manual data recording sheet is at Appendix C.

17.10 Blood sample data acquisition

As per Section 6.10.

17.10.1 Arterial blood gas

Immediately following the insertion of the left brachial artery 5 Fr catheter, an ABG sample was obtained, in order to calibrate the Swan-Ganz catheter. Following this, ABG samples were drawn from the brachial arterial line: before surgical preparation, at baseline (at the start of the ten-minute stabilisation period), and every ten minutes after the start of the intervention (t13, t23, t33, t43, t53, t63). An ABG was drawn from the SAAP catheter at the point of arrest (t0) as the severe state of haemorrhage prevented blood being drawn more peripherally from the brachial arterial line. The ABG sample included pH, partial pressures of oxygen and carbon dioxide, ionised calcium, potassium, sodium, glucose, lactate, and base deficit.

17.10.2 Full blood count and clotting profile

Blood samples, from the brachial arterial line, were collected into one EDTA specimen tube and one citrated specimen tube at each time point (baseline, t13,

t33, t63). Samples were additionally collected at the point of arrest (t0) via the SAAP catheter.

The EDTA sample was analysed for full blood count (including haemoglobin, white cell count, and platelet count). The citrated sample was analysed for clotting function (prothrombin time and fibrinogen).

17.10.3 Thromboelastography

At the same time points, and via the same catheters, one citrated specimen tube was drawn to undertake TEG. The output from TEG included R (the time to initial fibrin formation), K (the time to reach a specific level of clot strength), α (the rate of clot formation), MA (maximum clot amplitude), G (clot elasticity), CI (clot index), and LY30 (the rate of amplitude reduction from MA to 30 minutes, a measure of fibrinolysis).

17.11 Outcomes

17.11.1 Primary outcome

The primary outcome was survival to simulated hospital arrival, 60 minutes after the start of the REBOA intervention. Survival was defined as a systolic BP of 30 mmHg or greater at t63 (Section 17.8).

17.11.2 Secondary outcomes

In order to compare the physiological effects of receiving one (REBOA), two (REBOA and R-SAAP), or three (REBOA, R-SAAP, and SAAP-ECLS) of the

interventions, systolic BP, left carotid artery flow, and end-tidal carbon dioxide were compared between groups of animals at t63.

At the same time point (t63), comparison was made by ABG (pH, partial pressures of oxygen carbon dioxide and oxygen, ionised calcium, potassium, sodium, lactate, base deficit), by full blood count (haemoglobin, platelet count), by clotting analysis (fibrinogen, prothrombin time), and by TEG (R, K, alpha-angle, MA, G, CI, LY-30) between groups of animals that received one, two, or three of the interventions.

17.12 Comparison with Part One REBOA group

In order to evaluate the efficacy of escalating endovascular intervention against REBOA only, comparison was made between baseline measurements, arrest period physiology and blood samples, and survival at t63, between the Part Three animals and the Part One REBOA group.

17.13 Statistical Methods

Data are reported as number (percent), and mean (± standard deviation). Proportions are reported as number (percent, 95% confidence interval). The 95% confidence interval was calculated using the Wilcoxon-Brown method. Comparison between the means of two independent groups was analysed with an unpaired t-test with Welch's correction (without assumption of equal standard deviations). Comparison between the means of repeated measurements within the same group was analysed with a paired t-test. Three group analyses were by ordinary one-way ANOVA. Analysis of proportions

(survival and ROSC) was with Fisher's exact test, and survival curves were compared with the log-rank (Mantel-Cox) test. Correlation was determined with the Pearson correlation coefficient. Statistical significance was pre-defined as $p<0.05$.

Percentages and means (± standard deviation) have been calculated in Excel for for Mac (Microsoft, Redmond, WA, USA), version 15.36; standard deviation is reported as standard deviation of the sample. 95% confidence intervals, t-tests, analyses of proportions, correlation, and survival curves have been analysed in Prism (GraphPad, La Jolla, CA, USA), version 7.0c.

17.14 Exclusions and protocol deviations

Animals that convert into VF at any point during the protocol will be excluded. Protocol deviations and animals with VF were discussed with my primary laboratory supervisor (Dr. James Ross). All exclusion and protocol deviations are described.

CHAPTER EIGHTEEN

RESULTS – PART THREE

18.1 Overview and exclusions

Twelve animals were allocated to the Part Three experimental protocol. One animal, P3-1-7874, had a spontaneous cardiovascular collapse during the ten minute stabilisation period, did not recover, and was excluded. There were two protocol deviations: in P3-2-7893 intra-aortic epinephrine was not administered two minutes into the R-SAAP infusion per protocol, and P3-4-7918 had a systolic BP of 90 mmHg after 69 seconds of R-SAAP, the infusion was stopped, but when the systolic BP rapidly decreased to below 90 mmHg the R-SAAP infusion was not re-started per protocol. There was one occurrence of VF during Part Three – P3-5-7963 went into VF 73 seconds in the R-SAAP infusion. Subsequent to this animal, pressure line tubing was used in the calcium infusion setup for R-SAAP, and the intra-aortic arch ionised calcium concentration confirmed during the subsequent two R-SAAP infusions – data are presented in Section 18.5.2.1.

Exclusions were made within 24 hours of each experiment. As this was a descriptive analysis, further animals were not added to the schedule. Therefore, a total of 12 animals were used, with eight included in the data analysis.

18.2 Blood donor pool

A total of 32 swine were used in the blood donor pool, providing 166 units of FWB for experimental protocols. Each animal had up to three units of FWB

venesected 24 hours prior to each experimental protocol. After a minimum of 14 days, animals underwent a second venesection. The mean haemoglobin of donated product was 9.9 (±0.9) g/dl, and there was no statistical difference between first and second donations, p=0.81.

18.3 Animal baseline characteristics

18.3.1 Baseline physiology

All animals were within the required range of 70-90 kg, and the mean weight was 75.0 (±3.8) kg. At baseline, the mean physiological variables were systolic BP 91.6 (±17.3) mmHg, left carotid artery flow 357.4 (±128.7) ml/minute, and end-tidal carbon dioxide 5.5 (±0.2) kPa, Table 18.1

18.3.2 Baseline blood sample analysis

At baseline, an ABG, full blood count, clotting sample, and a TEG sample were drawn, Table 18.2.

Table 18.1 – Baseline physiological characteristics of Part Three animals (n=8), mean (± standard deviation).

Variable	Part Three
Animal weight (kg)	75.0 (±3.8)
Heart rate (beats/minute)	86.3 (±15.7)
Systolic blood pressure (mmHg)	91.6 (±17.3)
Mean arterial pressure (mmHg)	74.4 (±17.5)
Mean pulmonary artery pressure (mmHg)	23.8 (±2.1)
Cardiac output (l/minute)	5.2 (±1.0)
SaO_2 (%)	97.8 (±1.9)
End-tidal CO_2 (kPa)	5.4 (±0.2)
Left carotid artery flow (ml/minute)	357.4 (±128.7)
Central venous pressure (mmHg)	10.5 (±2.5)
Central venous saturation (%)	66.5 (±13.9)
Core temperature (^oC)	37.0 (±1.0)

Table 18.2 – Baseline blood sample analysis of Part Three animals (n=8), mean (± standard deviation).

Variable	Part Three
Arterial blood gas	
pH	7.49 (±0.03)
pO_2 (kPa)	13.9 (±2.3)
pCO_2 (kPa)	5.3 (±0.3)
Calcium (mmol/l)	1.2 (±0.1)
Potassium (mmol/l)	4.1 (±0.3)
Sodium (mmol/l)	138.8 (±2.0)
Glucose (mmol/l)	5.5 (±1.1)
Lactate (mmol/l)	1.5 (±0.6)
Base deficit	-6.3 (±2.0)
PaO_2:FiO_2 (mmHg)	497.3 (±82.1)
Full blood count	
Haemoglobin (g/dl)	11.0 (±0.8)
White cell count ($x10^9$/l)	19.1 (±4.8)
Platelets ($x10^9$/l)	277.6 (±93.5)
Clotting profile	
PT (seconds)	13.5 (±0.8)
Fibrinogen (g/l)	2.4 (±0.8)
Thromboelastography	
R (seconds)	4.7 (±1.1)
K (seconds)	1.4 (±0.5)
α angle (degrees)	71.8 (±4.7)
MA (mm)	77.3 (±4.5)
G	17.9 (±5.0)
CI	3.6 (±1.2)
LY-30 (%)	2.0 (±1.4)

18.4 Injury analysis

18.4.1 NCTH and controlled arterial haemorrhage

The hepatic injury was completed in a mean of 98.0 (±16.6) seconds, and was not significantly different to Part One – 105.2 (±17.4) seconds, p=0.29, or Part Two – 101.5 (±10.0) seconds, p=0.60. The mean percent excision of the left lateral lobe of the liver was 64.4 (±4.5) %, and was lower than Part One – 69.2 (±9.6) %, $p<0.05$, but not statistically different to Part Two – 66.6 (±7.1) %, p=0.36, Table 18.3.

The volume of the NCTH was measured during the post-experimental procedures, and therefore may have been affected by the interventions – it was a mean of 2,768.5 (±1,377.1) ml (a range of 878 ml to 4,251 ml), equating to 36.9 (±18.6) ml/kg, Table 18.3.

Table 18.3 – Injury characteristics of Part Three animals (n=8), mean (± standard deviation).

Variable	Part Three
Animal weight (kg)	75.0 (±3.8)
Spleen weight (g)	498.4 (±108.9)
Liver weight (kg)	1,641.8 (±305.3)
Injury time (seconds)	596.4 (±88.6)
NCTH	
Hepatic injury (seconds)	98.0 (±16.6)
Proportion of liver lobe excised (%)	64.4 (±4.5)
Abdominal haemorrhage (ml)	2,768.5 (±1377.1)
Abdominal haemorrhage (ml/kg)	36.9 (±18.6)
Controlled haemorrhage	
Controlled haemorrhage (ml)	1,381.6 (±388.1)
Controlled haemorrhage (ml/kg)	18.6 (±5.6)
Combined haemorrhage	
Total haemorrhage (ml)	4,150.1 (±1,481.9)
Total haemorrhage (ml/kg)	55.5 (±20.3)
Proportion of swine TBV (%)	82.4 (±30.2)

The volume of the controlled arterial haemorrhage was a mean of 1,381.6 (±388.1) ml, equating to 18.6 (±5.6) ml/kg, and was comparable to Part One – 22.9 (±11.1) ml/kg, p=0.41, and Part Two – 19.9 (±7.2) ml/kg, p=0.63, Table 18.3.

The injury period, from the start of the hepatic injury to the onset of TCA, (t0) was a mean of 596.4 (±88.6) seconds, and was not significantly different to Part One – 674.6 (±111.0) seconds, p=0.06, Table 18.3.

18.4.1.1 The correlation of resuscitative fluid and haemoperitoneum

In order to characterise the relationship between the total volume of resuscitative fluid administered and the volume of haemoperitoneum recorded after t63, these variables have been graphically presented, Figure 18.1.

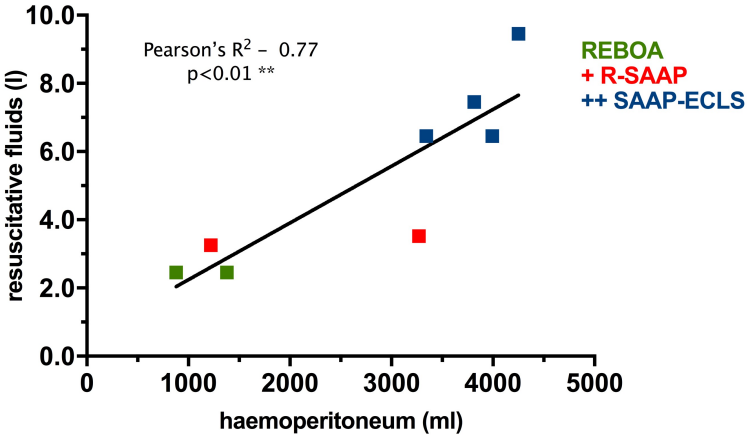

Figure 18.1 – Correlation of the total volume of resuscitative fluid and the volume of haemoperitoneum in Part Three animals (n=8).

There was a highly positive correlation between the total of volume of resuscitative fluid administered between t3 and t63 and the volume of haemoperitoneum recovered in post-experimental procedures.

18.4.2 Arrest period physiology

At the onset of TCA (t0), a mean of 596.4 (±88.6) seconds after the start of the hepatic injury, all animals had a systolic BP less than 10 mmHg, and a cardiac electrical rate lower than baseline measurement.

During the three minute arrest period, the mean systolic BP was 2.0 (±2.7) mmHg, the mean left carotid artery flow was 0.5 (±1.2) ml/minute, and the mean end-tidal carbon dioxide was 1.0 (±0.4) kPa.

The mean cardiac electrical rate during the last 30 seconds of the arrest period (t2.5 to t3) was 56.2 (±6.5) beats/minute. Three (37.5%) animals had ischaemic ECGs (ST segment depression), and two (25.0%) animals were in complete heart block. The mean cardiac electrical rate between t2.5 and t3 was significantly higher than Part One – 43.0 (±31.3) beats/minute, $p<0.05$. No Part Three animals were in cardiac electrical asystole during the arrest period, compared to 12 (30.0%) Part One animals.

18.4.3 Arrest point blood sample analyses

An ABG was drawn, via the SAAP catheter at the point of TCA (t0). Markers of tissue perfusion were examined (pH, lactate, base deficit); samples were obtained from all animals, n=8. The mean pH was 7.62 (±0.05), the mean lactate was 2.6 (0.8) mmol/l, and the mean base deficit was -5.4 (±1.6). All values were significantly elevated compared to baseline – pH and lactate $p<0.001$, base deficit $p<0.05$.

18.5 Intervention analysis

18.5.1 REBOA

The REBOA intervention (intra-aortic balloon inflation in Z1 of the aorta, and intravenous FWB infusion at 500 ml/minute) was started a mean of 181.7 (±1.5)

seconds after t0, and was not significantly different to the Part One REBOA group, 183.2 (±2.4) seconds, p=0.14.

Eight (100.0%) animals received 2,452 ml of intravenous FWB between t3 and t8. Pre-infusion blood samples of perfusate were obtained to check uniformity and to compare with the Part One REBOA group infusions. The mean pH, partial pressure of oxygen, and haemoglobin were not significantly different to the Part One REBOA group infusion. However, the Part Three REBOA infusion had a significantly higher arterial partial pressure of carbon dioxide compared to the Part One REBOA infusion, Table 18.4.

Table 18.4 – A comparison of the REBOA FWB pre-infusion blood samples between the Part One REBOA group (n=10), and Part Three animals (n=8), mean (± standard deviation).

Variable	Part One REBOA	Part Three	t-test
pH	7.04 (±0.02)	7.02 (±0.07)	p=0.56
pO_2 (kPa)	8.2 (±2.3)	7.2 (±1.6)	p=0.28
pCO_2 (kPa)	6.0 (±0.6)	10.0 (±2.3)	p<0.01**
Haemoglobin (g/dl)	7.9 (±0.9)	9.1 (±2.5)	p=0.23

Two (25.0%, 95%CI 4.4-59.1) animals had a return of cardiac output during the REBOA intervention – P3-11-8010, P3-12-8030. These animals had a systolic BP over 50 mmHg at t5.0 (8010) and t5.6 (8030), and a systolic BP over 90 mmHg at t5.6 (8010) and t5.8 (8030). Examination of the injury in these two animals demonstrated that the percentage of the hepatic injury, the arrest period duration, arrest period physiology, and the timing of the intervention were all within one standard deviation of the mean of the other six animals. The mean

cardiac electrical rate between t2.5 and t3 in the six animals without a ROSC was 56.9 (±7.4) beats/minute. P3-11-8010 and P3-12-8010 had mean cardiac electrical rates of 56.0 and 52.2 beats/minute respectively. There were therefore no observed differences between the two animals with a return of cardiac output and those without.

18.5.2 R-SAAP

Six (75.0%) Part Three animals had an R-SAAP infusion. The interval between the end of the REBOA FWB infusion and the start of R-SAAP was a mean of 9.2 (±4.6) seconds.

Pre-infusion blood samples were obtained from the R-SAAP infusion (n=6), and compared to the Part One SAAP-FWB group infusions (n=10), Table 18.5.

Table 18.5 – A comparison of the Part One SAAP-FWB group infusion (n=10), and the Part Three R-SAAP infusion (n=6), mean (± standard deviation).

Variable	P1 SAAP-FWB	P3 R-SAAP	t-test
pH	7.50 (±0.13)	7.53 (±0.16)	p=0.70
pO_2 (kPa)	82.9 (±7.5)	83.3 (±14.1)	p=0.94
pCO_2 (kPa)	1.4 (±0.6)	1.9 (±0.6)	p=0.14
Potassium (mmol/l)	4.6 (±0.5)	4.6 (±1.0)	p=0.96
Haemoglobin (g/dl)	7.0 (±0.6)	7.5 (±0.8)	p=0.23
Oxygen content (ml/100ml)	11.3 (±0.8)	12.0 (±1.2)	p=0.27

Four (66.7%) animals that received R-SAAP were administered 1 mg of intra-aortic epinephrine, per protocol, two minutes after the start of the infusion. All four had a return of intrinsic cardiac output within 30 seconds of epinephrine administration, but all fulfilled the protocol criteria to be commenced on SAAP-

ECLS on completion of the R-SAAP infusion. The same four animals had a systolic BP lower than 50 mmHg three minutes after the start of the infusion, and received 1,000 units of heparin via the left femoral 24 Fr vascular sheath.

18.5.2.1 Intra-aortic calcium concentration during R-SAAP

One excluded animal, P3-5-7963, went into VF 73 seconds in the R-SAAP infusion. Subsequent to this, and after review of the data during Part Three model development, the calcium infusion line was replaced with a pressure infusion line that does not have capacitance.

ABG samples were obtained from the left carotid artery 8.5 Fr catheter in two Part Three animals at 10, 30, 60, 90, and 180 seconds after the start of the R-SAAP infusion. The results demonstrate that there was still a delay in reaching normocalcaemia at the start of the R-SAAP infusion, but that the pressure infusion line may have reduced this (Section 16.9.1), Figure 18.2.

