Tissue perfusion and organ dysfunction following traumatic injury

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Tissue perfusion and organ dysfunction following traumatic injury

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For

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Abstract

Traumatic injury and associated traumatic haemorrhagic shock are important causes of preventable morbidity and mortality. A proportion of survivors of such injuries go on to develop multiple organ dysfunction and failure. Tissue perfusion, ultimately driven by flow in small micro vessels, may be important in determining which patients develop such sequelae. The aim of this thesis was to investigate the importance of microcirculatory perfusion to a range of outcomes following traumatic injury and hemorrhagic shock.

Dark Field video microscopy is a way of assessing the microcirculation in vivo. The first part of this thesis validates a new hand-held video microscope, the Cytocam, demonstrating that it produces comparable data to a precursor device, but with the advantage of improved image quality.

A series of porcine experimental studies were conducted in order to assess the impact of microcirculatory perfusion on immediate resuscitation outcomes. 30 animals were subjected to standardized limb injury, controlled blood loss and in some cases blast exposure. Different initial treatment with either 0.9% saline, blood products or MP4OX, a synthetic haemoglobin based oxygen carrier, reflected potential treatment options in the pre-hospital setting. Animals who had above average microcirculatory perfusion during shock and early resuscitation had improved lactate clearance and lower base deficit at the end of the experiment. There was wide inter individual variation in microcirculatory perfusion that was not predictable by assessment of traditional vital signs, especially blood pressure. The type of initial resuscitation fluid did not have a significant impact on

microcirculatory indices. MP4OX was effective at resuscitating the microcirculation and was not associated with any adverse effects at the microcirculatory level.

In the same porcine model, the effect of microcirculatory perfusion on the development of trauma induced coagulopathy was examined. Animals who had below average microcirculatory perfusion during shock and early resuscitation who received 0.9% saline as the initial resuscitation fluid became coagulopathic. However, no animal with above average microcirculatory perfusion became coagulopathic, nor did any animal treated with blood products, regardless of the state of the microcirculation.

A clinical observational study of patients presenting to three UK major trauma centers following traumatic injury and blood loss was conducted. Patients who developed multiple organ dysfunction at a week following injury had significantly worse microcirculatory variables following resuscitation. Measurements of systemic haemodynamic status, such as cardiac output and blood pressure, did not show an association with the development of persistent organ dysfunction. Microcirculatory perfused vessel density was the best predictor for the development of multiple organ dysfunction compared to lowest recorded systolic blood pressure, highest lactate and cardiac index.

Samples taken from 12 patients enrolled in the clinical study were examined to investigate biomarkers potentially associated with microcirculatory impairment. Patients exhibited an increased level of a variety of markers of inflammation, endothelial activation and glycocalyx injury but there was no observed correlation between these levels and the degree of microcirculatory impairment in this small cohort.

This thesis confirms that the microcirculation plays a key role in the response to traumatic hemorrhagic shock. Poor microcirculatory perfusion is associated with a slower reversal of the shock state, development of trauma induced coagulopathy and post traumatic organ dysfunction. The mechanism behind these changes remains to be fully elucidated. However, both the studies reported in this thesis show that there was wide inter individual variation in the microcirculatory response to trauma and resuscitation and that prediction of microcirculatory indices by an examination of global systemic indices, especially blood pressure lacked accuracy. Future work should focus on adapting current microcirculatory imaging technology for use as a reliable and reproducible point of care assessment tool alongside clinical interventional studies that target the microcirculation as an end point of resuscitation.

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Chapter 1

Introduction and Review of Relevant Literature

1.1 Traumatic Haemorrhagic Shock – Demographic and Historical Perspective

1.1.1 Traumatic Injury – a global problem

Traumatic injury is a major global healthcare issue. According to a recent report by the World Health Organisation approximately 6 million people a year die as a result of trauma[1]. The single largest cause of injury is road traffic accidents (23%) but violent crime and conflict related injury form the second largest group (14%)[1]. Furthermore, all types of traumatic injury are increasing in relative importance as causes of death. Traumatic injury is still predominantly an affliction of youth and is the leading cause of death in those aged 19 -25. [1] For this reason the impact on loss of life years and economic productivity is significantly magnified when compared to disease processes which predominantly affect the elderly. For every survivor of traumatic injury there are many others who require long term rehabilitation and may not return to full independent activity. [2] One of the leading causes of death following traumatic injury is severe haemorrhage. [3]

1.1.2 Traumatic Hemorrhagic Shock – A historical and contemporary perspective

Haemorrhage, derived from the Ancient Greek *haimorrhagia,* literally burst blood, has been recognized as a potentially fatal occurrence since historical records began. Probably the earliest precisely recorded death produced by traumatic haemorrhage was Julius Caesar (100 BC – 44BC). In what was possibly the first formally conducted forensic examination in

history, the Roman physician Antistius recorded twenty-three knife wounds, noting that none were fatal except for one piercing the thorax and entering the heart.

Figure 1.1 La morte di Cesare. Vincenzo Camuccini, 1804

Although traumatic haemorrhage as a cause of mortality and morbidity has been important throughout and before recorded history, the nature of the problem only began to be understood from the seventeenth century onwards. Prior to this time there was little concept of a circulating blood volume and the theories of Galen regarding the nature of blood had held sway for over a Millennia. Physicians routinely advocated bloodletting to treat symptoms, and the concept of blood loss as a cause of harm was not countenanced. The publication of de Motu Cordis – On the motion of the heart and blood in animals - by William Harvey (1578-1657) in 1628 was the first step in the recognition that blood circulated around the organism and that interruption of this circulation could potentially lead to harm. At the time this theory was widely ridiculed and it was not until the early

twentieth century that the work of Otto Frank (1865-1944) and Ernest Starling (1866-1927) accurately described the relationship between the circulating blood volume and cardiac output.

Figure 1.2 De Motu Cordis et Sanguinis in Animali, William Harvey 1648

The stimulus of armed conflict has produced significant advances in our understanding of the nature of traumatic haemorrhagic shock (THS). In his ground breaking work Traumatic Shock [4] Walter Cannon (1871-1945) described his observations of wounded soldiers injured on the Western Front during the First World War. By this time it was widely accepted that blood loss, leading to lowered blood pressure, was a key determinant of mortality, but the actual mechanism of this hypotension was still the subject of debate, with the various contributions of the heart, vascular tone and blood volume unclear. Cannon's work was prescient in a number of ways, first describing the situation where normotension could be associated with a reduction in tissue blood flow:

"Although a diminished arterial pressure may measure the degree of shock, and the course of the pressure changes may indicate the tendencies in the patient, there is strong probability that, in the development of the condition, especially in cases of secondary shock, a stage is passed through in which the arterial pressure, though maintained at a normal – level, is accompanied by such a serious disturbance of the blood flow as to render the individual's chances precarious."

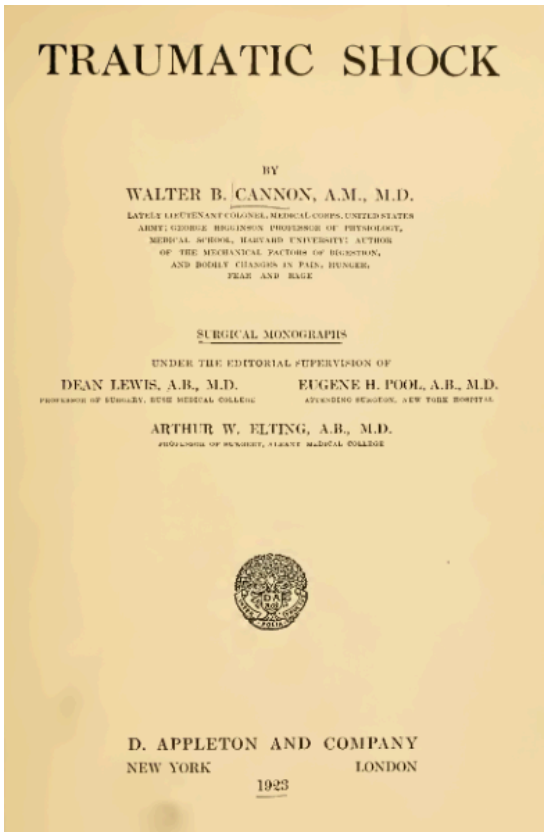
He also provides what must be one of the first descriptions of microcirculatory dysfunction following traumatic haemorrhagic shock (THS):

"When the capillary stagnation has become established it may not promptly disappear. I have seen a patient who had recovered from severe shock so far as arterial blood-pressure readings indicated, but whose hands, from wrists to finger tips, in spite of being warmed, were still bluish gray with stagnant blood."

Cannon also describes an area that is still widely debated today, that of the optimum blood pressure to maintain tissue perfusion. Through a series of large animal experiments that

produced hypotension through induced cardiac tamponade, Cannon and co-workers suggest that allowing the systolic blood pressure to fall below 80 mmHg produced an increasingly worse acidosis. Cannon is probably the first individual on record discussing what would subsequently be known as the theory of permissive hypotension:

"Injection of a fluid that will increase blood pressure has dangers in itself. Hemorrhage in a case of shock may not have occurred to a marked degree because blood pressure has been too low and the flow too scant to overcome the obstacle offered by a clot. If the pressure is raised before the surgeon is ready to check any bleeding that may take place, blood that is sorely needed may be lost."


TRAUMATIC SHOCK

BY

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NEW YORK LONDON

1923

Figure 1.3 Walter Cannon, President of the American Physiological Society (1914-1916), Higginson Professor of Physiology, Harvard University (1906-1942). Cannon's seminal work in this field was Traumatic Shock (1923)

The bloody course of the twentieth century produced further changes in the way traumatic injury was resuscitated. By the end of the Second World War the use of blood and plasma to treat blood loss was standard practice. However, a significant proportion of such casualties went on to develop acute renal failure. [5] The work of Tom Shires (1925-2007) in the 1960s led to a reappraisal of the nature of the volume losses in THS. Using isotopic preparations, he demonstrated that controlled hemorrhage in dogs led, not only to a contraction in the circulating blood volume, but also to a contraction of the extra-cellular space. Using shed blood alone to replace the deficit resulted in an 80% mortality; however, if the blood replacement was augmented with Ringers solution the mortality fell to 30%. [6] Widespread translation of these principals into mainstream clinical practice occurred and were later reflected in the Advanced Trauma Life Support (ATLS) guidance to provide aggressive large volume crystalloid infusions to trauma patients. [7] The use of such aggressive crystalloid resuscitation is widely accepted to have led to the emergence of a condition known as "shock lung" or "Da Nang lung" during the Vietnam War. [8] In reality, this condition was what we now term Acute Respiratory Distress Syndrome (ARDS), probably exacerbated by the large volume resuscitation strategy advocated at the time. Such an approach also led to an increased incidence of abdominal compartment syndrome in patients with traumatic injury, described by some as an epidemic, and almost certainly caused by visceral oedema resulting from the liberal usage of crystalloid resuscitative fluids. [9]

Figure 1.4 American World War 2 era poster encouraging blood donation. WW2 saw the first widespread use of blood and plasma to treat traumatic haemorrhage.

Figure 1.5 Vietnam war era casualty evacuation. The conflict saw the widespread use of crystalloid fluid based resuscitation and the emergence of ARDS.

THS remains an important clinical issue in the present day. Death from traumatic hemorrhagic shock (THS) usually occurs early, often in the pre-hospital phase and haemorrhage accounts for 80% of intra-operative deaths following traumatic injury. [10] Overall, only central nervous system injury is a more important cause of trauma related death. However, there are often limited treatment options available for patients with catastrophic traumatic brain injury. By contrast death from traumatic haemorrhage can frequently be prevented.

A study by *Eastridge* and co-workers [11] examined all fatalities amongst US service personal during the Iraq and Afghanistan conflicts between 2002 and 2012. Of the 4,596 deaths 32% were instantaneous, often from catastrophic bodily disruption as a result of explosive charges. 52% died within minutes to hours and of these 76% were judged to be non-survivable following expert panel review. However, 24% of these early deaths were judged preventable and of this group 90% of deaths resulted from haemorrhage, with only a small proportion attributed to airway obstruction or tension pneumothorax.

A similar cohort study examined the causes of death of UK service personal killed in Iraq and Afghanistan between 2003 and 2014. [12] Of the 632 deaths only 71 (11%) occurred after admission to a medical treatment facility. The majority of these were attributed to severe traumatic brain injury with hemorrhage implicated as the cause in 31% of those dying within a medical treatment facility.

Important evolutions in military medical practice, most notably the widespread use of point of wounding tourniquets, have reduced the incidence of early preventable haemorrhage related deaths by up to 85%. [13] Despite this traumatic haemorrhagic shock still remains the leading cause of preventable early mortality.

Figure 1.6 NATO involvement in the Afghanistan conflict (2001-14) saw the widespread use of physician led medical retrieval teams and extensive use of blood product resuscitation in the pre-hospital environment.

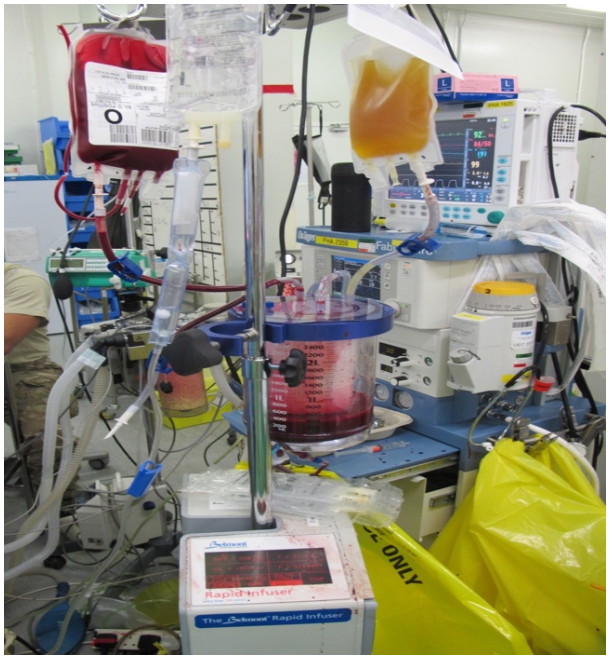

Figure 1.7 Haemostatic resuscitation using blood products at the UK Role 3 Hospital, Camp Bastion, Afghanistan

For patients who survive the initial minutes and hours following injury, THS can have significant sequalae. Shock is intrinsically linked to the development of Trauma Induced Coagulopathy (TIC) [14] which is itself an independent prognostic indicator. [15] Additionally, there are strong indications that the degree and duration of the initial shock state is important in determining organ dysfunction following traumatic injury. Such organ dysfunction remains an important cause of late mortality and morbidity following THS.

1.2 Multiple Organ Failure; a potential consequence of Traumatic Haemorrhagic Shock

1.2.1 Incidence and definition of post traumatic MOF

First described in 1970s by *Baue* [16] as a multiple, progressive or sequential systems failure; multiple organ failure (MOF) was interestingly felt to be a syndrome of its time caused by progress in medical therapy leading to longer periods of survival with the potential to develop sequalae.

The potential for traumatic injury to cause organ dysfunction was first recognized in the late 1970s and early 1980s. In a seminal paper that paved the way for a revolution in trauma care management *Trunkey* [17] described three peaks of mortality and ascribed the third peak of this model to the onset of MOF and sepsis. His paper describes a cohort of 862 patients presenting to a single US hospital over an undefined period and reports an incidence of late deaths of around 18%. The precise nature of the organ failure or the system used to define it is not reported.

The first description of an objective scoring system to classify MOF in a trauma cohort was produced by *Faist* and co-workers in 1983. [18] Using a bespoke scoring system that rated injury to the lungs, heart, liver, kidneys, coagulation and gastro intestinal systems the authors reported an incidence of MOF of 7.5% in a cohort of polytrauma patients presenting to a single German hospital between 1978 and 1982. Risk factors for the development of MOF were noted to include transfusion of more than 6 units of blood and the presence of shock on arrival at hospital. This paper was also the first to report the so called "second hit" hypothesis which postulated that the development of organ dysfunction was primed by the initial pattern of shock and injury but ultimately triggered by the subsequent later onset of sepsis.

Several studies have looked at the changes in the incidence of MOF in an individual trauma system over time. *Nast – Kolb* and co-workers examined a cohort of 1361 polytrauma patients treated in Essen, Germany from 1975 to 1999. [19] There was a relatively constant incidence of MOF (12 to 17%), but a striking reduction in MOF related mortality (73% to 22%) over the 24-year period which the authors attributed to general improvements in the management of critically ill patients over the period. *Probst* and colleagues, [20] studying a similar cohort of patients over a 29 year period from 1975 to 2004 found a similar rate of MOF (14-20%) but noted that the incidence was increasing over time, even as the associated mortality fell (38% to 18%). The largest of these database studies examined data from 31,154 patients recorded on the German National Trauma Network Registry. [21] In this cohort of critically ill patients MOF was reported in 16% and associated with a markedly higher mortality (34% v 7.5%). Risk factors for the development of MOF in this cohort

included older age, more severe injury pattern, the presence of hypotension or acidosis on arrival in hospital and the number of transfused blood products.

There are several issues with these studies. Although most only included patients with significant anatomical injury (typically taken to be an Injury Severity Score (ISS) of > 15) there was still a wide constellation of injury patterns producing heterogeneity in the data. One of the most important aspect of these analyses and comparisons is the inclusion or exclusion of patients with severe Traumatic Brain Injury (TBI). These patients typically have a much more prolonged period of critical care dependency and, often, associated higher organ failure scores. This makes answering the specific question of the relationship between THS and late organ dysfunction more problematic.

Several published studies have focused specifically on the role of THS in the development of MOF. *Minei* and colleagues [22] examined 1002 patients admitted to 7 US trauma centers between 2003 and 2007. All patients had suffered blunt trauma, were shocked (defined as Systolic Blood Pressure < 90 mmHg & Standard Base Excess > -6), and had received blood products. They found an incidence of MOF of 29.4%, higher than those found in the larger cohort studies. Patients who developed MOF were more likely to be older, have more severe injury, have received more crystalloid and blood products and had a higher recorded lactate and base deficit during the first 24 hours following injury, indicating that they had a more significant and persistent perfusion deficit. Interestingly, although patients with MOF had a higher incidence of developing subsequent sepsis, there was no suggestion that sepsis triggered MOF. Indeed, there was no second hit apparent in these patients whose incidence of MOF peaked at Day 3 following injury and was then observed to decline rapidly. This

finding is also reported in more contemporary studies, [23] perhaps suggesting that with modern critical care practice it is predominantly the initial shock insult and patient centric factors that determine the chances of developing MOF with the sepsis related second hit phenomena being relatively rare.

An interesting paper by *Morrison* and colleagues describes a cohort of 296 severely injured UK service personnel with non-compressible torso haemorrhage. The mortality was high with 75% dying before reaching a medical treatment facility and a further 10% dying prior to hospital discharge. [24]The authors report an overall MOF incidence of 4%. However, this equates to 14% of those surviving to hospital admission. A larger cohort study of all 632 UK military personal killed during combat operations in Iraq and Aghanistan between 2003 & 2014 reported that MOF was the cause of death in 2% of the overall total and 15% of those who survived to reach a medical treatment facility. In keeping with most other contemporary studies the authors noted no "late peak" of deaths. [12]

The majority of studies that have included predictive models of MOF have identified that the degree of shock in the early post injury period is an important determinant of later organ dysfunction and infective complications. [25] The single exception is a 2013 study by Dewar and colleagues. This paper found that only admission age and platelet count, as well as 24-hour creatinine and bilirubin levels, predicted the development of MOF. The authors argue that the development of more effective haemostatic resuscitation protocols and advances in targeted fluid resuscitation have produced a situation where even profoundly shocked patients have a good outcome, providing they survive to reach hospital.

A recently published study performed by Brohi's group at the Royal London Hospital examined 595 patients admitted to a single trauma center. [26] The authors divided patients into those with early MODS (ERMODS), which had resolved by day 7 post injury, and prolonged MODS (PRMODS) which persisted after 7 days. MODS was defined as a single SOFA score of > 5. There were clear distinctions between the groups, with PRMODS patients having a higher mortality, ICU length of stay and incidence of infection. Predictive factors for the development of PRMODS were increasing age, higher numbers of administered blood products and worse admission base deficit but, interestingly, not injury severity.

The studies discussed in this chapter use different scoring systems to assess for the presence of MOF. The most common scoring system is the Sequential Organ Failure Assessment (SOFA) score originally developed using a cohort of mostly septic critically ill patients [27] but also validated in a sub set of trauma patients. [28] An alternative eponymous score, has been developed by investigators in Denver specifically to assess for MOF in critically ill trauma patients. [29] The Denver score omits a central nervous system component, acknowledging the difficulty in applying this to an injured cohort, a substantial portion of whom have severe TBI, and also provides a more discriminating model for inotropic support. MOF defined by the Denver score has been shown to be more specific in predicting mortality when compared to SOFA. [30] Full details of the SOFA and Denver scores can be found in the appendices to this work.

In summary MOF following traumatic injury and especially THS remains an important clinical issue affecting approximately a fifth of severe poly-trauma patients and leading to a significant increase in mortality for those affected. Older patients and those with a

heightened injury burden are more affected. The incidence of MOF is high in the early phases following injury and falls abruptly. The previously seen bimodal peak of MOF appears to have disappeared, possibly because of advances in critical care practice leading to a reduction in iatrogenic insults and sepsis related complications. The majority of studies suggest a link between the initial degree of shock and hypo-perfusion and the development of MOF. However, the precise mechanism by which some patients develop MODs following traumatic injury remains to be fully elucidated.

1.2.2 The Inflammatory & Immune Response to Traumatic Injury – the motor of MOF.

Huber –Lang and co-authors described the inflammatory response to major trauma as akin to opening Pandora's box. [31] In Greek mythology Pandora inadvertently opens an ancient box releasing all the evils of the world. In a similar fashion major traumatic injury has the potential to flood the body with a seemingly bewildering array of inflammatory mediators which can lead to extensive detrimental effects; our understanding of this highly complex and pleotropic response to major traumatic injury continues to evolve. This section aims to provide a broad overview of the inflammatory and immune response to trauma and explore some of the theories that have been advanced to explain why some patients develop late organ dysfunction.

It is increasingly clear that the inflammatory / immune response to trauma is triggered very early, within minutes of the initial injury. [32-37] In contrast to sepsis, where inflammatory / immune responses are triggered by exogenous or "non-self" substances, traumatic injury is, at least initially, a sterile process. Despite this, the nature of the observed responses to

major trauma are similar in many ways to those seen in septic patients. The discovery that certain endogenous or "self" substances can, in certain situations, including following traumatic injury, trigger an immune response has improved our understanding of the processes. The "Danger Model"[38] [39,40] of immune activation postulates that cells killed by a number of injurious processes, including mechanical damage and ischaemia release substances termed Alarmins [41] or Damage Associated Molecular Patterns (DAMPS)[33] that activate downstream effectors involved in acute inflammation. Some DAMPS, such as HMGB-1 may also be directly released from cells involved in the immune response, especially monocytes / macrophages. [39]

Effectors of the inflammatory / immune response are legion. [42] At the center of the acute response are cytokines. These substances are released from immunologically potent cells such as macrophages/ monocytes and lymphocytes as well as from endothelial cells and play a crucial role in cell-cell communication. [43] They can be broadly divided into those with pro and anti-inflammatory characteristics. [44] Cytokines are involved in a multitude of actions following traumatic injury including regulation of immune system cells, production of fever, induction of apoptosis and alterations in endothelial permeability and surface characteristics, including expression of molecules responsible for leucocyte adhesion and migration. [35,45,46]

Once activated cellular components of the immune response produce effects, not only at the point of injury, but also at distant sites. Neutrophil adherence to endothelium is facilitated by the up regulation of adhesion molecules both on the surface of the neutrophil and the endothelium. [47,48] Elevated levels of such adhesion molecules correlate with

mortality following traumatic injury. [49] Once activated and extravasated into tissues, neutrophils produce a potent acute inflammatory response through degranulation releasing a host of active substances including proteases, leucotrienes, nitric oxide and pro-inflammatory cytokines. [50]

Although the prime role of the neutrophil is to attack and kill non-self substances, over-activation can lead to extensive tissue injury at sites remote from any immunological threat. Another important consequence of neutrophil activation is the release of reactive oxygen species (ROS).[51] These potent substances cause peroxidation of cell membranes and lead to tissue necrosis. In the context of THS, ROS are also an important contributor to ischaemia – reperfusion injury. This process is initiated by the buildup of hypoxanthine in ischaemic tissue. During tissue re-perfusion hypoxanthine reacts with molecular oxygen to produce superoxide anions ($\cdot O_2^-$).[52] These are further reduced to hydrogen peroxide (H_2O_2) which then reacts with superoxide anions to produce highly potent hydroxyl radicals (OH^-) capable of causing considerable disruption to cellular architecture. [44]

1.2.3 Hypotheses explaining the development of MOF following trauma

Why some patients mount a relatively exaggerated inflammatory response to a traumatic insult and go on to develop persisting organ dysfunction remains to be fully elucidated. Possible explanations have included overproduction of cytokines by macrophages, impaired microcirculatory perfusion, excessive leucocyte endothelial interactions, the role of bacterial translocation from the gut and the two hit theory.[53,54] However, given the complex nature of the immunological and inflammatory response to traumatic injury, such a

reductionist approach is unlikely to provide the full answer and a theory that links multiple causative factors is required.

Writing in 1996, Roger Bone expounded a theory that he termed immunologic dissonance to explain why some patients with an acute inflammatory response went on to develop multiple organ failure whilst others did not. [55] He noted that up to this point, research emphasis had been placed upon the identification and therapeutic targeting of pro-inflammatory mediators. Such trials had not met with success, possibly because of the pleotropic nature of the response to inflammation. However, Bone advanced a theory that patients with acute inflammation were in a constantly changing state of balance (or imbalance) between a pro and anti-inflammatory state. Bone termed this anti-inflammatory response the Compensated Anti-Inflammatory Syndrome (CARS), in contrast to the classically described pro-inflammatory Systemic Inflammatory Response Syndrome (SIRS). The presence of an anti-inflammatory response is an essential requirement to prevent over expression of acute inflammation in response to an insult. However, Bone postulated that overexpression of this response would lead to a state of relative immunodeficiency and potentiate the development of downstream complications, especially sepsis. This approach appeared to synergise with other authors who described MOF as essentially a second hit phenomena, [56] driven by late septic complications. In his original explanation Bone hypothesized that CARS temporally followed SIRS, rather than occurring synchronously. However, as we have already seen there is no convincing evidence for the presence of a late second hit to explain the development of MOF in trauma patients and this casts doubt on the simplicity of the temporal relationship outlined by Bone.

A paradigm shift in our understanding of post injury morbidity was advanced by *Xiao* et al in a seminal paper published in 2011.[36] The investigators studied patterns of genomic expression in the circulating leucocytes of 167 severely injured patients (median ISS 31.3). The results showed that >80% of gene signaling pathways were changed within 4-12 hours of injury. Genes responsible for the innate immune response, (e.g. leucocyte extravasation, toll like receptor signaling and production of both pro and anti-inflammatory cytokines) were up-regulated, whilst those responsible for adaptive immune responses (e.g. T-cell receptor signaling) were typically down regulated. Both of these responses happened in parallel rather than sequentially. Interestingly, and perhaps surprisingly, there was no difference in the nature of the gene pathways changed between patients who went on to make a rapid recovery and those with a more prolonged course, including those with MOF. However, patients with complications had a greater *magnitude* of genomic changes than those without. These findings led the authors to advance a new paradigm for the development of post trauma MOF, where the magnitude, rather than the temporal distribution or nature of both the pro and anti-inflammatory genomic changes were of importance. Interestingly, there was a signal in the data, albeit statistically insignificant, suggesting that the degree of tissue perfusion impairment, as evidenced by worst recorded base deficit, could differentiate between the groups with complicated and uncomplicated recovery.

As we have seen the presence of impaired tissue perfusion has been implicated in the development of post trauma MOF in a number of studies.[21,22,26,57-61] However, current resuscitation paradigms, along with most experimental and clinical studies conducted in this field place an emphasis on pressure based resuscitation targets. The next chapter of this

thesis will explore the rationale for this strategy alongside the argument that pressure based resuscitation may not be the most efficacious way to target traumatic hemorrhagic shock.

1.3 Trauma shock resuscitation; a pressure centric paradigm

1.3.1 Traditional vital signs and shock recognition

Traditionally resuscitation of patients presenting with traumatic haemorrhage has been based on assessment and attempted correction of traditional vital signs such as blood pressure. However the use of intra-arterial pressure to estimate the degree of blood loss is problematic, principally because the baroreceptor reflex effectively maintains blood pressure in the face of significant blood loss, possibly up to 30% of total circulating blood volume. [62] Furthermore, in complex traumatic haemorrhage, as opposed to simple blood loss, nociceptive factors cause an increase in sympathetic outflow leading to increased systemic vascular resistance and blood pressure. [63,64] Several studies have convincingly demonstrated that there is poor correlation between pressure based variables, such as intra- arterial pressure and central venous pressure, and total circulating blood volume. [65-67] Furthermore, experimental models of progressive haemorrhage have demonstrated that tissue perfusion parameters can be markedly reduced even in the presence of a maintained blood pressure. [68]

Traditional vital signs were first used in a formal clinical grading system for trauma shock severity in the ATLS Course in 1978. [69] Although still in widespread use, the ATLS grading system has been widely criticized for not reflecting clinical reality with some studies

suggesting that less than 10% of trauma patients can have their degree of blood loss accurately classified using such an approach. [70] However, a fundamental tenet of the ATLS approach was the suggestion that blood pressure was relatively maintained until eventual profound decompensation occurred after roughly 40% of the circulating blood volume had been lost. Whilst the accuracy of this figure has been challenged, [71] the underlying principal that changes in blood pressure cannot accurately predict the shock state are supported by evidence from several studies. A meta-analysis of studies, including 17 conducted in trauma patients, concluded that no individual traditional vital sign had an acceptable predictive value when estimating the degree of blood loss. [72]

Park and co-workers [73] showed, in a very large registry analysis of over 100,000 trauma patients, that whilst there was a trend to increases in base deficit in patients presenting with worsening hypotension the actual association was weak (r=0.28). Put another way there was a very wide variation in perfusion (measured using base deficit) for any given systolic blood pressure in patients presenting following traumatic haemorrhage. This relationship is shown graphically in Figure 1.8. *Mutschler* et al carried out an analysis of a large number of patients (16,305) from a German trauma registry showing a clear association between admission base deficit and mortality suggesting that such an approach was considerable more predictive of outcome than the use of traditional vital signs. [74]

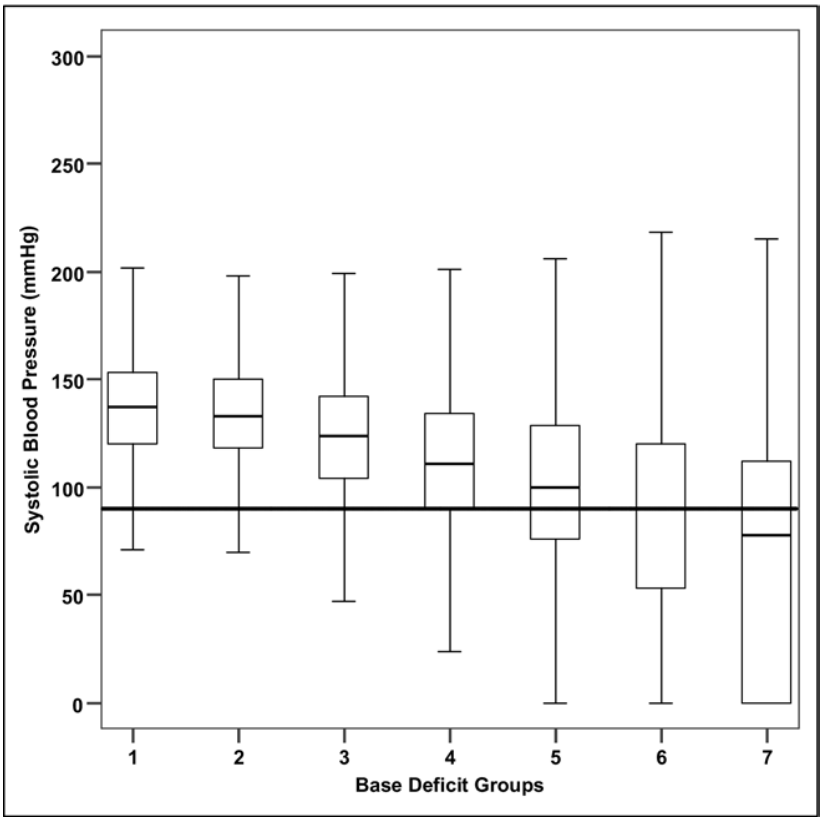

Figure 1.8 Data from a large trauma registry showing the relationship between admission systolic blood pressure (SBP) and base deficit. Box plots represent the median & IQR for base deficit groups. Group 1 represents BXS >0, groups 2-7 progressively negative BXS with each increment representing a change of -5, such that patients in group 7 have a BXS of -30. Taken from [75]

A similar association with plasma lactate has also been described. [76] Occult hypoperfusion, defined in one study as persistent lactataemia (>2.4 mmol/l) in the absence of abnormal traditional vital signs, is associated with worse outcomes following THS [77].