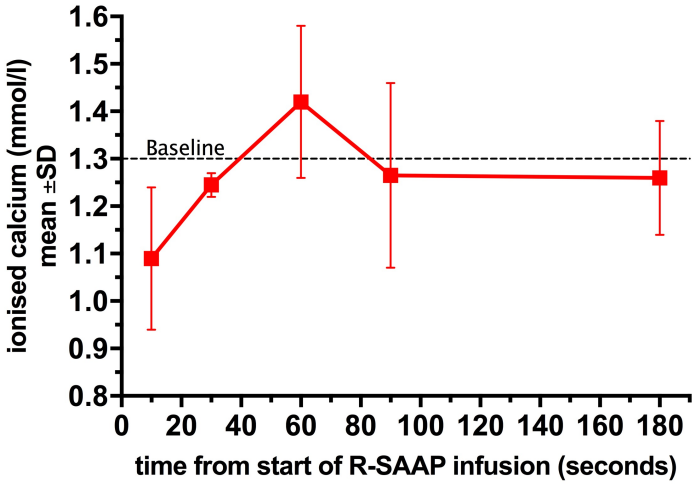

Figure 18.2 – Intra-aortic arch ionised calcium concentration of n=2 Part Three animals during the first 180 seconds of R-SAAP infusion with FWB.

Including these two animals, P3-7-7958 and P3-8-7975, four animals received R-SAAP using the calcium pressure infusion line – none converted into VF.

18.5.3 SAAP-ECLS

Four (50.0%) animals received SAAP-ECLS. The interval between the end of R-SAAP and the start of ECLS flow was a mean of 180.2 (±36.2) seconds. This interval was comparable to the interval between removing the SAAP catheter and starting ECLS flow in the Part Two SAAP-FWB2 group – 149.3 (±217.6) seconds, p=0.77.

Two (50.0%) animals that received SAAP-ECLS required no additional LR, one (25.0%) animal required 1,000 ml of LR owing to an ECLS venous pressure of less than -150 mmHg and venous cannula 'chatter'. During SAAP-ECLS in the fourth animal, P3-10-8007, the circuit came apart on two occasions (resulting in an undefined volume of extra-corporeal haemorrhage), and the animal required 3,000 ml of LR to maintain ECLS flow.

18.6 Primary outcome

Eight (100.0%, 95%CI 67.6-100.0) animals survived the pre-hospital period.

Two (25.0%, 95%CI 4.4-59.1) animals had a systolic BP of 90 mmHg or greater after the REBOA intervention, and did not receive any further intervention. Two (25.0%, 95%CI 4.4-59.1) animals had a systolic BP of 90 mmHg or greater after the subsequent R-SAAP intervention, and did not receive any further intervention. Four (50.0%, 95%CI 21.5-78.5) animals had a systolic BP lower

than 90 mmHg after REBOA followed by R-SAAP intervention and received SAAP-ECLS, Figures 18.3a, 18.3b, & 18.3c.

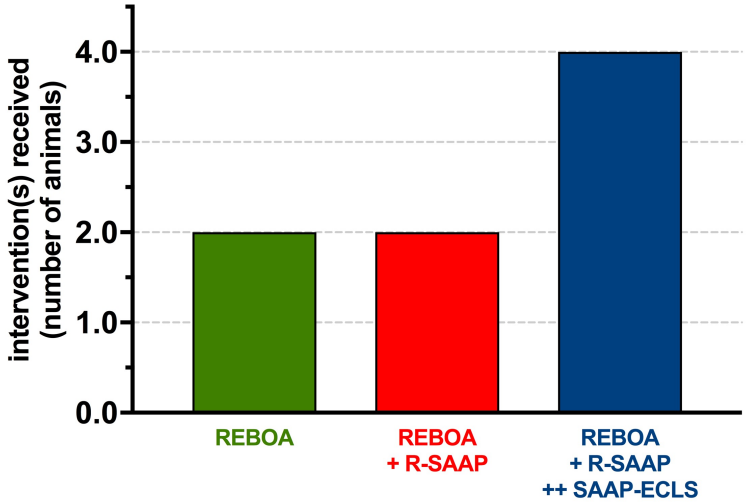

Figure 18.3a – The number of animals that required intervention with REBOA alone, REBOA followed by R-SAAP, and REBOA followed by R-SAAP, followed by SAAP-ECLS.

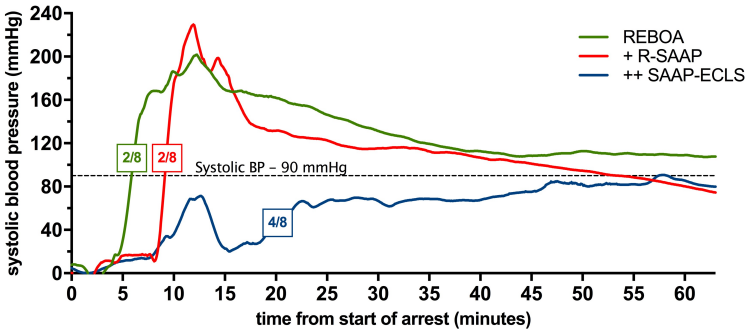

Figure 18.3b – Systolic BP from the start of TCA (t0) to the end of the protocol (t63). All, n=8, received REBOA, six animals received subsequent R-SAAP, four animals received subsequent SAAP-ECLS.

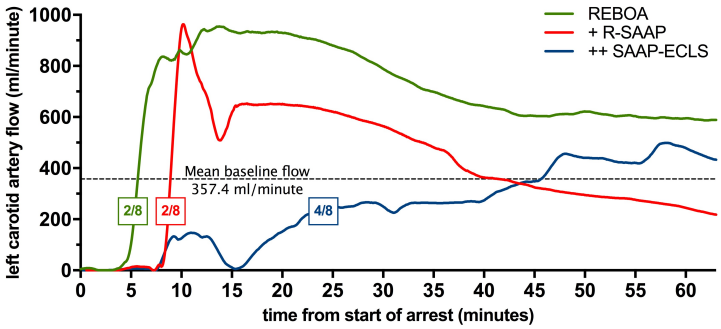

Figure 18.3c – Left carotid artery flow from the start of TCA (t0) to the end of the protocol (t63). All, n=8, received REBOA, six animals received subsequent R-SAAP, four animals received subsequent SAAP-ECLS.

18.7 Secondary outcomes

18.7.1 Physiology at t63

All animals were included in the analysis of physiological variables at t63.

18.7.1.1 Comparison of baseline and t63

At t63, the mean systolic BP was 85.4 (±26.1) mmHg, the mean left carotid artery flow was 416.3 (±236.4) ml/minute, and the mean end-tidal carbon dioxide was 3.9 (±1.6) kPa. The systolic BP and left carotid artery flow at t63 were not statistically different to baseline – 91.6 (±17.3) mmHg, p=0.66, and 357.4 (±128.7) ml/minute, p=0.50 respectively. However, the end-tidal carbon dioxide at t63 was significantly lower than baseline – 5.4 (±0.2) kPa, $p<0.05$.

18.7.1.2 Comparison by intervention

These physiological variables were compared at t63 between animals that only required REBOA intervention, those that required REBOA followed by R-SAAP, and those that required all three sequential interventions. The REBOA only

group had the highest mean value of systolic BP, left carotid artery flow, and end-tidal carbon dioxide of the three groups, but none of these reached statistical significance, Table 18.6.

Table 18.6 – A comparison of physiological variables at t63 between animals that received REBOA, animals that received REBOA followed by R-SAAP, and animals that received all three sequential interventions, mean (± standard deviation).

Variable	REBOA	REBOA + R-SAAP	REBOA + R-SAAP ++ SAAP-ECLS	ANOVA
Number of animals	2	2	4	
Systolic blood pressure (mmHg)	107.8 (±13.1)	74.1 (±44.1)	79.9 (±20.6)	p=0.43
Left carotid artery flow (ml/minute)	589.4 (±109.3)	216.0 (±229.1)	430.0 (±249.0)	p=0.33
End-tidal carbon dioxide (kPa)	5.1 (±1.5)	3.1 (±0.5)	3.7 (±1.9)	p=0.52

18.7.2 Blood sample analyses at t63

All animals were included in the analysis of blood samples at t63, n=8. Six (75.0%) clotting samples (prothrombin time, fibrinogen) were clotted and not analysed, therefore these variables have not been reported.

18.7.2.1 Comparison of baseline and t63

A comparison was made between baseline and t63 blood samples in all animals in order to describe the overall effect of the paradigm of escalating endovascular intervention. There was a significant decrease in pH, and a significant increase in lactate and base deficit between baseline and t63, Table 18.7.

Table 18.7 – A comparison of baseline and t63 blood samples in all Part Three animals (n=8), mean (±standard deviation).

Variable	Baseline	t63	t-test
Arterial blood gas			
pH	7.49 (±0.03)	7.24 (±0.12)	p<0.001***
pO_2 (kPa)	13.9 (±2.3)	50.1 (±20.9)	p<0.01**
pCO_2 (kPa)	5.3 (±0.3)	5.4 (±2.1)	p=0.95
Calcium (mmol/l)	1.2 (±0.1)	1.3 (±0.1)	p<0.05*
Lactate (mmol/l)	1.5 (±0.6)	13.8 (±2.0)	p<0.001***
Base deficit	-6.3 (±2.0)	11.3 (±2.3)	p<0.001***
Sodium (mmol/l)	138.8 (±2.0)	142.0 (±1.6)	p<0.001***
Potassium (mmol/l)	4.1 (±0.3)	4.9 (±0.5)	p<0.01**
PaO_2:FiO_2 (mmHg)	497.3 (±82.1)	375.5 (±157.0)	p<0.05*
Full blood count			
Haemoglobin (g/dl)	11.0 (±0.8)	7.6 (±2.1)	p<0.01**
White cell count ($x10^9$/l)	19.1 (±4.8)	8.2 (±3.5)	p<0.001***
Platelets ($x10^9$/l)	277.6 (±93.5)	170.5 (±51.1)	p<0.01**
Thromboelastography			
R (seconds)	4.7 (±1.1)	3.9 (±0.4)	p<0.05*
K (seconds)	1.4 (±0.5)	1.6 (±0.4)	p=0.41
α angle (degrees)	71.8 (±4.7)	69.2 (±3.9)	p=0.21
MA (mm)	77.3 (±4.5)	63.8 (±7.9)	p<0.001***
G	17.9 (±5.0)	9.4 (±3.3)	p<0.01**
CI	3.6 (±1.2)	1.8 (±1.5)	p=0.07
LY-30 (%)	2.0 (±1.4)	0.3 (±0.7)	p<0.05*

18.7.2.2 Comparison by intervention

Blood samples were also compared at t63 between animals that only required REBOA intervention, those that required REBOA followed by R-SAAP, and those that required all three sequential interventions. There was a significant difference between the three groups in base deficit (highest in the animals that required all three interventions), platelet count (lowest in the animals that required all three interventions), and TEG maximum amplitude, clot elasticity, and clot index – all lowest in the animals that required all three interventions, Tables 18.8 & 18.9.

Table 18.8 – A comparison of blood samples (arterial blood gas and full blood count) at t63 between animals that received REBOA, animals that received REBOA followed by R-SAAP, and animals that received all three sequential interventions, mean (±standard deviation).

Variable	REBOA	REBOA + R-SAAP	REBOA + R-SAAP ++ SAAP-ECLS	ANOVA
Number of animals	2	2	4	
Arterial blood gas				
pH	7.23 (±0.08)	7.31 (±0.04)	7.20 (±0.16)	p=0.65
pO_2 (kPa)	57.0 (±0.8)	36.3 (±19.6)	53.5 (±26.8)	p=0.63
pCO_2 (kPa)	6.2 (±1.2)	4.3 (±0.1)	5.5 (±2.9)	p=0.72
Calcium (mmol/l)	1.3 (±0.0)	1.3 (±0.0)	1.4 (±0.1)	p=0.06
Lactate (mmol/l)	11.7 (±2.1)	14.1 (±1.3)	14.8 (±1.8)	p=0.24
Base deficit	8.7 (±1.1)	10.4 (±1.5)	13.2 (±1.2)	p<0.05*
Sodium (mmol/l)	140.5 (±2.1)	142.5 (±2.1)	142.5 (±1.0)	p=0.36
Potassium (mmol/l)	5.1 (±0.1)	4.9 (±1.1)	4.9 (±0.2)	p=0.93
PaO_2:FiO_2 (mmHg)	427.5 (±6.4)	272.0 (±147.1)	401.3 (±201.2)	p=0.63
Full blood count				
Haemoglobin (g/dl)	9.0 (±0.8)	9.3 (±1.2)	6.2 (±1.9)	p=0.12
White cell count ($x10^9$/l)	10.1 (±5.6)	5.3 (±3.3)	8.7 (±2.6)	p=0.43
Platelets ($x10^9$/l)	176.0 (±49.5)	239.0 (±14.1)	133.5 (±15.5)	p<0.05*

Table 18.9 – A comparison of thromboelastography at t63 between animals that received REBOA, animals that received REBOA followed by R-SAAP, and animals that received all three sequential interventions, mean (±standard deviation).

Variable	REBOA	REBOA + R-SAAP	REBOA + R-SAAP ++ SAAP-ECLS	ANOVA
Number of animals	2	2	4	
Thromboelastography				
R (seconds)	4.0 (±0.2)	4.4 (±0.0)	3.7 (±0.3)	p=0.08
K (seconds)	1.5 (±0.4)	1.3 (±0.0)	1.8 (±0.4)	p=0.25
α angle (degrees)	71.8 (±3.7)	71.7 (±0.1)	66.7 (±3.8)	p=0.20
MA (mm)	70.6 (±3.4)	70.7 (±4.9)	56.9 (±2.5)	p<0.01**
G	12.1 (±2.0)	12.3 (±3.0)	6.6 (±0.7)	p<0.05*
CI	3.2 (±1.0)	3.0 (±0.6)	0.5 (±0.7)	p<0.05*
LY-30 (%)	0.1 (±0.1)	0.0 (±0.0)	0.5 (±1.1)	p=0.70

18.8 Comparison with Part One REBOA group

In order to evaluate the efficacy of an escalating endovascular intervention compared to intervention with REBOA and FWB only, comparison was made with the Part One REBOA group.

18.8.1 Baseline physiology

The Part Three animals had a significantly lower weight compared to the Part One REBOA group, but there were no other differences in measured physiological variables at baseline between groups, Table 18.10.

Table 18.10 – A comparison of baseline physiological characteristics of Part One REBOA animals and Part Three animals, mean (±standard deviation).

Variable	REBOA	Part Three	t-test
Number of animals	10	8	
Animal weight (kg)	82.5 (±4.7)	75.0 (±3.8)	p<0.01**
Heart rate (beats/minute)	89.8 (±16.6)	86.3 (±15.7)	p=0.65
Systolic blood pressure (mmHg)	86.3 (±6.2)	91.6 (±17.3)	p=0.43
Mean arterial pressure (mmHg)	68.9 (±5.0)	74.4 (±17.5)	p=0.42
Mean pulmonary artery pressure (mmHg)	23.7 (±2.5)	23.8 (±2.1)	p=0.96
Cardiac output (l/minute)	5.8 (±1.2)	5.2 (±1.0)	p=0.30
SaO_2 (%)	98.2 (±1.8)	97.8 (±1.9)	p=0.61
End-tidal CO_2 (kPa)	5.5 (±0.1)	5.4 (±0.2)	p=0.57
Left carotid artery flow (ml/minute)	395.1 (±96.0)	357.4 (±128.7)	p=0.50
Central venous pressure (mmHg)	9.9 (±1.4)	10.5 (±2.5)	p=0.56
Central venous saturation (%)	66.3 (±5.4)	66.5 (±13.9)	p=0.97
Core temperature (^oC)	37.4 (±0.7)	37.0 (±1.0)	p=0.44

18.8.2 Baseline blood samples

The Part Three animals had a significantly prolonged TEG R (the time from the start to when the waveform reaches 2 mm) that resulted in a significantly higher clot index compared to the Part One REBOA group. However, there were no

other significant differences in measured baseline blood sample analyses, Table 18.11.

Table 18.11 – A comparison of baseline blood samples of Part One REBOA animals and Part Three animals, mean (±standard deviation).

Variable	REBOA	Part Three	t-test
Number of animals	10	8	
Arterial blood gas			
pH	7.47 (±0.03)	7.49 (±0.03)	p=0.35
pO_2 (kPa)	14.2 (±3.1)	13.9 (±2.3)	p=0.85
pCO_2 (kPa)	5.4 (±0.3)	5.3 (±0.3)	p=0.46
Calcium (mmol/l)	1.3 (±0.1)	1.2 (±0.1)	p=0.79
Lactate (mmol/l)	1.7 (±0.4)	1.5 (±0.6)	p=0.44
Base deficit	-5.8 (±2.0)	-6.3 (±2.0)	p=0.59
Sodium (mmol/l)	140.2 (±2.2)	138.8 (±2.0)	p=0.16
Potassium (mmol/l)	4.2 (±0.3)	4.1 (±0.3)	p=0.30
Glucose (mmol/l)	5.8 (±1.5)	5.5 (±1.1)	p=0.62
PaO_2:FiO_2 (mmHg)	506.4 (±110.8)	497.3 (±82.1)	p=0.84
Full blood count			
Haemoglobin (g/dl)	11.3 (±0.1)	11.0 (±0.8)	p=0.51
White cell count ($x10^9$/l)	19.4 (±4.4)	19.1 (±4.8)	p=0.89
Platelets ($x10^9$/l)	260.5 (±68.1)	277.6 (±93.5)	p=0.67
Clotting profile			
PT (seconds)	13.6 (±0.4)	13.5 (±0.8)	p=0.80
Fibrinogen (g/l)	2.1 (±0.4)	2.4 (±0.8)	p=0.37
Thromboelastography			
R (seconds)	6.1 (±1.3)	4.7 (±1.1)	p<0.05*
K (seconds)	1.7 (±0.4)	1.4 (±0.5)	p=0.24
α angle (degrees)	69.5 (±3.3)	71.8 (±4.7)	p=0.27
MA (mm)	76.1 (±5.4)	77.3 (±4.5)	p=0.61
G	16.6 (±3.5)	17.9 (±5.0)	p=0.56
CI	2.2 (±1.0)	3.6 (±1.2)	p<0.05*
LY-30 (%)	1.6 (±0.8)	2.0 (±1.4)	p=0.43

18.8.3 Injury

In the Part Three animals, the hepatic injury was completed in a mean of 98.0 (±16.6) seconds, and was not significantly different to the Part One REBOA group – 89.5 (±16.0) seconds, p=0.29. There was no statistical difference between the

percentage excision of the left lateral lobe of the liver, or the time from the start of the hepatic injury to the arrest point (t0), p=0.09 and p=0.76 respectively.

During the arrest period (t0-t3), there were no significant differences between groups in systolic BP (Part Three animals – 2.0 (±2.7) mmHg, REBOA group – 1.6 (±1.7) mmHg, p=0.08), left carotid artery flow, (Part Three animals – 0.5 (±1.2) ml/minute, REBOA group – 0.8 (±1.0) ml/minute, p=0.45), or end-tidal carbon dioxide (Part Three animals – 1.0 (±0.4) kPa, REBOA group – 0.9 (±0.4) kPa, p=0.52).

The groups had similar profiles of systolic BP and left carotid artery flow from the start of the hepatic injury to the end of the arrest period (t3), Figures 18.4a, 18.4b.

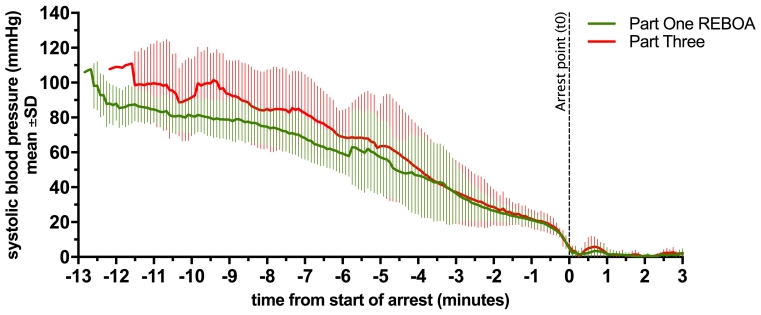

Figure 18.4a – A comparison of systolic BP from the start of the hepatic injury to the end of the arrest period (t3) between the Part One REBOA group and Part Three animals.

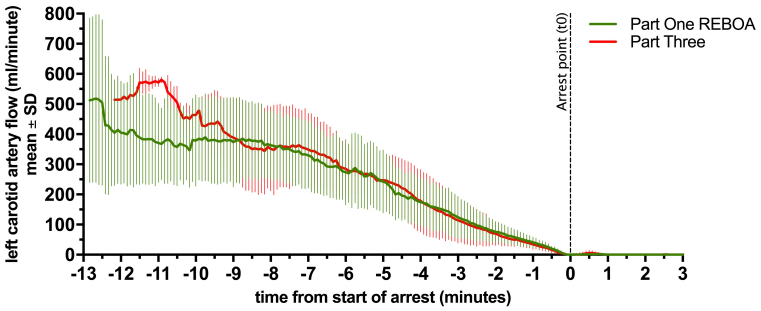

Figure 18.4b – A comparison of left carotid artery flow from the start of the hepatic injury to the end of the arrest period (t3) between the Part One REBOA group and Part Three animals.

18.8.4 Survival to t63

None (0.0%, 95%CI 0.0-27.8) of the ten Part One REBOA group animals had a return of spontaneous circulation, and none survived to t63, compared to eight of the Part Three animals (100.0%, 95%CI 67.6-100.0), p<0.001, Figure 18.5.

Figure 18.5 – A comparison of survival to t63 between the Part One REBOA group (n=10) and the Part Three animals (n=8).

18.9 Results of Part Three hypotheses

In a swine model of severe haemorrhagic TCA:

H9. *Intervention with REBOA and intravenous FWB will not result in a return of spontaneous circulation in any animal.*

Two (25.0%, 95%CI 4.4-59.1) animals had a return of spontaneous circulation with REBOA and intravenous FWB. There were no observed differences in injury characteristics between these two animals and the six animals that did not respond to REBOA.

H10. *R-SAAP (up to 4,000 ml of intra-aortic oxygenated FWB over five minutes), followed by SAAP-ECLS (venoarterial circulation and oxygenation of blood at 800 ml/minute) as required will result in 100% of animals surviving for 60 minutes after unsuccessful REBOA intervention.*

The paradigm of escalating endovascular intervention resulted in 100% (95%CI 67.6-100.0) of animals surviving for 60 minutes after the start of the initial REBOA intervention.

H11. *Escalating endovascular intervention, from REBOA to R-SAAP, to SAAP-ECLS as required will infer a significant 60 minute survival advantage over Part One historical controls that received REBOA and FWB only.*

The Part One REBOA group and the Part Three animals had comparable baseline

and injury characteristics, and the paradigm of escalating endovascular intervention inferred a significant 60 minute survival advantage over the Part One REBOA group.

CHAPTER NINETEEN

DISCUSSION – PART THREE

19.1 Summary of findings

The aim of this Part Three experiment was to demonstrate the efficacy of a translational paradigm of escalating endovascular intervention for the management of haemorrhagic TCA in large swine. The injury was similar to the Part One model, and resulted in a severe haemorrhagic TCA with a mean systolic BP of 2.0 (±2.7) mmHg prior to the intervention. The paradigm of escalating endovascular intervention, starting with REBOA and transitioning to R-SAAP and then SAAP-ECLS as required, resulted in all animals surviving the 60 minute simulated pre-hospital period. The baseline characteristics and injury model were comparable to the Part One REBOA group, and the paradigm of escalating endovascular intervention demonstrated a significant 60 minute survival advantage over Part One REBOA group controls. Part Three also provided further evidence that maintaining calcium homeostasis during initial SAAP infusion with citrated FWB is technically difficult, and that hypocalcaemia may be the cause of the high incidence of VF observed throughout this research.

19.2 Blood donor panel

As in Parts One and Two, the swine donor pool consistently provided a large volume of FWB that reduced the total number of animals required for this project. The data collected have demonstrated that up to three venesections of three units of FWB, with at least 14 days between donations, can be collected

without a significant change in haemoglobin concentration. To my knowledge this is the first description of the use of a dedicated swine blood donor panel in support of trauma haemorrhage translational research, and has potential utility in future large swine trauma protocols.

19.3 Haemorrhage model

The aim of the haemorrhage model was to achieve a standardised severe haemorrhagic TCA that was at the extremes of known survival. The injury was comparable to Part One by design, to allow more valid comparison with the Part One REBOA group that effected a 100% mortality ten minutes after the start of the intervention. There was a trend of a shorter time to complete the laparoscopic hepatic injury in Part Three (98.0 seconds), compared to Part One (105.2 seconds), but this did not reach significance. Previous reports of this injury have not included a description of the time to complete the hepatic excision, but that it was completed within two minutes.[101,118] The arrest point definition in all parts of this research has been by physiological measurement (accurately calibrated intra-aortic BP), compared to a time-based definition that has been used previously.[101,118] Therefore, the time to complete the hepatic injury will have had less effect on the resulting injury as compared to Ross et al and Morrison et al's models.

These previous descriptions of the laparoscopic hepatic injury report a percentage by weight left lateral lobe excision of between 74 (±6) % and 82 (±10) %.[101,118] The hepatic excision in Part Three (64.4 (±4.5)) % was significantly lower than both of these earlier descriptions, and also significantly

lower than Part One (69.2 (±9.6) %). The effect of this difference is unknown for several reasons: the volume of haemoperitoneum is potentially affected by the type and volume of resuscitative fluids, the hepatic injury used in this research is combined with a controlled arterial haemorrhage, and the end-points between this and previous studies use different variables to define the magnitude of the total injury (time versus BP), and result in different physiological states (a low-output state vs NOST). However, the swine hepatic vasculature is prominent, and it is unlikely that the difference in the proportion of left lateral lobe excision played an important role in defining the injury.

This theory is reinforced by the fact that both the volume by weight of the controlled arterial haemorrhage, and the time interval from the start of the hepatic injury to TCA (defined as an intra-aortic systolic BP of less than 10 mmHg) in Part One was not significantly different to Part Three.

The theory that the volume of haemoperitoneum is affected by subsequent resuscitative fluids was supported in Part Three animals – a range of 878 ml to 4,251 ml. The lowest volume was observed in one of the animals that only received 2,452 ml of IV FWB (REBOA only), and the four highest volumes were observed in the animals that received 2,452 ml of IV FWB, 4,000 ml of R-SAAP, and up to 3,000 ml of intravenous LR (SAAP-ECLS). There was a significant correlation between the total volume of resuscitative fluids and the volume of haemoperitoneum. This research has therefore determined that measuring the volume of the haemoperitoneum after the administration of resuscitative fluids,

and potentially the percentage excision of the hepatic injury, are not accurate methods of defining the hepatic injury in this model.

As previously discussed (Section 8.2), this hybrid model of severe haemorrhagic TCA is unique in the published literature, and no prior models have evaluated the use of endovascular resuscitation in this severity of TCA.[95,96,101,112] The arrest point in my model of haemorrhage was designed to simulate a clinical situation that is regarded as almost universally non-survivable.

19.4 Arrest period

The three minute arrest period was initially used in Part One with the aim of reducing the chance of spontaneous recovery of swine, and appeared to be effective in Part Three.

During the arrest period, no Part Three animals were in asystole despite a comparable haemorrhagic injury to Part One, in which asystole was observed in 30% of animals. In Part One animals there was not a linear decrease in heart rate prior to asystole – electrical activity abruptly ceased following an inappropriate bradycardia. The reason for the difference in the proportion of animals in asystole during the arrest period between Parts One and Three is unknown. However, there were no other significant differences observed in the injury model between groups, and no other large animal models of haemorrhage that include animals in asystole to which comparisons can be made (and therefore no data on the significance of asystole compared to an inappropriate bradycardia in haemorrhagic TCA).

The Part Three animals had a mean intra-aortic systolic BP of 2.0 (±2.7) mmHg and a mean left carotid artery flow of 0.5 (±1.2) ml/minute during the arrest period. Some animals therefore had a measurable cardiac output that means that they were not technically in NOST.[46,54] However, all had an inappropriate bradycardia, and therefore evidence of a failing myocardium. The inclusion of a cardiac electrical rate lower than baseline to the definition of arrest in this model provides a translatable physiological measurement that is readily available in the clinical setting.

19.5 Interventions

The REBOA intervention (inflation of a Z1 intra-aortic balloon and intravenous FWB infusion) was started a mean of 181.7 (±1.5) seconds after the onset of TCA, and was not significantly different to the Part One REBOA group.

19.5.1 REBOA

Per protocol, all animals received the REBOA intervention, and two (25.0%) had a return of cardiac output. Both of these animals had a systolic BP over 90 mmHg within three minutes of the start of the 2,452 ml intravenous FWB infusion. In the ten Part One animals, using the same injury and intervention methodology, no animals responded to the intervention, and it was concluded that the injury was too severe to be amenable to passive endovascular intervention (Section 8.9). Further analysis of these two animals did not demonstrate any observed differences in the injury or intervention compared to the animals without a return of cardiac output. Both of these animals had an inappropriate bradycardia secondary to haemorrhage, and prior to Part Three

would have been predicted to not respond to REBOA. However, these animals were not exposed to the additional risk of R-SAAP, and had an earlier ROSC that likely contributed to the trend of better physiology at t63 compared to the animals that had R-SAAP, and those that had R-SAAP and SAAP-ECLS. This was an unexpected result, and in translation further demonstrates the challenge in predicting which patients may be resuscitated with REBOA and intravenous fluid, and those that require active endovascular resuscitation. It also strengthens the argument for a clinical paradigm of escalating endovascular intervention in haemorrhagic TCA.

19.5.2 R-SAAP

Six (75.0%) animals met the criteria for R-SAAP. This intervention was started a mean of 9.2 seconds after the completion of the REBOA intravenous FWB infusion. This is an experimental concept with no prior translational or clinical data with which comparisons can be made. However, it is realistic that a clinical SAAP circuit could be prepared during the five minutes of initial REBOA intervention. The clinical feasibility of this transition would be greater if a SAAP catheter were used for the initial REBOA intervention, but as previously described there is a move towards smaller vascular sheaths for REBOA in clinical practice.[242]

The SAAP FWB infusion was comparable to the Part Two SAAP-FWB2 group infusion. As found in Part Two, the mean haemoglobin concentration in the FWB infusion was lower than swine baseline values.[120] This was caused by a combination of the blood product preservatives in donated FWB, and the LR

used to prime the SAAP circuit. The clinical effect of this is unknown, but it is tempting to suggest that the reduced viscosity of this infusion is beneficial in promoting vascular flow and tissue perfusion in haemorrhage with concomitant vasospasm.

Four animals did not initially respond to R-SAAP, and had a systolic BP lower than 50 mmHg after two minutes of the intervention. This failure to respond to R-SAAP is likely secondary to the prolonged period of TCA – at least five minutes longer than both the Part One SAAP-FWB group and the Part Two SAAP-FWB2 group. Per protocol these animals received 1 mg of intra-aortic epinephrine via the SAAP catheter. A previous swine translational model has used intra-aortic epinephrine in animals that did not initially respond to SAAP with LR.[112] Manning has also demonstrated an increase in aortic pressure and coronary perfusion pressure following intra-aortic epinephrine administration in medical cardiac arrest.[114] There is evidence that use of vasopressors early in the management of haemorrhagic shock is associated with worse outcomes.[177,178] However, there is limited clinical data on the use of inotropes in haemorrhagic shock, and no clinical data on the use of intra-aortic epinephrine in haemorrhagic TCA. In the Part Three animals that received intra-aortic epinephrine all had a return of cardiac output within one minute. This suggests that this technique may be effective, but without a comparison group it is not possible to define the effect of intra-aortic epinephrine. In clinical translation of SAAP / R-SAAP this could be an important question. None of these animals had a systolic BP greater than 90 mmHg at the end of the R-SAAP infusion (all received

4,000 ml of oxygenated intra-aortic FWB), and all met the experimental criteria for SAAP-ECLS.

19.5.2.1 Intra-aortic calcium concentration during R-SAAP

A significant technical challenge in the intervention of SAAP with citrated FWB is delivering a matched calcium infusion. Part Three model development suggested that even after removing dead-space within the calcium infusion line and pressurising the infusion system there was still a delay in delivering the correct rate of calcium. It is possible that this delay was caused by capacitance in the calcium infusion line. One Part Three animal was excluded owing to VF after 73 seconds of R-SAAP. Subsequent to this animal, a pressure infusion line was used for the calcium infusion, and no further occurrences of VF were observed. It was only feasible to collect intra-aortic samples during the R-SAAP from two animals, but the data suggest that the use of a pressure infusion line increased the rate of calcium chloride delivery during the first 60 seconds of SAAP. Prior to clinical translation further research is required to ensure that hypocalcaemic blood product is not delivered to the myocardium.

19.5.3 SAAP-ECLS

Four (50.0%) of the Part Three animals received SAAP-ECLS. This is an experimental concept, and there is no previous translational or clinical data with which to make comparisons. The mean interval between R-SAAP and the start of ECLS flow was 180.2 (±26.2) seconds, and was comparable to the interval between removal of the SAAP catheter and start of ECLS in Part Two. In the laboratory, a 24 Fr vascular sheath was partially inserted into the left femoral

vein during initial setup, making the time to insert a venous ECLS catheter very short, and certainly faster than it would be in the clinical setting. Personal communication with Dr. Lionel Lamhaut (SAMU de Paris) suggested that the fastest procedural setup time for ECLS in medical cardiac arrest is four minutes, but the expected setup time for conversion from R-SAAP to SAAP-ECLS is likely to be equal to or greater than the REBOA setup time quoted by Brenner et al (nine minutes).[238] Some of this interval would be during active resuscitation with R-SAAP, but there may still be a delay of a few minutes between R-SAAP and SAAP-ECLS in the clinical setting.

Evaluation of the volume of LR required to maintain ECLS flow was affected by the ECLS circuit becoming disconnected twice in one animal. These technical errors resulted in a large volume of extra-corporeal blood loss, and rapid decreases in systolic BP. However, they also demonstrated the power of SAAP-ECLS: restarting flow resulted in restoration of BP and left carotid artery flow in under one minute.

SAAP-ECLS demonstrated efficacy in supporting cardiac output, and the mean left carotid artery flow was higher in this group at t63 compared to baseline. Review of the left carotid artery flow in this group revealed another benefit of the escalating paradigm – although there was a prolonged period (at least five minutes) of no detectable left carotid artery flow, R-SAAP effected a carotid flow in the absence of an intrinsic cardiac output, preventing an even longer period of cerebral hypoperfusion prior to initiating ECLS.

19.6 Primary outcome

All animals survived the simulated pre-hospital period, 25.0% with REBOA and FWB alone, 25.0% with subsequent R-SAAP, and 50.0% with all three interventions sequentially. The paradigm of escalating endovascular intervention has therefore been demonstrated to be an effective strategy to resuscitate large swine in severe haemorrhagic TCA, while simultaneously reducing the risks and time delays associated with SAAP, and again with SAAP-ECLS.

When considering translation into clinical practice, this paradigm has the potential to influence some key endovascular resuscitation decisions. At present, there is no international consensus on how to differentiate patients who require REBOA and those who will survive to definitive haemostasis (and avoid the morbidity of REBOA). The risk benefit ratio of REBOA logically increases along the spectrum of haemorrhage, and knowledge that REBOA could be followed by R-SAAP (and further followed by SAAP-ECLS) may raise the threshold (for example by lowering the BP cut-off) for initiating REBOA. This is perhaps tenuous, and relies on a better understanding of the risks of REBOA, which may be reduced by the development of partial occlusion technology.

Haemorrhagic TCA is not widely recognised to be a spectrum of disease, and there are no clinically validated methods to differentiate a LOST from a NOST. This complicates the decision as to whether REBOA or SAAP is the most appropriate initial intervention in TCA, as was highlighted in the different responses to REBOA in Parts One and Three of this research. This paradigm

allows for REBOA to be used initially, giving time to review the response whilst setting-up a SAAP circuit – potentially mitigating the additional risks of SAAP over REBOA. It is also tempting to suggest that the use of this paradigm may have resulted in an increased survival in Brenner's recent REBOA case series, in which there was a 10% 30-day survival in TCA.[238]

There have been three clinical series of the use of ECLS in the acute management of haemorrhage.[206-208] Only one of these series (Huh et al) includes a mean time to start ECLS flow (34 minutes).[208] None of these studies used REBOA, or SAAP, prior to ECLS, and it is feasible that use of an escalating endovascular paradigm (REBOA > R-SAAP > SAAP-ECLS) would have resulted in more rapid normalisation of physiology, and potentially reduced the overall requirement for VA-ECLS.

In summary, this paradigm presents a theoretical approach to the clinical management of haemorrhagic shock and TCA, that is not without considerable logistical and technical challenges. It is likely that the utility of an escalating endovascular paradigm extends even further – starting with initial arterial access (more easily achieved prior to TCA, which also provides an invasive BP to guide further escalation),[238,242] transitioning through REBOA, R-SAAP, and SAAP-ECLS as required, and potentially on to EPR (deep hypothermic circulatory arrest).[202]

19.7 Secondary outcomes

In the eight Part Three animals there was no significant difference in mean systolic BP and left carotid artery flow between baseline and simulated hospital arrival (t63), but there was a significant difference in end-tidal carbon dioxide. The lower end-tidal carbon dioxide can be explained by the intra-aortic balloon occlusion resulting in only part of the swine being perfused. These results are encouraging, but should be balanced with the significantly lower pH, and higher base deficit and lactate at t63 compared to baseline.