Given these facts it is somewhat surprising that the question of blood pressure still dominates the discussion of treatment strategy for patients with THS in both the pre-hospital and in-hospital phases of resuscitation. An examination of national and international guidelines reveals recommendations on the specific blood pressure to be targeted during THS resuscitation[78,79] with much of the discussion focused on so called permissive or hypotensive resuscitation strategies. To date little clinical research has been conducted on the consequences of these pressure based resuscitation strategies on tissue

perfusion following THS. By contrast, many experimental and clinical studies have been conducted with the aim of answering the question as to the optimal blood pressure target during THS resuscitation.

1.3.2 Animal Experiments examining THS resuscitation strategies

Animal models examining resuscitation strategies following blood loss can be broadly grouped into two types. The first, pioneered by Wiggers in the 1950s [80] used a model of simple haemorrhage caused by controlled blood loss and then resuscitation, either with shed blood or other fluids. This model did not include an actual traumatic injury to the vasculature and thus did not address issues such as local haemostatic mechanisms or the response to tissue trauma. The second broad group uses a haemorrhage produced a standardised injury to a blood vessel or organ. Examples include aortic tears, splenic maceration or hepatic bolt gun injuries. Such injury patterns are, by their nature, uncontrolled and produce ongoing blood loss until local haemostatic mechanisms, or a loss of blood pressure cause the bleeding to stop.

A meta-analysis of animal studies conducted by *Mapstone* and colleagues in 2003 [81] examined nine studies that had investigated the issue of blood pressure targeted resuscitation. Four of these were conducted in a porcine model and the remainder in rats. All studies utilised an uncontrolled model of THS. All of these studies suggested that animals resuscitated to a normotensive target (systolic blood pressure (SBP)> 80mmHg) had a higher incidence of early death (within 2 hours) than those in whom the target was lower (typically 40 mmHg). Hypotheses advanced to explain these results include a reduction in physical clot

disruption associated with a lower intra-vascular pressure as well as a reduction in dilutional coagulopathy associated with the use of lower volumes of crystalloid solutions.

The issues with all these studies is that they have limited clinical translatability. Many do not utilize currently accepted strategies for fluid resuscitation, using a predominantly crystalloid based approach as opposed to blood products. Crucially none of the studies in this meta-analysis assess the longer term implications of hypotensive resuscitation in terms of tissue perfusion and late organ injury.

More recent animal experiments have attempted to address these issues. *Bai* and co-workers [82] subjected a series of 30 piglets to a complex traumatic injury involving femoral fracture, a grade III liver laceration and a small bowel crush injury followed by haemorrhage to a MAP of 40mmHg for one hour. They then conducted a laparotomy with small bowel resection and liver packing in a simulacrum of current damage control surgical techniques. Animals were then kept alive for twenty-four hours and subsequent resuscitation targeted to a MAP of either 60, 80 or 100 mmHg. The low MAP group had significantly worse perfusion (lactate, BXS) and organ function parameters as well as more histological organ damage post mortem. Interestingly the hypotension in this group appeared to result from a low cardiac index, rather than a low systemic vascular resistance. The high MAP group had an initially acceptable profile but after about 10 hours of resuscitation demonstrated a worsening of their lactate and BXS profiles. The authors attributed this to the effects of excessive volume administration. Best parameters were reported in the MAP 80 group. Despite the elegant design, which in many ways reflects current clinical practice this study

still has some issues with clinical translation, principally the use of crystalloids as the predominant resuscitation fluid.

Tao and colleagues [83] used a rat model to investigate the effect of changing MAP targets on survival following splenic injury and uncontrolled haemorrhagic shock. Initial resuscitation conducted using blood and Ringer's solution was targeted to a predetermined MAP of between 40 and 100 mmHg, in 10 mmHg increments. Blood loss and haemodilution progressively increased with each staged rise in blood pressure. A MAP of 50 mmHg was most associated with survival and a MAP of 100 produced the worst outcome. In the second phase of these experiments animals with the same injury profile were resuscitated at a MAP of 50 for either 60, 90 or 120 minutes. Although there was no significant evidence of harm with hypotension for 60 or 90 minutes, animals in the 120 minute group demonstrated increasing evidence of organ injury.

1.3.3 Clinical Studies examining permissive hypotension / pre-hospital fluid

Despite the large amount of interest in the "correct" blood pressure to target during trauma shock resuscitation there are surprisingly few clinical studies on which to draw for an evidence base. Only four studies are reported in the literature and of these only two have attempted to resuscitate to to a blood pressure target, the other two selecting patients to either receive or not receive fluids in the pre- hospital phase.

Bickel et al in a seminal study conducted at a single US trauma center in the early 1990s randomized patients with penetrating truncal trauma to receive either fluid or no fluid prior to operative intervention to control bleeding. [84] Mortality was 30% in the no fluid arm and

38% in the fluid arm. The postulated mechanism was an increase in blood pressure in patients who received fluid causing physical clot disruption and possibly a worsening of trauma induced coagulopathy via dilution with crystalloid fluids. Of note this study was conducted in an urban environment with short (around 30 minute) transit times to definitive care.

Carrick et al [85] conducted a trial examining the effects of intra operative blood pressure targets in patients with uncontrolled haemorrhage requiring laparotomy or thoracotomy. Although intended to include patients with both blunt and penetrating trauma only penetrating trauma patients were ultimately included. The target blood pressures were a MAP of 50 or 65 mmHg. However, there was little eventual difference between the two groups and both had an average MAP of > 65 mmHg during the study period. Unsurprisingly, this study showed no difference in either mortality or any other markers of morbidity. Only young patients were included in this study and those with CNS injury were excluded.

Dutton and co –workers [86] randomized a heterogeneous cohort of blunt and penetrating trauma patients, with at least one recorded episode of systolic hypotension (<90mmHg), to resuscitation targeted at a SBP of 70 or 100 mmHg, achieved through fluid boluses. Around half of the patients were victims of blunt trauma, in contrast to the other studies discussed. Again, the difficulty of actually achieving these targets in clinical practice was highlighted as both groups had average SBP of > 100 mmHg. Unsurprisingly, mortality was identical between the two groups (7.3%) and there were no differences in any other outcome measures.

Finally *Turner* and colleagues[87] conducted a study with the aim of investigating the effect of pre hospital fluid administration on outcome following traumatic injury. This was a large study with whole regions of pre-hospital services randomized *en bloc* to either administer or withhold pre-hospital fluid. Maybe unsurprisingly there were extensive protocol variations; for example, only a third of patients in the fluid arm actually received fluids and 20% of the patients in the no fluid arm had fluid administered. Again, no differences in outcome measures were demonstrated and no clear conclusions can be drawn from this study.

In summary, few clinical studies have been conducted around the question of resuscitation targets in traumatic injury and those that have utilised either no physiological measurable endpoints or a blood pressure target. There have been no interventional studies that have addressed the issue of maintenance of blood *flow* following traumatic haemorrhagic shock.

1.4 The Microcirculation in THS

This chapter has outlined the clinical importance of THS and described the experimental work undertaken in order to try and delineate the optimal resuscitation strategy. The preceding section examined the evidence suggesting that maintenance of blood flow and tissue perfusion are important elements in determining outcome following THS. Successful resuscitation is ultimately dependent on effective delivery of oxygen and substrate at a cellular level. Failure to achieve this results in a persistent oxygen debt and is one possible mechanism for the development of MOF. Although maintenance of cardiac output is a vital pre-requisite for adequate blood flow the final common pathway for substrate and oxygen delivery is the network of small blood vessels collectively termed the microcirculation.

1.4.1 Microcirculatory Structure and Function

Leonardo da Vinci (1452-1519) first described the small micro vessels he termed capillaries; but the discovery that these were the connecting vessels between the arterial and venous circulations, which had eluded William Harvey, was made in 1661 by the Italian anatomist and physicist Marcello Malpighi (1628-1694) using an early microscope. The pioneering Dutch scientist Antonie van Leeuwenhoek (1632-1723), sometimes described as the original microscopist, was the first individual to report the movement of red blood cells through capillaries in 1688. The study of the microcirculation has advanced greatly in the last 50 years, mainly due to developments in intra-vital microscopy and more latterly the development of hand held video microscopes which allow for the in vivo examination of the microcirculation without the use of complex preparations or intra vascular dyes.

The microcirculation is a collective description of the network of blood vessels with a diameter of less than 100 microns and comprises arterioles, capillaries and venules. Those vessels under 20 microns, principally representing capillaries and small venules / arterioles, are of most interest as they represent the key location of substrate transfer and because the small luminal diameter makes this a critical site for flow limitation during shock states.

The overall structure of a typical microcirculatory bed is shown in Figure 1.9. Inflow occurs through a branching system of arterioles from first generation (A1) to third generation (A3). [88,89] In terms of total surface area the capillaries are the most numerous vessel within the microcirculation and despite lacking the ability to constrict and dilate are far from passive tubular structures. Both the intrinsic and intravascular morphotic elements of capillaries play crucial roles in the regulation of blood flow through the microcirculation. Blood returns from the microcirculation via increasing sizes of venule.

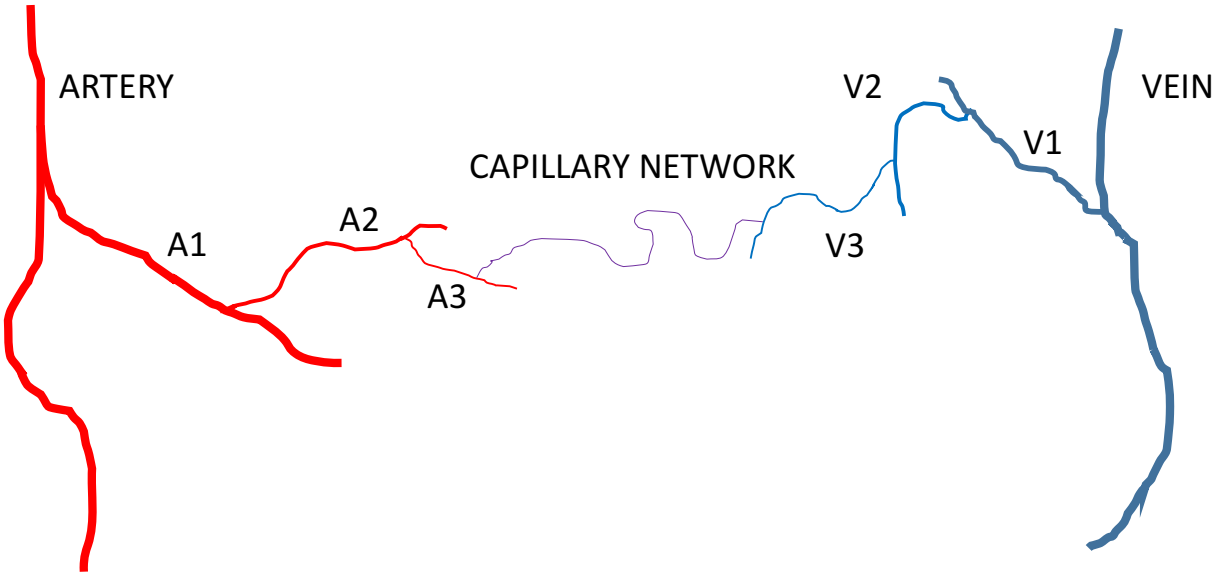

Figure 1.9 Schematic of generic microcirculatory network. A1 - A3 decreasing order arterioles. V3-V1 increasing order venules.

Although it is a gross simplification, given the heterogeneity found within the microcirculation, to ascribe importance to flow through a single capillary such a theoretical consideration can explain the mechanisms responsible for overall microcirculatory flow. The hydrodynamics of tubular flow were first described by the French physiologist Jean Poiseuille (1797-1869). His law, jointly credited to the German engineer Gotthilf Hagen (1797-1884), describes laminar flow through a rigid tubular structure.

$$Q = \frac{\pi r^4 \Delta P}{8 \mu L}$$

Equation 1.1 Hagen-Pouiselle equation. Q flow, r radius, ΔP pressure gradient between arteriolar and venous systems, μ fluid viscosity, L tubular length.

However, it is important to note that capillaries are not simple tubular structures and the above equation represents a simplification of flow dynamics within the microcirculation. [90] A feature of particular importance are the influence of the pressure gradient between the arteriolar and venular sides of the capillary, a reduction in ΔP may be brought about by a decrease in inflow pressure or an increase in outflow (venous) pressure. Also of note is the critical effect of reduction of the radius of an already small tubular structure by any pathological changes, such as that caused by endothelial cell swelling. It should be noted that although most of the general principles are still applicable, flow in capillaries does not adhere to the rules governing standard Newtonian fluid mechanics. [91] The viscosity of blood, for example has a disproportionately significant impact on flow within capillaries and this is principally determined by the haematocrit, which is substantially lower in the capillaries than in the systemic circulation. In small micro-vessels red blood cells flow in a laminar column in the center of the vessel with a cell free layer at the edges. This phenomena, termed the Fahraeus – Lindqvuist effect, [92] effectively decreases the blood viscosity at the vessel walls to close to that of plasma. Loss of this laminar flow stream can cause a local increase in viscosity which may reduce flow. [93] It is important to note, however, that micro-vessels are not inert tubes and local increases in endothelial wall stress can lead to an increase in flow by a process of mechano-transduction secondary to Nitric

Oxide (NO) release. [94 95] For these reasons, changes in fluid viscosity within micro-vessels can have complex and unpredictable effects on flow rates.

Hypoxic vasodilation and hyperoxic vasoconstriction are important factors in regulating microcirculatory blood flow. In an experimental model conducted by placing rabbit muscle in an isolated sealed environment, in which the oxygen tension could be changed, *Lindbom* and colleagues demonstrated that increasing oxygen tensions caused falls in functional capillary density and red cell velocity [96]. Hyperoxic vasoconstriction and hypoxic vasodilation in the microvessels is complex and mediated by the interplay of host of factors from both the endothelium and the vascular smooth muscle.[97]

1.4.2 Effect of haemorrhage on the microcirculation

A graphical representation of a capillary unit in a healthy state and after changes induced by THS is shown in Figure 1.10.

The initial impact of haemorrhage is vasoconstriction mediated through sympathetic nervous system activation. Vasoconstriction within the microcirculation occurs almost exclusively at the level of first order (A1) arterioles [98-100] and is, at least initially, the prime driver for the reduction in flow seen within the microcirculation. Changes in tone in lower order arterioles and venules appear to make a negligible impact. [101] The degree of vasoconstriction varies between different organ beds with some circulations, especially the splanchnic, particularly vulnerable to post-haemorrhagic vasoconstriction. [102,103]

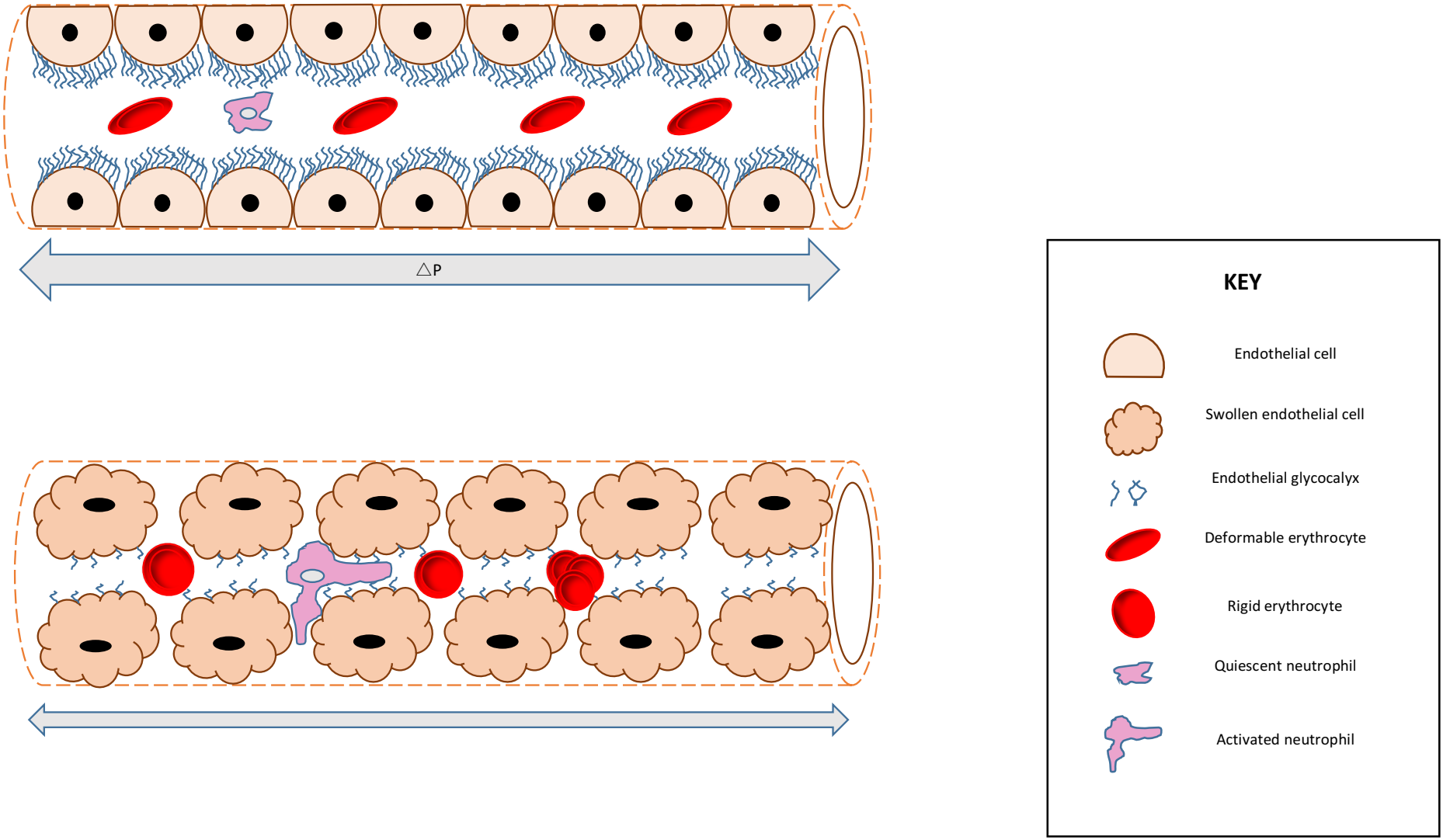

Figure 1.10 Schematic of capillary in steady state and following changes induced by traumatic hemorrhagic shock

Lack of perfusion and oxygen delivery to endothelial cells causes a switch to anaerobic metabolism and cell swelling, in part mediated through the Na^+/H^+ co-transporter. [104] Even a small increase in endothelial cell size can cause significant reduction in luminal diameter. [105]

The vascular endothelium is lined by a layer of proteoglycans and glycosaminolgycans termed the endothelial glyococalyx (EG). [106] The EG performs essential barrier functions and acts as a protective cage around a variety of surface molecules involved in leucocyte adhesion and thrombogenesis. [107,108] Animal studies have shown that THS results in substantial loss of the EG. [109,110]

Intravascular thrombosis and leucocyte adhesion, precipitated, in part, by loss of the EG may be a significant cause of microcirculatory flow reduction. [111] Shortly following THS leucocytes undergo a dramatic configurational transformation developing projecting pseudopods which facilitates adhesion to the endothelium. [112] This process causes physical "clogging" of the capillary and consequent microcirculatory flow reduction.

Erythrocytes play a key role in regulating microcirculatory blood flow. Conditions of local hypoxaemia result in the release of NO and ATP from erythrocytes leading to local vasodilatation. [113,114] Erythrocytes are typically larger (at 7μm) than the capillaries through which they pass (average diameter 4-5μm) and the ability to deform under pressure is therefore critical to avoid flow obstruction. Healthy erythrocytes have this ability but studies conducted on rats subjected to THS have demonstrated that post THS erythrocytes re-infused into healthy animals caused microcirculatory flow reduction. [115] Additionally,

banked and especially aged erythrocytes may lack deformability [116] and this is an important consideration in the context of a massive transfusion scenario.

Ultimately all of these factors can lead to a reduction in the number of the perfused capillary units within a microcirculatory bed, a factor which experimental evidence suggests may be an important determinant of outcome following hemorrhagic shock. In an important study *Kerger et al* [117] took hamsters and subjected them to a prolonged period (4 hours) of systemic hypotension before resuscitation with shed blood. Microcirculatory flow was assessed using intra-vital microscopy and animals were classified at 24 hours post resuscitation as survivors with good or poor outcome dependent on behavioral patterns. Interestingly there were significant difference in the functional capillary density (FCD) during the shock and resuscitation phases both between non-survivors and survivors but also between those with good and bad behavioral outcomes. This, despite the fact that the pattern of blood loss and resuscitation was uniform. The authors also noted that the diameter of the A1 arterioles was significantly smaller in those animals with poor outcomes and those who died, suggesting that the reduction in Functional Capillary Density (FCD) may be, at least in part, secondary to excessive vasoconstriction at the pre-capillary level.

1.4.3 The concept of haemodynamic coherence

Although there have been many studies examining the microcirculation in the context of sepsis there are far fewer exploring the effects of haemorrhage and traumatic injury. One key issue is the relationship, or degree of coherence, [118] between the systemic (macro) circulation and the microcirculation. If this relationship is intact then a strategy that targets

systemic flow (but possibly not pressure) could reasonably be expected to result in improvements at the microcirculatory level. However, if there is a loss of coherence between the two circulations then "optimization" of the macrocirculation may not lead to improvements in tissue perfusion and could potentially cause harm through over administration of fluid or vasoactive medications. [119] The phenomena of circulatory incoherence has been well documented in both experimental and clinical investigations of severe sepsis, [120-122] but the nature of the relationship during and after haemorrhage is less clear.

Dubin et al [123] reported the microcirculatory and macrocirculatory effects of blood loss in an ovine model. Using SDF videomicroscopy they demonstrated that there was coherence between systemic measures of blood flow (cardiac output) and microcirculatory measurements. The study terminated at the end of blood loss so the effects of any subsequent resuscitation were not addressed.

Van Iterson et al [124] studied the effects of microcirculatory oxygen delivery using a phosphoresence technique in a porcine model of blood loss. They found that haemodynamic coherence was preserved in both the mesenteric and coronary circulations.

Maier et al [125] investigated the effects of 3 different resuscitation fluids on the microcirculation using multi modal monitoring, including sublingual SDF videomicroscopy finding that haemodynamic coherence was maintained during shock and resuscitation.

Fang et al [126] examined the microcirculation using OPS imaging in a rat model of shock where groups included animals exposed to a septic insult as well as those subjected to blood loss. The investigators demonstrated that whilst haemodynamic coherence was lost in the septic rats it was preserved in the haemorrhage group.

A minority of studies show a loss of haemodynamic coherence during experimental blood loss. *Cryer* et al [127] demonstrated in a rat model of haemorrhage that whilst cardiac index returned to baseline following transfusion. Microcirculatory flow in muscle, gut and kidney (measured using IVM) remained suppressed. As we have seen previously *Kerger* et al [117] demonstrated that a subset of animals able to return to baseline FCD following resuscitation had better outcomes. Although not measuring systemic flow, these investigators did observe that all animals returned to baseline levels of blood pressure following transfusion. The conclusion here seems to be that restoring a normal blood pressure may not equate to a restoration in microcirculatory flow.

To date there has only been one clinical study examining the effects of traumatic haemorrhage on the microcirculation. *Tachon* et al [128] studied the sublingual microcirculation in critically ill trauma patients for 4 days following injury. In contrast to the majority of animal experimental work they found very significant microcirculatory impairment immediately following resuscitation that persisted throughout the entire study period. In contrast, cardiac output was not significantly impaired. The authors concluded that haemodynamic coherence may be lost following THS but that confirmatory studies were required to support or refute these findings.

1.4.4 Effect of differing resuscitation fluids on the microcirculation

A recent systematic review examined 71 pre-clinical studies to determine fluid characteristics affecting flow through the microcirculation. [129] 9 of these studies were performed in large animals and 62 in rodents. Only 5 studies utilised Dark Field video-microscopy with the majority using intra-vital microscopy, most commonly in a skin fold window. The studies included in this meta-analysis showed considerable heterogeneity, both in terms of the assessed microcirculatory endpoints as well as the amount of induced haemorrhage. No studies included a complex insult involving tissue trauma.

Fluids with relatively high viscosity and oncotic potential might be expected to improve microcirculatory haemodynamics through an increase in shear stress and local mechanotransduction leading to an up regulation in inducible nitric oxide[130] or by more efficacious expansion of the vascular compartment. However, the classic explanation for fluid retention in the vascular space has been questioned in the light of new evidence highlighting the importance of the endotheial glycocalyx [131] and it should be acknowledged that although fluids with high oncotic pressures have theoretical benefits in the context of volume expansion there is no convincing evidence of benefit in the clinical setting. Of the 19 studies included in the meta-analysis that examined the effects of differing oncotic pressure, 14 found that fluids with higher oncotic pressures had a greater effect on the measured microcirculatory endpoints.[100,102,127,132-142] 4 studies reported that the type of fluid had no effect on microcirculatory flow endpoints. [143,144] Of the 12 studies that examined the question of whether fluids with higher viscosity were more efficacious at

improving microcirculatory flow during haemorrhage resuscitation, 10 supported this hypothesis[145-153] whilst 2 reported no difference. [154,155]

There are a limited number of experimental studies that directly compare blood products with other resuscitation fluids, such as crystalloids or colloids, on microcirculatory flow in the context of hemorrhagic shock resuscitation. This is an area of obvious importance, given the logistic constraints inherent in the use of blood products, particularly in the early phases of resuscitation. *Casali* et al [156] reported that whole blood was more effective at restoring mesocaecal microcirculatory flow than either hypertonic saline and lactated Ringers in a rat model of controlled haemorrhage. *Kao* et al [157] demonstrated that blood was more effective than saline in restoring both blood pressure and microcirculatory flow in the gut and skeletal muscle of haemorrhaged rats. This improved macro and microcirculatory flow also resulted in a higher clearance of lactate. *Sakai* et al [158] demonstrated the superiority of shed whole blood over albumin in restoring microcirculatory flow in a hamster skin fold IVM chamber model.

In a recent study *Tanaki* et al [159] reported the effects of transfusion of a single unit of packed red blood cells on microcirculatory variables in patients within 12 hours of resuscitation from hemorrhagic shock. The results appeared to demonstrate that all microcirculatory variables were improved following transfusion. Macro haemodynamic parameters did not show significant changes in this small (n=15) cohort of patients. The authors postulate that this is further evidence of haemodynamic dissociation in the context of hemorrhagic shock. This study stands in contrast to previous work conducted by Weinberg and colleagues [160] where the investigators failed to show any change in

microcirculatory parameters following blood transfusion. Notably, this cohort of patient were haemodynamically stable with no evidence of hypoperfusion and minimal evidence of microcirculatory hypoperfusion at baseline. The difference between these two studies may lend credence to the theory, initially outlined by Christian Boerma and colleagues [161] that the baseline state of the microcirculation may aid in predicting patients for whom fluid expansion may prove beneficial.

1.5 Haemoglobin based oxygen carriers and the microcirculation

Transfusion of stored allogenic blood products is the current standard clinical treatment for traumatic haemorrhage. However, this is associated with risks including the potential for infectious agent transmission and immunomodulation. [162] The logistic difficulties and costs of blood storage, delivery and transfusion are considerable and this is magnified in austere and pre-hospital settings. In addition, and despite widespread adoption into practice by military healthcare providers, there is no convincing evidence of benefit associated with the administration of blood products in the pre-hospital environment. [163] These factors have led to considerable interest in the pursuit of a synthetic blood substitute.

Early attempts to transfuse cell free haemoglobin were unsuccessful and associated with significant adverse reactions characterized by acute renal failure [164]. Some of these early adverse reactions were caused by the presence of residual cellular stromal elements which provoked an endotoxic reaction [165]. Creation of stroma-free haemoglobin solutions was a first step in solving these initial problems but work to modify the structure of the haemoglobin molecule was needed to resolve other adverse reactions.

The 3-D structure of haemoglobin, first described by Max Perutz in 1953, is a tetramer of four protein subunits. [166] Extra cellular tetrameric and dimeric forms of haemeoglobin exist in equilibrium but the dimeric form has a short intra vascular half-life. [167] Delivery of large amounts of dimeric free haemoglobin to the renal tubules causes direct nephrotoxicity and clinical signs similar to those seen in an acute transfusion reaction. Increasing the effective size of the free haemoglobin units reduces the risk of these adverse effects and can be accomplished by crosslinking or polymerization to create chains of free haemoglobin molecules with significantly longer vascular retention. [168] Creation of these larger synthetic

and stroma free haemoglobin units greatly reduced the incidence of renal failure but a persisting problem caused by all early haemoglobin based oxygen carriers (HBOC) was vasoconstriction and associated hypertension.

Haemoglobin binds rapidly and irreversibly to nitric oxide (NO) to form methaemoglobin and nitrate compounds. [169] Such reactions are normally limited by diffusion barriers to NO found within the RBC membrane and by the fact that RBC flow in a laminar column separated from the vascular endothelium by the endothelial glycocalyx. However, the much smaller native free haemoglobin molecules react with NO in and around the endothelial layer approximately 600 times more avidly than when constrained within RBCs. [170] Scavenging of NO produces potent smooth muscle constriction and this has been shown to produce vasospasm. [171] Arteriolar vasoconstriction and consequent reduction in FCD has been reported in a number of HBOC experiments [172] [173,174] and has been primarily thought to result from NO scavenging.

Although NO scavenging is an important cause of vasoconstriction two other factors related to HBOC administration also play an important role. As previously stated oxygen partial pressure in arteriolar blood is important in the modulation of vasomotor tone with hypoxia producing local arteriolar dilatation. Conversely, local hyperoxia can cause vasoconstriction and a reduction in microcirculatory blood flow. [96] Early HBOCs were engineered to have low oxygen affinity to facilitate off-loading of oxygen in the tissues. However, the resultant increase in local oxygen concentrations and subsequent vasoconstriction can lead to a reduction in capillary blood flow and hence tissue perfusion and oxygenation. Finally, as previously mentioned, higher viscosity fluids have been shown to improve microcirculatory

flow, through a local mechanotransduction feedback mechanism. Early HBOCs had a low viscosity and may have attenuated flow through the microcirculation.

1.5.1 Malemide PEG Haemoglobin (MP4)

Advances in bioengineering has provided solutions to the early problems associated with HBOC administration. Modifications have been made to haemoglobin molecules to produce changes in the molecular size, viscosity and oxygen affinity. One of the more recently developed HBOCs is Malemide PEG Hb (MP4 *Hemospan*, Sangart Corporation, San Diego, California, USA). MP4 is produced by coupling six strands of maleimide activated polyethylene glycol (PEG) to purified human haemoglobin tetramers creating a molecule substantially larger than native haemoglobin (90 vs 60 kDa). When formulated in lactated Ringers solution it has a higher oncotic pressure than blood (70 v 27 mmHg) and a viscosity of 2.2 cPs (1.0 cPs = water, 4 cPs = whole blood). Crucially, the oxygen affinity of MP4 is substantially higher than native haemoglobin (p50 MP4 5.4 mmHg v p50 Hb 15 mmHg). [175] These physicochemical properties produce a molecule that is retained in the circulation, rather than diffusing into the endothelial / interstitial junction and which releases oxygen at the capillary rather than the arteriolar level. This ability to target capillary oxygen delivery was demonstrated in a hamster model of severe haemodilution where MP4 treated animals exhibited 70% of oxygen delivery at the capillary level in contrast to 30% of oxygen delivered at this level in animals treated with a polymerized bovine HBOC with a p50 of 54mmHg. [175] These factors may explain why MP4 does not appear to be associated with the vasoconstriction and hypertension observed with many other HBOC compounds. MP4 is presented in solution with an absolute haemoglobin concentration of 4.2 g/dl, substantially

lower than that of whole blood. MP4 can be prepared under oxygenated conditions in which case it is referred to as MP4OX.

1.5.2 Pre clinical studies involving MP4

Drobin et al conducted the first large animal study investigating the effects of MP4 administration on systemic pressure and flow parameters as well as metabolic markers of tissue perfusion. [176] Pigs were subjected to controlled blood loss prior to resuscitation with one of five tests fluids: 10% Pentastarch (PS), 4% MP4 in Ringer's acetate, 4% MP4 in 5% PS, 2% MP4 in 5% PS and stroma-free haemoglobin (SFH) in Ringer's acetate. Resuscitation was exclusively provided with the test fluid, rather than shed blood. Animals treated with SFH demonstrated markedly higher systemic vascular resistance and lower cardiac output than animals treated with the other fluids. Although there were no differences in haemodynamic parameters or markers of perfusion between the other groups, none of the MP4 treated animals showed evidence of vasoconstriction. The microcirculation was not specifically assessed in this experimental model.

In a study conducted by the same research group *Young* et al report the effects of 4 different initial resuscitation fluids, including MP4 on 20 hour survival in a swine model of uncontrolled haemorrhage.[177] Following 15 minutes of uncontrolled blood loss from an aortic tear, animals received 250 ml of either Ringer's acetate, 10% Pentastarch, 4 g/dl stroma free haemoglobin solution or MP4. On completion of this initial resuscitation fluid, shed blood was re-transfused followed by 0.9% saline to achieve set haemodynamic goals. Survival was significantly higher in the MP4 treated animals compared to those who

received starch, or stroma free haemoglobin and there was a trend to improved survival against crystalloid treated animals (p = 0.06). Systemic flow and pressure parameters were better in the MP4 group when compared to animals initially treated with crystalloid. Although there was no direct assessment of the microcirculation made in this study it was important in establishing that small volume MP4 administration appears free of significant hypertensive adverse events. Furthermore, the study design mirrors current clinical trauma resuscitation practice where the initial resuscitation fluid may be a crystalloid or colloid prior to resuscitation with blood products.

In a follow up study using the same model of uncontrolled blood loss in swine the same group investigated the effects of resuscitation using one of three fluids: MP4 in isolation, Ringer's acetate or Ringer's acetate plus shed autologous blood. [178] Again, there were no significant vasoconstriction effects observed, although the MP4 treated group did have the highest initial SVR and lowest cardiac outputs during initial resuscitation. Although all three treatment groups had similar lactate clearance profiles, the MP4 group received significantly less fluid (788 ml) to achieve this when compared to the blood & saline group (2507 ml) and the exclusive saline group (5161 ml).

Young et al investigated the effects of MP4 on volume expansion in rats, demonstrating that MP4 was more efficacious at producing an increase in plasma volume than two hydroxyethyl starch solutions. [179] In the second part of the experiment the investigators performed a controlled haemorrhage of 60% of blood volume on the previously haemodiluted animals and reported that animals haemodiluted with MP4 had a significantly reduced rate of lactate rise and improved survival profile at 2 hours after haemorrhage.

It should be noted that all of the experimental studies described were conducted by researchers funded by the manufacturer. Furthermore, none of these experimental studies specifically examined a microcirculatory endpoint.

1.5.3 Clinical Trials involving MP4

There have been several Phase I and II studies [180,181] undertaken to examine the safety profile of MP4 in patients undergoing elective orthopaedic surgery. In a phase 1b/2 study conducted by *Olofsson* et al, 20 patients received differing doses of MP4 immediately after the administration of spinal anaesthesia for joint arthroplasty surgery.[182] There were no serious adverse events and no difference in adverse events noted between the patients receiving MP4 and controls. The investigators studied the levels of plasma free haemoglobin and found a dose dependent relationship and an exponential decay curve. Patients who received the maximum dose of MP4 (1000 ml of 4.2% solution) had peak recorded levels of 13 g/dl of free Hb with a half life of 23 hours. Cardiovascular profiles were similar between MP4 patients and controls.

A multi center international randomized control trial investigating the use of MP4 as a peri-operative volume expander in patients undergoing hip arthroplasty was undertaken in 2007/8 and recruited 405 patients [183]. The findings show that MP4 treated patients had a significantly higher blood pressure following spinal anaesthesia and significantly reduced incidence of hypotensive episodes requiring intervention. The safety profile of MP4 appeared acceptable but there were a higher number of gastro-intestinal adverse events in the MP4 arm, potentially related to the effects of NO scavenging on smooth muscle relaxation in the gut.

These clinical studies, though interesting, are not directly relevant to the use of MP4 as a treatment for traumatic hemorrhagic shock, which is the main concern of this thesis. A multi- center study was undertaken between 2010 and 2012 investigating the use of MP4 in this context (Clinical trials ID NCT01262196). Inclusion criteria for this study were traumatic blood loss and an elevated lactate (>5 mmol/l). The study intervention was the administration of either 250 ml of MP4 or 0.9% saline (placebo). Unfortunately, the results of this study have not yet been reported.

1.6 Summary of introduction and basis for studies

Despite improvements in management traumatic injury remains associated with significant morbidity and mortality. Trauma associated haemorrhage is the leading cause of preventable death and is an important factor in the development of post injury sequelae, such as organ dysfunction.

Post traumatic multiple organ dysfunction remains an important clinical problem affecting up to 25% of survivors of major trauma. Although the precise mechanisms have not yet been fully elucidated there does appear to be an association with the state of tissue perfusion during the early phases of traumatic haemorrhagic shock. Estimation of the degree of tissue perfusion remains problematic and the commonly used methods, such as lactate and base deficit, are surrogate markers. There appears to be little relationship between the so called traditional vital signs, especially arterial pressure, and tissue perfusion in the context of traumatic hemorrhagic shock.

Five hypotheses are examined in this thesis:

Hypothesis One

An in vivo real-time measure of tissue perfusion can provide a more sensitive end point of resuscitation than arterial pressure with respect to both early markers of shock state reversal (e.g. lactate & base deficit) and late indices such as the development of organ dysfunction.

Blood flow can be assessed using global, whole body, approaches such as cardiac output estimation or regional, organ, or tissue, specific approaches. The final common pathway for

blood flow and oxygen and substrate delivery is the microcirculatory beds of end organs. The question of whether global and local blood flow are linked or coherent remains incompletely answered but probably depends on the pathophysiological disorder and specific region in question. The degree of haemodynamic coherence may also change over time. This may be particularly important within the microcirculation where pathophysiological changes involving endothelial dysfunction, loss of endothelial glycocalyx, leucocyte / endothelial interactions and erythrocyte dysfunction are potentially important contributors to flow impairment.

Hypothesis Two

The state of the sublingual microcirculation represents a more sensitive resuscitation endpoint than the global cardiac output for both early and late indices of resuscitation success.

Hypothesis Three

Haemodynamic coherence between macrocirculatory and microcirculatory flow is maintained during the initial stages of THS resuscitation, but is lost in the later stages (hours and days following initial resuscitation) as changes in the microcirculation produce local flow impairment.

The choice of resuscitation fluid is important in the context of THS resuscitation but experimental studies directly comparing the effects of blood products, haemoglobin based oxygen carriers and crystalloid fluids on microcirculatory perfusion are rare. Experimental evidence suggests that fluids with higher viscosity and oncotic potential are more effective

at resuscitating the microcirculation and that some haemoglobin based oxygen carriers may be associated with vasoconstriction. The HBOC MP4OX has been specifically developed to avoid vasoconstriction by reducing oxygen offloading at the arteriolar level and increasing the molecular size to restrict diffusion of NO scavenging free haemoglobin into the endothelial region. However, no studies have examined the specific issue of the relative microcirculatory effects of HBOCs, blood products and crystalloid fluids in an experimental model of THS or assessed the effects of MP4OX on in vivo microcirculatory parameters.

Hypothesis Four

Blood products are more effective at restoring microcirculatory perfusion during THS resuscitation than crystalloid fluids.

Hypothesis Five

MP4OX restores microcirculatory perfusion during THS resuscitation without inducing vasoconstriction.

1.7 Overall Aims of Thesis

1. To assess the relationship between systemic haemodynamic variables and microcirculatory perfusion in a porcine experimental model of traumatic haemorrhagic shock and resuscitation.

2. To assess the effects of different resuscitation fluids (blood products, MP4OX and 0.9% saline) on microcirculatory perfusion in a porcine experimental model of traumatic hemorrhagic shock and resuscitation.

3. To assess the relationship between microcirculatory dysfunction and the development of trauma induced coagulopathy in a porcine experimental model of traumatic haemorrhagic shock.

4. To assess the relationship between systemic haemodynamic variables and microcirculatory perfusion in a cohort of patients during and after traumatic haemorrhagic shock resuscitation.

5. To assess the impact of microcirculatory perfusion on the development of multiple organ dysfunction in a cohort of patients with traumatic hemorrhagic shock.

6. To explore the mechanisms behind the development of microcirculatory dysfunction in a cohort of patients with traumatic hemorrhagic shock.

7. To validate a new hand-held video microscope, for in vivo assessment of the microcirculation, against a precursor device.

Chapter 2

General Methods

2.1 Assessment of the Microcirculation

In general assessment of the microcirculation can be thought of as relying on two distinct approaches. The first makes an assessment of the end results of microcirculatory impairment, be that substrate or oxygen transport and exchange or markers of inflammation or coagulation. When applied to a specific target area such as the gastric [184] or sublingual [185 186] mucosa or the brain [187] such an approach can be of value. However, more global surrogates of tissue perfusion, such as lactate and base deficit estimation are less useful as pure markers of microcirculatory dysfunction. This is, in part, because they make no assessment as to whether impaired tissue perfusion is a result of impairment of global, regional or microcirculatory blood flow.

Lactate concentration is often used in clinical practice to inform decisions on tissue perfusion and there is evidence linking persistently elevated lactate levels to poor outcome in shock states, including THS. [188] However, there are several factors that reduce the utility of lactate assessment alone in assessing the state of the microcirculation. Beta adrenergic stimulation can increase the lactate concentration even in the absence of tissue hypo-perfusion, patients with high blood alcohol levels have altered lactate kinetics, as do those with acute liver injury. However, the main issue with lactate as a therapeutic target is the lag time between changes in tissue perfusion and a response in the observed lactate

concentration. Essentially lactate clearance is an indication of the effectiveness of previous therapy rather than an assessment of tissue perfusion at a discrete time point.

The second broad method of assessing the microcirculation relies on direct assessment of micro vessel flow and density. Here the endpoints are not markers of function, but rather measures that reflect the physical state of red cell flux within the microcirculation as well as an estimation of the density of these vessels. Laser Doppler flowmetry is one example of a technology that can assess the former but not the later. [189] Although it has advantages in providing an objective value for red cell flux it is limited by the fact that it samples a relatively large area and may be poor at detecting heterogeneity within the microcirculation. The studies included in this thesis utilised two methods of assessing the microcirculation, Sidestream or Incident Dark Field video microscopy (SDF/IDF) and, to a much lesser extent, Near Infra-Red Spectroscopy (NIRS).

2.2 Dark Field Video Microscopy

Traditionally assessment of the microcirculation in experimental models relied on the use of intra vital microscopy. This technique required trans-illumination of the target tissue and therefore the creation of a tissue window, typically a skin fold; often fluorescent dyes were also required. These preconditions effectively limited the use of intra vital microscopy for examination of the microcirculation to small animal experimental models.

In 1971 Sherman [190] described a method of tissue microscopy which utilised refracted incident, rather than trans-illuminated, light to outline a target tissue. This technique was a development of a principal widely in use in metallurgical microscopy. Although Sherman

reported success in producing images from a wide range of solid organs the technique did not achieve widespread adoption; direct imaging of the microcirculation remained confined to those vascular beds that were superficial and highly accessible, such as conjunctiva and nail folds.

1999 saw the dawn of the current era of in vivo video microscopy with the advent of Orthogonal Polarization Spectral (OPS) imaging. [191] Relying on refracted light, rather than trans-illumination, the technology was, crucially, small enough to be incorporated in a hand-held camera and for the first time this opened up the possibility of visualizing the microcirculation in a clinical setting. Validation studies demonstrated not only comparable optical characteristics when compared to intra vital microscopy but also well matched readings for relevant indices such as FCD. [191] A series of studies conducted using OPS imaging showed that microcirculatory parameters were important in determining outcome in patients with severe sepsis. [120,192,193] Despite these successes, there were disadvantages with OPS imaging; a mains power source was required to produce the degree of light intensity required and images of moving objects, such as erythrocytes, suffered from significant blurring. Researchers continued to seek refinements in the technology that would enable higher quality images to be produced and in 2007 a group at the Academic Medical Center, University of Amsterdam reported on a device that utilised Sidestream Dark Field (SDF) imaging. [194]

SDF imaging relies on incidental illumination provided by a ring of circumferential Light Emiting Diodes (LEDs) to illuminate a target tissue. The use of LEDs rather than higher intensity polarized light means that the device can be battery operated and liberates the

user from the requirement to provide mains power. The light produced by the LEDs is green with a wavelength of 530 nm, chosen as this wavelength of light is an isobestic point for haemoglobin (i.e. completely absorbed by both the oxygenated and deoxygenated forms). Thus, haemoglobin containing erythrocytes appear black on a background greyscale image, essentially highlighting blood vessels, assuming that they contain at least some erythrocytes. Light from the LEDs is absorbed by the target tissue but the central core of the microscope is optically isolated from this light and returning light is channeled through this core via an objective lens. The camera itself is analogue and a digital signal converter is required to generate files that can be viewed on a computer. Focusing is performed by a manual mechanism. A schematic of SDF imaging is shown in Figure 2.1. SDF imaging was validated against the precursor OPS technology using healthy volunteer nail fold capillary diameter estimation as well as an assessment of venular and capillary image sharpness. Vessel diameters were comparable between devices but the SDF device produced superior capillary image sharpness. [194]

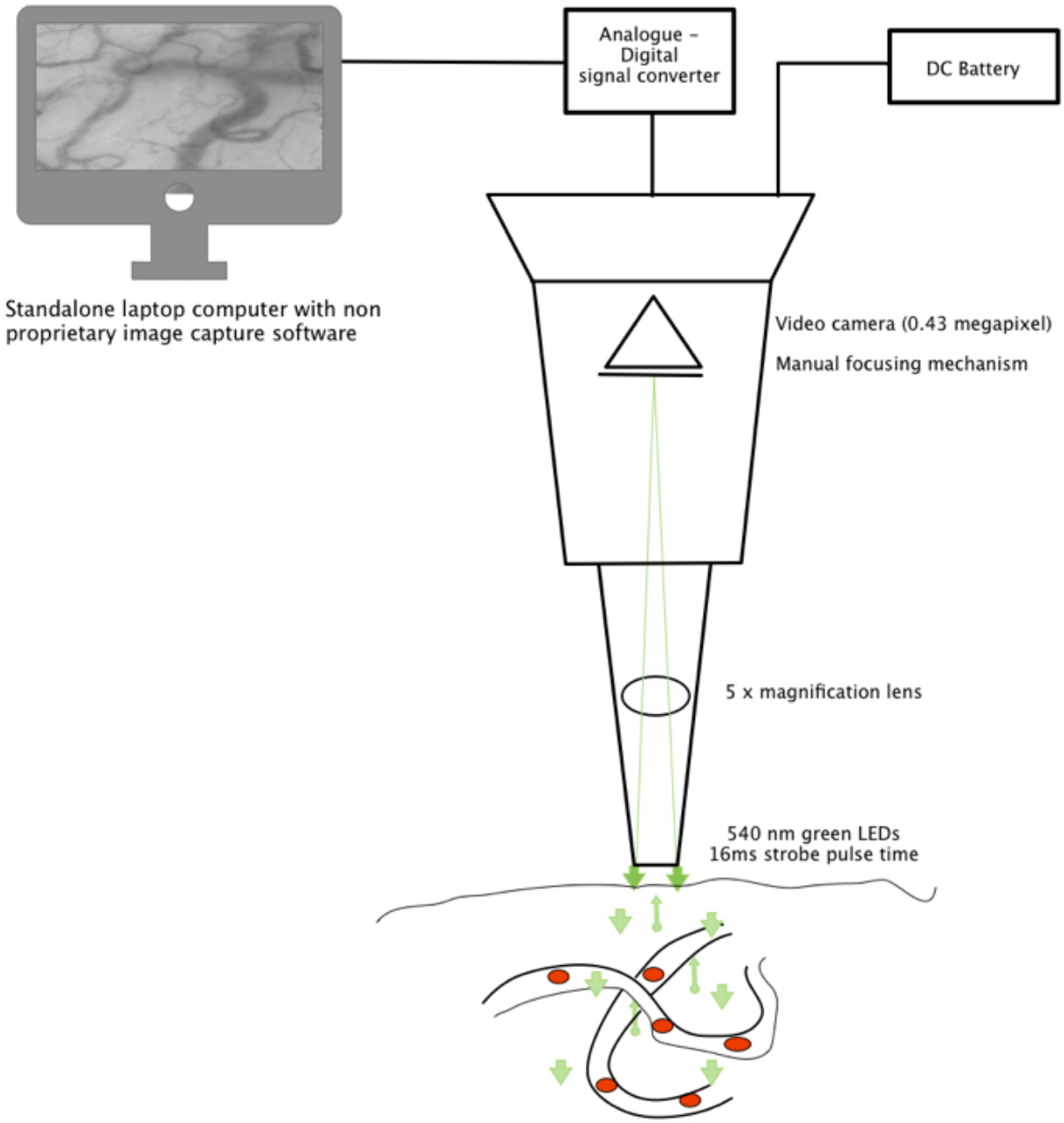

Figure 2.1 Schematic of SDF imaging using the Microscan device (Microvision Medical, Amsterdam, NL)

The most recent advance in microcirculatory handheld video microscopy is the introduction of the Cytocam (Braedius Medical, Huizen, NL). The Cytocam is conceptually similar to devices using SDF technology but there are several important differences. [195]

The device is lighter, making it easier to handle and possibly reducing some of the pressure artifact associated with SDF imaging. LED strobe pulse time is significantly shorter than with previous devices, potentially producing a sharper image. The field of view is significantly larger than previous devices, facilitating easier vessel recognition and image capture. Finally, the device is connected to a dedicated panel PC via a single cable, eliminating much of the supporting equipment required by previous devices and facilitating use in a clinical environment. A schematic of IDF imaging is shown in Figure 2.2.

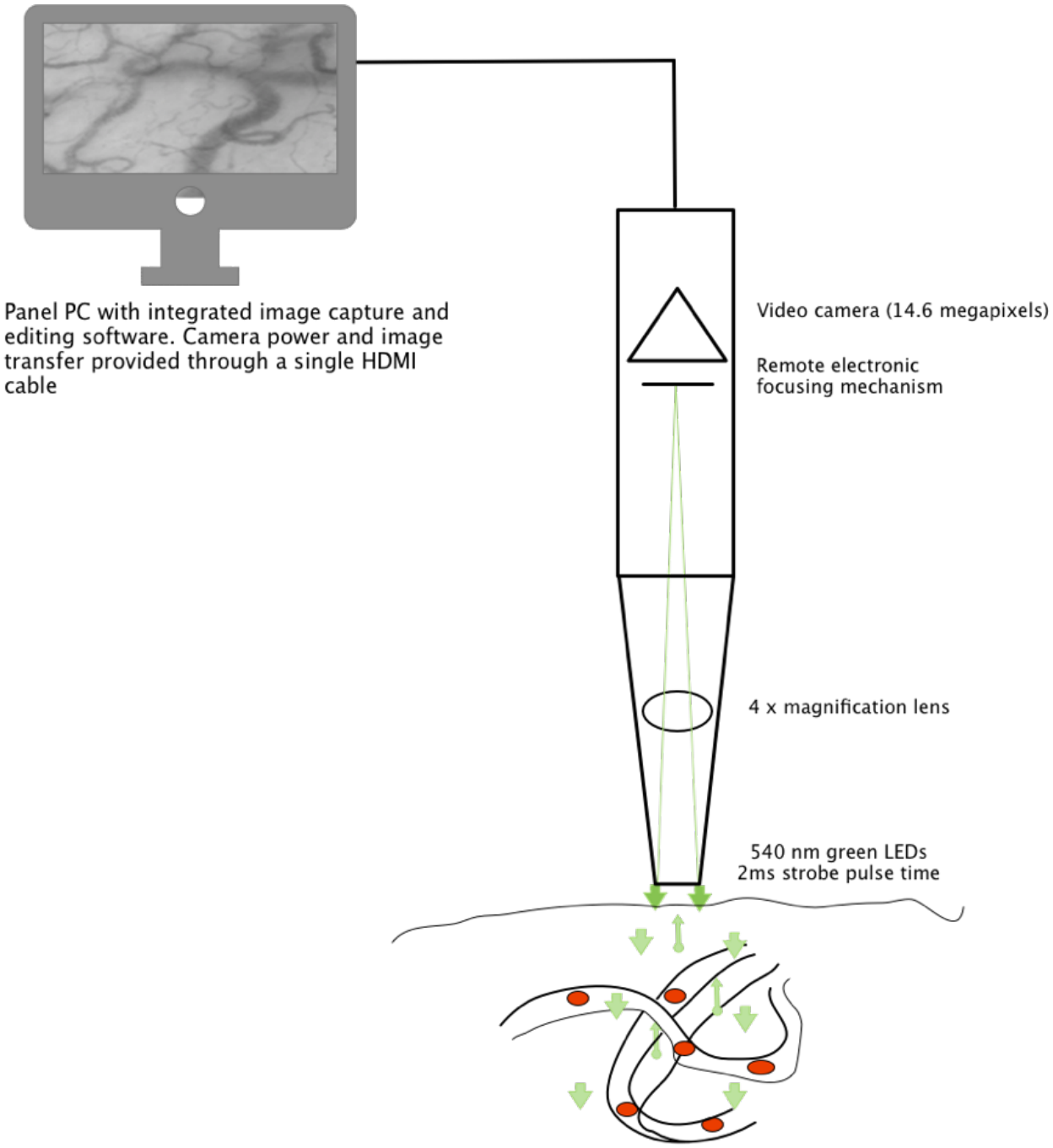

Figure 2.2 Schematic of IDF imaging using Braedius Cytocam hand held video microscope (Braedius Medical, Huizen, NL)

2.2.1 Image Acquisition

IDF videomicroscopy was used as the main method of assessing the microcirculation in both the animal experimental work and clinical studies reported in this thesis.

Microcirculatory images were obtained using a Cytocam video microscope (Braedius Medical, Amsterdam, NL) with the exception of some the images used for validation of the Cytocam which were obtained using a Microscan device (Microvision Medical, Amsterdam, NL). Full details of image acquisition with the Microscan are provided in Chapter 3. Image capture was carried out using Cytocam Tools v. 1.7.2 (Braedius Medical, Huizen, NL)

Videos were obtained from the sublingual area of both human and porcine subjects. Following suctioning of the oropharynx, a gauze swab was used to gently remove saliva from the mucosal surface. The camera probe was applied to the sublingual area and images were selected, taking care to exclude areas of buccal microcirculation with large numbers of looped vessels. The device was focused until individual erythrocytes could be visualized within capillaries. Brightness was adjusted to produce an acceptable degree of contrast between blood vessels and background tissue. At all times pressure artifact was scrupulously avoided by applying only the minimal amount of pressure necessary to obtain an image. Furthermore, flow was observed in larger venules and any stopped or reversed flow was taken to be a sign of pressure artifact, necessitating adjustment of the camera or selection of a different area of the microcirculation for observation. A minimum of three video images of the sublingual microcirculation were taken at each experimental time point

but five images was the preferred number. Each recorded clip consisted of 100 video frames at a rate of 20 frames per second.

Images were principally recorded by one investigator (SH). Other investigators recorded some porcine and human video sequences after training in the technique by SH. Given the heterogeneous nature of the microcirculation, both across time and within the same microcirculatory bed at each time point, it would be impossible to rate for inter-observer variation. Rather, all video sequences were rated for quality and subsequently analysed by one investigator (SH).

2.2.2 Image quality and analysis

Image quality is of vital importance prior to video sequence analysis and the guidance laid down by Massey [196] was used to determine which videos to reject prior to analysis. Of note however, is the fact that this guidance was written with the SDF Microscan device in mind. The lengthy video sequences of up to 20 seconds advocated by Massey were not required when using the Cytocam and other issues such as poor focus, illumination and stabilization were also uncommon findings. The overwhelming reason to reject videos was the presence of pressure artifact. For brevity, formal image quality scores for the over 1000 video sequences analysed as part of this thesis are not presented here.

Images obtained using SDF / IDF video microscopy must be interpreted before they can yield useful data. Broad consensus exists as to the variables that should be reported [197] and these were followed for the experimental work reported on in this thesis. Although the Cytocam

device is bundled with automated analysis software, Capillary Network Analysis, this has been shown to be unreliable, especially in terms of flow parameters. [198] An existing, proven method of vessel analysis, Automated Vascular Analysis (AVA) version 3.02 (Microvision Medical, Amsterdam, NL) was therefore used to analyse the video sequences.

The analysis method was as follows:

1. Prior to export from Cytocam Tools software video sequences were assessed for quality; videos with significant quality issues were not selected for analysis.
2. Video sequences were exported from Cytocam Tools using the AVA export function. At export files were assigned a random 5-digit number using Random Number Generator for iOS (Bice Applications). Videos were thus de-identified and subsequent analysis was blinded in terms of both subject and time point.
3. Video sequences were imported into AVA and stabilised.
4. All vessels less than 20 μm in diameter were traced by hand using the manual draw function as illustrated in Figure 2.3.
5. Each vessel segment was assigned a flow score of between 0 and 3 based on the Microvascular Flow Index score original described by Boerma: [199]
    - 0: No Flow (no movement of Red Blood Cells (RBC) in this vessel segment over the period of the video)
    - 1: Intermittent Flow (taken to be stop and start movement of RBC over the period of the video)
    - 2: Sluggish Flow (taken to be continuously forward movement of RBC but at a slower speed than normal)

- 3: Continuous Flow (continuous forward flow of RBC at normal speed for the duration of the video sequence)
- Each quadrant of the image was also assigned a flow score using the same method, but reflecting the overall flow for all vessels in the quadrant

6. Whole image analysis was run using AVA producing values for:
    a. Total Vessel Density (TVD): the length of vessel segments relative to the overall image field
    b. Perfused Vessel Density (PVD): the length of vessel segments with an assigned flow score of 2 or 3 relative to the overall image field
    c. Microvascular Flow Index (Quadrant) (MFI_q): the mean value for MFI from the four quadrants of the video image
    d. Microvascular Flow Index (Vessels) (MFI_v): the mean value of all assigned vessel segment MFI scores across the entire video image
    e. Proportion of Perfused Vessels (PPV): the percentage of vessel segments with an assigned MFI of 2 or 3
    f. Microcirculation Flow Heterogeneity Index (MHI) [120] calculated for each time point as follows:

$$\frac{MFIq\ (\max) - \ MFIq(\min)}{MFIq(mean)}$$

There are several approaches used for calculating MFI but taking the average of all the vessels (MFI_v) in a particular video sequence, despite being more time consuming than the quadrant method (MFI_q), appears to be better correlated with other parameters of microcirculatory flow such as the proportion of perfused vessels. [200]

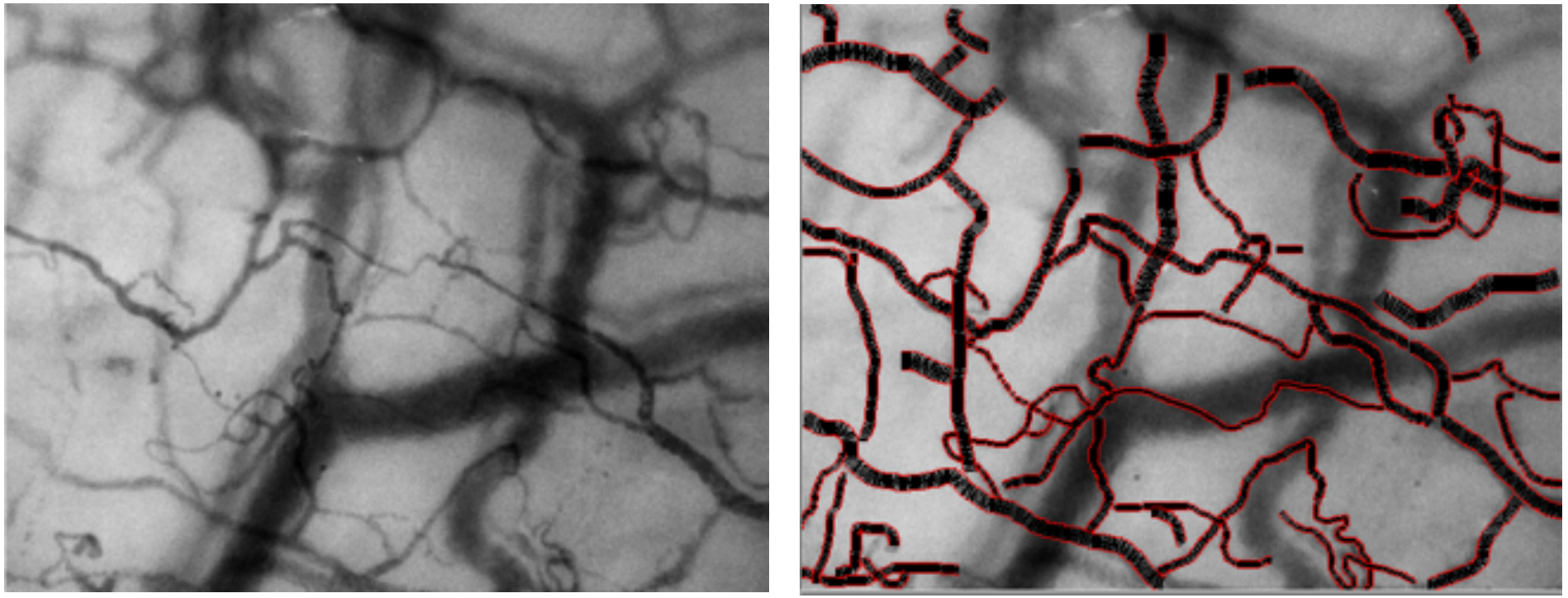

Figure 2.3 Single frame image of sublingual microcirculation obtained using the Cytocam video microscope. All vessels less than 20μm in diameter have been traced by hand using AVA software

2.2.3 Critical appraisal of the use of hand held video microscopy for microcirculatory assessment

Regional variations in the microcirculation

A key question relating to the use of sublingual video microscopy is to what extent the state of the sublingual microcirculation is reflective of the microcirculation in other organs. The sublingual site is commonly chosen as it is easy to access and the microcirculatory architecture is amenable to analysis using existing software. Blood supply to the sublingual area is plentiful and derived from the external carotid artery. The sublingual region therefore probably represents a central circulation that should, in theory, be relatively preserved during shock states. However, the embryological derivation of the sublingual region is from the gut and this poses the question as to whether there may be a relationship between splanchnic and sublingual blood flow. It is often difficult to directly compare the microcirculatory architecture of the sublingual region with other organs, which in reality limits any comparisons to flow rather than density parameters. These flow scores, such as MFI, are inherently subjective, further compounding the problem.

Several animal studies have addressed the question of correlation between the sublingual and splanchnic microcirculations using SDF imaging. Results are contradictory and should also be interpreted with caution given the potential differences between species. *Verdant* et al induced cholangitis in pigs and measured jejunal and sub lingual blood flow using OPS imaging during shock showing a good correlation between the two circulations. [201] However other studies in pigs [202,203] showed a lack of precise correlation, although the trends in the

observed changes was similar. In contrast *Dubin* et al [204] reported that whilst both sublingual and ileal perfusion fell following endotoxic shock, fluid resuscitation fully restored sublingual perfusion, but a persisting deficit in villous perfusion was observed.