There was no significant difference between the measured physiological variables at t63 between the three groups. However, the animals that only required REBOA had the highest mean systolic BP and left carotid artery flow, and the lack of statistical significance between groups is likely an effect of the small sample and wide variation between animals. It is logical that the animals with a shorter time in TCA (the two animals that responded to REBOA) would have a better physiological state at t63 compared to those that required further intervention. This is represented by the fact that the two animals that received REBOA only had the lowest mean base deficit and mean lactate between groups at t63, which further strengthens the argument for a paradigm of escalating endovascular intervention.

The SAAP-ECLS group had a significantly lower platelet count and lower TEG values (MA, G, CI) compared to the animals that received REBOA alone or REBOA and R-SAAP. ECLS is known to cause a combination of activation, destruction, and dysfunction of platelets and consumption of clotting factors.[197,198] This

group also received 1,000 units of heparin for ECLS circuit anti-coagulation, and up to 3,000 ml of LR to maintain ECLS flow. It was not possible to compare the fibrinogen concentration in Part Three animals owing to a large proportion of clotted samples. However, it is likely that the fibrinogen concentration would have also been significantly reduced in the animals that received SAAP-ECLS compared to those that did not (Section 13.10.3). It is not known what proportion of this observed coagulopathy is due to the prolonged period of tissue hypoperfusion during the TCA, blood contact with the ECLS circuit, the administration of heparin, or the dilution with LR, but is likely to be reduced in clinical practice with the use of a heparin-bonded circuit.[198]

19.8 Comparison with Part One REBOA group

In order to evaluate the efficacy of the escalating endovascular paradigm against intervention with REBOA only, comparison was made with the Part One REBOA group. The same injury model was used in both groups of animals, and the only differences observed were that the Part Three animals had a lower mean weight, a shorter TEG R, and a higher TEG clot index. The animal inclusion in both groups was identical, and required swine between 70 and 90 kg. Therefore, the difference in mean swine weight was dependent on the supply of animals, but is unlikely to have affected the validity of the comparison. The difference in TEG R is more difficult to explain, and the difference in clot index is a function of this. However, there were no other differences observed in clotting function between groups so this is unlikely to have affected the validity of the comparison.

There were no statistical differences in the injury between groups, and the systolic BP and left carotid artery flow were comparable from the start of the hepatic injury and the start of the REBOA intervention.

In summary, although these experiments were undertaken almost a year apart, there were few differences between groups. This demonstrates the reproducibility of the model, and allows for a valid comparison in survival proportions.

There was a significant difference in survival to simulated hospital arrival (t63) between the Part One REBOA group (0.0%, 95%CI 0.0-27.8), and the Part Three animals with escalating endovascular intervention (100.0%, 95%CI 67.6-100.0). This result further strengthens the argument for the paradigm. As previously discussed, it is tempting to suggest that the Part One REBOA group represent the patients in Brenner's recent case series that did not respond to REBOA,[238] and that the Part Three group represent the potential to improve survival in haemorrhagic TCA.

19.9 Conclusion – Part Three

The haemorrhage injury was comparable to Part One, and resulted in a very severe haemorrhagic TCA. The paradigm of escalating endovascular intervention was both a feasible and efficacious method of resuscitating large swine in severe haemorrhagic TCA whilst reducing the risks, logistical demands, and technical challenges of more advanced interventions. The limited data on calcium delivery during SAAP suggests that hypocalcaemia during the first 30

seconds of the infusion may have been the cause of VF observed throughout this project, and that further research is required to ensure matched calcium delivery before clinical implementation.

CHAPTER TWENTY

SUMMARY AND CONCLUSIONS

20.1 Introduction

There is arguably a social acceptance of traumatic death that confers an impression of inevitability. However, as the leading cause of death in young people in the UK and US, traumatic injury is a major public health issue.[2,244,245] Despite this, US government research funding for traumatic injury in 2016 was only US$ 1.38 per citizen – less than eight percent of the cancer research budget.[246] The recent armed conflicts in Iraq and Afghanistan have raised the public awareness of traumatic death, led to improvements in trauma care,[25] and generated research outputs that have relevance to both military and civilian healthcare.[247,248] The background chapters to this thesis have therefore reviewed both civilian and military data, and identified that torso haemorrhage presents the greatest opportunity to improve trauma survival, and that a high-proportion of patients with this injury pattern are in TCA on arrival at hospital. They also demonstrated the current uncertainty around the optimal management of patients in haemorrhagic TCA, and discussed the evidence underpinning the evolving intervention of endovascular resuscitation. These chapters are supported by my own secondary research: a REBOA gap-analysis of UK civilian trauma patients,[35] an analysis of civilian TCA in England and Wales,[46] and an analysis of military TCA during recent conflicts.[54]

The aim of this research was to develop a large animal translational model of severe haemorrhagic TCA in which to compare current, evolving, and potential future interventions. The results of Part One experiments led to further development of the animal injury model, and generated additional questions about active endovascular resuscitation with non-blood fluids, the potential for ECLS to mitigate the effects of IABO, and the feasibility of a paradigm of escalating endovascular intervention that were addressed in Parts Two and Three. In addition, the experiments required a large volume of donor swine FWB that was managed with a novel donor pool, and explored some of the technical challenges in delivering SAAP. This chapter summarises the main findings with reference to the existing literature, discusses the limitations and implications of these findings, and makes recommendations for further research and clinical translation.

20.2 The animal model

Previous translational research in the field of traumatic haemorrhage has primarily utilised swine models of haemorrhage.[95-99,101] Swine have many similarities to humans, and importantly in studying NCTH, similar cardiothoracic and abdominal anatomy and physiology.[116,117] The original animal model was developed by Ross et al, and subsequently utilised by Morrison et al to demonstrate the efficacy of REBOA in NCTH.[101,118] This research was undertaken in the same animal laboratory (Clinical Research Division, 59th Medical Wing USAF, JBSA Lackland, Texas), and Dr. James Ross was my primary laboratory supervisor, providing prior expertise in this swine model. Although the original model of laparoscopic hepatic injury was highly lethal,[118] the model had already

demonstrated survivability with REBOA.[101] However, in Morrison et al's swine study, three out of 16 (19%) of animals were not amenable to resuscitation with REBOA,[101] which suggests there is capacity for a more advanced intervention. Prior translational research by Manning et al had demonstrated that SAAP was capable of resuscitating swine from a significantly more severe injury than that incurred in Ross et al's model (a mean arterial pressure of less than 10 mmHg).[112] Manning et al used an open hepatic injury that reduced the validity of translation to the clinical setting. Part One model development demonstrated that the original model would not systematically result in an intra-aortic BP that was comparable or more severe than Manning's model, and a controlled arterial haemorrhage was added to effect this. Therefore, a combination of the two models was used to produce a translational model of severe haemorrhagic TCA secondary to NCTH and a controlled arterial haemorrhage.

In Part One, the model produced a very severe TCA that resulted in 30% of the forty animals being in cardiac electrical asystole. Previous animal models have used different cut-offs to define death-in-protocol, but range from a systolic BP of less than 30 mmHg to asystole.[96,101] This model is uniquely severe, and uses an injury cut-off that is more severe or equal to previous definitions of animal death.

Part Two initially aimed to use cardiac electrical asystole as the injury cut-off prior to intervention. However, during model development it became evident that asystole was not systematically achievable even after extending the injury until no intra-aortic pressure fluctuations were observed. Therefore, the injury

model in Part Two used the cut-off of no intra-aortic pressure fluctuations in which to evaluate SAAP with FWB, and also evaluated a larger volume of LR with SAAP. Part Three used the same injury model as Part One, and there were no significant differences in measured injury characteristics – providing evidence of model reproducibility.

The difference in BP effected by Ross et al's model and the models used in this research are easily differentiated in the animal laboratory. However, this relies on the use of accurately calibrated intra-aortic BP monitoring, that is extremely unlikely to be available in the clinical setting, and therefore affects the clinical applicability of results. For this reason, the definition of TCA in all parts of my research additionally included a cardiac electrical rate lower than baseline measurement. This physiological variable is easily measured in patients, and potentially provides a cut-off that translates this severity of haemorrhagic injury to clinical practice.

Throughout this research and in background publications,[46,54] I have made reference to the spectrum of haemorrhage, and the concept of the low-output state (LOST) and the no-output state (NOST) in haemorrhagic TCA (Section 8.2.3). The benefit of animals being in cardiac electrical asystole is that they can accurately be described as being in NOST, and therefore assumed to have no cardiac output. The Part Three animals had a mean intra-aortic systolic BP of 2.0 (±2.7) mmHg and a mean left carotid artery flow of 0.5 (±1.2) ml/minute during the arrest period. Some animals therefore had a laboratory-measurable cardiac output that means that they were not technically in NOST. This is of little

relevance to clinical practice, where this fidelity of physiological observation is not readily available. Consequently, when considering the clinical application of the interventions studied in this research, it may be more appropriate to characterise LOST as 'TCA with a tachycardia or normal heart rate', and NOST as a 'TCA with an inappropriate bradycardia or asystole'. However, this concept is theoretical and further clinical data would be required to test its applicability; particularly in reference to management options.

In summary, the final animal model produced a severe haemorrhagic TCA, that was uniquely severe, highly-lethal, included a translatable indicator of severity, and was reproducible. These features allowed testing of the experimental hypotheses at the extremes of survival, and allow translation of the results to a clinical situation that is generally regarded as non-survivable. Furthermore, the descriptions of the model have utility in the planning of future swine haemorrhage protocols.

20.3 The blood donor pool

This research required a large volume of FWB; up to 11,500 ml (19 units) per animal in the Part Two SAAP-FWB2 group. This is a significantly higher volume than previous swine translational studies,[96,101,168] and required a novel approach to reduce total animal use and to obtain ethical clearance. The donor pool for experimental protocols produced a total of 714 units of FWB from 126 swine, a mean of 5.7 units per animal. Some donor animals had up to four episodes of venesection of up to three units per session, with at least fourteen days between donations. The data collected demonstrated that there was no significant fall in

haemoglobin concentration between the first and subsequent venesections. On retirement, the donor animals were used in other protocols wherever possible, made possible by the level of research activity in this laboratory. It would have been feasible to venesect this volume per donor animal in non-survival experiments, but it would have resulted in a large number of additional animals used to answer the hypotheses, and is not in keeping with the international convention of refinement, reduction, and replacement in translational research.

Banked swine whole blood was used by Morrison et al in their swine REBOA experiments, but this product was stored for up to seven days (and therefore did not meet the definition of FWB),[129] and they did not describe the use of a donor pool.[101] Furthermore, there was no reference to cross-matching blood antibodies to ensure compatibility with recipients.[101] Swine are known to have an AO blood group system, but there are limited data on methods to cross-match and on swine allogenic transfusion reactions.[249] Over 700 units of FWB were transfused in this research, and there were no observed transfusion reactions. However, owing to the inherent physiological instability of the swine in these protocols it is feasible that transfusion reactions did occur and were not recognised. Using blood donors that are genetically identical to the experimental swine is a potential solution to this issue in future translational models.[249]

In summary, the use of a swine donor pool was an effective method to provide large volumes of FWB for experimental protocols, with no significant fall in haemoglobin concentration between venesections. This description is therefore of utility to future translational swine studies of haemorrhage.

20.4 CPR

The use of closed chest compressions has recently been de-emphasised in the clinical management of haemorrhagic TCA in favour of interventions aimed at reversing the aetiology.[41] However, prior to this research there was only a single animal study that aimed to provide data to inform this clinical scenario. Luna et al demonstrated that in a baboon model of controlled haemorrhage, intravenous filling was superior to compressions, and that compressions were likely to paradoxically reduce the chance of ROSC by reducing intra-aortic diastolic pressure.[67] More recently, Jeffcoach et al used a canine model of controlled haemorrhage to evaluate compressions compared to intravenous filling, and came to the same conclusions as Luna et al.[68] Both of these animal studies support the de-emphasis of compressions in haemorrhagic TCA, and it makes logical sense to prioritise interventions that may reverse the cause of the arrest, for example via oxygenation, chest decompression, and intravenous filling.

Both of these animal studies employed a model of controlled haemorrhage that effected a LOST haemorrhagic TCA, in which the animals had ongoing cardiac output.[67,68] It is intuitive in this clinical situation that intravenous filling alone is likely to be effective, and that compressions will reduce the rate of filling (by making vascular access more challenging, and by increasing intra-thoracic pressure; causing rapid-infusers to stop delivering the infusion and increasing the required pressure of a manual infusion). However, in the setting of NOST TCA, intravenous filling alone is unlikely to be effective, as there is no intrinsic vascular flow. This phenomenon was observed in the Part One REBOA group, that received a Z1 IABO occlusion and 2,452 ml of intravenous FWB over five

minutes – no animal had a ROSC, and all were dead ten minutes after the start of the intervention. In comparison, the Part One CPR group received the same volume of FWB together with closed chest compressions – 20.0% of animals had a ROSC, and 10.0% survived the 60 minute pre-hospital period. This small sample did not produce a significant difference, but review of the cardiac electrical rate in the two CPR animals with a ROSC revealed that they were both in asystole prior to a return of cardiac output. The combination of data from the REBOA and CPR group in this thesis suggests that the preload effected by the high-volume FWB infusion was sufficient to move blood through the low-resistance pulmonary circulation, increase the intra-aortic diastolic pressure, and followed by compressions, produced enough coronary flow to effect a ROSC. This phenomenon was only observed in a small number of animals, but may have significant implications for the clinical management of haemorrhagic TCA. Further data are ideally needed, but it would be sensible to advocate the use of compressions in haemorrhagic TCA after high-volume filling, oxygenation, and chest decompression have not rapidly reversed the arrest, in order to effect vascular flow.

20.5 REBOA

REBOA is a term that has been coined in the last decade, but was first described in the management of combat trauma over 60 years ago.[88] There is a growing body of both translational and clinical evidence that demonstrates that REBOA is an effective management strategy in NCTH.[96,101,104,250] There are also limited data on its effectiveness in the management of haemorrhagic TCA. Brenner et al's most recent REBOA case series included 50 patients in haemorrhagic TCA, of

whom 29 (58%) had a ROSC, and five (10%) survived for 30 days.[238] Data from the Part One REBOA group suggest that the patients who responded to REBOA were in a LOST TCA (with an ongoing cardiac output), and those who didn't respond were in a NOST TCA (with very little or no cardiac output). The cardiac electrical rate prior to REBOA was not reported by Brenner et al so it is not possible to evaluate this theory. However, in Brenner's series, all of the 30-day survivors had REBOA placed in the operating theatre, which supports the hypothesis that they were in LOST; having only recently progressed along the haemorrhage spectrum from severe shock to TCA.

Brenner et al's REBOA case series raises another point – patients in TCA received closed chest compressions during REBOA placement.[238] This seems to be counterintuitive to the evidence presented in the animal studies of Luna and Jeffcoach,[67,68] and to the contemporaneous understanding of the physiology of LOST, and is likely to have made REBOA placement more challenging. However, there is no clinically validated method of differentiating NOST and LOST, and in the setting of NOST this approach presents a hypothetical advantage over REBOA and intravenous filling alone. Part One demonstrated that REBOA and filling were ineffective in NOST, but that compressions and filling produced a 20.0% ROSC rate. The combination of REBOA, filling, and compressions was not evaluated in this large animal model, but the combination of haemorrhage control, high-volume filling, and flow effected by compressions presents an attractive theoretical management plan in this setting. In the absence of further data it is not possible to determine whether concomitant compressions in Brenner et al's series contributed to the ROSC rate observed with REBOA, but it

is tempting to suggest that they may have. There are no published translational studies aimed at answering this question, and this presents a further avenue of research. Using the same argument as that for compressions made in Section 20.4, pending translational data, it would be logical to advocate for the delivery of compressions alongside REBOA in patients suspected of being in NOST TCA.

20.6 SAAP

SAAP is an experimental active endovascular resuscitation technique that was first described in an animal model by Manning et al in 1992.[107] The initial aim of this technology was to resuscitate medical cardiac arrest by increasing CPP and allowing intra-aortic administration of epinephrine.[107] However, this technique, involving a large volume intra-aortic infusion above a Z1 IABO, is well suited to the setting of haemorhagic TCA with NCTH, and this application was first described by Manning et al in 2001.[112] Prior published SAAP translational studies have used lactated Ringer's and novel oxygen carriers as the perfusate,[111,112] but the Part One experiments are the first description of SAAP with FWB.[251]

In Part One, SAAP with FWB was demonstrated to infer a significant survival advantage over intervention with REBOA, compressions with intravenous filling, and SAAP with LR in a large swine model of severe haemorrhagic TCA. The animal injury model was designed to test these interventions at the extremes of known survival (NOST), and based upon previous research the differences observed between SAAP, REBOA, and compressions were anticipated. However, there were two unexpected results in the Part One SAAP experiments: the high

proportion of SAAP-LR group animals that had a ROSC, and the ability of SAAP with FWB to resuscitate asystole secondary to haemorrhagic TCA.