Only two studies in patients have used SDF imaging to examine regional differences in the microcirculation, both contrasted the differences between the sublingual and splanchnic regions. *Boerma* et al examined patients with sepsis resulting from faecal peritonitis who had a stoma formed during emergency abdominal surgery. [205] There was initially no correlation between gut and sublingual microcirculations in terms of absolute values but the trend over time was consistent between the two sites. Furthermore, microcirculatory flow was consistently lower in the gut. *Edul* et al also studied a similar cohort of patients and showed a lack of coherence between gut and sublingual microcirculations, again gut microcirculatory flow was consistently lower than that of the sublingual area. [206] Interestingly, changes in the gut microcirculation, but not the sublingual microcirculation, were able to predict mortality. This is in contrast to other studies in septic patients where sublingual microcirculatory changes were predictive of outcome. [207-209]

The studies discussed all relate to either septic patients or animals and not those with traumatic hemorrhagic shock. This is potentially a major limitation in drawing conclusions since there is a suggestion that animals with septic shock display a greater loss of haemodynamic coherence and greater heterogeneity of flow than those with THS. [126] It may be reasonable to suppose that this heterogeneity could also be applied to differences between regional microcirculatory beds as well as within individual beds. Another limitation is that the studies discussed so far have only assessed differences between the sublingual

and splanchnic beds. There is no data relating to the correlation between the sublingual and other important microcirculatory beds, such as the kidney.

Overall from the available evidence it seems reasonable to conclude that:

I. Flow in the sublingual microcirculation is higher than that in the splanchnic circulation during periods of reduced systemic flow.
II. There is no precise correlation of flow parameters between the sublingual and splanchnic circulations during induced septic shock but the dynamic trends within these circulations are comparable.

The sublingual circulation probably does, therefore, represent a central, preserved circulation, at least compared to the gut. Whilst changes in the sublingual microcirculation may be less pronounced than changes elsewhere the evidence points to it being an easily accessible and reasonable marker of whole body tissue perfusion with impairment correlating with outcome. Any impairment in microcirculatory flow in the sublingual area should alert the clinician to the potential of more severe changes in other circulations.

Subjective nature of the analytical process

Another criticism of the use of video-microscopy to assess the microcirculation is that the resulting data contains a considerable subjective component. Of the two broad elements that describe the microcirculation, density and flow, only density is currently objectively measurable using video-microscopy. Tracing of vessels by hand, ideally by a single operator, is still the gold standard method for producing density values and one that was used for all the experiments within this thesis.

Measurement of flow using video microscopy is more problematic. The consensus criteria for examining the microcirculation recommend a subjective approach to assessing flow in individual vessel segments and this has been adopted for the present experiments. Both AVA and the software bundled within the Cytocam purport to be able to measure flow using a technique that assesses the movement of red blood cells by changes in pixel intensity along the center line of a blood vessel. However, this method has not been shown to correlate with subjective flow values (MFI) as observed by the human eye [198] and the development of a truly objective measure of directly observed flow through microcirculatory vessels is still elusive.

The subjective nature of current flow analysis methods opens up the potential for bias if the researcher is aware of the experimental time point or nature of any interventions undertaken. For this reason, all video sequences recorded as part of this thesis were de-identified prior to analysis.

Issues relating to video acquisition

A significant issue with the use of video-microscopy for the assessment of tissue perfusion is that the technique is intermittent rather than continuous and therefore is reliant on frequent operator dependent interventions in order to produce clinically relevant information. A system that provided continuous tracking of tissue perfusion and oxygenation such as sublingual capnometry or near infra-red spectroscopy would have more utility in this regard but these techniques rely on averaging a signal from a wide range of microcirculatory vessels and in the process of averaging this data information on flow

heterogeneity may be lost. However, there is evidence that sublingual capnometry may track changes in organ blood flow during hemorrhagic shock [210] and the development of clinically accessible devices may allow adoption of this technology in the future.

Another important issue is the question of image quality during video acquisition. Given that subsequent analysis depends on high quality images it is vital that only such videos are recorded. A large study, conducted by *Damiani* et al, of over 2000 individual images collected from 100 patients using the SDF Microscan device showed that only 56% of videos met acceptable quality criteria with only 27% being rated as excellent. [211] Interestingly, poor quality video images were associated with worse values of both vessel density and flow. The authors also noted that good quality images were more likely in those who were intubated and heavily sedated and that the use of handheld video-microscopy in non-intubated patients with a reduced level of consciousness was associated with lower quality images. Finally, images collected by the experienced senior investigator were significantly more likely to be of high quality when compared to other investigators. These findings highlight the vital importance of ensuring good image quality and appropriate patient selection. In the clinical study reported in this thesis the inclusion criteria specifically called for intubated patients, partly because it was recognized that image acquisition was easier in this group. Although images were collected by several investigators every image was reviewed for quality by the principal investigator (SH).

2.3 Near Infra-Red Spectroscopy

Near-Infrared Spectroscopy (NIRS) is a non-invasive technique for examining tissue oxygenation that has been used in a range of critically ill and injured patients. [212,213] In

principal the technique relies on the differential absorbtion of infrared light by oxygen carrying compounds. In contrast to visible light, light waves in the near- infrared spectrum (700-1100nm) have reasonable tissue penetration of up to several cm and can therefore penetrate the immediate subcutaneous tissues, and even bone, to pass through structures such as skeletal muscle and brain tissue. Compounds that have variable NIRS absorbance characteristics dependent on the degree of oxygen binding include haemoglobin, myoglobin and cytochrome oxidase. Of these cytochrome oxidase is of a level of magnitude lower than the other compounds and its effect is probably negligible. Myoglobin is potentially more important when using NIRS to assess skeletal muscle oxygenation. Myoglobin possesses a much higher affinity for oxygen than haemoglobin and will therefore remain saturated until tissue oxygen tensions become extremely low. Although haemoglobin concentrations in skeletal muscle are considerable higher than myoglobin, the effect of the myoglobin on NIRS signal and interpretation is not clear with some investigators describing its effect as negligible [214] whilst others have suggested that it may constitute up to 60% of the NIRS signal. [215]

NIRS may be utilised in two broad ways. The first is to continuously record the trend in the values from a particular site in order to attempt to detect an imbalance in tissue oxygen supply and demand. [216] The second method is to occlude the local circulation using a tourniquet induced vascular occlusion test (VOT) and to assess the rate of change of tissue oxygen saturation (StO2), a steeper rate indicating more efficient micro vessel response to local hypoxia. [217,218]

Cohn et al applied NIRS within 6 hours of injury to 383 severely injured patients and continuously recorded the StO2 signal for the first 24 hours. They found that the lowest recorded StO2 predicted death or development of MODS with a reasonable degree of accuracy but that the test was essentially equivalent to highest base deficit or lowest systolic blood pressure. *Moore* et al used a multivariate logistic regression model to identify risk factors for MODS in 114 patients who received a massive transfusion following trauma. StO2 was the only indicator capable of accurately predicting the risk of MODS in this model. [219] *Duret* et al applied both static NIRS and a dynamic VOT to the thenar eminence of 54 patients with THS. The baseline StO2 reading at 6 hours after injury, as well as the gradient of the VOT downslope, both predicted organ failure, defined as an improvement of the SOFA score at day 3 post injury. [220]

NIRS was used to assess hind limb muscle StO2 during the animal experimental work reported in this thesis.

2.4 Assessment of Coagulation using thromboelastography

Thrombolelastography, a technique used to assess coagulation based on the viscoelastic properties of clotting blood, was first described by Hartert in 1948, with the first clinical uses in cardiac surgery and orthotopic liver transplantation [221]. The technology was commercialized as TEG® by the Haemoscope corporation.

The principal steps of a TEG® assay are as follows. Whole blood is placed in a cup and heated to 37^{o}C. A pin, suspended on a torsion wire is placed into the cup and remains static, whilst the cup is rotated clockwise and anticlockwise (Figure 2.4). As the blood clots and fibrin stands form between the cup and the pin the amount of torque reduces. The degree of torque generated by the wire is transduced and displayed producing a characteristic waveform. Viscoelastic tests are commonly conducted in the presence of a contact initiator of coagulation; for the experiments conducted as part of this thesis dilute Innovin was used for this purpose. [222]

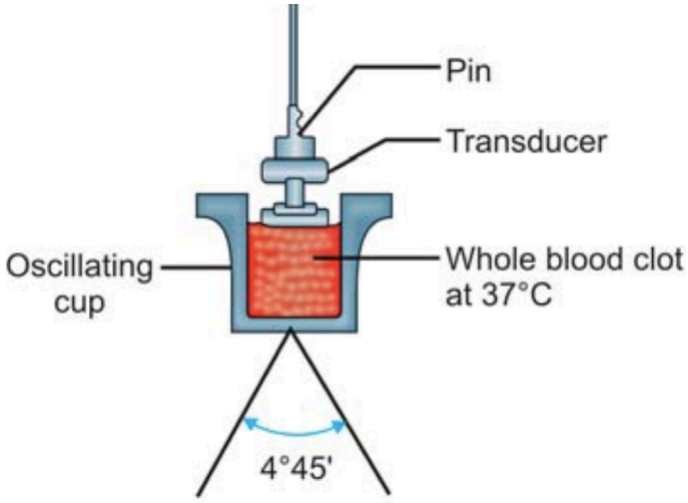

Figure 2.4 Thromboelastograph (TEG®) (Haemoscope Corp, Niles,IL, USA). The essential principle is that as blood clots it changes the torque on a rotating pin in cup. The transduced output is related to the strength of the clot.

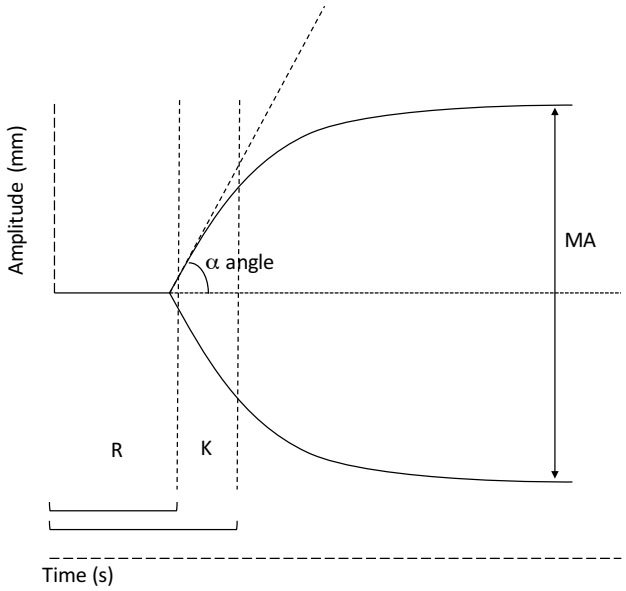

Figure 2.5 TEG® trace. MA, maximum amplitude; R, R time; K, K time. Explanation given in text.

The characteristics of this waveform provide information on the character of the clot produced as shown in Figure 2.5.

- R (Reaction) Time - time to reach 2 mm amplitude. An increased R time indicates a deficiency of thrombin generation which may be caused by a lack of clotting factors, anticoagulant drugs or an intrinsic pathological activation of anticoagulant factors such as activated Protein C
- K Time – time elapsed between 2 mm and 20 mm of amplitude
- α angle – tangent angle between the divergence of the two lines on the trace. Both the α angle and the K time relate to the speed of clot formation
- Maximum Amplitude (MA) – the peak amplitude reached, dependent on factors involved in initial clot generation but also on platelet function and fibrinogen levels

During the animal experiments that form part of this thesis thromoboelastography was performed on fresh un-citrated whole blood drawn from the femoral cannula of a porcine subject and analyzed immediately using 1:50,887 dilution Dade Innovin (Dade Behring, marketed by System UK Ltd, Milton Keynes, UK) as the initiator. All analyses were performed in triplicate at 37^{o}C.

2.5 Assessment of Cardiac Output and Intra Vascular Volume

2.5.1 Oesophageal Doppler

Stroke volume may be calculated by an assessment of the velocity of blood in the descending aorta using a Doppler based measurement. To utilize this technique an oesophageal Doppler probe (ODM Cardio Q, Deltex Medical, Chichester UK) was placed in the oesophagus and rotated until a characteristic trace was identified (Figure 2.6). The device automatically calculates the velocity time integer (VTi) of the Doppler signal, which it labels as the flow time. Derivation of the stroke volume from this value requires calculation of the cross-sectional area of the aorta at the point of the measurement. Rather than perform a direct measurement, the ODM device uses a nomogram to produce this value which relies on information relating to the patients age, gender, height and weight. Although this introduces potential inaccuracy into the measurement, validation studies have indicated an acceptable degree of agreement with other measures of cardiac output. [223] The ODM device is minimally invasive with a very low risk of complications. However, one disadvantage is the fact that it is only tolerated in sedated patients.

The ODM device was used to measure cardiac output in patients enrolled in the clinical study reported in this thesis.

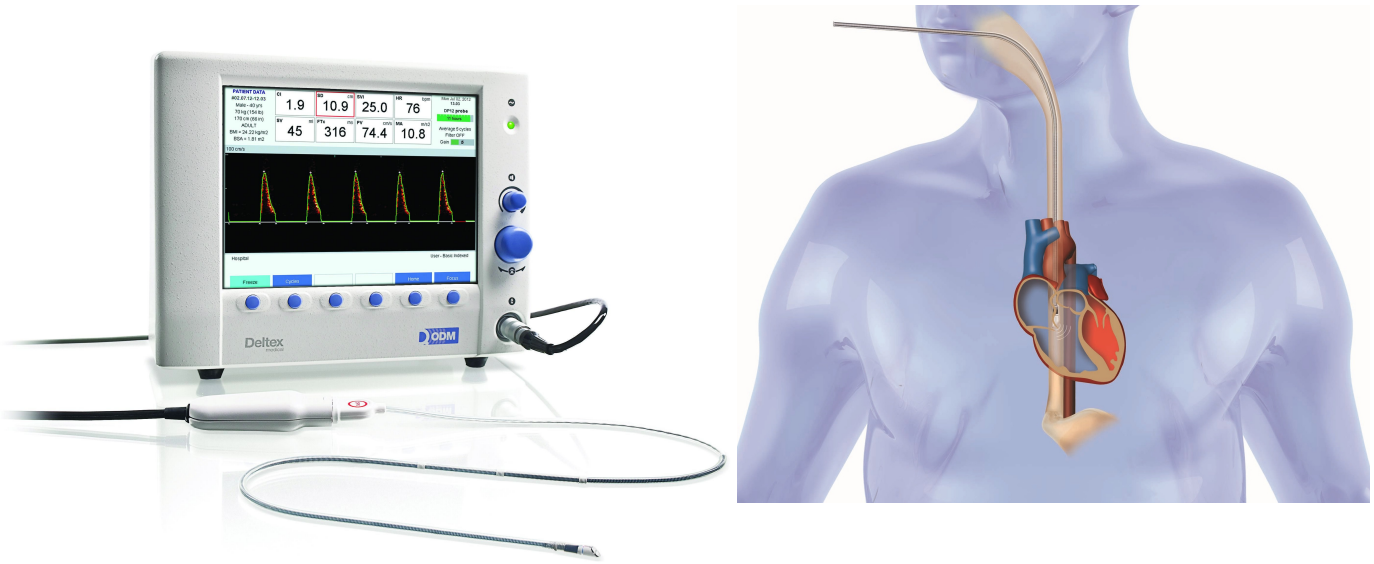

Figure 2.6 Oesophageal Doppler Monitor (ODM) (Deltex Medical, Chichester, UK). A probe inserted into the oesophagus is rotated until a characteristic aortic waveform is produced. Velocity is converted into flow using a nomogram based on patient height, weight, age and gender.

2.5.2 Echocardiography

Focused Trans Thoracic Echocardiography (TTE) was used as a technique to assess intra vascular volume status during the clinical study reported in this thesis. The method chosen utilized the fact that the inferior vena cava (IVC) undergoes a dynamic change during respiration. In patients receiving positive pressure mechanical ventilation the IVC expands during inspiration and collapses during expiration, whilst during spontaneous breathing the reverse situation occurs. Furthermore during spontaneous negative pressure respiration the magnitude of IVC collapse is usually greater and the reproducibility of the measurement more difficult to assure due to changes in tidal volume with each breath [224]. For this reason, the mode of ventilation was recorded at the time of measurement.

All measurements were obtained using a Sparq ultrasound machine with an S4-2 cardiac sector probe (Philips Healthcare, Guildford, UK).

The IVC was visualized in the subcostal long axis window. An M mode image was obtained, ideally at 90 degrees to the longitudinal axis, 2cm distal to the right atrial / IVC junction. Measurements were taken across a full respiratory cycle and the minimum and maximum values were determined. IVC collapsibility index (CI) was calculated using the following formula:

$$CI = \frac{\left(IVC\ diameter\ (\max) - IVC\ diameter\ (\min)\right)}{IVC\ diameter\ (\max)}$$

In line with the best available evidence an IVC CI of greater than 40% in spontaneously breathing patients (including those receiving pressure support ventilation) [225] or greater than 20% in those receiving mandatory positive pressure ventilation [226] was taken to be an indicator of potential hypovolaemia. An M Mode ultrasound image of the IVC from one of the patients enrolled in the MICROSHOCK study is shown in Figure 2.7.

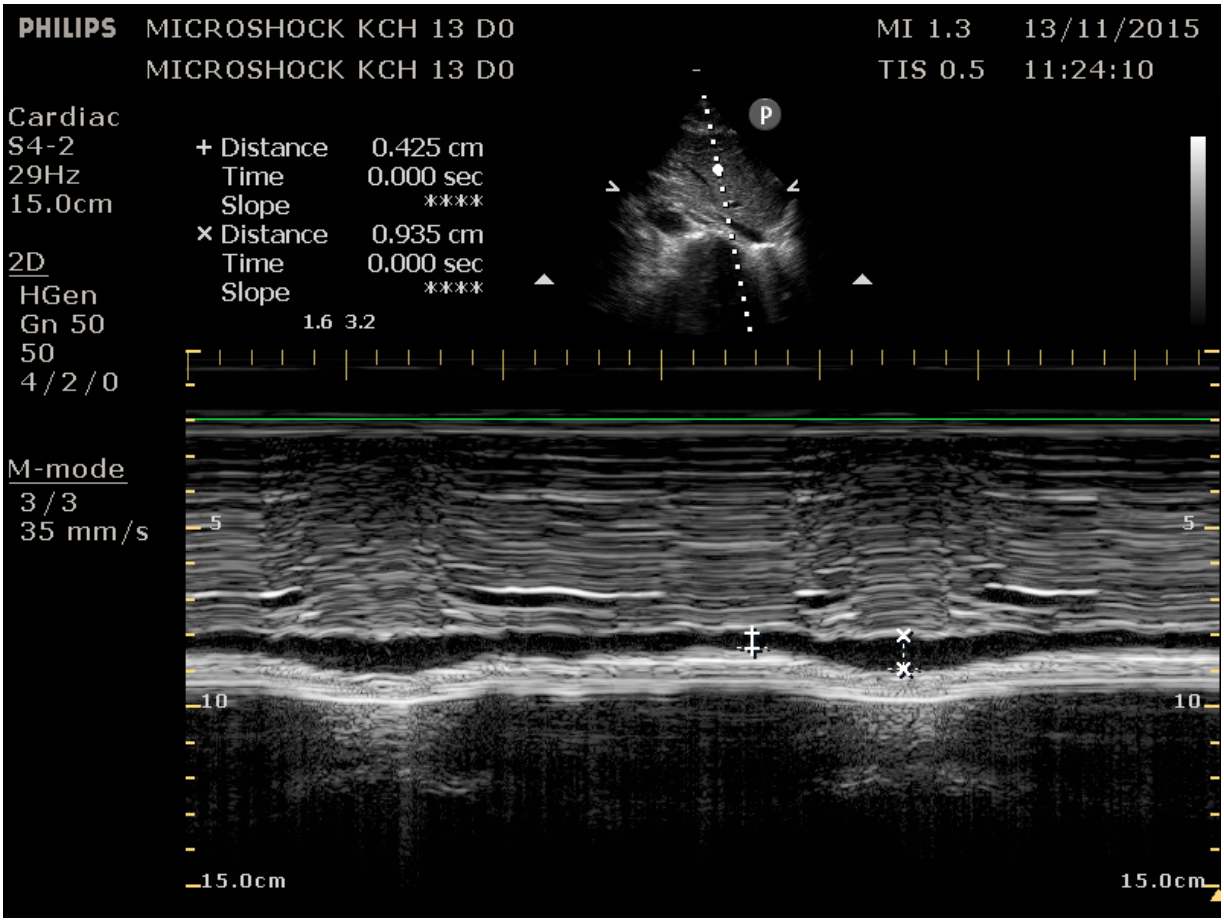

Figure 2.7 M mode image of the inferior vena cava in a patient receiving mechanical ventilation. There is dynamic insufflation and collapse of the IVC with respiration. The collapsibility index is 55% indicating potential hypovolaemia

2.6 Assessment of plasma markers of inflammation and endothelial activation

Assessment of several biomarkers of inflammation and endothelial activation / damage were conducted on plasma samples obtained from participants enrolled in the clinical study reported in this thesis; inclusion criteria and further details are given in Chapter 6. Blood samples were collected in EDTA tubes. As soon as possible following collection samples were centrifuged at 10,000 rpm for 10 minutes and multiple aliquots of plasma prepared and frozen at -80^{0}C. Prior to analysis samples were returned to room temperature.

Analysis of a panel of relevant biological markers was undertaken using an *in vitro* sandwich Enzyme Linked Immunoabsorbant Assay (ELISA) technique. Full details of the kits used is given in Chapter 7. Specific manufacturers instructions were followed when performing each ELISA but the basic principal was the same for all the assays and is shown in diagrammatic form in Figure 2.8.

Essentially, standards (prepared by serial dilution), controls and samples were added to a 96 well plate, coated with detection antibodies. The target substance antigen bound to the antibody in the well and was immobilized. Following incubation and washing, biotinylated antibody was added to the wells which bound to the antigen – antibody complex. After a further period of incubation and washing away of unbound antibody, Streptavidin horse radish peroxidase (HRP) solution was added. Following a final incubation and further wash, substrate solution was added, resulting in a colour change proportional to the amount of bound antibody /streptavidin HRP solution present. The addition of a stop solution

(sulphuric acid) halted the reaction. Light absorbance at a 450 nm wavelength was measured immediately using a dedicated microplate reader (POLARstar Omega, BMG Labtech Ltd, Aylsbury, UK). Construction of a standard curve, using a four parameter best fit approach allowed calculation of sample concentrations for each well. All standards, controls and samples were analyzed in duplicate.

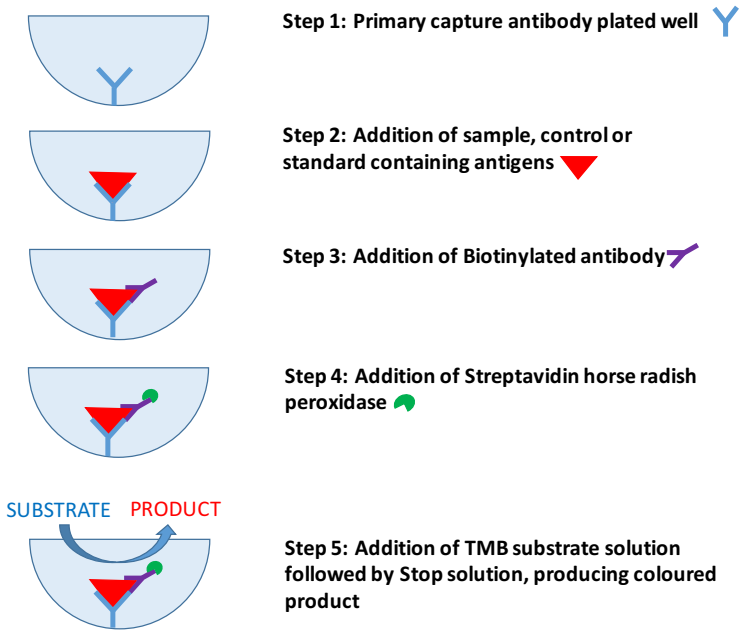

Figure 2.8 Schematic showing the principals of sandwich ELISA technique

Chapter 3

Validation of the Cytocam Dark Field Video Microscope

3.1 Introduction

Historically direct visualization of the microcirculation required the use of large microscopes and tissue dyes, so called intra vital microscopy, and was therefore mainly limited to experimental work in small animal models. The development of handheld microscopes has revolutionized this area of research. The first generation handheld devices used Orthogonal Polarised Spectroscopy (OPS) imaging [191] and were superseded by a second generation of devices that utilised Sidestream Dark Field (SDF) imaging. [194] The technical details of Dark Field microscopy are discussed in Chapter 2.

The latest optical device for assessing the microcirculation is the Braedius Cytocam, which utilises an Incident Dark Field (IDF) technique. This technique, originally described by Sherman and colleagues in the 1970s [190] is similar to SDF in that it uses a ring of circumferential LEDs to illuminate the target tissue tangentially, the illuminating light being excluded from the central column of the microscope. However, the LED strobe speed is significantly slower in the Cytocam device (2 vs 16ms); this theoretically produces less distortion of the erythrocyte image, potentially allowing more accurate automated flow analysis. The Cytocam is a small handheld camera, weighing considerably less than the

previous generation Microscan SDF device (115 vs 350 grams). It is also less bulky than the SDF device and this has the effect of making it easier for the operator to manipulate. In turn this has the potential to reduce pressure artifacts caused by the tip of the device occluding flow in microcirculatory vessels. The Cytocam has a higher optical resolution than the Microscan (3.12 versus 4.54μm) and a wider field of view (1.79 versus 0.84 mm^2). This larger field of view enables the user to more quickly identify suitable areas of microcirculation for analysis. The Cytocam has an electronically driven focusing motor as opposed to the analogue focusing mechanism of the Microscan making it easier to carry out fine focusing actions. Additionally, the device returns to the same focus depth for subsequent measurements, speeding up the time taken to acquire images. The Cytocam is linked to a dedicated computer with integral software which controls image acquisition and editing. This is in contrast to previous devices which required analogue to digital signal conversion through a signal adapter as well as bulky separate batteries and a non-proprietary laptop computer with adapted software.

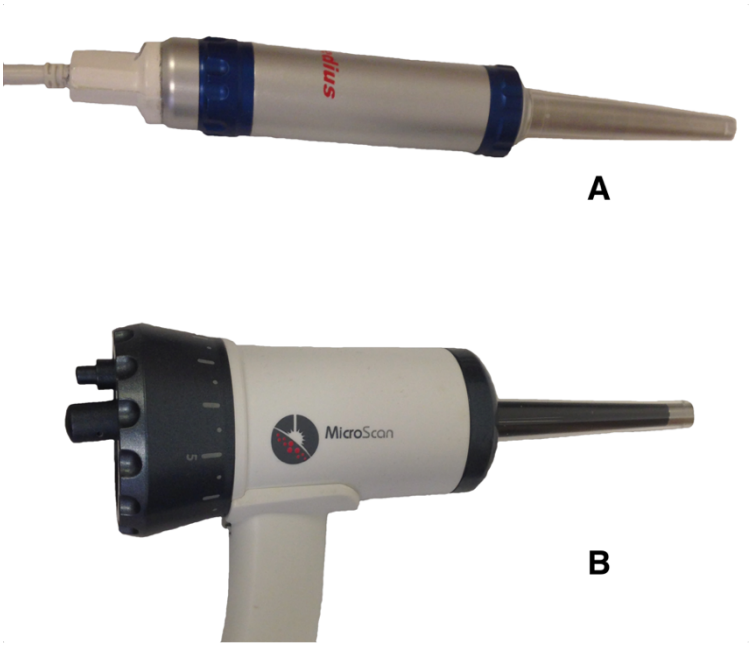

Figure 3.1 Cytocam (A) & Microscan (B) hand held video microscopes

The series of experiments presented in this chapter were designed to assess the performance of the Cytocam IDF device and to compare and contrast it to an immediate predecessor, the Microscan SDF device. In the first part of the study, the Cytocam was directly compared with the Microscan in an existing large animal model of haemorrhagic shock and complex injury. The aim was to compare the performance of the devices, with a particular focus on vessel identification. The second part of study was a direct comparison of image quality between the two devices, using matched sublingual microcirculatory images, obtained from the present author.

3.2 Methods

Conduct of this research was approved under license from the United Kingdom Home Office Animals (Scientific Procedures) Act, 1986. There was no clinical component to the study requiring ethical approval.

3.2.1 Subjects

Data was collected from six Large White pigs involved in the experiments described in Chapter 4. This data was used to examine vessel identification and flow characteristics.

The aspect of the study examining image contrast required two closely matched microcirculatory images, recorded using the subject devices. These images were obtained from the sublingual region of the present author.

3.2.2 Vessel Identification and flow assessment

Two devices were used to record video images of the sub lingual microcirculation, utilising Incident Dark Field (IDF) technology (Cytocam, Braedius Medical, Huizen, NL) or Sidestream Dark Field (SDF) technology (Microscan, Microvision Medical, Amsterdam, NL).
Serial video images of the sublingual microcirculation were recorded at the following experimental time points: Baseline (a steady state time point prior to injury or blood loss) and Shock (15 minutes after extremity trauma, controlled blood loss and blast wave injury). The Shock time point represents a low flow state, with a degree of dynamic response within the microcirculation in contrast to the predictable steady state seen at baseline. At each time point at least three, and ideally five, ten second sequences were recorded using each device, in accordance with accepted consensus opinion on assessment of the

microcirculation. [197] Images were recorded using the Microscan, immediately followed by the Cytocam. Images were either directly recorded onto a dedicated panel PC using integral software (Cytocam Tools v. 7.1, Braedius Medical, Huizen, NL) or via an analogue to digital signal converter (Canopus, ADVC 110) onto a stand-alone laptop computer in the case of the Microscan device. Microscan acquired images were saved as digital DV-AVI files, whilst those acquired by the Cytocam were converted to this format during export using integral software. As previously mentioned the field of view from the Cytocam is considerably wider than that of the Microscan necessitating a reduction in the field of view during export to DV-AVI format. Video captures were all performed by the present author. All images were recorded from the same part of the sublingual area, to the left side of the midline. Care was taken to minimise pressure artefact, by applying the probe tip and pulling back until contact was just lost and then reapplying with the minimum amount of pressure required to produce an image. Focus was adjusted by the operator in order to produce the best possible vessel definition, either manually (Microscan) or via the integral software linked to the electronic focusing mechanism (Cytocam). Videos were assigned a five-digit random number prior to archiving in a non-sequential order to reduce bias during interpretation. The videos were linked to study time point and device by reference to a separate database. Video files were saved on an external hard drive prior to off line analysis.

3.2.3 Analysis of video images

Analysis of video sequences was carried out by a single operator using dedicated software (AVA 3.02, Automated Vascular Analysis, Microvision Medical, NL). The operator was blinded to both the device used for recording and the time point of the video sequence. Only video sequences that conformed to pre-determined standards of stability, focus,

illumination, length and absence of pressure artefacts were included in the analysis. These standards were detailed by *Massey* et al in a previous study. [196] All visible vessels were manually traced by the operator and flow characteristics were assigned to each individual vessel segment, using the Microcirculatory Flow Index (MFI) scoring system. [199] The mean flow across all segments was calculated in order to produce an overall MFI for each video sequence. Analysis of each video sequence produced data for the total number of vessel segments compared to the size of the analysis area (Total Vessel Density, TVD).

3.2.4 Vessel contrast assessment

Vessel contrast and sharpness was analysed using a technique originally described by *Goedhart* et al. [194] To compare vessel contrast between the two devices the present author obtained two near identical images from his own sublingual microcirculation, using the two subject devices. Optimal image characteristics were obtained with respect to focus, illumination and avoidance of pressure. Video sequences were examined and one video frame, demonstrating the highest quality of focus and illumination, was selected for each device. This video frame was saved as a Portable Network Graphic (PNG) file. In each video frame image, ten venular segments and ten capillary segments were selected for quality analysis. To determine vessel contrast and sharpness, cross sectional grey scale profiles (greyscale value 0 corresponding to black and 255 to white) were obtained using Image J software (Freeware, US National Institute for Health). The contrast was defined as the absolute difference between the minimum value within the vessel and the maximum value on either side of the vessel wall (average of the two sides of the vessel).

3.2.5 Statistical Analysis

Statistical Analysis was performed using Prism v. 6.0 (Graph Pad Inc. California, USA). Data was tested for normality using D'Agostino & Pearson omnibus normality test. Paired t tests were used to compare vessel density data which was normally distributed. Mann Whitney U tests were used to compare vessel flow data, which did not have a normal distribution. Bland - Altman distribution analysis was performed on the TVD results from the two devices. Contrast values for the two devices were analysed using paired t tests after confirming normal distribution. A p value of < 0.05 was considered to be statistically significant.

3.3 Results

3.3.1 Vessel Identification and Flow Assessment

Results from 6 animals were included in the study. 44 video sequences were obtained with each device, giving a total of 88 matched video sequences. In order to assess for possible variance at different flow states video sequences were subdivided into Baseline (n=40) and Shock (n=48) time points. As expected there was a wider spread of results during the shock time point as the microcirculation exhibited increased heterogeneity. By contrast the baseline time point was more homogenous representing a relatively steady state at this stage of the experiment. One experimental time point was excluded as at least three video sequences of sufficient quality could not be obtained.

There were no significant differences in vessel detection, expressed as the Total Vessel Density, recorded by the two devices at either the Baseline or Shock time points (Table 3.1).

There was also no difference in the observed MFI, recorded by the two devices at either experimental time point (Table 3.2). These findings are consistent when the data is aggregated as shown in Figure 3.2, or examined for each individual animal.

Bland Altman analysis confirms the comparability of data collection from each device and shows minimal evidence of bias (Figure 3.3).

		IDF Cytocam	SDF Microscan	p value
Animal 1	BASELINE	13.1±0.2	12.2±0.9	0.40
	SHOCK	11.1±2.6	7.2±2.5	0.33
Animal 2	BASELINE	13.3±1.0	13.1±1.0	0.88
	SHOCK	9.2±1.0	9.8±1.6	0.79
Animal 3	BASELINE	15.6±1.4	15.1±1.3	0.80
	SHOCK	13.1±1.0	13.3±1.6	0.96
Animal 4	BASELINE	15.7±1.0	14.2±0.3	0.31
	SHOCK	14.2±1.8	14.9±0.2	0.73
Animal 5	BASELINE	Not analysed - insufficient quality video sequences		
	SHOCK	8.7±0.7	10.5±1.2	0.22
Animal 6	BASELINE	12.9±1.0	11.2±0.8	0.19
	SHOCK	12.8±1.0	14.0±1.9	0.60

Table 3.1 Total Vessel Density (TVD) values obtained from each animal at the Baseline and Shock experimental time points using the two subject devices. Units are mm/mm^2 and expressed as mean±SD.

		IDF Cytocam	SDF Microscan	p value
Animal 1	BASELINE	2.9 (2.9-3.0)	3.0 (3.0)	0.40
	SHOCK	0.6 (0-1.7)	0.5 (0-2.0)	0.74
Animal 2	BASELINE	2.9 (2.4-3.0)	2.9 (2.1-3.0)	0.41
	SHOCK	0.2 (0.1-0.6)	0.8 (0 -1.7)	0.86
Animal 3	BASELINE	2.9 (2.7-3.0)	3.0 (3.0)	0.99
	SHOCK	2.7 (2-2.9)	3.0 (3.0)	0.63
Animal 4	BASELINE	3.0 (2.9-3.0)	2.9 (2.8-3.0)	0.99
	SHOCK	2.7 (2.6-2.7)	2.3 (2.2-2.4)	0.10
Animal 5	BASELINE	Not analysed - insufficient quality video sequences		
	SHOCK	1.1 (0.9-2.0)	0.6 (0 - 2.2)	0.69
Animal 6	BASELINE	2.6 (2.4-2.9)	2.9 (2.9-3.0)	0.99
	SHOCK	2.7 (2.4-2.8)	3 (2-3)	0.70

Table 3.2 Microvascular Flow Index (MFI) values obtained from each animal at the Baseline and Shock experimental time points using the two subject devices. Units are AU and expressed as median (IQR).

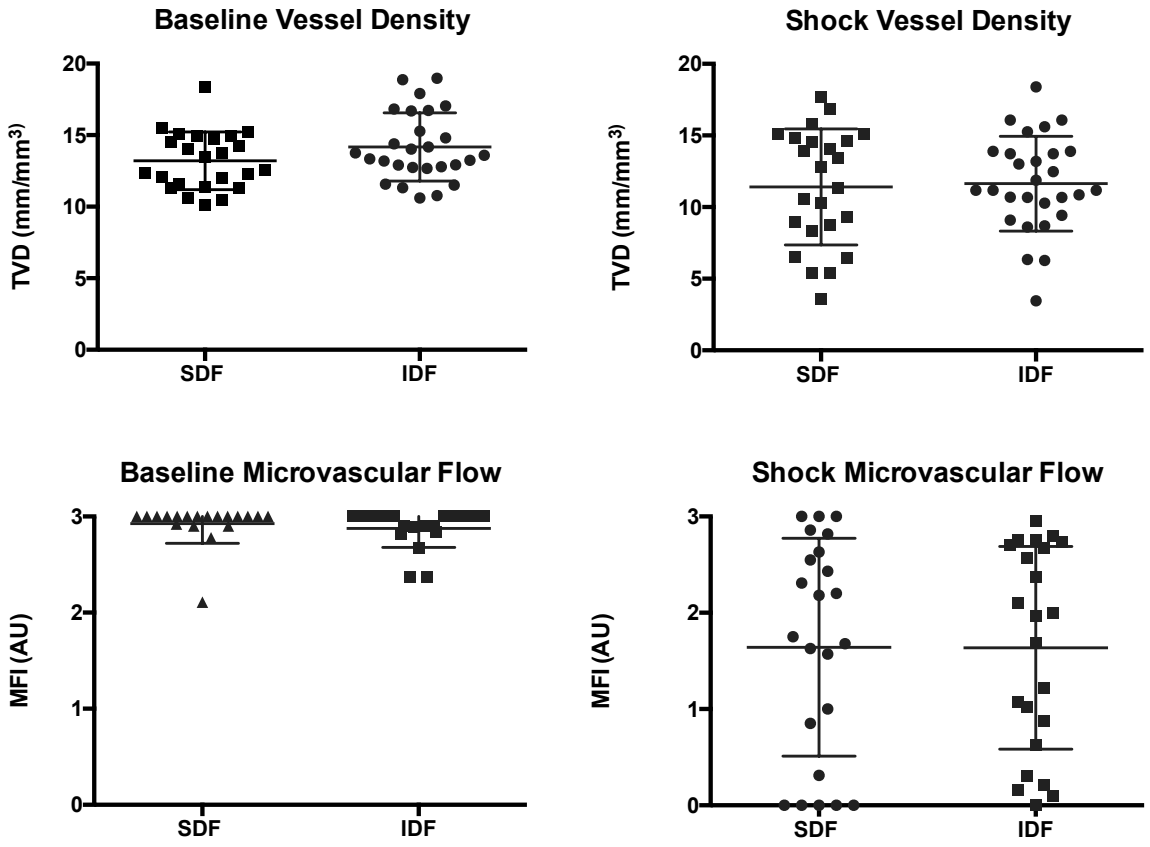

Figure 3.2 Aggregated results showing Total Vessel Density (TVD) and Microvascular Flow Index (MFI) data from all animals at both experimental time points. Each data point represents the average value obtained from all video sequences at a particular time point. No significant differences between groups

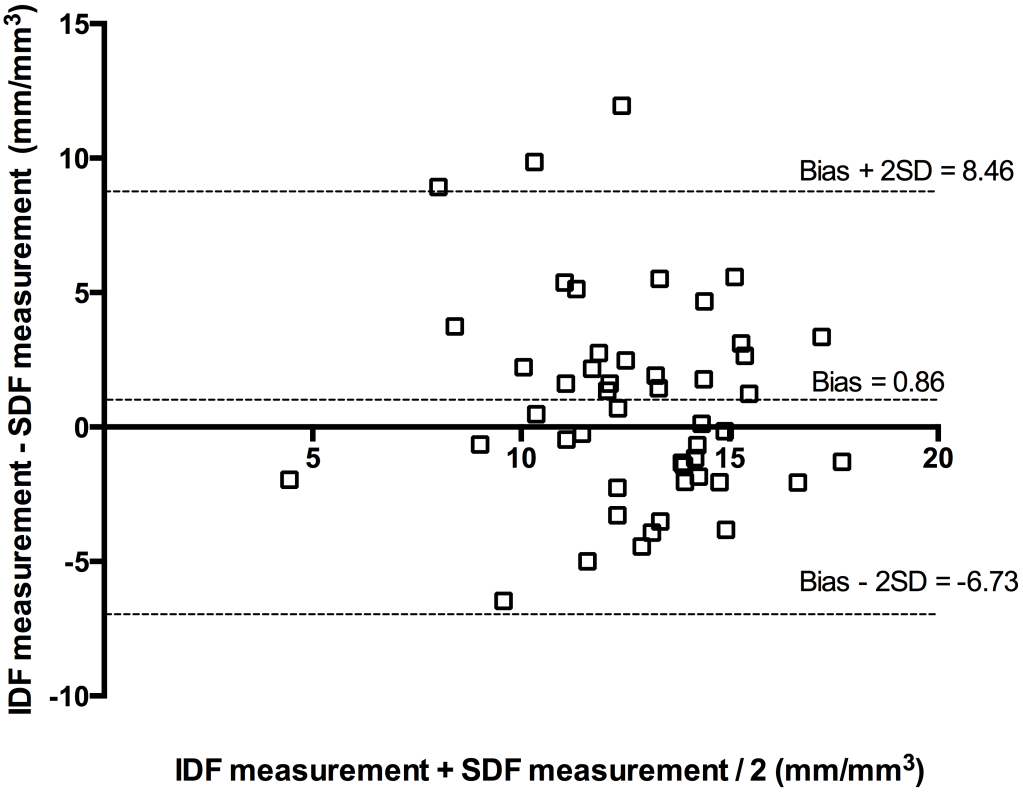

Figure 3.3 Bland-Altman plot showing comparison of Total Vessel Density readings obtained using subject devices at both experimental time points

3.3.2 Vessel contrast and sharpness

Matched microcirculatory images from the sublingual region of the author are shown in Figure 3.4. Vessel contrast was significantly higher in the image obtained from the Cytocam IDF device. This was true for both capillaries and venules. (Table 3.3).

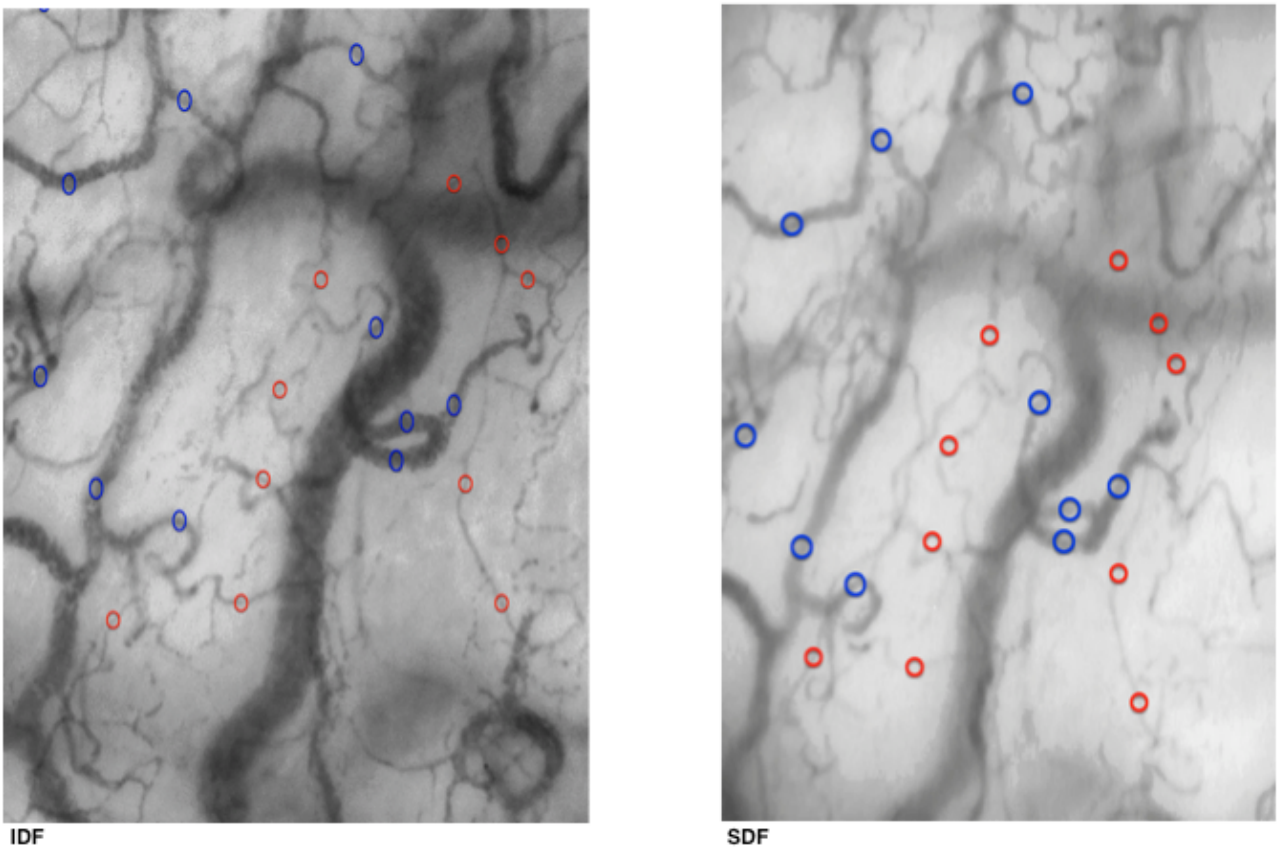

Figure 3.4 Dark Field microscopy images of the sublingual microcirculation of the author obtained using the Cytocam IDF device and Microscan SDF device. Sites selected for contrast comparison are shown by blue circles (venules) and red circles (capillaries).

	IDF Cytocam	SDF Microscan	p value
Contrast (capillaries)	17.1±3.9	9.4±3.6	0.0006
Contrast (venules)	36.1±11.4	26.4±7.1	0.014

Table 3.3 Pixel contrast intensity between image background and micro vessel. Higher values indicate a greater degree of contrast and hence a sharper vessel image. Units are arbitrary measures of intensity and expressed as mean±SD.

3.4 Discussion

This series of experiments was designed to assess the performance of a new device for assessing the microcirculation, the Braedius Cytocam. Although other studies have compared the Cytocam and Microscan in healthy volunteers [195] [227] this is the first evaluation of the Cytocam IDF device performed over a range of flow states, in an experimental model of shock. Including the low flow images obtained in this study produces a comparison between the two devices over the full operating range in which they would be used in clinical practice.

The first part of the study demonstrated that, in the pragmatic setting of an existing trial, the two devices performed in a comparable fashion and the data obtained, with respect to both vessel density and flow, was virtually indistinguishable. By taking some readings at the baseline (steady state) timing point any potential changes in the microcirculation, seen between the recording of the SDF and the IDF acquired images, were minimized. The addition of the shock timing point is important because it allows a comparison of the two devices at a low flow state, where vessel identification may potentially be more difficult. However, at this time point the microcirculation was in a state of flux, induced by haemorrhage and injury, and this can be seen in the much wider spread of data. Despite this dynamism and heterogeneity there is still a remarkably similarity between the results collected from the two devices. As far as possible the time interval between image acquisition was kept as short as possible and in practice was achieved within five minutes from the start of SDF imaging to the end of IDF imaging.

It is acknowledged that this experimental approach has limitations and it would be optimal to compare identical images derived from the two devices. However, in practical terms it is extremely difficult to find even two such images, given the magnification and field of view constraints as well as the instability inherent in the use of handheld cameras. In reality, it is impossible to obtain matched images in a dynamic study that includes animals or patients with shocked microcirculations. The first part of the study therefore represents an attempt to answer the question of comparability of data generated by the two devices, rather than a completely objective assessment of image identification under perfect operating conditions.

The second part of the study examined whether the image quality produced by the IDF device was superior to that of the SDF device. Prior to the study it was felt that, subjectively, the IDF images of the microcirculation were sharper and had greater definition than comparable SDF images. The current study confirmed this objectively using pixel analysis software. Further studies are needed to replicate this finding which is of potential importance as sharper images and improved contrast between blood vessels and background tissue potentially make the automated analysis of such images by computer software more achievable. Automated analysis programs cannot yet reliably measure vessel density in video microscopy images and improvements in image quality are a necessary pre-requisite for the development of this technology.

Two other studies have been conducted to compare and contrast these two subject devices. In contrast to the findings of the current study *Aykut* et al reported that the Cytocam identified 30% more micro vessels, at the same time point, than the Microscan. Using an identical technique to that utilised in the current study they also reported a higher vessel

contrast when using the Cytocam. [195] This study was conducted on healthy human volunteers and therefore only included baseline figures. Gilbert-Kawai and colleagues compared the two subject devices, finding that the Cytocam produced images with higher quality, defined using the Microcirculatory Image Quality Score. [227] Although this comparison was not formally carried out in the current study, the findings are in keeping with our experience of using the Cytocam.

3.5 Conclusion

The Braedius Cytocam is a new device for imaging the microcirculation which the present study has validated against a precursor device. The findings of this study, conducted during an existing experiment of traumatic haemorrhage and resuscitation, suggest that vessel detection rates are similar between the two devices. This contrasts with some other studies, conducted in healthy volunteers, suggesting that the Cytocam has a significantly higher vessel detection rate. Researchers transitioning between the two devices should therefore be cautious about direct comparisons of microcirculatory data, particularly that relating to vessel density. There are several technical advantages of the Cytocam over the precursor device and vessel image quality appears sharper which may aid in the quest for fully automated microcirculatory analysis.

Chapter 4

Microcirculatory perfusion is important in determining shock reversal in an experimental model of traumatic haemorrhagic shock

4.1 Introduction

Haemorrhage is a leading preventable cause of death following traumatic injury. [3] Resultant traumatic haemorrhagic shock (THS) may lead to trauma induced coagulopathy and multiple organ failure. [128,228] Timely reversal of the resulting shock state is essential in reducing post injury morbidity and mortality. Traditional vital signs, such as heart rate, respiratory rate and systolic blood pressure are normally used in a clinical setting to guide resuscitation. However, they can have poor sensitivity as markers of perfusion during haemorrhagic shock, and predict outcomes relatively poorly. [72,73] [77] Resuscitation strategies targeted at these markers may lead to under or over resuscitation.

Studies from other clinical settings such as sepsis [229] [208] and major surgery [230] have suggested that targeting microcirculatory flow, as opposed to more traditional parameters such as blood pressure pressure, may be beneficial. This is likely to be because the microcirculation is the final common pathway of cellular oxygen and substrate delivery and therefore of paramount importance to tissue perfusion. There is a paucity of clinical evidence from patients with THS, with only one clinical study suggesting a link between early microcirculatory impairment and later organ dysfunction. [128]

Whether pressure or flow are targeted, the initial treatment for haemorrhagic shock will always involve the administration of resuscitative fluid. Whilst it is logical to aim to replace blood loss with blood products this is often difficult to achieve in the early stages of resuscitation, particularly in the pre-hospital setting. The last two decades have seen a paradigm shift in the choice of initial resuscitative fluid used; away from a predominantly crystalloid based approach towards the use of early blood component therapy. [231] This approach, whilst appearing logical and physiologically sound has not been rigorously tested for efficacy. Additionally, early delivery of blood products poses considerable logistic challenges which are further magnified in the austere setting of military operations. One potential alternative approach is to use a synthetic haemoglobin based fluid that combines the volume expanding properties of a colloidal solution with the oxygen carrying capacity of haemoglobin. Such agents have a chequered history, in part because of the ability of free haemoglobin to avidly scavenge nitric oxide leading to systemic vasoconstriction and a presumed reduction in microcirculatory perfusion. [232] A newer compound, MP4OX, which is composed of pegylated human derived haemoglobin may avoid these potential adverse effects. [233] MP4OX has been tested in animal [178] and clinical [183] trials and found to have an acceptable safety profile. However, to date, the direct effect of MP4OX on the microcirculation has not been assessed.

The present study uses a porcine experimental model designed to mimic battlefield injury leading to haemorrhagic shock. The aims of the study were to:

i) Investigate the relationship between blood pressure, cardiac output and microcirculatory flow following THS.

ii) Explore the effects of differing initial resuscitation fluids (0.9% saline, component blood products and MP4OX) on microcirculatory perfusion.

4.2 Methods

The experiment was approved and conducted in accordance with the Animals (Scientific Procedures) Act 1986.

4.2.1 Animal Preparation

The study was conducted on terminally anaesthetized Large White pigs. Prior to the experiment the animals were fasted for 18 h but were allowed water *ad libitum*. Animals were sedated with i.m. Midazolam (0.1mg/kg) prior to induction of anaesthesia with Isoflurane (5%), oxygen and nitrous oxide. Animals were intubated and initially ventilated (Blease Manley MP3 ventilator). The left femoral artery was cannulated to allow for withdrawal of blood. The left common carotid artery was cannulated to allow for measurement of systemic blood pressure. The left internal jugular vein was cannulated and a pulmonary artery flotation catheter was inserted with the tip positioned in the pulmonary artery. A midline laparotomy was performed. Because of the ability of the pig to mobilise large amounts of blood from the spleen in the event of haemorrhage and the fact that this is not a feature of human physiology, a splenectomy was performed. The bladder was catheterized by open suprapubic cystostomy. The abdomen was then closed with

interrupted loose mass closure sutures. Anaesthesia was continued with intravenous Alphaxalone (Alfaxan; Jurox (UK) Ltd, Malvern Link UK) and the Isoflurane discontinued. Animals were allowed to breath spontaneously for the remainder of the experiment. A 1 h recovery period was mandated following surgery before the experimental protocol was instituted.

4.2.2 Monitoring and blood gas analysis

Arterial blood pressure was recorded via the carotid arterial cannula. Central venous pressure and pulmonary artery pressure were recorded via the pulmonary artery catheter (Vigilance Volumetrics CEDV; Edwards Lifesciences Ltd), which was also used to determine cardiac output using a continuous (6 min average) thermodilution technique. Zero pressure for all invasive haemodynamic measurements were set at the level of the heart. Arterial and venous blood samples were taken from the carotid and pulmonary artery catheters, respectively, for blood gas, base excess, and lactate analysis (Gem Premier 3000 Blood Gas Analyzer; Instrumentation Laboratories, Warrington, UK).

4.2.3 Blood Products

Blood for transfusion was collected by exsanguination from terminally anaesthetised female Large White pigs; these animals were not involved in the experimental protocol. Briefly, standard units (1 unit represents approximately 450 mL) of blood were collected from a carotid cannula. The blood was processed within 90 minutes according to standard UK blood transfusion protocols to separate the red blood cells from the plasma. PRBCs were leucodepleted and stored with SAG-M. The platelets were discarded. The resulting units of PRBCs were stored at 4 ^{o}C (LabCold Blood Bank, Basingstoke, UK) and used within 14 days of

collection. The plasma (FFP) was fast-frozen (MP1100 Plasma Freezer System; Thermogenesis, Noblesville, US), stored at -30 ^{o}C (LabCold Plasma Freezer; LabCold), and used within 6 months of collection. FFP was thawed at 37 ^{o}C in a dry plasma thawer (Sahara III Maxitherm; Sarstedt, Germany) immediately before use. Prior to use, all donor products were forward and reverse matched to recipient blood. In addition, because PRBC and FFP from different donors were used for resuscitation, they were also cross-matched with each other.

4.2.4 Experimental Protocol

The experimental protocol is shown in Figure 4.1.

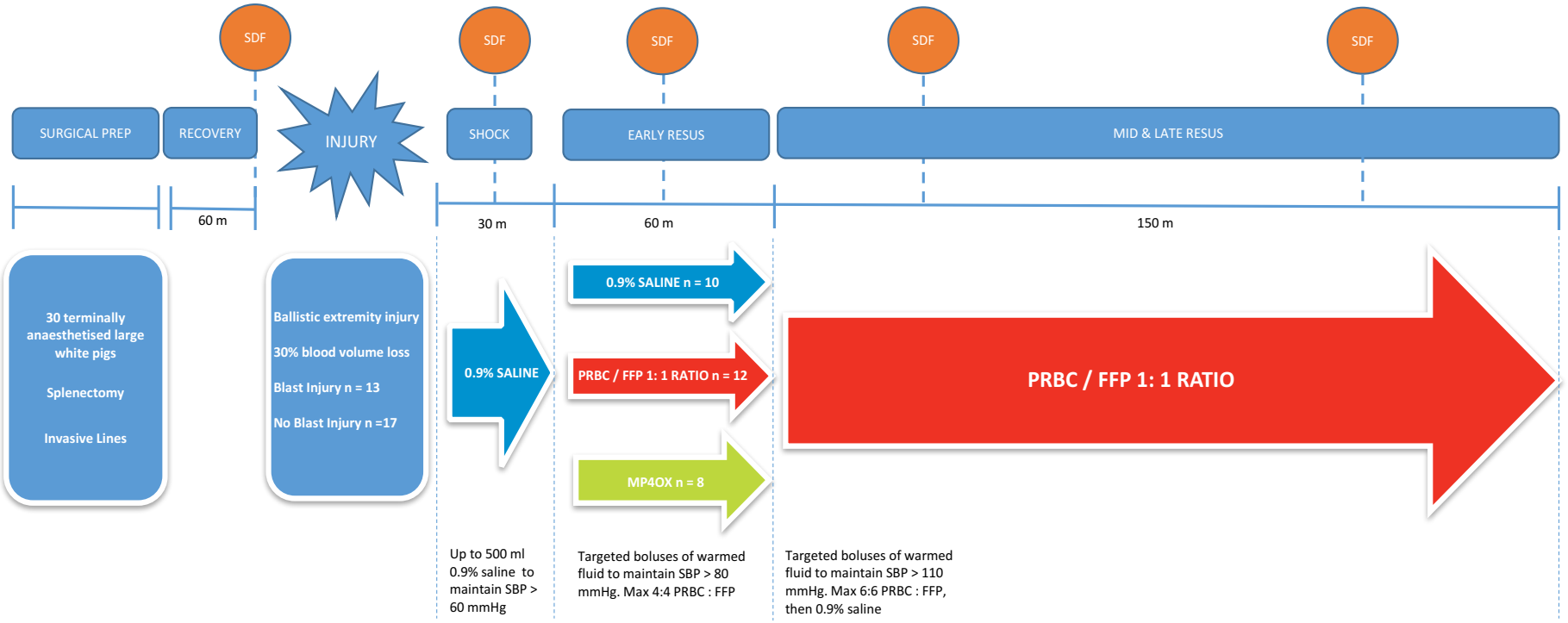

Figure 4.1 Experimental Protocol. (SDF represents microcirculatory assessment points)

Soft tissue injury

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

Blast Injury

A proportion of animals in each treatment group also received a blast wave injury, in addition to the soft tissue injury. These animals were completely wrapped in 12- ply Kevlar® blankets for protection from minor debris and secured supine onto a wheeled trolley on a fixed steel platform in an open-air blast range. The right side of the animal faced a bare charge of 2.2 kg of EDC 1 commercial explosive (QinetiQ, Fort Halstead, UK) mounted on a 1-m cardboard tube 2.0–2.5 m from the animal. The blast arena and explosive charge were such that there were no fragments or major debris to cause secondary blast injury. Anesthesia was maintained by continuous infusion from a protected battery-powered syringe driver. After remote detonation of the charge, the animal was removed from the blast trolley and returned to the laboratory.

Haemorrhage and fluid resuscitation

Animals were randomised to receive initial resuscitation with either 0.9% saline, component blood therapy, or a synthetic haemoglobin based oxygen carrier (MP4OX, Sangart Inc., San Diego, California, USA).

Following injury, controlled removal of blood, up to 30 % estimated blood volume by

weight, was commenced through the femoral arterial cannula via a computer controlled pump. At the conclusion of blood removal animals were left in a shocked state for 30 min prior to the commencement of resuscitation. However, boluses of 0.9% saline, up to a total of 500ml, could be used during this period if systolic blood pressure fell below 60 mmHg. Fluid boluses were not given in a set amount but rather tailored to blood pressure response. After 30 min resuscitation was commenced with the specific initial resuscitation fluid. Resuscitation was initially targeted to a systolic blood pressure of 80 mmHg. Following 60 min of initial resuscitation animals in all treatment groups then received blood products as the resuscitation fluid and the systolic blood pressure target was increased to 110 mmHg. Blood product usage was capped at 6 units PRBC and 6 units FFP after which animals received 0.9% saline, if further fluid was required. The experiment concluded after 210 min of resuscitation at which point animals were euthanised.

4.2.5 Microcirculatory monitoring

Sublingual video microscopy was conducted at 5 specific time points (Baseline, Early Resus, Shock, Mid Resus & Late Resus). The present author collected the majority of the images (23/30 animals) with 2 other trained investigators collecting images for the remaining animals. All images were reviewed for quality and analysed by the author. Video microscopy techniques are fully described in Chapter 2.

In addition to sublingual assessment, microcirculatory flow was measured in the splachnic circulation of five animals during the early and late resuscitation time points. A section of jejunum was exteriorised and the video microscope tip applied to the serosal surface. Images were scrutinised for quality and rejected if there was any evidence of pressure

artefact. Image acquisition was performed as closely as possible to the time when sublingual images were acquired. Images were de-identified and analysed using the same technique as that applied to the sublingual circulation. Given the difference in microcirculatory anatomy between the sublingual and splanchnic beds only flow and not density data was used when comparing the two sites.

Tissue oxygenation was assessed using near infrared spectroscopy (NIRS), the principles of which are described in Chapter 2. NIRS electrodes (Hutchinson Technology Inc, Hutchinson, MN, USA) were applied to the uninjured hind limb of all animals and values recorded every six seconds, throughout the entire experimental protocol. The mean tissue oxygen saturation (StO2) value for 5 minutes before and 5 minutes after each video microscopy measurement was used as the basis for comparative analysis.

4.2.6 Measures of resuscitation outcome

The study was designed to keep animals alive until the end of resuscitation. Outcome measures were therefore chosen that reflected the effects of resuscitation on restoring tissue perfusion parameters. The primary measures of immediate resuscitation outcome was taken as the lactate clearance, defined as the percentage reduction in lactate concentration between the early resuscitation and late resuscitation time points.

Secondary outcome measures taken to reflect tissue perfusion were:

i) Lactate concentration and standard base excess at the late resuscitation time point.

ii) Mixed venous oxygen saturation, analysed from blood drawn from the pulmonary artery catheter, at the late resuscitation time point.

iii) Mixed venous carbon dioxide - arterial carbon dioxide partial pressure difference, analysed from blood drawn from pulmonary artery and femoral artery catheters at the late resuscitation time point.

iv) Urine output between baseline and late resuscitation time points.

4.2.7 Data Analysis and statistical methods

Animals were classified *post hoc* as having above or below average microcirculatory perfusion during shock and early resuscitation.

The method used to dichotomise animals into these groups was as follows:

1. For each animal, the lowest recorded PVD value at either the shock or early resuscitation time points was selected. Individual animals reached a nadir of microcirculatory perfusion during either the shock or the early resuscitation phases and for this reason it was necessary to consider values at both these time points.
2. The mean of these lowest values was calculated for the entire cohort of animals. Individual animals were classed as having above or below average perfusion based on the relationship of their individual lowest value to the mean lowest value of the cohort.

Statistical analysis was performed using Prism v. 6.0 (GraphPad Software Inc., California, USA). Data was assessed for normality using the D'Agostino-Pearson omnibus normality test. Parametric data is expressed as mean (standard deviation), non-parametric data is expressed as median (interquartile range). Comparison between groups over time was performed using repeated measures two-way ANOVA with respect to time and treatment,

Tukey's correction for multiple comparisons was applied. For non-parametric data Friedman's test with Dunn's correction for multiple comparisons was used. Direct comparison between two groups at specific time points was made using two tailed t tests for parametric data or Mann-Whitney tests for non-parametric data. Categorical data was analysed using Chi squared or Fisher's Exact tests. p values of <0.05 were taken to indicate significance.

4.3 Results

Details of animals and treatment groups is shown in Figure 4.2. There was an investigator trained in the acquisition of microcirculatory images present during 55 experiments. 20 animals died prior to the end of the experiment, mostly from immediate complications arising from blast exposure. 2 animals were not included in the study because of a lack of high quality video images and 3 because digital video data was corrupted prior to analysis. Data from 30 animals who all survived to the conclusion of the experiment was included. There were 7 groups of animals who received homogenous treatments and injury patterns. Preliminary analysis of the data failed to show any convincing effect of blast exposure on systemic or microcirculatory haemodynamic variables and it was therefore decided to analyse blast and non-blast injured animals together.