The SAAP-LR group had a 60% ROSC rate, and a 30% 60 minute survival. Manning et al's prior SAAP-LR study used a similar severity of haemorrhage, but reported a 33% ROSC rate, and a 0% 60-minute survival. Furthermore, the animals received a larger volume of SAAP infusion, and none had a ROSC until after administration of intra-aortic epinephrine.[112] The difference in outcomes in these two small studies is not statistically significant, but the trend may be explained by a different definition of ROSC, and a different injury model – Manning et al used an open hepatic injury to all four lobes and removed shed blood during the haemorrhage, which is likely to have prevented clot formation and encouraged ongoing venous haemorrhage.[112] Resuscitation with LR has significant technical and logistical advantages over resuscitation with blood product, particularly in the resource-constrained pre-hospital environment. However, with reference to the substantial body of evidence in support of haemostatic resuscitation with balanced blood products in traumatic hypovolaemia,[252] the administration of large volumes of LR in TCA is unlikely to gain clinical traction. The SAAP-LR results in Part One do have relevance, in that the animals acted as a non-blood control group for SAAP-FWB, provided supporting data concerning the observation of VF during SAAP, more than doubled the volume of reported data on SAAP with LR, and demonstrated utility in the planning of future swine translational research. The development of dried plasma and novel oxygen carriers (HBOCs and perfluorocarbons) present a potential solution to some of the technical and logistic challenges of

SAAP.[111,112,216,229] These evolving technologies may revolutionise haemostatic resuscitation, and have the potential to significantly increase the feasibility of pre-hospital SAAP. These novel blood substitutes are transported in LR, further increasing the importance to understand the characteristics of this fluid with SAAP. In Part One, the mean time to ROSC in the SAAP-LR group was 110 seconds – only ten seconds prior to the cessation of the infusion. Therefore, in Part Two SAAP-LR was administered for up to five minutes (a maximum volume of 4,000ml), with the hypothesis that a longer SAAP infusion would increase the ROSC rate. This comparison was impaired by the difference in the injury model between Parts One and Two, but the longer SAAP infusion did not result in an increased ROSC rate. Three animals in the SAAP-LR2 group without a ROSC at t13 had a return of cardiac output during the infusion, that with ongoing SAAP was lost. The cause of this unknown, and this phenomenon is not reported by Manning et al.[112] However, together with the data from the SAAP-LR animal that had a ROSC after the SAAP-LR infusion was stopped, the cause is likely to be secondary to a combination of increased cardiac afterload effected by SAAP increasing myocardial workload, further LR infusion reducing the haemoglobin concentration (thereby reducing oxygen delivery to the myocardium) and reducing the calcium concentration (thereby impairing myocardial contractility). A similar phenomenon was observed in the one SAAP-FWB2 animal that did not have a ROSC at t13, and supports the theory that the increased cardiac afterload of SAAP was responsible. This phenomenon requires further SAAP translational research, but potential solutions include reducing the rate of SAAP infusion, or converting to central venous fluid administration, once an intrinsic cardiac

output is observed, or using partial aortic occlusion – either initially, or once an intrinsic output is detected.

Cardiac electrical asystole has previously been used in swine translational research to signify animal death,[101] and in the clinical setting of haemorrhagic TCA is regarded as almost universally non-survivable.[47,52] There are no published reports of the resuscitation of asystole secondary to haemorrhage in translational models, and a single case report of successful resuscitation following ultrasound confirmed cardiac standstill.[131] The finding in Part One that SAAP with FWB can effect ROSC from cardiac electrical asystole is therefore a novel finding, and demonstrates the resuscitative power of this intervention.

However, this must be balanced against the finding that five Part One SAAP-FWB animals died shortly after removal of the IABO – there was only a 20.0% survival to the end of the protocol in this group, and no survivors that were initially resuscitated from asystole. Cardiovascular instability after IABO removal has been described in previous translational studies, and clinical reports.[95,101,103] These five animals received a mean volume of 2,500 ml of intravenous FWB after IABO removal without a significant increase in BP, but with a significant increase in central venous pressure and a significant fall in systemic vascular resistance. The most striking biochemical feature of re-perfusion in this group was a fall in arterial pH to 7.10 (±0.03). This effect has been described in Morrison's REBOA model,[101] but not to this magnitude; neither has the high proportion of death following IABO removal. These differences are likely to be due to the increased severity of the initial injury compared to Morrison et al's model, and without

further investigation potentially limit the applicability of SAAP. Hypothetical strategies to mitigate the effects of IABO following severe haemorrhagic TCA in this model were reviewed in Chapter 9, and ECLS was demonstrated to extend survival in the Part Two SAAP-FWB2 group – this is summarised in Section 20.7.

20.6.1 Intra-aortic calcium delivery during SAAP

One technical challenge in the intervention of SAAP with citrated FWB is in delivering a matched calcium infusion.[226] Calcium cannot be added to the FWB more than a few seconds prior to the infusion, otherwise it will clot – in Part One model development a membrane oxygenator was destroyed by re-circulating FWB mixed with a small volume of calcium. The ratio of 10% calcium chloride to FWB was determined *in vitro* in Part One model development, Figure 5.1, and checked *in vivo* during experimental protocols (Section 13.15.2.2). However, the SAAP infusion of 800 ml/minute through a 7.5 Fr (2.3 mm) effects high pressure, and even using a pressure infusion pump for the calcium infusion, there was a delay of up to 30 seconds before normocalcaemia was observed in the SAAP infusion, Figure 16.1. These data suggest that there was capacitance in the calcium infusion system, and the infusion line was therefore replaced with a pressure line. Subsequent *in vivo* data demonstrated that this improved the rate of initial SAAP calcium delivery, Figure 18.2. Prior to the clinical translation of SAAP, further research is required to avoid the delivery of hypocalcaemic blood to the myocardium.

20.6.2 VF

VF was observed 18 times throughout this research; twice during the haemorrhagic injury, and 16 times during SAAP with FWB. There is therefore some evidence that VF occurs more readily in swine compared to humans, in which VF is an extremely rare haemorrhage arrhythmia,[45,52] and personal correspondence has revealed that other research teams pre-dose swine with amiodarone to reduce the chance of VF. Five animals in Part Two went into VF during SAAP-FWB infusion, and presented an opportunity to investigate the cause of this arrhythmia.

In animals with VF, the SAAP infusion had a significantly higher pH, and a significantly lower partial pressure of carbon dioxide and potassium compared to those without VF. It is likely that this combination of differences in the infusion was caused by a longer circuit circulation time in the animals in the VF group. Hypokalaemia has been demonstrated in swine to effect VF at concentrations of 1.1 mmol/l and lower,[224] but the lowest SAAP infusion potassium concentration in these five animals was 3.1 mmol/l, and the mean value for this group was within swine reference values.[120] It is therefore unlikely that hypokalaemia was the cause of the VF observed.

During this research, there were no cases of VF during SAAP-LR infusion, and Manning et al did not report VF during their previous SAAP experiments with LR.[112] The mean potassium concentration in the Part One SAAP-LR infusion was 3.6 (±0.1) mmol/l, providing further evidence that hypokalaemia is an unlikely candidate for the cause of VF during SAAP. The mean time to VF in the Part Two

group of five animals was 44.4 (±21.7) seconds, meaning that the stimulus for VF was present during the first 60 seconds of SAAP. This observation makes the t5 aortic arch blood samples unhelpful in determining the cause of VF (Section 13.15.2.3). However, in reference to the observation that prior to Part Three the SAAP-FWB infusion delivered 30 seconds of hypocalcaemic blood, the observation that the VF stimulus was present during the first 60 seconds of SAAP becomes more interesting. All Part Two animals (n=13) that received SAAP-FWB had a significantly prolonged ECG QTc at t3.5 compared to t3. There are numerous causes of a prolonged QTc,[227] but the most likely cause in these animals is hypocalcaemia. There was no significant difference in the QTc prolongation between animals with VF and animals without, which is in keeping with the theory that all SAAP-FWB infusions prior to adaption to the calcium infusion in Part Three delivered hypocalcaemic blood. The theory that hypocalcaemia was the cause of VF in these animals is reinforced by a mean ionised calcium of 0.9 (±0.0) mmol/l in the Part One SAAP-LR infusion (that was not associated with VF), and by Manning et al's more recent SAAP experiments that demonstrated systematic VF after delivery of hypocalcaemic SAAP-FWB.[226] It is likely therefore that a failure to infuse the correct rate of 10% calcium chloride in the initial 30 seconds of SAAP during Parts One and Two was the cause of VF. Further translational research is required to definitively answer this question before clinical implementation. The use of SAAP with novel oxygen carriers partially mitigates this requirement, but these are unlikely to be clinically approved in the UK or US in the near future.[229]

In summary, SAAP with FWB has been demonstrated to be an efficacious intervention for the management of severe haemorrhagic TCA in large swine, but further data on the delivery of calcium with citrated blood products and strategies to mitigate cardiopulmonary dysfunction after SAAP catheter removal are needed prior to clinical translation.

20.7 ECLS to mitigate the effects of IABO

From the available large animal and clinical data, it is evident that IABO has some significant negative physiological effects.[96,101,103,139,160,168] In previous animal models of NCTH and IABO, using a less severe injury than that described in this thesis, these effects were managed with intravenous fluid and vasopressors.[101] However, a large proportion of animals in the Part One SAAP-FWB group died shortly after balloon deflation, and were unresponsive to large volumes of intravenous FWB. This difference is likely to be caused by the severity of the injury and the occlusion time of 75 minutes – this is a likely clinical scenario for SAAP, and potentially limits its utility.[37,46,119]

The data from the Part One SAAP-FWB animals that died after SAAP catheter removal showed evidence of cardiac and pulmonary dysfunction (Section 7.11.3). This could be separated into two overlapping aetiologies; the first is myocardial injury secondary to acute left ventricular dilation, leading to a reduction in subendocardial blood flow,[142,144,145] and the second is the more general syndrome of ischaemia and reperfusion injury.[147] The myocardial injury is caused by a rapid increase in afterload with IABO that may be mitigated with development of partial aortic occlusion. A current guideline recommends

considering partial occlusion after initial complete occlusion.[160] This approach of delayed partial occlusion is unlikely to mitigate the initial myocardial injury, but may reduce the magnitude of the subsequent cardiovascular dysfunction secondary to the ischaemia and reperfusion injury. Prior IABO animal data has demonstrated a significant increase in cardiac troponin I between baseline and the end of the study, but did not differentiate the timing of this injury.[101,168] Data from the Part Two SAAP-FWB2 group in this thesis have demonstrated that the myocardial injury occurs before balloon deflation, and suggests it is likely to be secondary to the balloon occlusion (Section 13.12.1). This theory is supported by Avaro et al, who reported a significant difference in cardiac histology between 40 minutes and 60 minutes of aortic occlusion.[96]

However, SAAP delivers a high-volume infusion directly into the aortic arch, which introduces another element to this consideration. In this thesis, SAAP infusion was immediately preceded by a 50 ml intra-aortic flush of LR that was designed to close the aortic valve, preventing retrograde filling of the left ventricle, and maximising coronary artery flow (Figure 5.4). However, in the setting of a beating heart (either initially, or after successful SAAP resuscitation) the aortic valve will open, creating the potential for dilation of the left ventricle, and a reduction in sub-endocardial blood flow. This may have been the phenomenon observed in the four Part Two animals that had a return of cardiac output, but re-arrested with ongoing SAAP infusion (Section 13.7.3). SAAP therefore has the theoretical potential to effect a more severe myocardial injury than REBOA. Potential solutions to this phenomenon include reducing the rate of SAAP infusion, or converting to central venous fluid administration, once an

intrinsic cardiac output is observed, or using partial aortic occlusion – either initially, or once a cardiac output is detected – but further data are required.

Ischaemia and reperfusion injury is a recognised complication of aortic cross clamping.[147] This injury is modulated by a large number of pro-inflammatory mediators and metabolites, and results in cardiovascular dysfunction (complement, hypoxanthine, acidaemia, hyperkalaemia), and pulmonary dysfunction (IL-6, TNF-α, C3a and C5a complement).[148-155] Some of these effects were observed in Part One SAAP-FWB animals, and measured in Part Two SAAP-FWB2 animals (Sections 13.12.1 & 13.12.2). These data provide a further description of the increase in IL-6 and TNF-α associated with IABO,[168] and provide novel data on IL-1B, IL-2, and IL-8.

There are numerous potential strategies to mitigate the negative effects of IABO, that include intermittent or partial occlusion, blood purification, and pharmacological adjuncts. However, none of these strategies have either a proven benefit in reducing organ dysfunction in the setting of IABO, or target every negative effect of IABO. ECLS is an endovascular intervention that provides both exogenous oxygenation and vascular flow, and as such has the potential to mitigate the known negative effects of IABO. Furthermore, it is a logical extension to SAAP, and can be used in conjunction with other extra-corporeal interventions, for example blood purification. Therefore, in Part Two of this thesis, ECLS was commenced in SAAP-FWB2 group animals after SAAP catheter removal.

This is the first description of ECLS to mitigate the negative effects of IABO, and it demonstrated a significant three hour survival advantage over Part One historical controls (Figure 13.14). However, the key unknown is whether ECLS can improve long-term outcome or whether it simply extends survival. The most striking biochemical feature of re-perfusion in the SAAP-FWB groups in Parts One and Two was acidaemia, a pH of 7.10 (±0.03) and 7.01 (±0.03) respectively. There is limited evidence in Part Two that one mechanism by which ECLS may mitigate the ischaemia and reperfusion injury is by the exogenous removal of carbon dioxide, effecting an increase in pH. This feature of ECLS is made more important by the significant fall in pulmonary gas exchange function observed on re-perfusion in these models, which may itself necessitate VV or VAV ECLS. The description also provides data on ECLS setup, maintenance, and anticoagulation that is of utility to future swine translational research.

20.8 The paradigm of escalating endovascular intervention

In recognition of the clinical difficulty of predicting whether patients in haemorrhagic TCA require REBOA or SAAP (demonstrated further by the unexpected response to REBOA intervention by two animals in Part Three), the setup time and logistical burden differences between REBOA, SAAP, and ECLS, and the increased risk associated with more advanced interventions, Part Three aimed to test the feasibility of a paradigm of escalating endovascular intervention. Since this research was undertaken, publication of the REBOA case series by Brenner et al that includes 50 TCA patients, of whom almost half do not respond to REBOA, and in those that do only 10% survived to 30 days has further increased the importance of this strategy.[238]

The paradigm was demonstrated to be both a feasible and efficacious method of resuscitating large swine in severe haemorrhagic TCA whilst reducing the risks, logistical demands, and technical challenges of more advanced interventions. The timing of interventions used in this paradigm were aimed at achieving a balance between maintaining the validity of comparison with Part One REBOA group controls, avoiding fluid overload by limiting R-SAAP infusion to five minutes, and clinical feasibility – based on data from other parts of this thesis and Brenner's REBOA case series.[238] In view of the growing experience in endovascular resuscitation,[232,233] the large numbers of US hospitals offering ECLS,[231] the example of pre-hospital ECLS in Paris,[209] and the publication of three case series on the early use of ECLS in haemorrhage,[206-208] this paradigm may be clinically feasible. In future, this paradigm may extend further, starting with arterial vascular access, then to partial REBOA, followed by R-SAAP, followed by SAAP-ECLS, potentially to VA-ECLS, and finally with the experimental intervention of EPR.[203]

20.9 Conclusions

A dedicated swine blood donor pool can provide large volumes of FWB over time without a significant decrease in haemoglobin concentration, but further data on clotting function and allogenic transfusion reactions are required. The hybrid model of laparoscopic hepatic injury and controlled arterial haemorrhage is reproducible and effects a very severe TCA that it is at the extremes of known survivability. There is limited evidence that compressions and high-volume intravenous FWB are an effective intervention in severe haemorrhagic TCA. SAAP with FWB is capable of resuscitating swine in haemorrhagic cardiac

electrical asystole, and infers a superior short-term survival compared to intervention with REBOA, but further data are needed to ensure normocalcaemia during infusion of citrated blood products. ECLS prolongs survival in the setting of cardiopulmonary dysfunction secondary to TCA and IABO. An escalating paradigm of endovascular intervention is a feasible and efficacious method of resuscitating large swine in haemorrhagic TCA that may be translatable to clinical practice.

Appendix A – SAAP challenge-response checklist

Challenge-Response SAAP Checklist

Millar
- **Is DAQ recording ON?**
- Is the aortic pressure trace ok and ZEROED (on 25)?

Calcium Pump – checked with saline and pumping correctly?
Perfusion ABG taken?

Place defib pads / 4-lead ECG on pig – print sample ECG

Circuit
- How much fluid is in the bucket?
- Is the oxygen attached and turned on?

DEFLATE balloon to check liver clamps!!

Masterflex
- What is the rate set?
- Is the tubing size correct?

Calcium
- Is the syringe inserted correctly?
- Tell me the rate?

ARREST
- ISOFLURANE OFF
- Take t0 blood sample (20ml)
- Keep bleeding at 1 ml/kg/min for 2 minutes – rate =……
- note time
- write obs
- Start stopwatch
- Prepare to ECG print t2.5 to t3.5
- Prepare to take blood sample

INTERVENTION
- balloon up (at t2.75)
- Start SAAP (at t3.0)
- note time
- write obs
- OXYGEN to 100%
- is calcium running?
- at end of SAAP, flush system

Appendix B – Manual data collection sheet – Part One

AHR FWH20140020A: VITALs DATA SHEET

Date:	Animal #:	EX	Weight (kg): Length (in):	CPR	REBOA	SAAP-LR	SAAP-FWB	(circle protocol)

Timer	Exp Time	HR	Et CO2	SpO2	CVP	SVR	Sys/Dia BP	MAP	PAP	mean PAP	Carotid Flow	NIRS above	NIRS below	SvO2	CCO	SV	Temp	NOTES
TIME	**BL1**																	SYNCHRONSE ALL CLOCKS
TIME	**BL2**																	
0:00	T0																	
0:03	T3																	
0:13	T13																	
00:23	T23																	
00:33	T33																	
00:43	T43																	
00:53	T53																	
01:03	**T63**																	
01:13	T73																	
01:23	T83																	
01:33	**T93**																	
02:03	T123																	
02:33	T153																	
03:03	T183																	
03:33	T213																	
04:03	T243																	
04:33	T273																	

Time	Enter all times as hours:minutes:seconds!
hh:mm:ss	Time of LIVER CUT
hh:mm:ss	Duration of LIVER CUT
hh:mm:ss	Time of ARREST (T=0)

v 3.11 | EBGB

Spleen, liver, and prehospital blood weights *** in grams		TOTALS
Spleen weight	GRAMS	
Liver excised lobe weight	GRAMS	
Lobe remaining weight	GRAMS	
Liver remaining weight	GRAMS	
Volume of blood drawn (fem)	GRAMS	
Volume of prehosp suction	GRAMS	
Volume prehosp laps / clots	GRAMS	

Appendix C – Manual data collection sheet – Parts Two and Three

AHR FWH20140020A: VITALs DATA SHEET

Date:	Animal #:	EX	Weight (kg): Length (in):	AHR PHASE 2/3 EXPT (please circle): FWB-SAAP LR-SAAP REBOA+R-SAAP

Timer	Exp Time	HR	Et CO2	SpO2	CVP	SVR	Sys/Dia BP	MAP	PAP	mean PAP	Carotid Flow	NIRS above	NIRS below	SvO2	CCO	SV	Temp	ECLS flow	ACT	NOTES
TIME	**BL1**																			SYNCHRONSE ALL CLOCKS
TIME	**BL2**																			? HAVE PERFUSION SAMPLES FOR REBOA AND SAAP?
0:00	T0																			
0:03	T3																			
0:13	T13																			
00:23	T23																			
00:33	T33																			
00:43	T43																			
00:53	T53																			
01:03	**T63**																			FWB-SAAP ANIMALS ONLY PAST HERE
01:13	T73																			
01:23	T83																			
01:33	**T93**																			ECLS ABG WITH EVERY BLOOD TEST
02:03	T123																			
02:33	T153																			
03:03	T183																			
03:33	T213																			
04:03	T243																			
04:33	T273																			

Time	Enter all times as hours:minutes:seconds!
hh:mm:ss	Time of LIVER CUT
hh:mm:ss	Duration of LIVER CUT
hh:mm:ss	Time of ARREST (T=0)

v 4.3 | EBGB

Spleen, liver, and prehospital blood weights *** in grams		TOTALS
Spleen weight	GRAMS	
Liver excised lobe weight	GRAMS	
Lobe remaining weight	GRAMS	
Liver remaining weight	GRAMS	
Volume of blood drawn (fem)	GRAMS	
Volume of prehosp suction	GRAMS	
Volume prehosp laps / clots	GRAMS	

REFERENCES

1. World Health Organization. Injuries and Violence: the Facts. 2014.

2. Office for National Statistics. Deaths registered in England and Wales. 2014.

3. Centers for Disease Control and Prevention. National Center for Health Statistics. Health, United States. 2016.

4. National Audit Office. Major Trauma Care in England. 2010.

5. Kauvar DS, Lefering R, Wade CE. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. *J Trauma* 2006;60(6):S3–11.

6. Kolias AG, Guilfoyle MR, Helmy A, Allanson J, Hutchinson PJ. Traumatic brain injury in adults. *Pract Neurol* 2013;13(4):228–35.