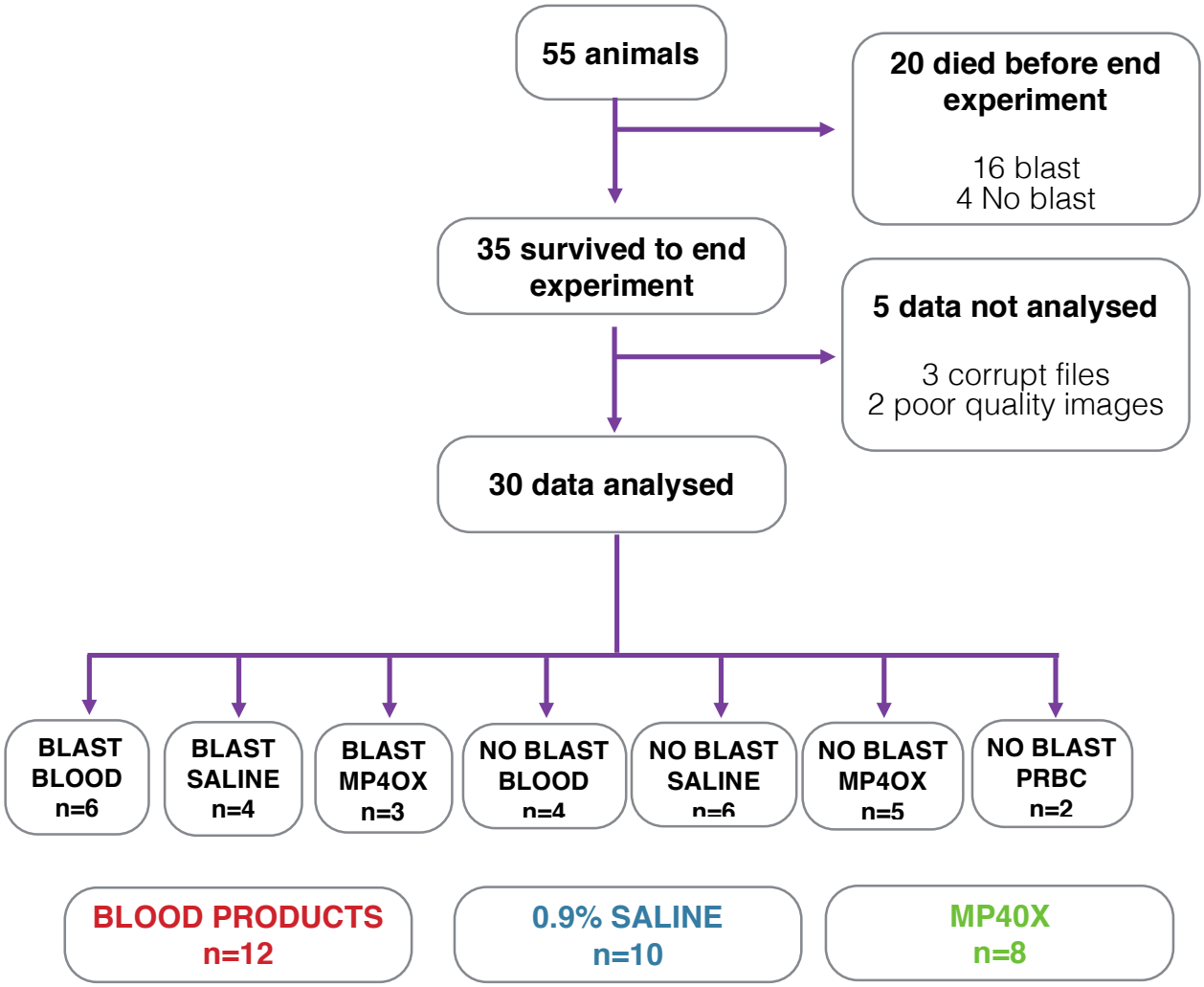

Figure 4.2 Details of experimental animals. Of the 55 animals for whom there was an investigator trained in video microscopy present, 30 survived to the end of the experiment and had complete data for analysis. There were 7 discrete groups dictated by the type of initial resuscitation fluid and the presence of blast injury. Analysis was conducted using three groups, based solely on the resuscitation fluid, after preliminary analysis showed that the presence of blast injury had little or no effect on the variables under investigation

4.3.1 Effect of microcirculatory perfusion on resuscitation outcomes

Using the method described in section 4.2.7 the mean of lowest recorded PVD values for all animals during the shock and early resuscitation time points was 6.35 mm/mm^2. This was therefore the value by which animals were dichotomized into groups with above average PVD (Group A) and below average PVD (Group B).

Table 4.1 shows the characteristics of these two groups. When compared to Group B, Group A appeared to contain a lower proportion of animals treated with 0.9% saline and a higher proportion treated with MP4OX during the early resuscitation phase but these differences did not reach statistical significance. Animals in Group B appeared to receive a larger amount of 0.9% saline over the course of the experiment but this difference did not reach statistical significance. In addition, such a finding may be explicable by the larger proportion of animals in this group who received 0.9 % saline as the initial fluid therapy. There was no discernible difference in blast exposure between the two groups.

In terms of measures of resuscitation outcome, Group A had a lower lactate and smaller base deficit at the conclusion of resuscitation than Group B as well as a higher clearance of lactate over the course of the experiment. Group A had a smaller mixed venous – arterial CO2 gradient than Group B at the end of resuscitation but there was no observed difference in the mixed venous oxygen saturation. Urine outputs were not significantly different between the two groups.

	Group A Above average PVD (n=14)	Group B Below average PVD (n=16)	p value
Initial resuscitation fluid			
0.9% Saline	3 (22%)	7 (44%)	
Blood products	6 (42%)	6 (37%)	
MP4OX	5 (36%)	3 (19%)	0.37
Blast Injury	7 (47%)	6 (40%)	1.0
Volume of Fluid Administered* **			
0.9% Saline	467±512	1128±1190	0.17
Blood Products	1657±541	1437±393	0.36
MP4OX	341±164	367±172	0.85
Tissue Perfusion Parameters (values at late resus time point unless otherwise stated)			
Lactate (mmol/l)	4.6±2.2	8.9±3.6	0.001
Standard base deficit (mEq/l)	2.1±4.1	-4.6±5.5	0.001
PvCO2-PaCO2 (kPa)	1.4±0.6	1.9±0.7	0.03
SvO2 (%)	56.1±9.5	51.3±10.2	0.27
Lactate clearance (%)*	73.4±10.3	40.5±29.9	<0.001
Urine output (ml)**	260±163	168±152	0.25

Table 4.1 Characteristics of animals with above and below average microcirculatory perfused vessel density (PVD) during shock / early resuscitation. Values given as mean±SD. Differences between groups assessed using 2 tailed t tests or Fisher's exact test. PvCO2 mixed venous CO2 tension, PaCO2 arterial CO2 tension, SvO2, mixed venous oxygen saturation. * % decrease between Early and Late Resus time points, ** cumulative between Baseline and Late Resus time points, * during entirety of experiment.**

4.3.2 Changes in microcirculatory and macrocirculatory variables during shock and resuscitation

Both macro haemodynamic parameters (cardiac output & mean arterial pressure) and microcirculatory parameters (PVD, PPV, MHI) followed the same broad trends during shock and resuscitation, with both macro and microcirculatory variables falling during blood loss and early resuscitation before recovering during the later stages of resuscitation. However, there was a notable degree of variation in this general trend with respect to microcirculatory variables recorded for each individual animal. Figure 4.3 shows the trends in macro and micro circulatory variables for the two previously described groups of animals with above and below average PVD during the shock and early resuscitation phase. Animals in Group B showed significantly more marked falls in microcirculatory flow (PPV) and density (PVD) indices during the shock and early resuscitation phases of the experiment as well as exhibiting a greater degree of heterogeneity within the microcirculation, reflected in a higher MHI value. Unsurprisingly there was no difference in the targeted parameter of blood pressure between the groups but there was also no difference in the observed cardiac output. Interestingly, systemic vascular resistance index (SVRI) during the shock phase was noted to be significantly higher in Group B than in Group A (1503±319 v 1086±296 dynes s cm^{-5}, $p < 0.01$)

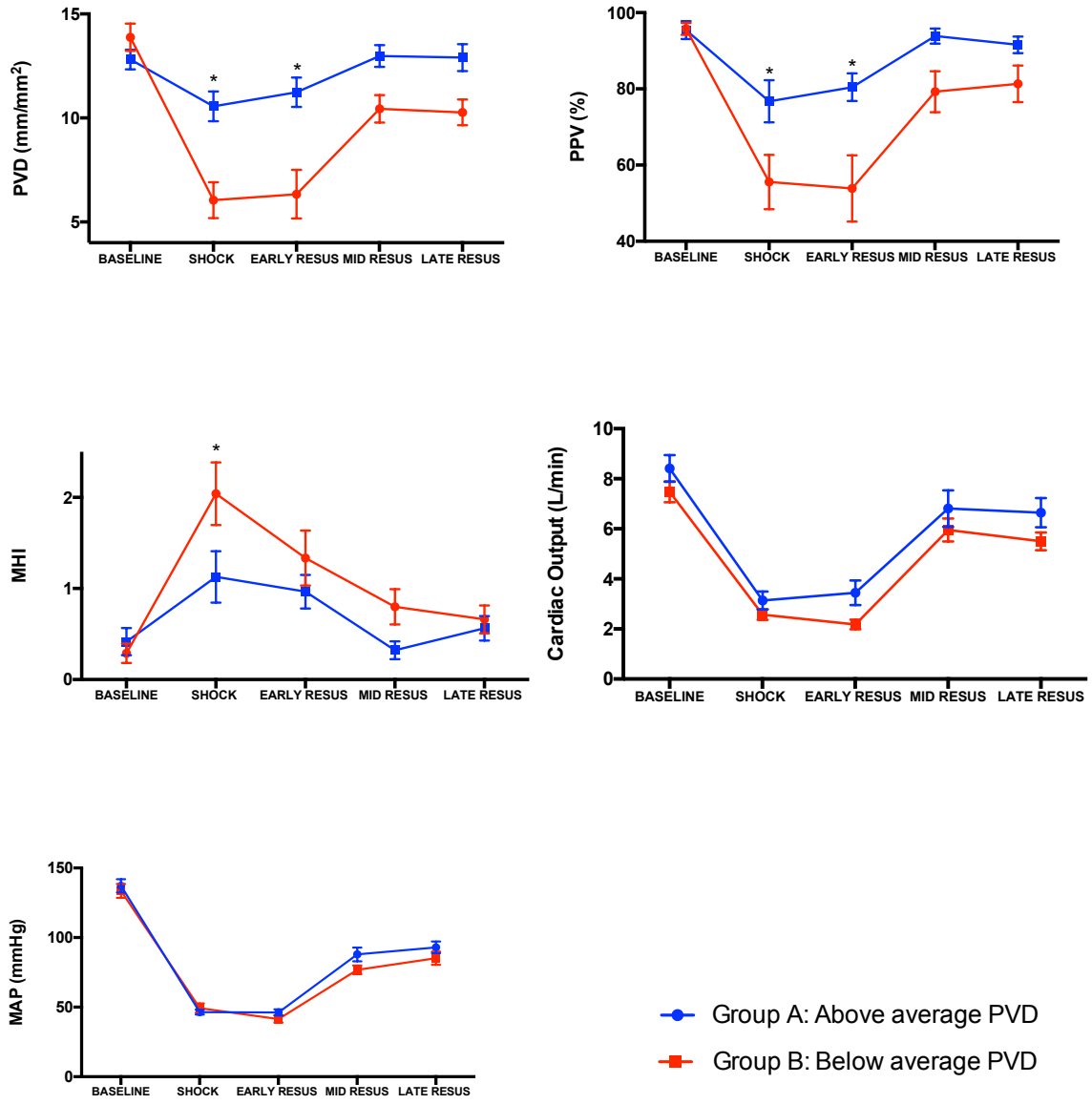

Figure 4.3 Trends in macrocirculatory and microcirculatory variables during shock and subsequent resuscitation. Animals grouped into those with above and below average PVD during shock / early resuscitation. PVD Perfused Vessel Density, PPV Proportion of Perfused Vessels, MHI Microcirculatory Heterogeneity Index, MAP Mean Arterial Pressure,

*** p<0.05 between groups at time point (2 way repeated measures ANOVA). No significant differences detected between groups for measures of cardiac output or blood pressure**

Figure 4.4 illustrates the wide inter-individual variation in microcirculatory perfusion in an alternative way. Each data point represents an experimental time point (Baseline, Shock etc.) with corresponding values for PVD and MAP plotted. It can be observed that under conditions of marked hypotension, there was nonetheless a group of animals able to maintain relatively preserved microcirculatory flow and vessel density. This figure also illustrates the apparent lack of uniform effect of resuscitation fluid on microcirculatory parameters, which will be discussed in detail in section 4.3.4.

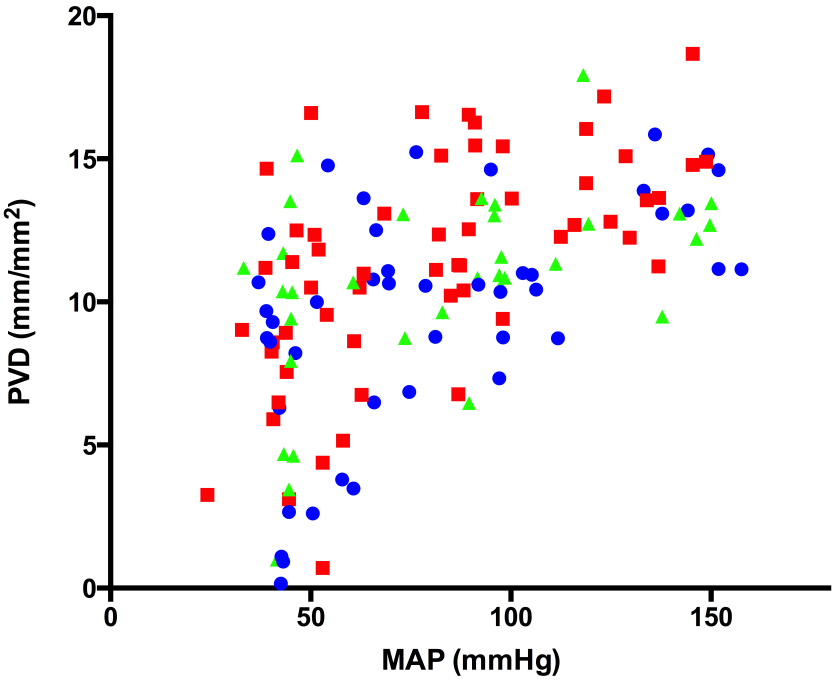

Figure 4.4 Relationship between Mean Arterial Pressure (MAP) and Perfused Vessel Density (PVD). Each point represents a single time point for one animal. Saline treated animals, blue circles; blood product treated animals, red squares; MP4 treated animals, green triangles. Note the very wide range of microcirculatory perfusion (PVD) for animals with marked hypotension

4.3.3 Effects of microcirculatory perfusion on resuscitation outcome measures

Table 4.1 showed the effects of microcirculatory perfusion on the chosen resuscitation outcome measures relating to tissue perfusion, Figure 4. 5 illustrates the changes in these parameters over the course of the experiment. Animals in Groups A & B had significant differences in lactate and base deficit kinetics during the resuscitation phases of the experiment. There was also a wider mixed venous – arterial CO2 difference in Group B. No differences were observed in the mixed venous oxygen saturation values or in the hind limb tissue oxygen saturation recorded using NIRS.

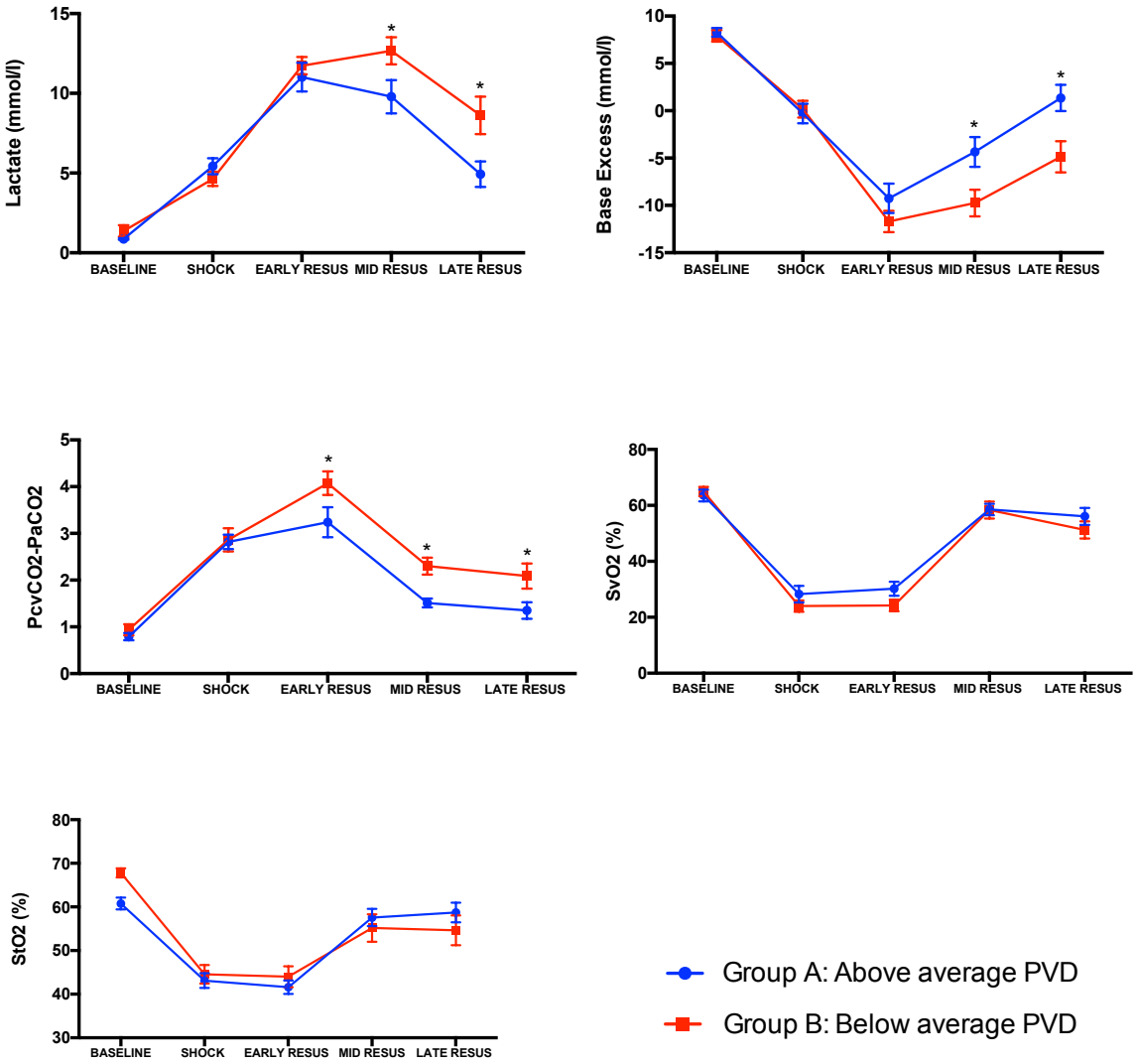

Figure 4.5 Changes in tissue perfusion parameters during shock and subsequent resuscitation. Animals grouped into those with above and below average PVD during shock / early resuscitation. PvCO2 mixed venous CO2 tension, PaCO2 arterial CO2 tension, SvO2 mixed venous oxygen saturation, StO2 tissue oxygen saturation.

*** p <0.05 between groups at time point (2 way repeated measures ANOVA). No significant differences detected between groups with respect to measures of SvO2 or StO2**

4.3.4 Effects of initial resuscitation fluid

Figure 4.6 illustrates the effect of initial resuscitation fluid on haemodynamic and tissue perfusion variables. Animals treated with 0.9% saline as the initial resuscitation fluid had significantly lower PVD during the shock phase (i.e. prior to any differences in resuscitation fluid) than those who received blood products or MP4OX. Although there is a suggestion from the data that animals initially treated with 0.9% saline continued to have a lower PVD throughout subsequent resuscitation, further meaningful analysis was precluded by this lack of homogeneity prior to treatment. There were no discernible differences in lactate kinetics based on the type of initial resuscitation fluid used. Base deficit was significantly greater in the 0.9% saline group during mid and late resuscitation, perhaps explicable by a difference in chloride ion concentrations, unfortunately data relating to this variable was not collected during the experiment.

Examining changes in PVD for each individual animal between the shock and early resuscitation time points failed to reveal any discernible pattern resulting from the type of resuscitation fluid used (Figure 4.7).

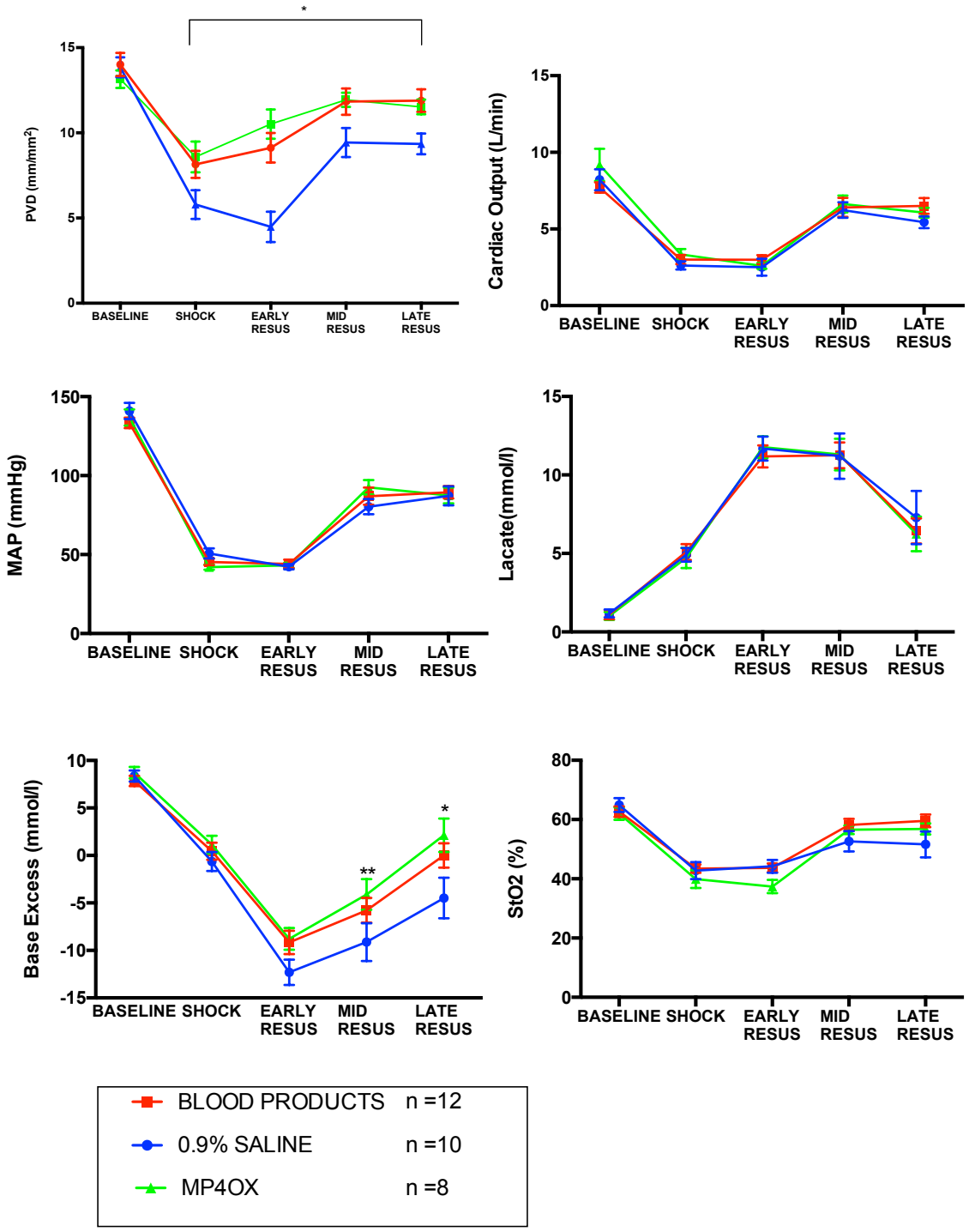

Figure 4.6 Effect of initial resuscitation with either 0.9% saline, blood products or MP4OX on macro and microcirculation and tissue perfusion parameters. * p <0.05 0.9% Saline v MP4OX & blood. ** p<0.05 0.9% Saline v MP4OX (2 way repeated measures ANOVA).

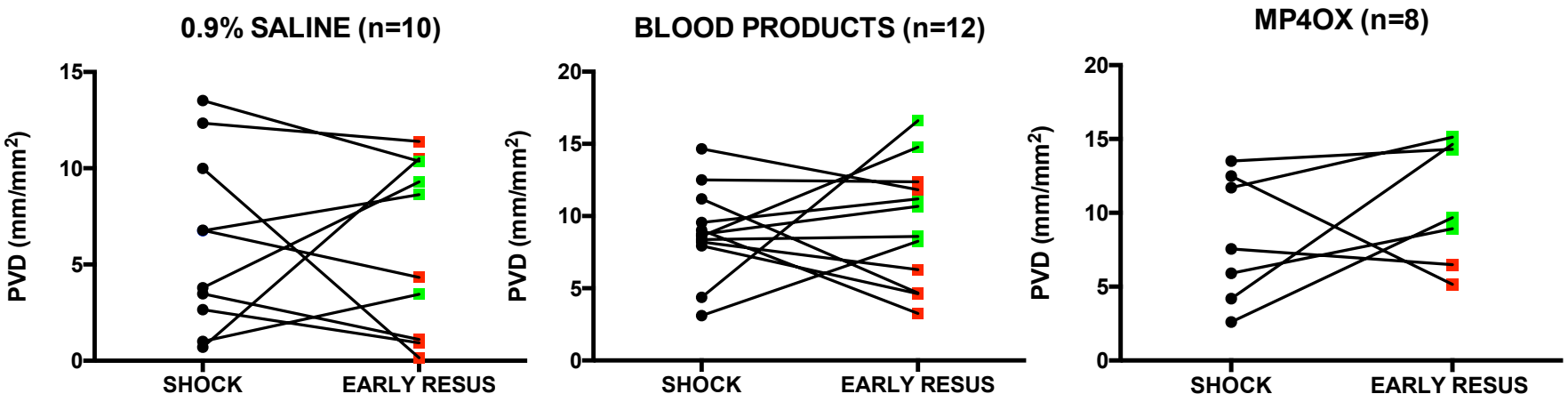

Figure 4.7 PVD for individual animals before and after administration of initial resuscitation fluid. Animals were exclusively given only the subject fluid during this time period. Green squares indicate an increase in PVD and red squares a decrease in PVD between the two measurements. There were no significant differences and no clear trends in the data

4.3.5 Effect of blast injury

13 of the 30 animals who survived to the conclusion of the experiment were exposed to blast injury. Overall those animals surviving the blast injury to the point of the commencement of resuscitation exhibited minimal differences in macro or microvascular parameters or markers of perfusion when compared to animals not exposed to blast (Figure 4.8).

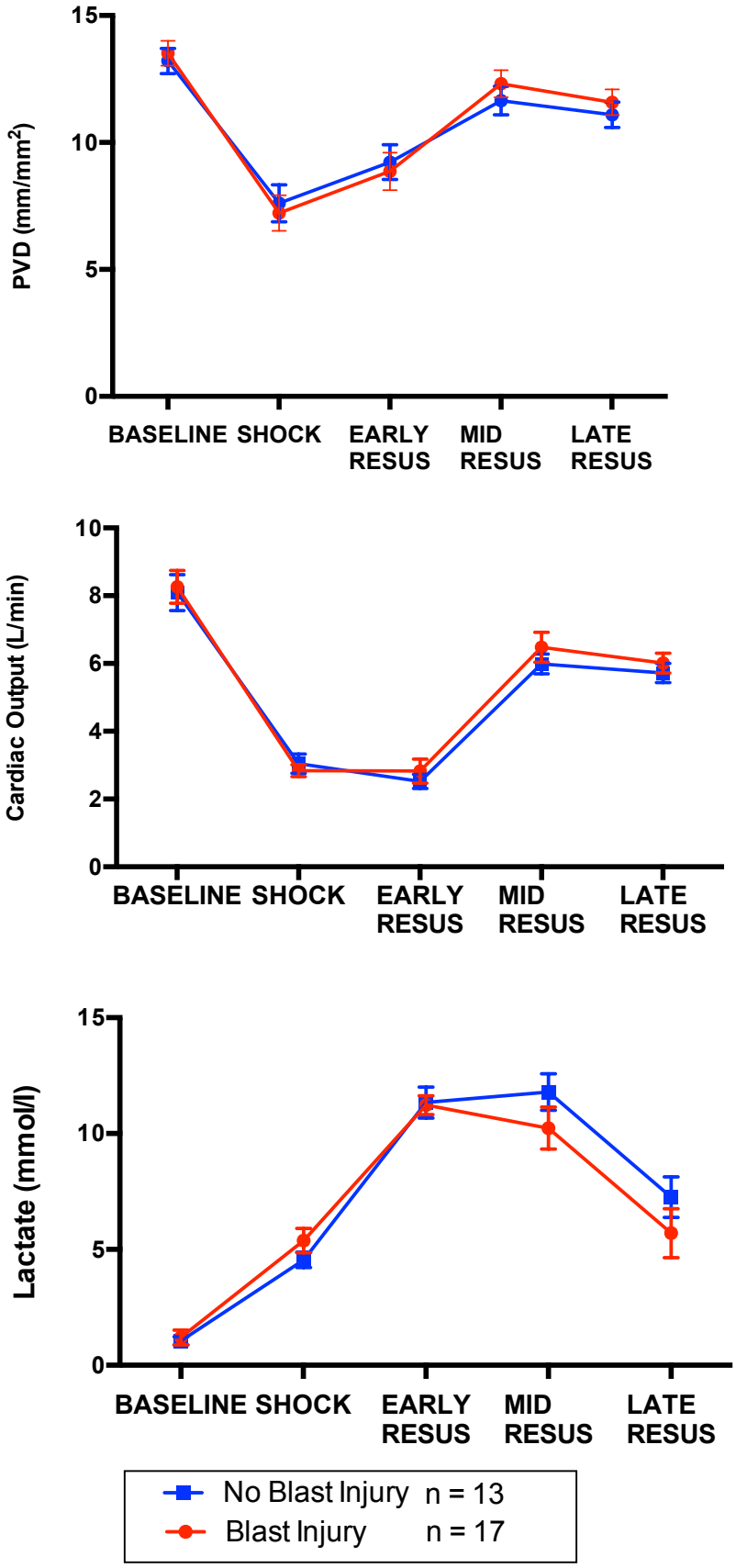

Figure 4.8 Selected macro and microcirculatory parameters and lactate profile for animals exposed or not exposed to blast injury. No significant differences between groups.

4.3.6 Differences between sublingual and splachnic microcirculation

A total of 36 time matched video sequences were obtained from the gut (ileal) and sublingual microcirculations of five animals. Gut microcirculatory flow was significantly lower than that of the sublingual region. Differences were significant in 4 of the 5 animals and persisted through to the end of the experiment as shown in Figure 4.9.

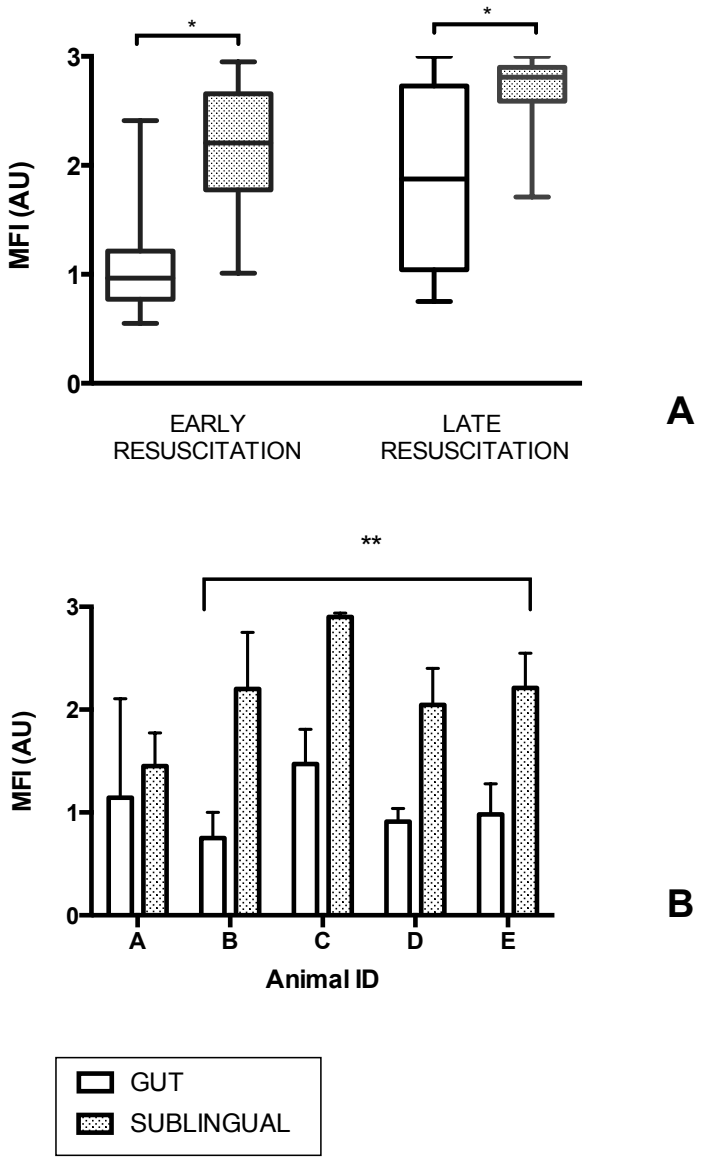

Figure 4.9 Comparison of gut and sublingual microcirculations recorded at time matched points during early and late resuscitation. A, aggregated data from all 5 animals; B,

individual animal data. * p <0.05, ** p < 0.05 for each animal (B-E) (2 tailed t tests or Mann-Whitney test)

4.4 Discussion

4.4.1 Microcirculatory perfusion is a key determinant of resuscitation outcome

The main finding of the present study is that animals who can maintain a higher degree of microcirculatory perfusion in the face of haemorrhagic shock and traumatic injury have a better outcome, in terms of markers of tissue perfusion measured at the conclusion of resuscitation.

Whilst the overall trends in systemic flow, microcirculatory flow and blood pressure were similar when the entire cohort of animals was considered, the most striking finding of the present study was the wide inter-individual variation in observed microcirculatory perfusion. It was possible for two animals at the same experimental time point receiving identical treatments to have markedly different perfused vessel densities, despite no significant differences in blood pressure or cardiac output. Previous studies in septic critically ill patients have demonstrated that there is considerable inter individual variation in the microcirculatory response to changes in systemic blood pressure caused by vasopressor agent administration. [229] The present study confirms that there is significant inter individual variation in microcirculatory performance, in the context of traumatic haemorrhagic shock, and further shows that it is very difficult, in the absence of flow, especially microcirculatory flow, monitoring to determine the adequacy of tissue perfusion.

One hypothesis for these findings may be that those animals with below average microcirculatory perfusion during shock and early resuscitation are responding to a fall in cardiac output by excessive vasoconstriction at the arteriolar level compared to animals

with above average microcirculatory perfusion. These results show that those animals with below average microcirculatory perfusion during shock and early resuscitation had a significantly higher systemic vascular resistance than better perfused animals. This excessive vasoconstriction, above that required to maintain organ perfusion pressure, leads to microcirculatory impairment and consequent tissue hypo-perfusion, as evidenced by the increased lactate in this group of animals. In a clinical setting identifying patients who have a similar response to haemorrhage may lead to important changes in management. For example, a strategy of controlled vasodilatation, combined with targeted volume expansion, would appear physiologically sound for this group. However, this strategy may lead to over aggressive volume expansion, with negative sequeale, if carried out in patients who are not excessively vasoconstricted. The ability to tailor resuscitation to microcirculatory perfusion rather than systemic blood pressure may reduce the total volume of resuscitative fluid required, elegantly demonstrated by Xu and colleagues in a large animal model [234]. Why some animals, and individuals, respond to an equivalent insult with differing degrees of vasoconstriction remains unclear, but could potentially be inherent to that individual and linked with the genomic response to traumatic injury or a low flow state.

4.4.2 Haemodynamic coherence can be lost during THS and resuscitation

It is common in critically ill septic patients to see a loss of haemodynamic coherence between the macro and micro circulations. Clinical studies have demonstrated that failure to normalise the microcirculation following resuscitation has significant detrimental effects on organ function. [128,193,208] Some previous animal experiments, involving simple haemorrhage, have reported that there is no persisting microcirculatory impairment following restoration of systemic blood flow and pressure,[125,126,235,236] whilst others have

demonstrating persistent abnormalities in several key microcirculatory beds. [128,237] Whilst the results of the present study show that, when the entire cohort of animals are considered *en bloc,* microcirculatory perfusion and flow broadly follow systemic haemodynamic parameters there are a group of animals in whom the microcirculation is relatively preserved even in the face of marked reductions in systemic flow and pressure. Furthermore, these animals appear to have improved markers of tissue perfusion following resuscitation. Such findings point to a loss of haemodynamic coherence during THS and resuscitation, if not for all animals, then at least for a proportion. Recognising this loss of coherence is potentially important during resuscitation; for example, the administration of excess resuscitation fluid to patients with reduced systemic blood flow and pressure but preserved microcirculatory flow may be unnecessary and perhaps even detrimental.

Although sublingual videomicroscopy was the main measures of microcirculatory performance used in the present study, data was also collected using NIRS electrodes applied to the hind limbs of the subject animals. There were no discernible differences in tissue oxygenation between animals with above and below average microcirculatory perfusion at the sublingual level. This is somewhat surprising as it would be expected that tissue oxygenation should follow perfusion. A possible explanation for this is the regional differences in measurements obtained by the two techniques. In the present experimental model the NIRS electrode was applied to an extremity and was thus measuring peripheral, potentially vasoconstricted, muscle rather than microcirculatory perfusion in a more centrally derived bed. Additionally, the use of continuous NIRS derived values may not be sufficiently discriminating and it may be that the dynamic change in the NIRS signal is of

more relevance. In the present experimental model, sublingual video-microscopy proved a better tool for detecting clinically relevant impaired tissue perfusion than NIRS.

There is increasing evidence that the gradient between mixed venous (or central venous) and arterial partial pressure of CO_2, sometimes termed the "CO_2 gap", may be an important marker of tissue perfusion [238-240] and that this value may be correlated with measurements made using sublingual video-microscopy. [241] The present study provides further evidence for this. Interestingly, the present findings suggest that the CO_2 gap was also a better discriminator of microcirculatory impairment than the mixed venous oxygen saturation (SvO_2), which is more commonly used to guide resuscitation in the clinical setting.

4.4.3 Effect of Resuscitation Fluids on the Microcirculation

A stated aim of the present study was to investigate the impact on the microcirculation of different fluids, and in particular MP4OX, a synthetic haemoglobin based oxygen carrier. However, the group of animals initially treated with 0.9% saline during initial resuscitation had a significantly lower PVD following injury and blood loss than the other two treatment groups. This lack of pre-treatment homogeneity made further analysis problematic. There is a suggestion from the data that 0.9% saline may not be as effective at restoring microcirculatory perfusion than blood products or colloidal fluids (such as MP4OX) and this would be in keeping with previous experimental studies that suggest that fluids with higher viscosity and oncotic potential have a more pronounced effect on microcirculatory flow. [129] However, the differences in PVD between different fluid treatment groups in the present study did not convincingly translate into improved resuscitation outcomes such as improved lactate clearance. The saline group did exhibit a worse base deficit at the end of

resuscitation but given the similarity in lactate concentrations it is probable that this resulted from hyperchloraemia rather than a tissue perfusion deficit. As discussed previously, the key determinant of outcome in this experimental model appears to be the ability of animals to maintain a greater degree of microcirculatory perfusion during the shock and early resuscitation phases. More of the MP4OX animals and less of the saline treated animals were found in this above average perfusion group. However, the data does not suggest that fluid type is the reason for this increased perfusion. Were this the case one would expect there to be a more convincingly uniform response of the microcirculation to individual fluid resuscitation which was not found, at least in this experimental model.

Free haemoglobin is a potent nitric oxide scavenger and previous experience with stroma free haemoglobin based oxygen carrying solutions have raised concerns about the systemic vasoconstriction these agents can produce. Previous studies involving haemoglobin based oxygen carriers have demonstrated a reduction in small vessel diameter and functional capillary density consistent with vasoconstriction. [172,242] PEGylated haemoglobin solutions are reported to be free from this problem; however, data from the microcirculation of PEG - Hb treated individuals is limited to small animal studies using intra vital microscopy or large animal trials that only reported systemic haemodynamic variables. The results of the present study appear to demonstrate that animals treated with MP4OX exhibited no evidence of systemic or microcirculatory vasoconstriction.

4.4.4 Effect of blast injury on the microcirculation

Blast related trauma has been an important problem for the military over the past decade. In addition, terrorist attacks on civilians frequently involve the use of explosive devices

capable of producing blast wave injury. Patterns of injury related to blast waves may include damage to air filled viscera, such as the lungs, as well as direct contusional damage to solid organs, such as the heart. Some investigators have also suggested that blast exposure may have an impact on haemodynamics, independent of cardiorespiratory impairment and that over expression of nitric oxide may have a role in mediating this. [243] The present study was designed to mimic the pattern of injury seen in military trauma and therefore included a proportion of animals exposed to a blast wave. Animals so exposed had a much higher chance of dying before the commencement of resuscitation. At post mortem, these animals usually had evidence of gross cardiorespiratory impairment, particularly severe lung contusions or evidence of coronary air embolism. However, those animals that survived the blast exposure exhibited minimal differences when compared to non-blast exposed animals. This was true for all systemic haemodynamic variables and reported outcome measures. There was therefore no evidence from the present study to support the theory of widespread Nitric Oxide induced vasodilatation as a consequence of blast injury.

4.4.5 Differences between sublingual and splanchnic microcirculations

The present study used the sublingual area for the assessment of the microcirculation. To investigate the applicability of these results to other organs measurements were also taken from the ileal microcirculation during the early and late resuscitation time points. These results show that flow within the sublingual microcirculation is higher than that within the small intestine at matched time points during early resuscitation. This time point reflects a point of the experiment where animals were still shocked, with systemic hypotension. The trend in improvement of the sublingual microcirculation was in line with the trend seen in the gut supporting the findings of several previous studies. [202,203] Based on these limited

findings it seems reasonable to postulate that the sublingual circulation represents a central circulation which is relatively preserved during shock states but can act as a “bell weather” microcirculation for the detection of worse flows in more vulnerable organ beds, such as the gut. It would have been useful to more fully assess whether there was a similar trend in the responses of the two regional microcirculations to shock and resuscitation but access to the abdominal cavity was not possible at all time points.

4.4.6 Limitations of the experimental model

The main limitation of the present study was its opportunistic nature. The protocol was primarily designed to investigate changes in coagulation parameters in a simulated model of battlefield trauma and the primary outcome measures were markers of coagulation. Outcome measures, such as lactate clearance, were adopted to answer the research questions posed, but a trial designed to exclusively assess the posed questions would probably use survival, or functional status following resuscitation, as more relevant outcome measures.

As recent battlefield trauma has involved significant numbers of casualties exposed to blast, this was an important component of the experimental injury profile. Blast injury appeared to have little, if any, effects on the variables assessed in the current study but it did introduce a further variable into the study. It was not felt that separating blast and non-blast injured animals in each treatment group would materially affect the results but it would have made the group sizes disproportionate and too small for meaningful analysis.

Because the study was designed to mimic clinical conditions, differences in administered fluid were only applied during the initial phase of resuscitation, simulating current therapeutic options in pre-hospital practice. All animals received blood products after an hour of resuscitation, in keeping with established in hospital resuscitation norms. This can only have minimised any differences caused by the choice of initial fluid. The principal finding of the study, the critical effect of early microvascular hypo-perfusion on outcome would be easier to defend had there been less heterogeneity between animals with above and below average perfused vessel densities. Additionally, a protocol that exclusively used a specific fluid would have made the question of which fluid is "best" for the microcirculation easier to answer.

Another significant limitation of the present study was the lack of data relating to tissue oxygenation. The principal aim of resuscitation is to restore oxygen delivery to tissues and cells. Although an intact microcirculation is a key precursor to this it is not the only factor. A key feature of haemoglobin based oxygen carriers, in particular, is their purported ability to restore not only blood flow but also tissue oxygenation. The current experimental protocol attempted to assess this factor using NIRS but it proved to be an insensitive monitoring tool in this experimental model, unable to differentiate between animals with above and below average tissue perfusion during the shock and early resuscitation phases. There are several possible explanations for this. Firstly, NIRS probes were applied to extremities that may have been critically compromised by peripheral vasoconstriction. Secondly, static NIRS measurements may be poorly predictive of microcirculatory function in contrast to dynamic measurements made following a limb vascular occlusion test. Future studies investigating

the effect of haemoglobin based oxygen carriers on the microcirculation should utilise an effective marker of tissue oxygen delivery alongside in vivo visualisation of microcirculation.

4.5 Conclusion

In a porcine experimental model of complex traumatic haemorhage, shock and resuscitation there was wide inter-individual variation in sublingual microcirculatory perfusion which predicted lactate clearance during resuscitation. The state of the microcirculation was not predictable from an observation of blood pressure or cardiac output. The choice of resuscitation fluid did not appear to have a significant impact on microcirculatory perfusion. MP4OX was an effective initial treatment for haemorrhagic shock in this experimental model with no discernible detrimental effect on vasomotor tone or microcirculatory perfusion.

Chapter 5

Poor microcirculatory perfusion combined with early fluid resuscitation using 0.9% saline predisposes to the development of trauma induced coagulopathy in a porcine model of complex haemorrhagic shock

5.1 Background

Major haemorrhage is the leading cause of preventable death following traumatic injury [10]. A contributing factor to a proportion of these deaths is Trauma Induced Coagulopathy (TIC) which is independently associated with increased mortality and morbidity, including post traumatic multiple organ failure. [15 244]

An evolving understanding of the pathogenesis of TIC has led to the realization that central to the development of TIC are the combined insults of tissue injury and hypo-perfusion. [14] As the critical final common pathway of oxygen and substrate delivery the microcirculation, the network of small blood vessels < 25 microns in diameter, plays a key role in tissue perfusion. Despite evidence showing the importance of microcirculatory impairment following THS with impacts both on initial lactate clearance [245] and later organ dysfunction [128] there is a paucity of experimental or clinical data linking the directly observed state of the microcirculation with the development of TIC.

The choice of resuscitation fluid may have an impact on the development of TIC. A number of clinical service providers, including NATO military medical services, have adopted a strategy of pre-hospital administration of blood products for patients with traumatic injury and major blood loss. Such a strategy is physiologically plausible but there is currently a lack of high quality evidence to support this approach, which is associated with significant logistic implications.

The present study examines the influence and relationship between the initial resuscitation fluid (blood products or 0.9% saline) and the state of microcirculatory perfusion, visualised using Incident Dark Field (IDF) video-microscopy, on the development of TIC in a porcine experimental model of complex THS. The hypothesis was that animals with poor microcirculatory perfusion during shock and subsequent resuscitation would develop an exaggerated degree of TIC when compared to those with better perfusion.

5.2 Methods

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

5.2.1 Animal preparation

The present study uses data obtained from animals involved in the experimental work outlined in Chapter 4 of this thesis and full animal preparation has been described in detail therein. In summary, female Large White pigs (43–56 kg) were terminally anaesthetised, and allowed spontaneous respiration following tracheal intubation. The left femoral artery and left common carotid arteries were cannulated to allow withdrawal of blood and infusion of resuscitative fluid respectively. Cardiac output monitoring was performed using a

pulmonary artery catheter (Vigilance Volumetrics CEDV; Edwards Lifesciences Ltd, UK). The bladder was catheterised. A midline laparotomy and splenectomy were performed, and the abdomen was closed.

5.2.2 Experimental protocol

The full experimental protocol has been fully described in Chapter 4 and is shown in Figure 4.1. The protocol was designed to replicate battlefield injury, shock and subsequent resuscitation and to keep all animals alive until the end of the experiment. A proportion of animals received a controlled blast injury, and all animals received a controlled hind limb injury from a captive bolt gun. Following a 30 min shock phase animals underwent 60 min of resuscitation targeted to a systolic blood pressure of 80 mmHg. During this phase, which was designed to simulate pre- hospital care, animals were randomized to receive either 0.9% saline or component blood therapy consisting of cross-matched, leucodepleted porcine red blood cells and plasma (delivered in a 1:1 ratio). Following this phase all animals received blood product resuscitation to a target systolic blood pressure of 110 mmHg for a further 150 minutes.

5.2.3 Microcirculatory monitoring

The microcirculation was assessed using Incident Dark Field (IDF) video-microscopy. (Cytocam, Braedius Medical, Huizen, NL). Techniques for image acquisition and analysis are described in detail in Chapters 2 and 4 of this thesis. Animals were divided *ex priori* into two groups, classed as having above average and below average PVD using the method described in Chapter 4.

5.2.4 Assessment of Coagulopathy

Thromboelastography was performed using a TEG® 5000 Hemostasis Analyzer (Haemonetics Ltd, Coventry, UK) on fresh, un-citrated whole blood. Arterial blood was taken from the femoral cannula and analyzed immediately using dilute Innovin (1:50,887 dilution Dade Innovin; Dade Behring, marketed by System UK Ltd, Milton Keynes, UK) as the initiator. All TEG analyses were performed in triplicate at 37^{o}C.

Prothrombin and activated partial thromboplastin time assays were performed on arterial blood samples taken into citrated tubes and centrifuged at 1,500g for 10 min. The plasma was separated and stored at -80^{o} C for determination of prothrombin time (PT) and activated partial thromboplastin time (aPTT) by turbidometry and fibrinogen concentration (Clauss method) using an ACL Elite analyzer (Instrumentation Laboratories).

5.2.5 Statistical Analysis

Statistical analysis was performed using Prism v. 6.0 (Graph Pad, San Diego, California, USA). All data was tested for normality using the D'Agostino & Pearson omnibus normality test. Normally distributed data are presented as mean±SEM. Differences between groups over time was assessed using repeated measures 2-way ANOVA with Sidak's correction for multiple comparisons. A p value of <0.05 was taken to indicate significance.

5.3 Results

Data was analysed from the same 30 animals presented in Chapter 4. Using the methodology reported in Chapter 4, 14 animals had above average PVD and 16 below average PVD during shock and early resuscitation. Details of animals based on received treatments and microcirculatory perfusion are shown in Table 5.1.

	0.9 % Saline	Blood Products	MP4OX
Above average PVD*	3	6	5
Below average PVD*	7	6	3

Table 5.1 Characteristics of animals in terms of initial resuscitation fluid and state of microcirculatory perfusion during shock and early resuscitation time points. * relation to mean of lowest recorded values during shock / early resuscitation

For animals initially treated with blood products there were no differences in any of the measured parameters of coagulation between the group with above average microcirculatory perfusion and the group with below average perfusion (Fig. 5.1). Notably, none of the animals treated with blood products, regardless of the degree of microcirculatory impairment, developed a coagulopathy, as evidenced by changes in TEG® profile and PT/APPT from baseline.

By contrast the degree of coagulopathy for 0.9% saline treated animals appeared to be dependent on the state of the microcirculation during shock and early resuscitation (Fig 5.2). Animals with below average microcirculatory perfusion who were initially treated with 0.9% saline exhibited significant impairment in viscoelastic measures of coagulation. TEG Maximum Amplitude (MA), representing maximal clot strength, was the variable that exhibited the most difference over time between the two groups of animals. R time, representing clot initiation, was initially significantly longer in animals with below average perfusion, but this difference was lost as resuscitation progressed and animals received blood products as the resuscitation fluid. Interestingly, animals with above average perfusion initially treated with 0.9% saline did not exhibit any signs of coagulopathy.

Animals initially treated with MP4OX exhibited a notable pattern of derangement on viscoelastic testing with some animals appearing to develop greatly prolonged R times and reduced MA which persisted until the end of the experiment (Fig. 5.3) However, there was a very wide spread in these results. Notably, however abnormalities in viscolelastic parameters appeared confined to animals with below average microcirculatory perfusion.

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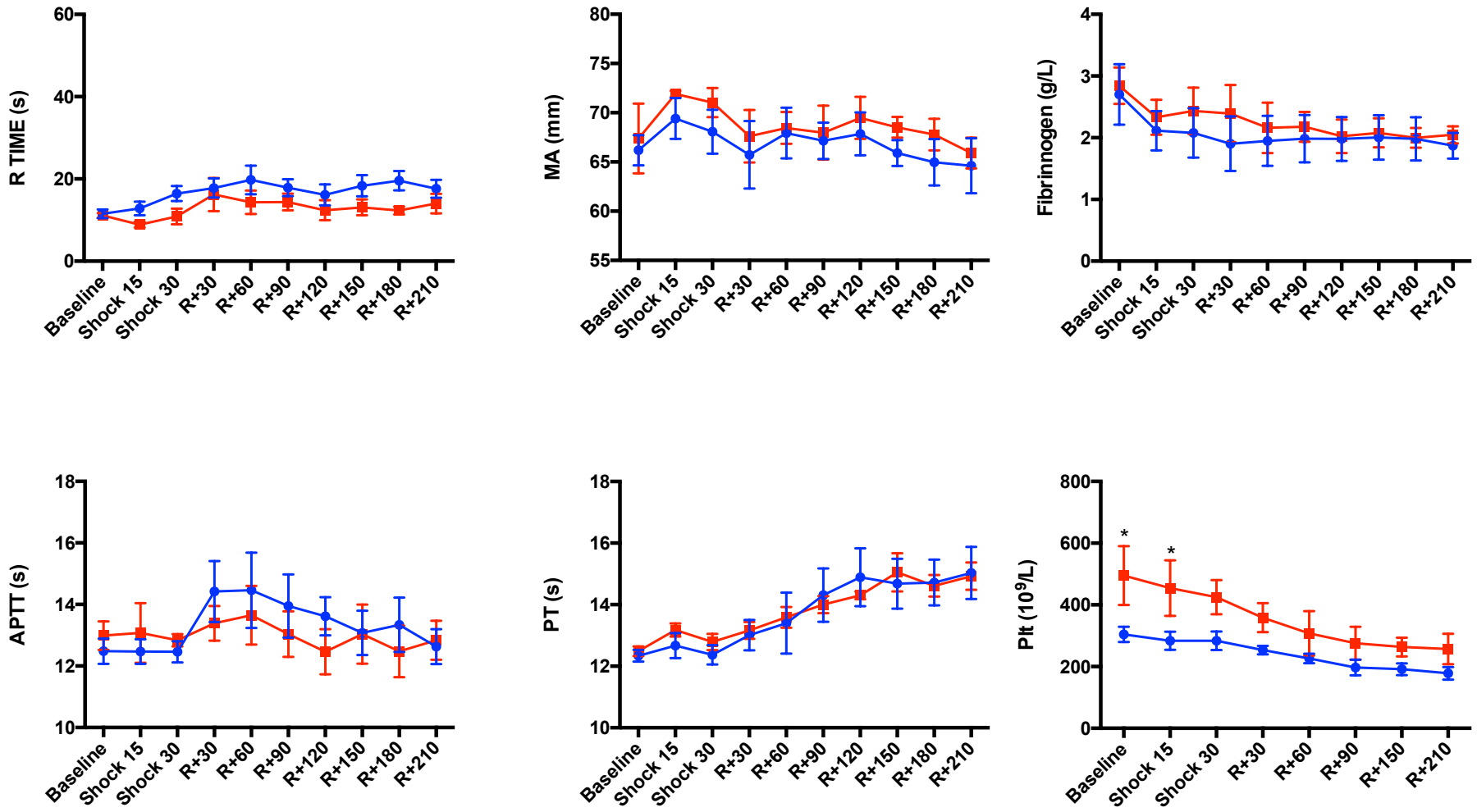


Figure 5.1 Coagulation profiles assessed using viscoelastic and conventional measures for animals treated with blood products (PRBC/FFP) during the early resuscitation phase (Shock 30-R60)

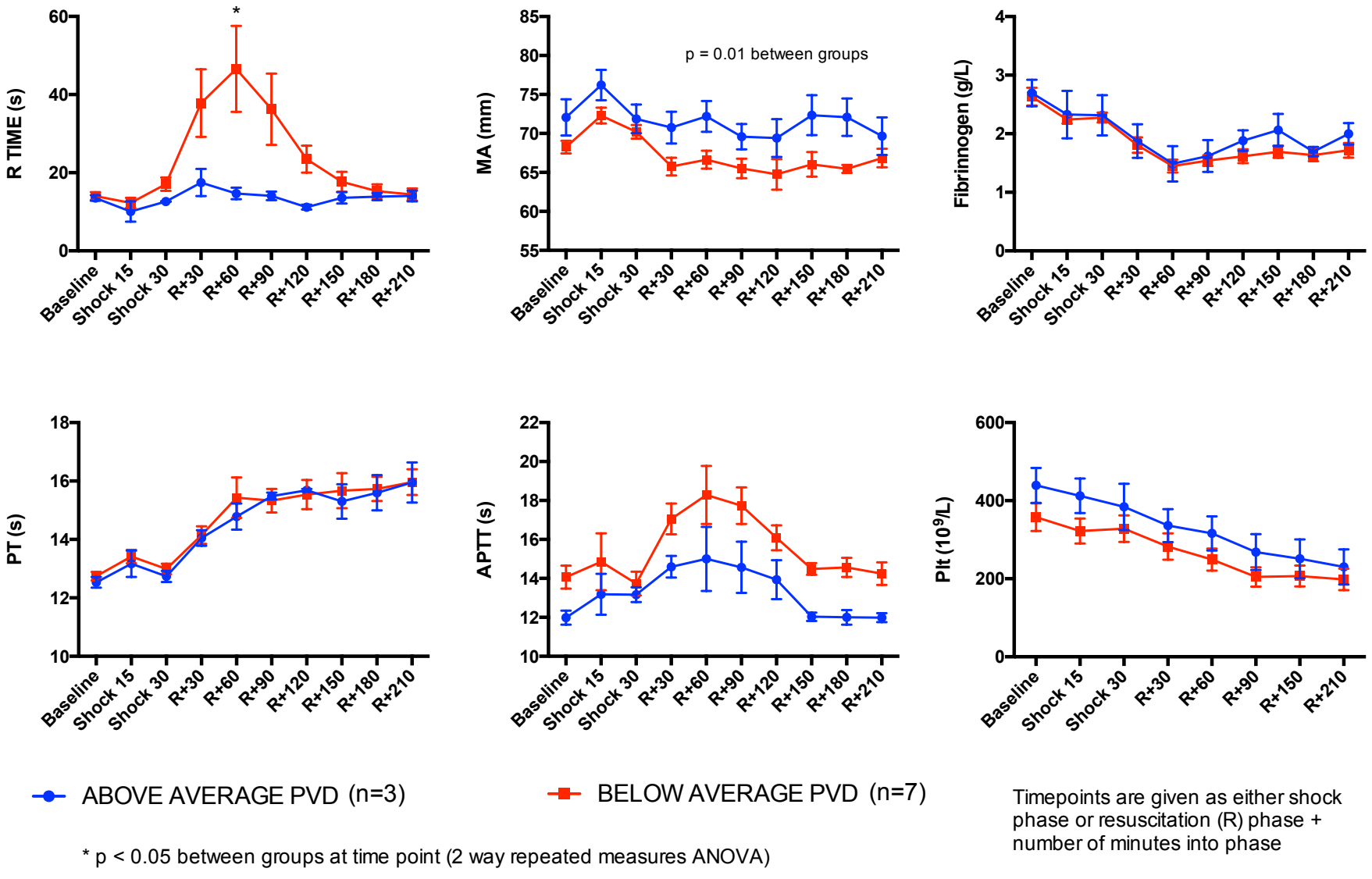
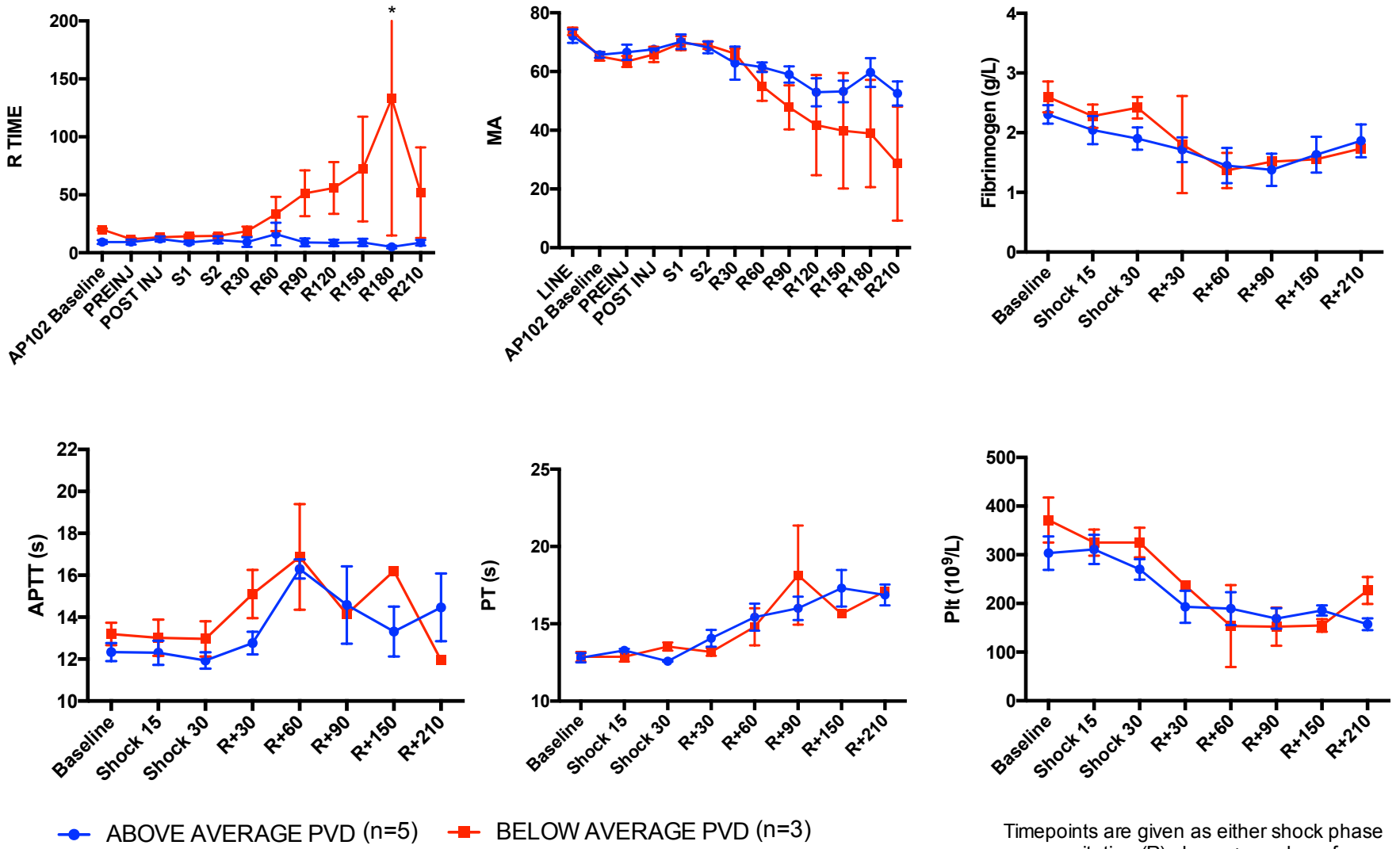


Figure 5.2 Coagulation profiles assessed using viscoelastic and conventional measures for animals treated with 0.9% saline during the early resuscitation phase (Shock 30-R60)



Timepoints are given as either shock phase or resuscitation (R) phase + number of minutes into phase

* $p<0.05$ between groups at timepoint (2 way repeated measures ANOVA)

Figure 5.3 Coagulation profiles assessed using viscoelastic and conventional measures for animals treated with MP4OX during the early resuscitation phase (Shock 30-R60)

Although PT and APTT increased following shock in all animals no differences were observed based on the degree microcirculatory perfusion.

There were no significant differences in Fibrinogen levels relating to the type of initial resuscitation fluid. Additionally, animals with above and below average perfusion, treated with the same fluid exhibited no differences in Fibrinogen levels.

Platelet counts were higher at baseline in those with below average microcirculatory perfusion treated with initial blood products and this difference persisted throughout the shock and initial resuscitation phases. Despite this there was no difference in clot strength, as measured by TEG MA between these animals and this finding is probably of no significance. Other than this finding there was no difference in platelet counts between groups that could explain the differing coagulation profiles.

5.4 Discussion

The principal finding of the present study is that animals with poor microcirculatory perfusion following THS who are treated with 0.9% saline for an hour following injury and blood loss exhibit a significantly greater coagulopathy than identically injured and treated animals with above average levels of microcirculatory perfusion. Furthermore, this difference did not apply to animals who received blood products as the initial resuscitation fluid. Some animals with poor microcirculatory perfusion who received MP4OX appeared to exhibit a marked and persistent degree of derangement in viscoelastic measure of coagulopathy but there was wide heterogeneity in this group of animals.