7. Sapsford W. Penetrating brain injury in military conflict: does it merit more research? *J R Army Med Corps* 2003;149(1):5–14.

8. Davis JS, Satahoo SS, Butler FK, Dermer H, Naranjo D, Julien K, et al. An analysis of prehospital deaths: Who can we save? *J Trauma Acute Care Surg* 2014;77(2):213–8.

9. Chiara O, Scott JD, Cimbanassi S, Marini A, Zoia R, Rodriguez A, et al. Trauma deaths in an Italian urban area: an audit of pre-hospital and in-hospital trauma care. *Injury* 2002;33(7):553–62.

10. Tien HC, Spencer F, Tremblay LN, Rizoli SB, Brenneman FD. Preventable deaths from hemorrhage at a level I Canadian trauma center. *J Trauma* 2007;62(1):142–6.

11. Teixeira PGR, Inaba K, Hadjizacharia P, Brown C, Salim A, Rhee P, et al. Preventable or potentially preventable mortality at a mature trauma center. *J Trauma* 2007;63(6):1338–47.

12. Carpenter D, Fuller T, Roberts L. WikiLeaks and Iraq Body Count: the sum of parts may not add up to the whole-a comparison of two tallies of Iraqi civilian deaths. *Prehosp Disaster Med* 2013;28(3):223–9.

13. Obermeyer Z, Murray CJL, Gakidou E. Fifty years of violent war deaths from Vietnam to Bosnia: analysis of data from the world health survey programme. *BMJ* 2008;336(7659):1482–6.

14. Iraq body count. The public record of violent deaths following the 2003 invasion of Iraq. https://www.iraqbodycount.org. Accessed Nov 2017.

15. Crawford NC. War-related Death, Injury, and Displacement in Afghanistan and Pakistan 2001-2014. Watson Institute for International Studies. Brown University. 2015.

16. Eastridge BJ, Mabry RL, Seguin P, Cantrell J, Tops T, Uribe P, et al. Death on the battlefield (2001-2011): implications for the future of combat casualty care. *J Trauma Acute Care Surg* 2012;73(6):S431–7.

17. Russell RJ, Hodgetts TJ, McLeod J, Starkey K, Mahoney P, Harrison K, et al. The role of trauma scoring in developing trauma clinical governance in the Defence Medical Services. *Philos Trans R Soc Lond B Biol Sci* 2011;366(1562):171–91.

18. Iraq and Afghanistan Coalition Military Fatalities by Year. icasualties.org. Accessed Nov 2017.

19. The Care Quality Commission. Defence Medical Services: a review of compliance with the essential standards of quality and safety. https://www.cqc.org.uk/sites/default/files/documents/20120621_dms_report_summary_final.pdf. Accessed Nov 2017.

20. Hodgetts TJ, Mahoney PF. Military pre-hospital care: why is it different? *J R Army Med Corps* 2009;155(1):4–8.

21. Champion HR, Bellamy RF, Roberts CP, Leppaniemi A. A profile of combat injury. *J Trauma* 2003;54(5):S13–9.

22. Maughon JS. An inquiry into the nature of wounds resulting in killed in action in Vietnam. *Mil Med* 1970;135(1):8–13.

23. Kelly JF, Ritenour AE, McLaughlin DF, Bagg KA, Apodaca AN, Mallak CT, et al. Injury severity and causes of death from Operation Iraqi Freedom and Operation Enduring Freedom: 2003-2004 versus 2006. *J Trauma* 2008;64(2):S21–6.

24. RN JS, Hodgetts T, Mahoney P, Russell R, Davies S, McLeod J. Trauma Governance in the UK Defence Medical Services. *J R Army Med Corps* 2007;153(4):239–42.

25. Penn-Barwell JG, Roberts SAG, Midwinter MJ, Bishop JRB. Improved survival in UK combat casualties from Iraq and Afghanistan: 2003-2012. *J Trauma Acute Care Surg* 2015;78(5):1014–20.

26. Holcomb JB, McMullin NR, Pearse L, Caruso J. Causes of death in US Special Operations Forces in the global war on terrorism 2001–2004. *Ann Surg* 2007;245:986-991.

27. Holcomb JB, Stansbury LG, Champion HR, Wade C, Bellamy RF. Understanding combat casualty care statistics. *J Trauma* 2006;60(2):397–401.

28. Hodgetts T, Davies S, Midwinter M, Russell R, Smith J, Clasper J, et al. Operational Mortality of UK Service Personnel in Iraq and Afghanistan: A One Year Analysis 2006-7. *J R Army Med Corps* 2007;153(4):252–4.

29. Singleton JAG, Gibb IE, Hunt NCA, Bull AMJ, Clasper JC. Identifying future 'unexpected' survivors: a retrospective cohort study of fatal injury patterns in victims of improvised explosive devices. *BMJ Open* 2013;3:e003130. doi: 10.1136/bmjopen-2013-003130.

30. Russell R, Hunt N, Delaney R. The Mortality Peer Review Panel: a report on the deaths on operations of UK Service personnel 2002-2013. *J R Army Med Corps* 2014;160(2):150–4.

31. Keene DD, Penn-Barwell JG, Wood PR, Hunt N, Delaney R, Clasper J, et al. Died of wounds: a mortality review. *J R Army Med Corps*. 2016;162(5):355–60.

32. Breeze J, Baxter D, Carr D, Midwinter MJ. Defining combat helmet coverage for protection against explosively propelled fragments. *J R Army Med Corps* 2015;161(1):9–13.

33. Morrison JJ, Rasmussen TE. Noncompressible torso hemorrhage: a review with contemporary definitions and management strategies. *Surg Clin North Am* 2012;92(4):843–58.

34. Kisat M, Morrison JJ, Hashmi ZG, Efron DT, Rasmussen TE, Haider AH. Epidemiology and outcomes of non-compressible torso hemorrhage. *J Surg Res* 2013;184(1):414–21.

35. Barnard EBG, Morrison JJ, Madureira RM, Lendrum R, Fragoso-Iñiguez M, Edwards A, et al. Resuscitative endovascular balloon occlusion of the aorta (REBOA): a population based gap analysis of trauma patients in England and Wales. *Emerg Med J* 2015;32(12):926–32.

36. Stannard A, Morrison JJ, Scott DJ, Ivatury RA, Ross JD, Rasmussen TE. The epidemiology of noncompressible torso hemorrhage in the wars in Iraq and Afghanistan. *J Trauma Acute Care Surg* 2013;74(3):830–4.

37. Morrison JJ, Stannard A, Rasmussen TE, Jansen JO, Tai NRM, Midwinter MJ. Injury pattern and mortality of noncompressible torso hemorrhage in UK combat casualties. *J Trauma Acute Care Surg* 2013;75(2):S263–8.

38. Hayashi M, Shimizu W, Albert CM. The spectrum of epidemiology underlying sudden cardiac death. *Circ Res* 2015;116(12):1887–906.

39. Shimazu S, Shatney CH. Outcomes of trauma patients with no vital signs on hospital admission. *J Trauma* 1983;23(3):213–6.

40. Battistella FD, Nugent W, Owings JT, Anderson JT. Field triage of the pulseless trauma patient. *Arch Surg* 1999;134(7):742–5.

41. Truhlář A, Deakin CD, Soar J, Khalifa GEA, Alfonzo A, Bierens JJLM, et al. European Resuscitation Council Guidelines for Resuscitation 2015. *Resuscitation* 2015;95:148–201.

42. McManus J, Yershov AL, Ludwig D, Holcomb JB, Salinas J, Dubick MA, et al. Radial pulse character relationships to systolic blood pressure and trauma outcomes. *Prehosp Emerg Care* 2005;9(4):423–8.

43. Poulton TJ. ATLS paradigm fails. *Ann Emerg Med* 1988;17(1):107.

44. Deakin CD, Low JL. Accuracy of the advanced trauma life support guidelines for predicting systolic blood pressure using carotid, femoral, and radial pulses: observational study. *BMJ* 2000;321(7262):673–4.

45. Smith JE, Rickard A, Wise D. Traumatic cardiac arrest. *J R Soc Med* 2015;108(1):11–6.

46. Barnard E, Yates D, Edwards A, Fragoso-Iñiguez M, Jenks T, Smith JE. Epidemiology and aetiology of traumatic cardiac arrest in England and Wales - A retrospective database analysis. *Resuscitation* 2017;110:90–4.

47. Rosemurgy AS, Morris, Olson SM, Hurst JM, Albrink MH. Prehospital Traumatic Cardiac Arrest: The cost of futility. *J Trauma*. 1993;35(3):468.

48. Soar J, Perkins GD, Abbas G, Alfonzo A, Barelli A, Bierens JJLM, et al. European Resuscitation Council Guidelines for Resuscitation 2010 Section 8. Cardiac arrest in special circumstances: Electrolyte abnormalities, poisoning, drowning, accidental hypothermia, hyperthermia, asthma, anaphylaxis, cardiac surgery, trauma, pregnancy, electrocution. *Resuscitation* 2010;81(10):1400-33.

49. Lockey D, Crewdson K, Davies G. Traumatic cardiac arrest: who are the survivors? *Ann Emerg Med* 2006;48(3):240–4.

50. Huber-Wagner S, Lefering R, Qvick M, Kay MV, Paffrath T, Mutschler W, et al. Outcome in 757 severely injured patients with traumatic cardiorespiratory arrest. *Resuscitation* 2007;75(2):276–85.

51. Leis CC, Hernández CC, Blanco MJG-O, Paterna PCR, Hernández R de E, Torres EC. Traumatic cardiac arrest: should advanced life support be initiated? *J Trauma Acute Care Surg* 2013;74(2):634–8.

52. Morrison JJ, Poon H, Rasmussen TE, Khan MA, Midwinter MJ, Blackbourne LH, et al. Resuscitative thoracotomy following wartime injury. *J Trauma Acute Care Surg* 2013;74(3):825–9.

53. Tarmey NT, Park CL, Bartels OJ, Konig TC, Mahoney PF, Mellor AJ. Outcomes following military traumatic cardiorespiratory arrest: A prospective observational study. *Resuscitation* 2011;82(9):1194–7.

54. Barnard EBG, Hunt PAF, Lewis PEH, Smith JE. The outcome of patients in traumatic cardiac arrest presenting to deployed military medical treatment facilities: data from the UK Joint Theatre Trauma Registry. *J R Army Med Corps* 2017. doi: 10.1136/jramc-2017-000818.

55. Soar J, Deakin CD, Nolan JP, Abbas G, Alfonzo A, Handley AJ, et al. European Resuscitation Council guidelines for resuscitation 2005. Section 7. Cardiac arrest in special circumstances. *Resuscitation* 2005;67(1):S135-70.

56. Resuscitation Council UK. Traumatic Cardiac Arrest Treatment Algorithm. 2015.

57. Lockey DJ, Lyon RM, Davies GE. Development of a simple algorithm to guide the effective management of traumatic cardiac arrest. *Resuscitation* 2013;84(6):738–42.

58. Sherren PB, Reid C, Habig K, Burns BJ. Algorithm for the resuscitation of traumatic cardiac arrest patients in a physician-staffed helicopter emergency medical service. *Crit Care* 2013;17(2):308.

59. Soto-Ruiz KM, Varon J. Resuscitation great. George W. Crile: a visionary mind in resuscitation. *Resuscitation* 2009;80(1):6-8.

60. Kouwenhoven WB, Jude JR, Knickerbocker GG. Closed-chest cardiac massage. *JAMA* 1960;173:1064–7.

61. Georgiou M, Papathanassoglou E, Xanthos T. Systematic review of the mechanisms driving effective blood flow during adult CPR. *Resuscitation* 2014;85(11):1586–93.

62. Rudikoff MT, Maughan WL, Effron M, Freund P, Weisfeldt ML. Mechanisms of blood flow during cardiopulmonary resuscitation. *Circulation* 1980;61(2):345–52.

63. Hackl W, Simon P, Mauritz W, Steinbereithner K. Echocardiographic assessment of mitral valve function during mechanical cardiopulmonary resuscitation in pigs. *Anesth Analg* 1990;70(4):350–6.

64. Paradis NA, Martin GB, Rivers EP, Goetting MG, Appleton TJ, Feingold M, et al. Coronary perfusion pressure and the return of spontaneous circulation in human cardiopulmonary resuscitation. *JAMA* 1990;263(8):1106–13.

65. Ministry of Defence. Clinical Guidelines for Operations. Joint Service Publication 950 Vol 11. www.clinicalguideforops.co.uk. Accessed Nov 2017.

66. Deakin CD, Nolan JP, Soar J, Sunde K, Koster RW, Smith GB, et al. European Resuscitation Council Guidelines for Resuscitation 2010 Section 4. Adult advanced life support. *Resuscitation* 2010;81(10):1305-52.

67. Luna GK, Pavlin EG, Kirkman T, Copass MK, Rice CL. Hemodynamic effects of external cardiac massage in trauma shock. *J Trauma* 1989;29(10):1430–3.

68. Jeffcoach DR, Gallegos JJ, Jesty SA, Coan PN, Chen J, Heidel RE, et al. Use of CPR in hemorrhagic shock, a dog model. *J Trauma Acute Care Surg* 2016;81(1):27–33.

69. Mattox KL, Feliciano DV. Role of external cardiac compression in truncal trauma. *J Trauma* 1982;22(11):934–6.

70. Hermreck AS. The history of cardiopulmonary resuscitation. *Am J Surg* 1988;156(6):430–6.

71. Beck CS. Wounds of the heart: The technic of suture. *Arch Surg* 1926;13(2):205–27.

72. Burlew CC, Moore EE, Moore FA, Coimbra R, McIntyre RC Jr, Davis JW, et al. Western Trauma Association critical decisions in trauma: resuscitative thoracotomy. *J Trauma Acute Care Surg* 2012;73(6):1359-63.

73. Wise D, Davies G, Coats T, Lockey D, Hyde J, Good A. Emergency thoracotomy: "how to do it". *Emerg Med J* 2005;22(1):22–4.

74. Rhee PM, Acosta J, Bridgeman A, Wang D, Jordan M, Rich N. Survival after emergency department thoracotomy: review of published data from the past 25 years. *J Am Coll Surg* 2000;190(3):288–98.

75. Keller D, Kulp H, Maher Z, Santora TA, Goldberg AJ, Seamon MJ. Life after near death: long-term outcomes of emergency department thoracotomy survivors. *J Trauma Acute Care Surg* 2013;74(5):1315–20.

76. Morgan BS, Garner JP. Emergency Thoracotomy – The Indications, Contraindications and Evidence. *J R Army Med Corps* 2009;155(2):87–93.

77. Lyon M, Johnson D, Gordon R. Use of a Novel Abdominal Aortic and Junctional Tourniquet to Reduce or Eliminate Flow in the Brachial and Popliteal Arteries in Human Subjects. *Prehosp Emerg Care* 2015;19(3):405–8.

78. Lawrence W, Kuehn P, Masle ET, Miller DG. An abdominal tourniquet for regional chemotherapy. *J Surg Res* 1961;1:142–51.

79. Taylor DM, Coleman M, Parker PJ. The evaluation of an abdominal aortic tourniquet for the control of pelvic and lower limb hemorrhage. *Mil Med* 2013;178(11):1196–201.

80. Anonymous. Abdominal aortic tourniquet? Use in afghanistan. *J Spec Oper Med* 2013;13(2):1–2.

81. Kheirabadi BS, Terrazas IB, Miranda N, Voelker AN, Grimm R, Kragh JF, et al. Physiological Consequences of Abdominal Aortic and Junctional Tourniquet (AAJT) Application to Control Hemorrhage in a Swine Model. *Shock* 2016;46(3 Suppl 1):160–6.

82. Rall JM, Ross JD, Clemens MS, Cox JM, Buckley TA, Morrison JJ. Hemodynamic effects of the Abdominal Aortic and Junctional Tourniquet in a hemorrhagic swine model. *J Surg Res* 2017;212:159–66.

83. Chang JC, Holloway BC, Zamisch M, Hepburn MJ, Ling GSF. ResQFoam for the Treatment of Non-Compressible Hemorrhage on the Front Line. *Mil Med* 2015;180(9):932–3.

84. Zdrahala RJ, Zdrahala IJ. Biomedical applications of polyurethanes: a review of past promises, present realities, and a vibrant future. *J Biomater Appl* 1999;14(1):67–90.

85. Duggan M, Rago A, Sharma U, Zugates G, Freyman T, Busold R, et al. Self-expanding polyurethane polymer improves survival in a model of noncompressible massive abdominal hemorrhage. *J Trauma Acute Care Surg* 2013;74(6):1462–7.

86. Stannard A, Morrison JJ, Sharon DJ, Eliason JL, Rasmussen TE. Morphometric analysis of torso arterial anatomy with implications for resuscitative aortic occlusion. *J Trauma Acute Care Surg* 2013;75:S169–72.

87. Edwards WS, Salter PP, Carnaggio VA. Intraluminal aortic occlusion as a possible mechanism for controlling massive intra-abdominal hemorrhage. *Surg Forum* 1953;4:496–9.

88. Hughes CW. Use of an intra-aortic balloon catheter tamponade for controlling intra-abdominal hemorrhage in man. *Surgery* 1954;36(1):65–8.

89. Low RB, Longmore W, Rubinstein R, Flores L, Wolvek S. Preliminary report on the use of the percluder® occluding aortic balloon in human beings. *Ann Emerg Med* 1986 Dec;15(12):1466–9.

90. Gupta BK, Khaneja SC, Flores L, Eastlick L, Longmore W, Shaftan GW. The role of intra-aortic balloon occlusion in penetrating abdominal trauma. *J Trauma* 1989;29(6):861–5.

91. Hesse FG, Kletschka HD. Rupture of abdominal aortic aneurysm: control of hemorrhage by intraluminal balloon tamponade. *Ann Surg* 1962;155(2):320–2.

92. Tsilimparis N, Saleptsis V, Rohlffs F, Wipper S, Debus ES, Kolbel T. New developments in the treatment of ruptured AAA. *J Cardiovasc Surg (Torino)* 2016;57(2):233–41.

93. Paull JD, Smith J, Williams L, Davison G, Devine T, Holt M. Balloon occlusion of the abdominal aorta during caesarean hysterectomy for placenta percreta. *Anaesth Intensive Care* 1995;23(6):731–4.

94. Wang Y-L, Su F-M, Zhang H-Y, Wang F, Zhe R-L, Shen X-Y. Aortic balloon occlusion for controlling intraoperative hemorrhage in patients with placenta previa increta/percreta. *J Matern Fetal Neonatal Med* 2017;60:1–5.

95. White JM, Cannon JW, Stannard A, Markov NP, Spencer JR, Rasmussen TE. Endovascular balloon occlusion of the aorta is superior to resuscitative thoracotomy with aortic clamping in a porcine model of hemorrhagic shock. *Surgery* 2011;150(3):400–9.

96. Avaro J-P, Mardelle V, Roch A, Gil C, de Biasi C, Oliver M, et al. Forty-Minute Endovascular Aortic Occlusion Increases Survival in an Experimental Model of Uncontrolled Hemorrhagic Shock Caused by Abdominal Trauma. *J Trauma* 2011;71(3):720–6.

97. Morrison JJ, Percival TJ, Markov NP, Villamaria C, Scott DJ, Saches KA, et al. Aortic balloon occlusion is effective in controlling pelvic hemorrhage. *J Surg Res* 2012;177(2):341–7.

98. Markov NP, Percival TJ, Morrison JJ, Ross JD, Scott DJ, Spencer JR, et al. Physiologic tolerance of descending thoracic aortic balloon occlusion in a swine model of hemorrhagic shock. *Surgery* 2013;153(6):848–56.

99. Scott DJ, Eliason JL, Villamaria C, Morrison JJ, Houston R 4th, Spencer JR, et al. A novel fluoroscopy-free, resuscitative endovascular aortic balloon occlusion system in a model of hemorrhagic shock. *J Trauma Acute Care Surg* 2013;75(1):122-8.

100. Hodgetts T, Mahoney P, Kirkman E. Damage Control Resuscitation. *J R Army Med Corps* 2007;153(4):299–300.

101. Morrison JJ, Ross JD, Houston R, Watson JDB, Sokol KK, Rasmussen TE. Use of resuscitative endovascular balloon occlusion of the aorta in a highly lethal model of noncompressible torso hemorrhage. *Shock* 2014;41(2):130–7.

102. Morrison JJ, Percival TJ, Markov NP, Spencer JR, Rasmussen TE. Endovascular Balloon Occlusion of the Terminal Aorta is an Effective Hemorrhage Control Maneuver in a Porcine Model of Non-Compressible Pelvic Hemorrhage. *J Surg Res* 2012;172(2):324–4.

103. Martinelli T, Thony F, Decléty P, Sengel C, Broux C, Tonetti J, et al. Intra-aortic balloon occlusion to salvage patients with life-threatening hemorrhagic shocks from pelvic fractures. *J Trauma* 2010;68(4):942–8.

104. Brenner ML, Moore LJ, DuBose JJ, Tyson GH, McNutt MK, Albarado RP, et al. A clinical series of resuscitative endovascular balloon occlusion of the aorta for hemorrhage control and resuscitation. *J Trauma Acute Care Surg* 2013;75(3):506–11.

105. Stannard A, Eliason JL, Rasmussen TE. Resuscitative endovascular balloon occlusion of the aorta (REBOA) as an adjunct for hemorrhagic shock. *J Trauma* 2011;71(6):1869–72.

106. Norii T, Crandall C, Terasaka Y. Survival of severe blunt trauma patients treated with resuscitative endovascular balloon occlusion of the aorta compared with propensity score-adjusted untreated patients. *J Trauma Acute Care Surg 2015;78(4):721-8*.

107. Manning JE, Murphy CA, Hertz CM, Perretta SG, Mueller RA, Norfleet EA. Selective aortic arch perfusion during cardiac arrest: a new resuscitation technique. *Ann Emerg Med* 1992;21(9):1058–65.

108. Paradis NA, Rose MI, Gawryl MS. Selective aortic perfusion and oxygenation: an effective adjunct to external chest compression-based cardiopulmonary resuscitation. *J Am Coll Cardiol* 1994;23(2):497–504.

109. Paradis NA. Dose-response relationship between aortic infusions of polymerized bovine hemoglobin and return of circulation in a canine model of ventricular fibrillation and advanced cardiac life support. *Crit Care Med* 1997;25(3):476–83.

110. Manning JE, Batson DN, Gansman TW, Murphy CA, Perretta SG, Norfleet EA. Selective aortic arch perfusion using serial infusions of perflubron emulsion. *Acad Emerg Med* 1997;4(9):883–90.

111. Manning JE, Batson DN, Payne FB, Adam N, Murphy CA, Perretta SG, et al. Selective aortic arch perfusion during cardiac arrest: enhanced resuscitation using oxygenated perflubron emulsion, with and without aortic arch epinephrine. *Ann Emerg Med* 1997;29(5):580–7.

112. Manning JE, Katz LM, Pearce LB, Batson DN, McCurdy SL, Gawryl MS, et al. Selective aortic arch perfusion with hemoglobin-based oxygen carrier-201 for resuscitation from exsanguinating cardiac arrest in swine. *Crit Care Med* 2001;29(11):2067–74.

113. Puchwein P, Sommerauer F, Clement HG, Matzi V, Tesch NP, Hallmann B, et al. Clamshell thoracotomy and open heart massage--A potential life-saving procedure can be taught to emergency physicians: An educational cadaveric pilot study. *Injury* 2015;46(9):1738–42.

114. Manning JE. Feasibility of blind aortic catheter placement in the prehospital environment to guide resuscitation in cardiac arrest. *J Trauma Acute Care Surg* 2013;75:S173–7.

115. Cross AM, Davis C, Penn-Barwell J, Taylor DM, De Mello WF, Matthews JJ. The incidence of pelvic fractures with traumatic lower limb amputation in modern warfare due to improvised explosive devices. *J R Nav Med Serv* 2014;100(2):152–6.

116. Hughes GC, Post MJ, Simons M, Annex BH. Translational physiology: porcine models of human coronary artery disease: implications for preclinical trials of therapeutic angiogenesis. *J Appl Physiol* 2003;94(5):1689–701.

117. Wolf P, Meyer C, Boudjema K, Kieny R, Cinqualbre J, Jaeck D, et al. The pig as a model in liver xenotransplantation. *Vet Res* 1997;28(3):217–22.

118. Ross JD, Burns CJ, Sagini EM, Zarzabal L-A, Morrison JJ. A laparoscopic swine model of noncompressible torso hemorrhage. *J Trauma Acute Care Surg* 2014;77(3 Suppl 2):S77–82.

119. Morrison JJ, Ross JD, Rasmussen T, Midwinter MJ, Jansen JO. Resuscitative Endovascular Balloon Occlusion of the Aorta (REBOA): A Gap Analysis of Severely Injured UK Combat Casualties. *Shock* 2014;41(5):388-93.

120. Hannon JP, Bossone CA, Wade CE. Normal physiological values for concious pigs used in biomedical research. *Lab Anim Sci* 1990;40(3):293-8.

121. Li K, Xu Y. Citrate metabolism in blood transfusions and its relationship due to metabolic alkalosis and respiratory acidosis. *Int J Clin Exp Med* 2015;8(4):6578-84.

122. Bebarta VS, Daheshia M, Ross JD. The significance of splenectomy in experimental swine models of controlled hemorrhagic shock. *J Trauma Acute Care Surg* 2013;75(5):920.

123. Barnard EBG, Potter DL, Ayling RM, Higginson I, Bailey AG, Smith JE. Factors affecting blood sample haemolysis: a cross-sectional study. *Eur J Emerg Med* 2016;23(2):143–6.

124. Liao Q, Sjoberg T, Paskevicius A, Wohlfart B, Steen S. Manual versus mechanical cardiopulmonary resuscitation. An experimental study in pigs. *BMC Cardiovasc Disord* 2010;10:53.

125. HARKINS GA, BRAMSON ML. Mechanized external cardiac massage for cardiac arrest and for support of the failing heart. A preliminary communication.*J Surg Res* 1961;1:197–200.

126. Harrison-Paul R. Resuscitation great. A history of mechanical devices for providing external chest compressions. *Resuscitation* 2007;73(3):330–6.

127. Semenas E, Nozari A, Wiklund L. Sex differences in cardiac injury after severe haemorrhage and ventricular fibrillation in pigs. *Resuscitation* 2010;81(12):1718–22.

128. Ledgerwood AM, Kazmers M, Lucas CE. The role of thoracic aortic occlusion for massive hemoperitoneum. *J Trauma* 1976;16(08):610–5.

129. Spinella PC. Warm fresh whole blood transfusion for severe hemorrhage: U.S. military and potential civilian applications. *Crit Care Med* 2008;36(7):S340–5.

130. Secher NH, Sander Jensen K, Werner C, Warberg J, Bie P. Bradycardia during severe but reversible hypovolemic shock in man. *Circ Shock* 1984;14(4):267–74.

131. Kinnear-Mellor R, Newton K, Woolley T, Rickard R. Predictive utility of cardiac ultrasound in traumatic cardiac arrest in a combat casualty. *J R Army Med Corps* 2015;162(1):68-70.

132. Jani VP, Yalcin O, Williams AT, Popovsky MA, Cabrales P. Rat red blood cell storage lesions in various additive solutions. *Clin Hemorheol Microcirc* 2017;67(1):45-57.

133. Sparrow RL. Time to revisit red blood cell additive solutions and storage conditions: a role for “omics” analyses. *Blood Transfus* 2012;10(2):S7–11.

134. Steenbergen C, Deleeuw G, Rich T, Williamson JR. Effects of acidosis and ischemia on contractility and intracellular pH of rat heart. *Circ Res* 1977;41(6):849–58.

135. Yang S-W, Park K-H, Zhou Y-J. The Impact of Hypoglycemia on the Cardiovascular System: Physiology and Pathophysiology. *Angiology* 2016;67(9):802–9.

136. Spinella PC, Perkins JG, Grathwohl KW, Beekley AC, Holcomb JB. Warm fresh whole blood is independently associated with improved survival for patients with combat-related traumatic injuries. *J Trauma* 2009;66(4):S69–76.

137. Stengl M, Ledvinova L, Chvojka J, Benes J, Jarkovska D, Holas J, et al. Effects of clinically relevant acute hypercapnic and metabolic acidosis on the cardiovascular system: an experimental porcine study. *Crit Care* 2013;17(6):R303.

138. Sadek S, Lockey DJ, Lendrum RA, Perkins Z, Price J, Davies GE. Resuscitative endovascular balloon occlusion of the aorta (REBOA) in the pre-hospital setting: An additional resuscitation option for uncontrolled catastrophic haemorrhage. *Resuscitation* 2016;107:135-8.

139. Sadeghi M, Nilsson KF, Larzon T, Pirouzram A, Toivola A, Skoog P, et al. The use of aortic balloon occlusion in traumatic shock: first report from the ABO trauma registry. *Eur J Trauma Emerg Surg* 2017. doi: 10.1007/s00068-017-0813-7.

140. Matsumura Y, Matsumoto J, Kondo H, Idoguchi K, Ishida T, Kon Y, et al. Fewer REBOA complications with smaller devices and partial occlusion: evidence from a multicentre registry in Japan. *Emerg Med J* 2017;34(12):793–9.

141. Paravastu SCV, Jayarajasingam R, Cottam R, Palfreyman SJ, Michaels JA, Thomas SM. Endovascular repair of abdominal aortic aneurysm. *Cochrane Database Syst Rev* 2014. doi: 10.1002/14651858.CD004178.pub2.

142. Anrep VG. On the part played by the suprarenals in the normal vascular reactions of the body. *J Physiol* 1912;45(5):307–17.

143. Sarnoff SJ, Mitchell JH, Gilmore JP, Remensnyder JP. Homeometric autoregulation in the heart. *Circ Res* 1960;8:1077–91.

144. Gamble WJ, LaFarge CG, Fyler DC, Weisul J, Monroe RG. Regional coronary venous oxygen saturation and myocardial oxygen tension following abrupt changes in ventricular pressure in the isolated dog heart. *Circ Res* 1974;34(5):672–81.

145. Roizen MF, Beaupre PN, Alpert RA, Kremer P, Cahalan MK, Shiller N, et al. Monitoring with two-dimensional transesophageal echocardiography. *J Vasc Surg* 1984;1(2):300–5.

146. Zammert M, Gelman S. The pathophysiology of aortic cross-clamping. *Best Pract Res Clin Anaesthesiol* 2016;30(3):257–69.

147. Nielsen VG, McCammon AT, Tan S, Kirk KA, Samuelson PN, Parks DA. Xanthine oxidase inactivation attenuates postocclusion shock after descending thoracic aorta occlusion and reperfusion in rabbits. *J Thorac Cardiovasc Surg* 1995;110(3):715–22.

148. Bhatia M, Moochhala S. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *J Pathol* 2004;202(2):145–56.

149. Utsunomiya T, Krausz MM, Dunham B, Mannick JA, Allen PD, Shepro D, et al. Maintenance of cardiodynamics with aspirin during abdominal aortic aneurysmectomy (AAA). *Ann Surg* 1981;194(5):602–8.

150. Knudsen F, Andersen PT, Nielsen LK, Jersild C. Complement and leukocyte changes during major vascular surgery. *J Cardiothorac Anesth* 1988;2(5):646–9.

151. Bengston A, Heideman M. Altered Anaphylatoxin Activity during Induced Hypoperfusion in Acute and Elective Abdominal Aortic Surgery. *J Trauma* 1986;26(7):631–7.

152. Fattahi F, Ward PA. Complement and sepsis-induced heart dysfunction. *Mol Immunol* 2017;84:57–64.

153. Parks DA, Granger DN. Ischemia-induced vascular changes: role of xanthine oxidase and hydroxyl radicals. *Am J Physiol* 1983;245(2):G285–9.

154. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev* 2014;94(3):909–50.

155. Baldus S, Müllerleile K, Chumley P, Steven D, Rudolph V, Lund GK, et al. Inhibition of xanthine oxidase improves myocardial contractility in patients with ischemic cardiomyopathy. *Free Radic Biol Med* 2006;41(8):1282–8.

156. Gal TJ, Cooperman LH, Berkowitz HD. Plasma Renin Activity in Patients Undergoing Surgery of the Abdominal Aorta. *Ann Surg* 1974;179(1):65–9.

157. Grindlinger GA, Vegas AM, Manny J, Bush HL, Mannick JA, Hechtman HB. Volume loading and vasodilators in abdominal aortic aneurysmectomy. *Am J Surg* 1980;139(4):480–6.

158. Breckwoldt WL, Mackey WC, Belkin M, O'Donnell TF. The effect of suprarenal cross-clamping on abdominal aortic aneurysm repair. *Arch Surg* 1992;127(5):520–4.

159. Russo RM, Neff LP, Lamb CM, Cannon JW, Galante JM, Clement NF, et al. Partial Resuscitative Endovascular Balloon Occlusion of the Aorta in Swine Model of Hemorrhagic Shock. *J Am Coll Surg* 2016;223(2):359–68.

160. Johnson MA, Neff LP, Williams TK, DuBose JJ, EVAC Study Group. Partial resuscitative balloon occlusion of the aorta (P-REBOA): Clinical technique and rationale. *J Trauma Acute Care Surg* 2016;81(5 Suppl 2):S133–7.

161. Davidson AJ, Russo RM, Ferencz S-AE, Cannon JW, Rasmussen TE, Neff LP, et al. Incremental balloon deflation following complete resuscitative endovascular balloon occlusion of the aorta results in steep inflection of flow and rapid reperfusion in a large animal model of hemorrhagic shock. *J Trauma Acute Care Surg* 2017;83(1):139–43.

162. Reva VA, Matsumura Y, Hörer T, Sveklov DA, Denisov AV, Telickiy SY, et al. Resuscitative endovascular balloon occlusion of the aorta: what is the optimum occlusion time in an ovine model of hemorrhagic shock? *Eur J Trauma Emerg Surg* 2016. doi: 10.1007/s00068-016-0732-z.

163. Harel Z, Kamel KS. Optimal dose and method of administration of intravenous insulin in the management of emergency hyperkalemia: a systematic review. *PLoS One* 2016;11(5):e0154963. doi: 10.1371/journal.pone.0154963.

164. Allon M, Copkney C. Albuterol and insulin for treatment of hyperkalemia in hemodialysis patients. *Kidney Int* 1990;38(5):869–72.

165. Aronson PS, Giebisch G. Effects of pH on potassium: new explanations for old observations. *J Am Soc Nephrol* 2011 Nov;22(11):1981–9.

166. Kimmoun A, Novy E, Auchet T, Ducrocq N, Levy B. Hemodynamic consequences of severe lactic acidosis in shock states: from bench to bedside. *Crit Care* 2015;19(1):175.

167. Acute Respiratory Distress Syndrome Network, Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT, et al. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 2000;342(18):1301–8.

168. Morrison JJ, Ross JD, Markov NP, Scott DJ, Spencer JR, Rasmussen TE. The inflammatory sequelae of aortic balloon occlusion in hemorrhagic shock. *J Surg Res* 2014;191(2):423–31.

169. Friesecke S, Träger K, Schittek GA, Molnar Z, Bach F, Kogelmann K, et al. International registry on the use of the CytoSorb® adsorber in ICU patients : Study protocol and preliminary results. *Med Klin Intensivmed Notfmed* 2017;355(10):1018–9.

170. Joannes-Boyau O, Rapaport S, Bazin R, Fleureau C, Janvier G. Impact of high volume hemofiltration on hemodynamic disturbance and outcome during septic shock. *ASAIO J* 2004;50(1):102–9.

171. Laurent I, Adrie C, Vinsonneau C, Cariou A, Chiche J-D, Ohanessian A, et al. High-volume hemofiltration after out-of-hospital cardiac arrest: a randomized study. *J Am Coll Cardiol* 2005;46(3):432–7.

172. Sykora R, Chvojka J, Krouzecky A, Radej J, Karvunidis T, Varnerova V, et al. High versus standard-volume haemofiltration in hyperdynamic porcine peritonitis: effects beyond haemodynamics? *Intensive Care Med* 2009 Feb;35(2):371–80.

173. Hosgood SA, Nicholson ML. Hydrogen sulphide ameliorates ischaemia-reperfusion injury in an experimental model of non-heart-beating donor kidney transplantation. *Br J Surg* 2010;97(2):202–9.

174. Causey MW, Miller S, Singh N, Martin M. Pharmacologic attenuation of the hyperdynamic response to supraceliac aortic clamping. *J Vasc Surg* 2015;61(1):224–30.

175. Sparks MA, Crowley SD, Gurley SB, Mirotsou M, Coffman TM. Classical Renin-Angiotensin system in kidney physiology. *Compr Physiol* 2014;4(3):1201–28.

176. Hong SA, Gelman S, Henderson T. Angiotensin and adrenoceptors in the hemodynamic response to aortic cross-clamping. *Arch Surg* 1992;127(4):438–41.

177. Voelckel WG, Raedler C, Wenzel V, Lindner KH, Krismer AC, Schmittinger CA, et al. Arginine vasopressin, but not epinephrine, improves survival in uncontrolled hemorrhagic shock after liver trauma in pigs. *Crit Care Med* 2003;31(4):1160–5.

178. Sperry JL, Minei JP, Frankel HL, West MA, Harbrecht BG, Moore EE, et al. Early use of vasopressors after injury: caution before constriction. *J Trauma* 2008;64(1):9–14.

179. Cohn LH. Fifty years of open-heart surgery. *Circulation* 2003;107(17):2168-70.

180. Bartlett RH. Extracorporeal life support: history and new directions. *ASAIO J* 2005;51(5):487–9.

181. Hill JD, O'Brien TG, Murray JJ, Dontigny L, Bramsom ML, Osborn JJ, et al. Prolonged extracorporeal oxygenation for acute post-traumatic respiratory failure (shock-lung syndrome). Use of the Bramson membrane lung. *N Engl J Med* 1972;286(12):629–34.

182. Zapol WM, Snider MT, Hill JD, Fallat RJ, Bartlett RH, Edmunds LH, et al. Extracorporeal membrane oxygenation in severe acute respiratory failure. A randomized prospective study. *JAMA* 1979;242(20):2193–6.

183. Bartlett RH, Andrews AF, Toomasian JM, Haiduc NJ, Gazzaniga AB. Extracorporeal membrane oxygenation for newborn respiratory failure: forty-five cases. *Surgery* 1982;92(2):425–33.

184. Toomasian JM, Snedecor SM, Cornell RG, Cilley RE, Bartlett RH. National experience with extracorporeal membrane oxygenation for newborn respiratory failure. Data from 715 cases. *ASAIO Trans* 1988;34(2):140–7.

185. O'Rourke PP, Crone RK, Vacanti JP, Ware JH, Lillehei CW, Parad RB, et al. Extracorporeal membrane oxygenation and conventional medical therapy in neonates with persistent pulmonary hypertension of the newborn: a prospective randomized study. *Pediatrics* 1989;84(6):957–63.

186. UK collaborative randomised trial of neonatal extracorporeal membrane oxygenation. *Lancet* 1996;348(9020):75–82.

187. Squiers JJ, Lima B, DiMaio JM. Contemporary extracorporeal membrane oxygenation therapy in adults: Fundamental principles and systematic review of the evidence. *J Thorac Cardiovasc Surg* 2016;152(1):20–32.

188. Frank JA, Matthay MA. Science review: mechanisms of ventilator-induced injury. *Crit Care* 2003;7(3):233–41.

189. Khoshbin E, Roberts N, Harvey C, Machin D, Killer H, Peek GJ, et al. Poly-methyl pentene oxygenators have improved gas exchange capability and reduced transfusion requirements in adult extracorporeal membrane oxygenation. *ASAIO J* 2005;51(3):281–7.

190. Peek GJ, Elbourne D, Mugford M, Tiruvoipati R, Wilson A, Allen E, et al. Randomised controlled trial and parallel economic evaluation of conventional ventilatory support versus extracorporeal membrane oxygenation for severe adult respiratory failure (CESAR). *Health Technol Assess* 2010;14(35):1–46.

191. Stub D, Bernard S, Pellegrino V, Smith K, Walker T, Sheldrake J, et al. Refractory cardiac arrest treated with mechanical CPR, hypothermia, ECMO and early reperfusion (the CHEER trial). *Resuscitation* 2015;86:88–94.

192. King CS, Roy A, Ryan L, Singh R. Cardiac Support: Emphasis on Venoarterial ECMO. *Crit Care Clin* 2017;33(4):777–94.

193. Mosier JM, Kelsey M, Raz Y, Gunnerson KJ, Meyer R, Hypes CD, et al. Extracorporeal membrane oxygenation (ECMO) for critically ill adults in the emergency department: history, current applications, and future directions. *Crit Care* 2015 Dec;19(1):431.

194. Jayanthi NVG, Dabke HV. The effect of IV cannula length on the rate of infusion. *Injury* 2006;37(1):41–5.

195. Rupprecht L, Lunz D, Philipp A, Lubnow M, Schmid C. Pitfalls in percutaneous ECMO cannulation. *Heart Lung Vessel* 2015;7(4):320–6.

196. Toomasian JM, Bartlett RH. Hemolysis and ECMO pumps in the 21st Century. *Perfusion* 2011;26(1):5–6.

197. Buscher H, Vukomanovic A, Benzimra M, Okada K, Nair P. Blood and Anticoagulation Management in Extracorporeal Membrane Oxygenation for Surgical and Nonsurgical Patients: A Single-Center Retrospective Review. *J Cardiothorac Vasc Anesth* 2017;31(3):869–75.

198. Bedeir K, Seethala R, Kelly E. Extracorporeal life support in trauma. *J Trauma Acute Care Surg* 2017;82(2):400–6.

199. Extracorporeal Life Support Organization. ELSO Guidelines for Cardiopulmonary Extracorporeal Life Support, v1.4. https://www.elso.org/resources/guidelines.aspx. Accessed Nov 2017.

200. Nishinaka T, Tatsumi E, Katagiri N, Ohnishi H, Mizuno T, Shioya K, et al. Up to 151 days of continuous animal perfusion with trivial heparin infusion by the application of a long-term durable antithrombogenic coating to a combination of a seal-less centrifugal pump and a diffusion membrane oxygenator. *J Artif Organs* 2007;10(4):240–4.

201. Larsson M, Talving P, Palmér K, Frenckner B, Riddez L, Broomé M. Experimental extracorporeal membrane oxygenation reduces central venous pressure: an adjunct to control of venous hemorrhage? *Perfusion* 2010;25(4):217–23.

202. Tisherman SA, Safar P, Radovsky A, Peitzman A, Sterz F, Kuboyama K. Therapeutic deep hypothermic circulatory arrest in dogs. *J Trauma* 1990;30(7):836–47.

203. Kutcher ME, Forsythe RM, Tisherman SA. Emergency preservation and resuscitation for cardiac arrest from trauma. *Int J Surg* 2016;33(Pt B):209–12.

204. True NA, Siler S, Manning JE. Endovascular resuscitation techniques for severe hemorrhagic shock and traumatic arrest in the presurgical setting. *J Spec Oper Med* 2013;13(2):33–7.

205. Ried M, Bein T, Philipp A, Müller T, Graf B, Schmid C, et al. Extracorporeal lung support in trauma patients with severe chest injury and acute lung failure: a 10-year institutional experience. *Crit Care* 2013;17(3):R110.

206. Arlt M, Philipp A, Voelkel S, Rupprecht L, Mueller T, Hilker M, et al. Extracorporeal membrane oxygenation in severe trauma patients with bleeding shock. *Resuscitation* 2010;81(7):804–9.

207. Bonacchi M, Spina R, Torracchi L, Harmelin G, Sani G, Peris A. Extracorporeal life support in patients with severe trauma: an advanced treatment strategy for refractory clinical settings. *J Thorac Cardiovasc Surg* 2013;145(6):1617–26.

208. Huh U, Song S, Chung SW, Kim S. Is Extracorporeal Cardiopulmonary Resuscitation Practical in Severe Chest Trauma?: A Systematic Review in Single Center of Developing Country. *J Trauma Acute Care Surg* 2017;83(5):903-907.

209. Lamhaut L, Jouffroy R, Soldan M, Phillipe P, Deluze T, Jaffry M, et al. Safety and feasibility of prehospital extra corporeal life support implementation by non-surgeons for out-of-hospital refractory cardiac arrest. *Resuscitation* 2013;84(11):1525–9.

210. MAQUET CARDIOHELP System. https://www.maquet.com/int/products/cardiohelp-system/. Accessed Nov 2017.

211. MAQUET. HLS Cannulae. Solutions from tip to tip. https://www.maquet.com. Accessed Nov 2017.

212. Horton S, Augustin S. Activated clotting time (ACT). *Methods Mol Biol* 2013;992:155–67.

213. Vandenberk B, Vandael E, Robyns T, Vandenberghe J, Garweg C, Foulon V, et al. Which QT Correction Formulae to Use for QT Monitoring? *J Am Heart Assoc* 2016;5(6):e003264. doi: 10.1161/JAHA.116.003264.

214. Davis JW. The relationship of base deficit to lactate in porcine hemorrhagic shock and resuscitation. *J Trauma* 1994;36(2):168–72.

215. Smith IM, James RH, Dretzke J, Midwinter MJ. Prehospital Blood Product Resuscitation for Trauma: A Systematic Review. *Shock* 2016;46(1):3–16.

216. Gokhale SG, Scorer T, Doughty H. Freedom from frozen: the first British military use of lyophilised plasma in forward resuscitation. *J R Army Med Corps* 2016 Feb;162(1):63–5.

217. Cinar Y, Demir G, Pac M, Cinar AB. Effect of hematocrit on blood pressure via hyperviscosity. *Am J Hypertens* 1999;12(7):739–43.

218. Choi J, Kim S, Kim Y, Kim S-Y, Kim K-S, Joo S-J, et al. Application of veno-arterial-venous extracorporeal membrane oxygenation in differential hypoxia. *Multidiscip Respir Med* 2014;9(1):55.

219. Antman E, Bassand J-P, Klein W, Ohman M, Sendon JLL, Rydén L, et al. Myocardial infarction redefined—a consensus document of The Joint European Society of Cardiology/American College of Cardiology committee for the redefinition of myocardial infarction. *J Am Coll Cardiol* 2000;36(3):959–69.

220. Harruff RC. Subendocardial hemorrhages in forensic pathology autopsies. *Am J Forensic Med Pathol* 1993;14(4):284–8.

221. Fanelli V, Vlachou A, Ghannadian S, Simonetti U, Slutsky AS, Zhang H. Acute respiratory distress syndrome: new definition, current and future therapeutic options. *J Thorac Dis* 2013;5(3):326–34.

222. Walcott GP, Kroll MW, Ideker RE. Ventricular fibrillation: are swine a sensitive species? *J Interv Card Electrophysiol* 2015;42(2):83–9.

223. Stankovicová T, Bito V, Heinzel F, Mubagwa K, Sipido KR. Isolation and morphology of single Purkinje cells from the porcine heart. *Gen Physiol Biophys* 2003;22(3):329–40.

224. Grumbach L, Howard JW, Merrill VI. Factors related to the initiation of ventricular fibrillation in the isolated heart; effect of calcium and potassium. *Circ Res* 1954;2(5):452–9.

225. Patel RB, Tannenbaum S, Viana-Tejedor A, Guo J, Im K, Morrow DA, et al. Serum potassium levels, cardiac arrhythmias, and mortality following non-ST-elevation myocardial infarction or unstable angina: insights from MERLIN-TIMI 36. *Eur Heart J Acute Cardiovasc Care* 2017;6(1):18–25.

226. Manning JE, Ross JD, McCurdy SL, True NA. Aortic Hemostasis and Resuscitation: Preliminary Experiments Using Selective Aortic Arch Perfusion With Oxygenated Blood and Intra-aortic Calcium Coadministration in a Model of Hemorrhage-induced Traumatic Cardiac Arrest. *Acad Emerg Med* 2016;23(2):208–12.

227. Pourmand A, Mazer-Amirshahi M, Chistov S, Sabha Y, Vukomanovic D, Almulhim M. Emergency department approach to QTc prolongation. *Am J Emerg Med* 2017;35(12):1928-1933.

228. Jones JL, Snyder DE, Morgan C. Predictions from misleading pig model are potentially harmful to humans. *Resuscitation* 2003;59(3):365–7.

229. Lewis CJ, Ross JD. Hemoglobin-based oxygen carriers: an update on their continued potential for military application. *J Trauma Acute Care Surg* 2014;77(3 Suppl 2):S216–21.

230. Manley JD, Mitchell BJ, DuBose JJ, Rasmussen TE. A Modern Case Series of Resuscitative Endovascular Balloon Occlusion of the Aorta (REBOA) in an Out-of-Hospital, Combat Casualty Care Setting. *J Spec Oper Med* 2017;17(1):1–8.

231. Tonna JE, Johnson NJ, Greenwood J, Gaieski DF, Shinar Z, Bellezo JM, et al. Practice characteristics of Emergency Department extracorporeal cardiopulmonary resuscitation (eCPR) programs in the United States: The current state of the art of Emergency Department extracorporeal membrane oxygenation (ED ECMO). *Resuscitation* 2016;107:38–46.

232. Davidson AJ, Russo RM, Reva VA, Brenner ML, Moore LJ, Ball C, et al. The Pitfalls of REBOA: Risk Factors and Mitigation Strategies. *J Trauma Acute Care Surg* 2017. doi: 10.1097/TA.0000000000001711.

233. Endovascular Resuscitation and Trauma Management. Estimated world-wide Trauma REBOA to 2017. http://www.jevtm.com/estimated-world-wide-trauma-reboa-to-2017/. Accessed Nov 2017.

234. Scerbo MH, Holcomb JB, Taub E, Gates K, Love JD, Wade CE, et al. The trauma center is too late. *J Trauma Acute Care Surg* 2017;83(6):1165-1172.

235. Biffl WL, Fox CJ, Moore EE. The role of REBOA in the control of exsanguinating torso hemorrhage. *J Trauma Acute Care Surg* 2015;78(5):1054–8.

236. Holcomb JB, Fox EE, Scalea TM, Napolitano LM, Albarado R, Gill B, et al. Current opinion on catheter-based hemorrhage control in trauma patients. *J Trauma Acute Care Surg* 2014;76(3):888–93.

237. Brenner M, Hoehn M, Pasley J, DuBose J, Stein D, Scalea T. Basic endovascular skills for trauma course: bridging the gap between endovascular techniques and the acute care surgeon. *J Trauma Acute Care Surg* 2014;77(2):286–91.

238. Brenner M, Teeter W, Hoehn M, Pasley J, Hu P, Yang S, et al. Use of Resuscitative Endovascular Balloon Occlusion of the Aorta for Proximal Aortic Control in Patients With Severe Hemorrhage and Arrest. *JAMA Surg* 2018;153(2):130-135.

239. Kudo D, Yoshida Y, Kushimoto S. Permissive hypotension/hypotensive resuscitation and restricted/controlled resuscitation in patients with severe trauma. *J Intensive Care* 2017. doi: 10.1186/s40560-016-0202-z.

240. Duchesne JC, McSwain NE Jr, Cotton BA, Hunt JP, Dellavolpe J, Lafaro K, et al. Damage Control Resuscitation: The New Face of Damage Control. *J Trauma* 2010;69(4):976–90.

241. Reva VA, Hörer TM, Makhnovskiy AI, Sokhranov MV, Samokhvalov IM, DuBose JJ. Field and en route resuscitative endovascular occlusion of the aorta: A feasible military reality? J Trauma Acute Care Surg. *J Trauma Acute Care Surg* 2017;83(1 Suppl 1):S170–6.

242. Qasim Z, Brenner M, Menaker J, Scalea T. Resuscitative endovascular balloon occlusion of the aorta. *Resuscitation* 2015;96:275–9.

243. Harvard Apparatus UK. MRI Compatible Syringe Pump (#702131). https://www.harvardapparatus.co.uk/webapp/wcs/stores/servlet/haisku2_10001_11051_53701_-1_HAUK_ProductDetail_N_37295_37313. Accessed Nov 2017.

244. Heron M. Deaths: Leading Causes for 2011. *Natl Vital Stat Rep* 2015;64(7):1-96.

245. Carrico CJ. Scientific Priorities and Strategic Planning for Resuscitation Research and Life Saving Therapy Following Traumatic Injury: Report of the PULSE Trauma Work Group. *Acad Emerg Med* 2002;9(6):621–6.

246. U.S. Department of Health & Human Services. Estimates of Funding for Various Research, Condition, and Disease Categories (RCDC). Available from: https://report.nih.gov/categorical_spending.aspx. Accessed Nov 2017.

247. Bailey JA, Morrison JJ, Rasmussen TE. Military trauma system in Afghanistan: lessons for civil systems? *Curr Opin Crit Care* 2013;19(6):569–77.

248. Perkins JG, Brosch LR, Beekley AC, Warfield KL, Wade CE, Holcomb JB. Research and analytics in combat trauma care: converting data and experience to practical guidelines. *Surg Clin North Am* 2012;92(4):1041–54–x.

249. Martínez-Alarcón L, Ramis G, Majado MJ, Quereda JJ, Herrero-Medrano JM, Ríos A, et al. ABO and RH1 blood group phenotyping in pigs (Sus scrofa) using microtyping cards. *Transplant Proc* 2010;42(6):2146–8.

250. Morrison JJ, Galgon RE, Jansen JO, Cannon JW, Rasmussen TE, Eliason JL. A systematic review of the use of resuscitative endovascular balloon occlusion of the aorta in the management of hemorrhagic shock. *J Trauma Acute Care Surg* 2016;80(2):324–34.

251. Barnard EBG, Manning JE, Smith JE, Rall JM, Cox JM, Ross JD. A comparison of Selective Aortic Arch Perfusion and Resuscitative Endovascular Balloon Occlusion of the Aorta for the management of hemorrhage-induced traumatic cardiac arrest: A translational model in large swine. *PLoS Med* 2017;14(7):e1002349. doi: 10.1371/journal.pmed.1002349.

252. Midwinter MJ, Woolley T. Resuscitation and coagulation in the severely injured trauma patient. *Philos Trans R Soc Lond B Biol Sci* 2011;366(1562):192–203.