Traditional teaching on the development of coagulopathy following massive blood loss has focused on the role of acidosis, hypothermia and dilution, the so called lethal triad [246]. However, the finding that an established coagulopathy often occurred very early following injury highlighted the limitations of such an explanation. It is now accepted that TIC is an intrinsic biological process [228,247]. Key determinants for the initial development of TIC are the presence of both tissue injury and a reduction in tissue perfusion.[14]

The vascular endothelium plays a key role in the development of TIC. Although the precise mechanism is still opaque several key features associated with the pathogenesis have been determined. Firstly, there is increasing evidence that activated Protein C (aPC) plays a key role in the process with high circulating levels of aPC being associated with increased mortality following traumatic injury.[15] Hypoperfusion and damage to the vascular endothelium leads to the exposure of thrombomodulin, which complexes with thrombin to transform Protein C to aPC. aPC reduces the levels of Va and VIIIa and also consumes

plasminogen activator inhibitor 1 (PAI-1). PAI-1 is a major antagonist of the natural fibrinolytic tissue plasminogen activator (tPA) and hence reduction in levels can lead to excessive fibrinolysis.[248] Whilst aPC pathways probably exist to ensure that periods of physiological low flow do not lead to inappropriate clot formation in small vessels the widespread activation of this process, leading to the systemic overexpression of aPC, with the consequences listed above, will produce an inappropriate coagulopathy.

An additional potential mechanism for the development of TIC is the destruction of the endothelial glycocalyx. [249] This layer of bio-polymers, including glycosamoninoglycans, glycoproteins and proteoglycans not only performs an essential vascular barrier function, preventing capillary leak but also retains coagulation inhibition factors. Damage to the glycolcalyx therefore promotes coagulopathy by exposing thrombomodulin and endothelial Protein C receptors and activating the protein C pathway. Additionally, several components of the glycocalyx are heparin like substances which when released into the circulation are capable of inducing a coagulopathy via activation of anti-thrombin 3. [250] High levels of circulating Syndecan-1, a glycocalyx breakdown product, have been shown to be associated with a worse outcome following traumatic injury [251] and work recently completed by our group in a cohort of patients with THS has suggested that the degree of glycocalyx shedding is related to the degree of microcirculatory flow impairment. [252]

Given these pathophysiological considerations factors which affect the integrity of the vascular endothelium, for example impairment of blood flow through the microcirculation, are therefore likely to influence the development of TIC. The findings of the present study

show a link between directly observed microcirculatory flow and vessel density and the development of TIC in animals treated with 0.9% saline.

The most visually striking difference in coagulation parameters for saline treated animals with above and below average microcirculatory perfusion is in the TEG® R time which represents clot initiation. Initiation of a clot is critically dependent on the initial thrombin burst and subsequent propagation, a process that is intimately associated with the vascular endothelium. One explanation for these findings is that significant impairment of microcirculatory flow during the shock and initial resuscitation phases following injury causes damage to vascular endothelial cells, which augments the development of TIC. Two other factors are known to be crucial in the development of TIC; abnormal platelet function and low levels of circulating fibrinogen.[253] Although undoubtedly of importance these factors do not explain the differences in the development of TIC in this experimental model as there were no significant differences in either platelet count or fibrinogen concentration between the studied groups.

The present study found that the use of early blood products was protective against the development of TIC, even in animals with poor microcirculatory perfusion. Prevention and amelioration of the effects of TIC have largely focused on the choice of resuscitation fluid. Several retrospective observational studies suggested a reduction in mortality associated with the use of high ratio PRBC: FFP transfusions following traumatic injury and such a policy was adopted by many military and civilian trauma services. However, the largest RCT conducted in this area, the PROPPR trial, did not show an improvement in 24 hour or 30 day mortality in those treated with 1:1 ratio versus a 1:2 ratio. [254] Work by our group showed

that in an experimental model of traumatic haemorrhagic shock, used in the present study, animals treated with blood products had improved markers of coagulation after an hour of resuscitation compared to those treated with 0.9% saline.[255] Interestingly, in this study PRBC alone, without the addition of plasma also prevented coagulopathy. This finding is superficially surprising as several other investigators have reported that use of plasma may reduce the incidence of TIC through a restorative effect on the endothelial glycocalyx. [256] [109] The present study only included data from animals treated with both PRBC and plasma rather than PRBC alone so it is not possible to discriminate between these two fluids when examining the present findings. It may be that the potential improvement in oxygen delivery to the vascular endothelium, associated with the use of early PRBC may be the factor responsible for the reduction in TIC, but further work is needed to answer this important question.

MP4OX is a product for which there is limited data and to date there have been no reports relating to the effects of MP4OX administration on coagulation variables. The data presented in the present study suggest that some animals treated with MP4 exhibited very marked derangements in both R time and MA but that there is wide inter-individual variation in the results. Notably, however, such abnormalities were confined to animals with below average microcirculatory perfusion and these results add some weight to the argument that the state of microcirculatory perfusion is a critical determinant for the development of TIC. Why some animals with poor microcirculatory perfusion treated with MP4OX should exhibit such prolonged and marked abnormalities in TEG variables is not clear. Unfortunately, further work in this area is unlikely to occur as the product is no longer available following the commercial failure of the manufacturer.

The findings outlined in Chapter Four of this thesis demonstrated that the state of microcirculatory perfusion, both during shock and initial resuscitation is important in determining the subsequent speed of resolution of the shock state. The present study builds on this and demonstrates, for the first time using a point of care measure, that microcirculatory perfusion is also a key determinant of ATC, at least for those animals treated with crystalloid initial resuscitation. Such a finding could be clinically important. The use of blood products during initial resuscitation in the pre –hospital environment can be logistically challenging and this is especially the case in the deployed military setting. If a point of care device can reliably discriminate between patients with poor microcirculatory perfusion, who may benefit from the early use of blood products, and those with a relatively preserved microcirculation, blood product use could be more rationally prescribed on a targeted basis. Such point of care assessment may be within reach as the evolution of new, more portable video microscopes, combine with the development of more rapid scoring systems, which can accurately assess the microcirculation at the bedside within minutes. [257] [258]

5.4.1 Limitations of the experimental model

This study has several limitations. Most importantly findings from a porcine model should be cautiously translated into clinical practice. Although the porcine and human response to hemorrhage are similar there may be differences in viscoelastic variables such could limit the direct translation of results. [259] Reproducing these findings in a clinical setting would add weight to the conclusions. Secondly, although significant differences were seen in some TEG® related variables, principally the R Time, these variables tended to normalise once saline treated animals received blood products. Arguably, the clinical impact of initial resuscitation with 0.9% saline, even in patients with impaired tissue perfusion may therefore be limited, if that patient then goes on to be resuscitated with blood products.

Although the present study findings show that clot strength, measured as TEG® MA, was reduced in saline treated hypoperfused animals, correlating these findings with more clinically relevant endpoints of resuscitation, such as mortality or amount of blood loss / replacement would add weight to the findings presented here.

There are several mechanisms through which microcirculatory hypoperfusion may lead to ATC and many unanswered questions remain regarding the precise mechanism. Correlation of the findings presented here with biomarkers of glycocalyx degradation (e.g. Syndecan-1) or endothelial activation (e.g. Angiopoetin-2) may shed further light on the mechanisms behind these findings. Unfortunately, porcine assays for many of these biomarkers are not currently available.

Finally, it would be of significant interest to determine which component of blood products (PRBC or plasma) are principally responsible for the putative protective effect seen in the findings of this study. Limited animal numbers precluded the examination of a third group, treated purely with PRBC during initial resuscitation, but given the logistic implications of transporting plasma into the pre-hospital environment it would be of value to explore this question further.

Conclusion

In conclusion, the findings of the current study demonstrate that microcirculatory impairment, when combined with initial resuscitation with 0.9% saline is associated with a heightened degree of coagulopathy. Blood products may attenuate, or even prevent this process. The precise mechanisms behind these findings remain unclear and further work and clinical studies are required before these findings can be translated into clinical practice.

Chapter 6

Microcirculatory impairment is associated with multiple organ dysfunction following traumatic haemorrhagic shock

6.1 Introduction

Haemorrhage remains the leading cause of preventable mortality following major trauma [3]. Whilst some of these deaths result from uncontrolled blood loss and overt exsanguination others occur later, as a result of multiple organ failure (MOF); the incidence of which has been reported as between 7 - 29 %. [20-22,24] The mechanism behind MOF resulting from traumatic haemorrhage is yet to be clearly elucidated. Previous hypotheses espousing the view that MOF occurred mainly as a "second hit" phenomenon, driven by secondary sepsis have been largely refuted by more recent evidence which shows that the temporal distribution of post traumatic organ dysfunction peaks at around 3 days following injury and that there is no obvious second peak. [30]

A repeated finding from observational cohort studies of severe trauma patients is that the degree of shock, assessed using surrogate markers such as lactate concentration or base deficit, is associated with the magnitude of organ dysfunction.[21,22,57,260,261] The overriding focus on pressure based management during trauma shock resuscitation has meant that maintenance of organ perfusion is often only considered later in the resuscitation pathway, if at all.

A key element of effective tissue perfusion during traumatic shock and resuscitation is the maintenance of blood flow through the network of microcirculatory vessels which represent the final pathway for cellular oxygen and substrate delivery. Data presented in Chapter 4 of this thesis suggest that maintenance of microcirculatory perfusion is an important factor in determining immediate resuscitation outcomes, such as lactate clearance. In the only clinical study reported to date *Tachon* and colleagues demonstrated that in patients with resuscitated traumatic hemorrhagic shock there is a persisting microcirculatory deficit for up to 4 days following injury and that those patients with more organ dysfunction, defined in that study as a SOFA score > 6 on day 4 post injury, had significantly worse post resuscitation microcirculatory parameters. [128]

The aim of the present study was to confirm the link between microcirculatory impairment and organ dysfunction in a larger cohort of patients managed in three UK major trauma centers.

6.2 Methods

The study received ethical approval from Leeds & the Humber Research Ethics Committee (14/YH/0078) and was registered at Clinical Trials.gov (NCT02111109). The full study protocol was published. [262]

6.2.1 Inclusion and exclusion criteria

Patients were eligible for inclusion if they had suffered a traumatic injury, received blood products during resuscitation and had a plasma lactate concentration of > 2 mmol/l at any stage prior to study recruitment. In order to recruit patients with the severest injury

patterns and to facilitate the use of IDF video-microscopy only patients who were intubated and ventilated were included. Study enrollment had to occur within 12 hours of admission to the intensive care unit.

Patients were excluded from the study if they had un-survivable injuries with a palliative focus of care or if they had facial injuries that made IDF video-microscopy impossible.

6.2.2 Recruitment and consent

As informed consent was not possible at study inclusion a process of deferred consent was used. Consent for enrollment was initially provided by a nominated consultee, a senior clinician responsible for the patient's management who was not involved in the study. Families of study participants were then approached to act as personal consultees and provide consent for continuing involvement in the study. Finally, when patients recovered they were approached to provide retrospective informed consent. If patients died or did not recover to a position where they had capacity to provide informed consent their data was included in the study in accordance with the wishes of their personal consultee. No patients or relatives withdrew consent for the use of data after study enrollment.

6.2.3 Study measurements

At the initial (D0) time point (within 12 hours of ICU admission) an assessment was made of microcirculatory perfusion using IDF video-microscopy (Cytocam, Braedius Medical, Huizen, NL). Cardiac output was concurrently assessed using an oesophageal Doppler monitor (Deltex Medical, Chichester, UK). For a selection of study patients an assessment of intra-vascular volume status was made using trans- thoracic echocardiography (Sparq, Philips, United States) if the investigator was trained in the technique. Specific details of the methods used for these monitoring methods are provided in Chapter 2. IDF

videomicroscopy was performed by either the present author (SH), or an operator trained by SH, All video sequences were assessed for quality by SH. Blood was drawn from arterial and central venous catheters (once placed) and used to determine plasma lactate, base deficit, hemoglobin oxygen saturation and arterial and central venous carbon dioxide tensions. Standard physiological parameters were recorded using existing monitoring devices. The clinical monitoring and blood gas analysis devices varied between the 3 study sites but were the devices in standard clinical use at that center. Measurements were repeated at D0 + 24 hours (D1) and D0+72 hours (D2). However, if the patient was extubated during this time then no further microcirculatory or cardiac output measurements were taken. Organ failure was assessed using SOFA and Denver scores every day for the first 7 days following injury. Details of SOFA and Denver scores are provided in the appendices to this thesis. The primary outcome of the study was the presence or absence of multi organ dysfunction (defined as a SOFA score of 6 or more) on day 7 following injury. Patients who had died by day 7 following injury were assigned a maximal SOFA and Denver score (24 and 12 respectively).

6.2.4 Video Analysis

Microcirculatory videos were collected from the sublingual region of recruited patients and analysed using the methods outlined in Chapter 2. Operators were all trained by the present author. Videos from all 3 study sites were assessed for quality by the present author. AVA Analysis from 2 of the study sites were conducted by the present author whilst those from a third site was conducted by the local principal investigator.

6.2.45 Statistical Analysis

Statistical analysis was conducted using Prism v 6.0 (GraphPad Software Inc., California, USA). Data was assessed for normality using the D'Agostino & Pearson omnibus normality test. Parametric data is presented as Mean (SD) and non-parametric data as Median (IQR).

Patients were dichotomized into 2 groups based on the development of MODS at day 7. Differences in variables at the initial (D0) time point for these patients was assessed using unpaired t-tests or Mann-Whitney tests depending on distribution of the data. Differences in quantitative variables over time, for patients with measurements at all experimental time points, was assessed using repeated measures 1-way ANOVA with post-test for linear trend or Friedman's test with Dunn's multiple comparison correction depending on the distribution of the data.

Differences in daily organ failure rates over time between patients based on initial microcirculatory perfusion were assessed using repeated measures 2-way ANOVA with Sidak's multiple comparison test.

Receiver Operator Characteristic (ROC) curves were constructed to predict the incidence of multiple organ dysfunction (SOFA >6 at day 7 post injury) by plotting sensitivity versus (1-specificity) for different threshold values of a series of variables. In order to determine the best fit predictive value for determining MODS, the microcirculatory variable exhibiting the largest area under the ROC curve was selected. The Youden index for this variable was calculated by determining the J value (Sensitivity +Specificity-1) for each threshold

measurement. The threshold measurement with the largest J value was selected as the best fit or cut off value for determining MODS.

A p value of < 0.05 was taken to indicate significance.

6.3 Results

6.3.1 Patient characteristics

60 patients were recruited between July 2014 and April 2016. Data from 2 patients was rejected as there were insufficient high quality microcirculatory videos, leaving 58 patients with data suitable for analysis. Characteristics of study patients are presented in Table 6.1. Patients were enrolled in the study on average 9 (3-13) hours following traumatic injury and 1.5 (0-6) hours after admission to intensive care. The mechanism of injury in the majority of patients was blunt (75%) with transport related accidents commonest (53%) followed by falls (17%) and crush injuries (7%). Patients with penetrating trauma represented 24% of those enrolled with one patient injured by a firearm and the remainder knife injuries. The median number of PRBC transfused during initial resuscitation (up to D0 time point) was 6 (4-11) and 29% of patients received a massive transfusion (> 10 units PRBC in the first 24 hours). Enrolled patients exhibited signs of shock and haemodynamic compromise; the mean highest recorded lactate concentration prior to ICU admission was 7.3±6.1 and mean lowest recorded systolic blood pressure 69±27 mmHg. 8 of the patients suffered a traumatic brain injury, representing 13% of the cohort. 28-day mortality was 12% and the mean ICU stay was 17±14 days. 29% of patients had persisting multiple organ dysfunction (defined as a SOFA score of 6 or more) at day 7 after injury.

Age	43±19
Gender	47 male, 11 female
Mechanism of injury **Blunt** **Penetrating**	 44 (75%) 14 (25%)
ISS	29±14
APACHE II	23±13
PRBC before D0 (units)	6 (4-11)
FFP before D0 (units)	4.5 (2-9)
Crystalloid fluid before D0 (ml)	2500 (250-4200)
Colloidal fluid before D0 (ml)	0 (0)
Initial Management **Surgery** **Surgery + IR** **IR alone** **Conservative**	 47 (81%) 6 (10%) 1 (2%) 4 (7%)

Table 6.1 Characteristics of study patients. ISS Injury Severity Score, PRBC Packed Red Blood Cells, FFP Fresh Frozen Plasma, IR Interventional Radiology. D0 = initial study time point. Values shown as mean±SD or median (IQR)

6.3.2 Microcirculatory and macrocirculatory responses

Microcirculatory and systemic haemodynamic variables at study enrollment and subsequent time points are shown in Figure 6.1 and 6.2. The number of such measurements made at the D0, D1 & D2 time points were 58, 31 & 25 respectively. Missing values were caused by patients being extubated prior to the time point, except for 3 cases in which the patient died prior to measurements being obtained.

Patients were dichotomized into those with or without MODS (n=24 & n=34 respectively) based on their SOFA score at Day 7 following injury. Those patients who developed MODS had significantly lower values for TVD, PVD & MFI at D0 than those who did not go on to develop MODS. In addition, patients who went on to develop MODS had a significantly higher MHI at DO, indicating a greater degree of flow heterogeneity within the microcirculation. Those patients who went on to develop MODS exhibited an improvement in microcirculatory variables over the 48 hours of the study period, perhaps indicating a degree of persistant impairment. By contrast, those who never developed MODS had no differences in their recorded microcirculatory indices between D0 and D2.

There were no differences in observed macrocirculatory values (Cardiac Index & MAP) between the two groups. Vasopressor usage and dose was also not significantly different between the groups. Median Noradrenaline dose at the D0 time point was minimal 0 (0-0.14) mcg/kg/min of ideal body weight.

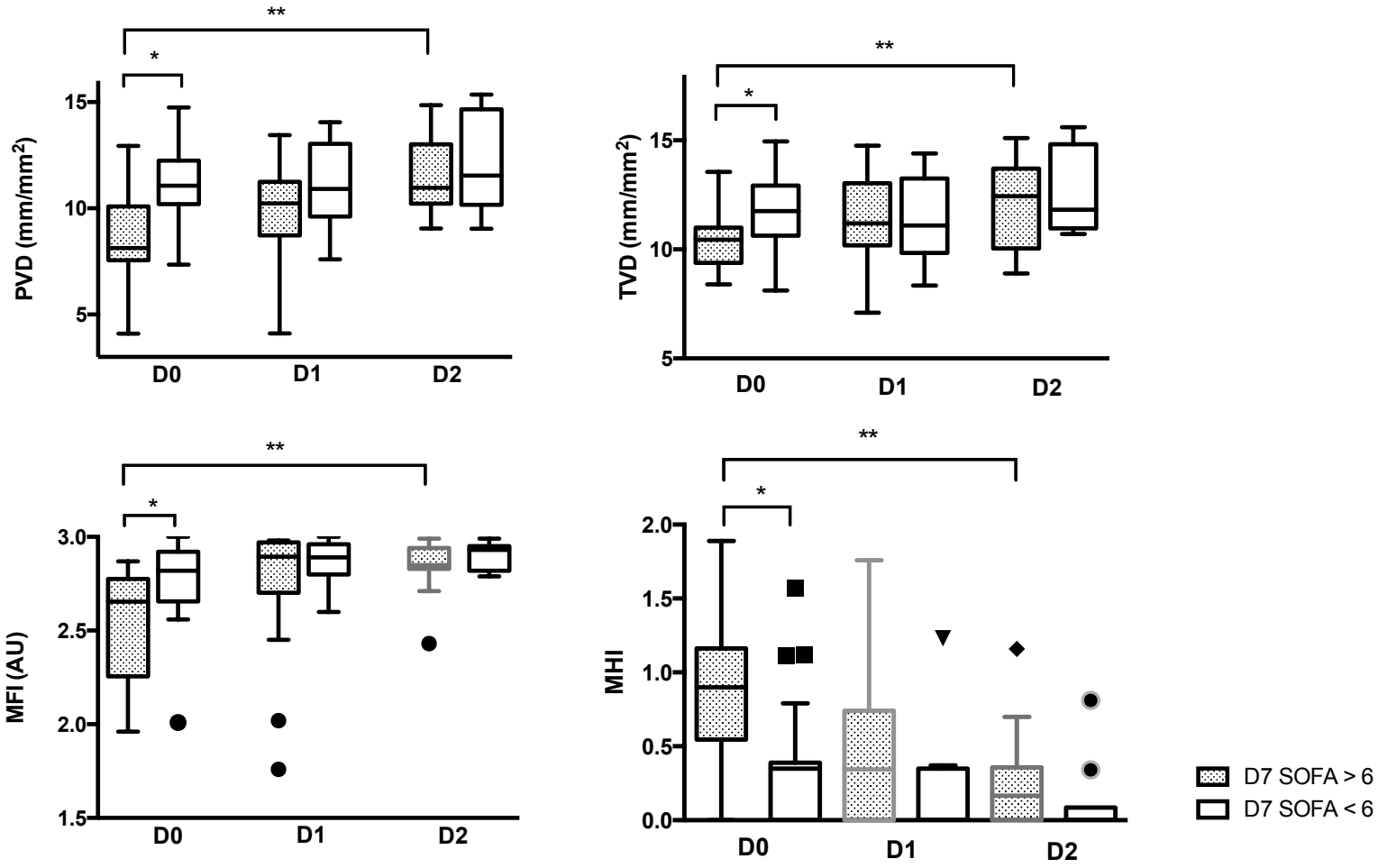


Figure 6.1 Microcirculatory variables at study inclusion (D0 n=58), +24 h (D1 n=31)) and +48 h (D2 n=25)). Patients are grouped into those who did or did not develop MODS by Day 7 after injury. PVD Perfused Vessel Density, TVD Total Vessel Density, MFI Microcirculatory Flow Index, MHI Microcirculatory Heterogeneity Index. * $p < 0.05$ between groups (t test / Mann-Whitney) ** $p < 0.05$ over time (1-way ANOVA)

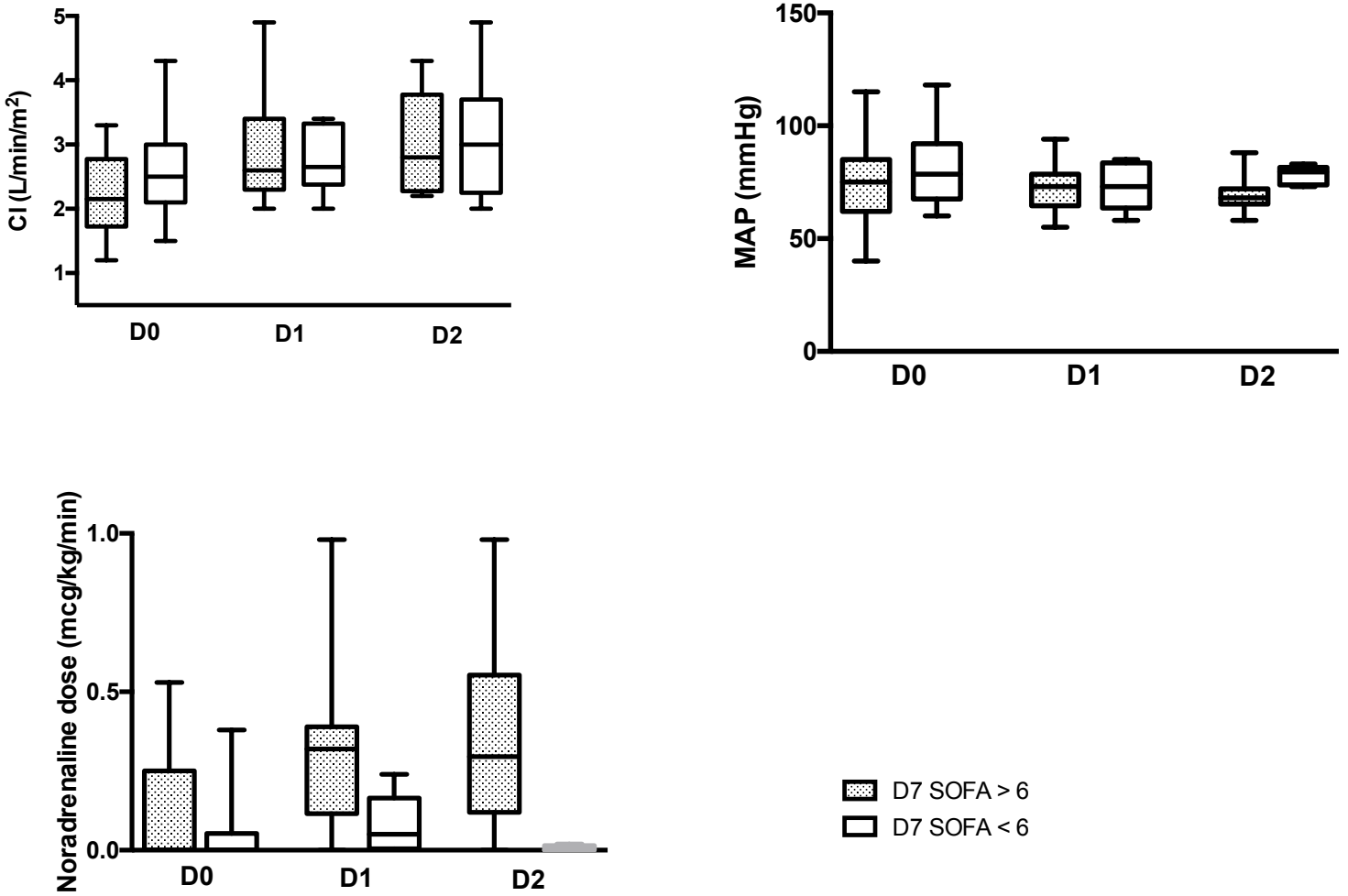


Figure 6.2 Haemodynamic variables at study inclusion (D0 n=58), +24 h (D1 n=31) and +48 h (D2 n=25). Patients are grouped into those who did or did not develop MODS by Day 7 after injury. CI Cardiac Index, MAP Mean Arterial Pressure. No significant differences between groups or over time (1-way ANOVA)

6.3.3 Characteristics of patients with and without MODS

Table 6.2 shows the characteristics of patients based on their subsequent development of MODS. MODS defined as SOFA score > 6 at day 7 after injury.

	SOFA > 6 (n=23)	SOFA < 6 (n=35)	p value
Age	49 (28-74)	36 (27-46)	0.06
ISS	35±12	25±14	0.01
PRBC prior to D0 (units)	6 (4-13)	6 (3-8)	0.20
FFP prior to D0 (units)	5 (4-10)	4 (2-8)	0.39
Crystalloid fluid prior to D0 (ml)	2900 (225-6125)	2000 (0-3500)	0.19
Noradrenaline dose (mcg/kg/min)	0 (0-0.25)	0 (0-0.05)	0.10
TVD (mm/mm2)	10.6±1.6	11.8±1.6	<0.01
PVD (mm/mm2)	8.6±1.8	11.2±1.8	<0.01
MFI (AU)	2.6 (2.2-2.8)	2.8 (2.6-2.9)	<0.01
PPV (%)	88 (76-96)	96 (92-99)	<0.01
MHI (AU)	0.9 (0.5-1.2)	0.35 (0-0.39)	<0.01
Cardiac Index (L/min/m2)	2.1±0.7	2.5±0.6	0.11
MAP (mmHg)	76±18	81±15	0.26
Lactate (mmol/l)	4.1 (2.1-6.9)	2.2 (1.4-3.8)	<0.01
Base Deficit (mmol/l)	-5.6 (-8.9 to -2.1)	-2.6 (-5.6 to -0.2)	0.05
ScvO2 (%)	71.6±5.7	68.1±7.8	0.20
PcvCO2-PaCO2 (kPa)	1.27±0.7	1.1±0.5	0.51
Lowest recorded Systolic Blood Pressure pre-ICU (mmHg)	66±24	71±28	0.53
Highest recorded lactate pre-ICU (mmol/l)	8.5 (4.5-13.6)	4 (2.8-5.1)	<0.01
ICU length of stay (days)	28±14	12±11	<0.01
28-day mortality (%)	25	0	<0.01

Table 6.2 Characteristics of study patients with or without MODS at day 7 after injury. Variables shown are those recorded at D0 unless otherwise stated. Values are given as mean±SD or median (IQR). Differences between groups assessed using 1 way ANOVA or Mann Whitney tests. TVD Total Vessel Density, PVD Perfused Vessel Density, MFI Microvascular Flow Index, PPV Proportion of Perfused Vessels, MHI Microcirculatory Heterogeneity Index, MAP Mean Arterial Pressure, ScvO2 central venous oxygen saturation, PcvCO2 central venous CO2 tension, PaCO2 arterial CO2 tension

6.3.4 Comparison of variables used for the prediction of MODS

Receiver operator characteristics curves constructed to predict the development of MODS are shown in Figure 6.3. Post resuscitation microcirculatory variables, such as PVD and MFI: AUC 0.87 (0.76-0.99) & 0.83 (0.71-0.95) respectively provided a more accurate prediction of MODS at Day 7 than cardiac output: AUC 0.66 (0.49-0.83) and highest recorded lactate concentration prior to ICU admission: AUC 0.69 (0.53-0.84). The lowest recorded systolic blood pressure appeared to be an insensitive predictive measure for the development of MODS: AUC 0.54 (0.39-0.70).

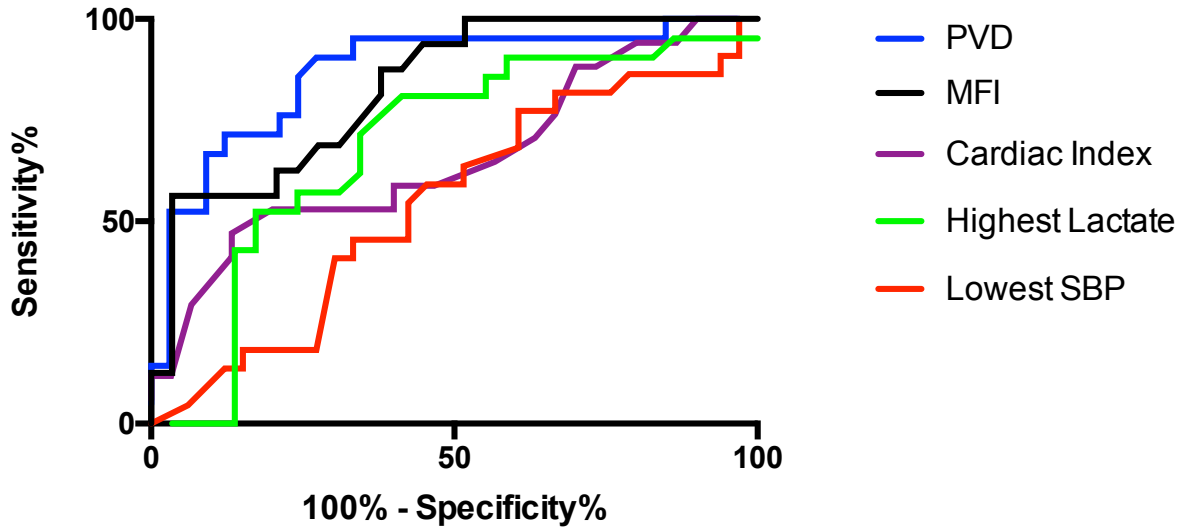


Figure 6.3 Receiver Operator Characteristic curves constructed to predict the development of MODS at Day 7 post injury. PVD Perfused Vessel Density at D0, MFI Microcirculatory Flow Index at D0, SBP Systolic Blood Pressure. The value for lactate represents the highest recorded pre-ICU measurement and that for blood pressure the lowest recorded pre-ICU measurement

6.3.5 Factors associated with post resuscitation microcirculatory impairment

Based on the ROC curve analysis PVD was selected as the microcirculatory variable with the best discrimination at predicting MODS. The threshold value for PVD which represented the best fit for MODS prediction was 10.33 mm/mm^2

Figure 6.4 shows mean daily organ failure scores for all patients with D0 PVD scores of greater than or less than 10.33 mm/mm^2. At each day from Day 4 to Day 7 after injury patients with initial (D0) PVD values of < 10.33 mm/mm2 had significantly higher organ failure scores than those with PVD values of > 10.33 mm/mm^2.

For those patients with a SOFA score of > 6 at day 7 the greatest contributor to the score was the central nervous system component (28 total points), followed by the cardiovascular and respiratory components (19 &17 points respectively). The renal, liver and haematology components were relatively small contributors to an elevated SOFA score (scores of 8,4 & 4 respectively).

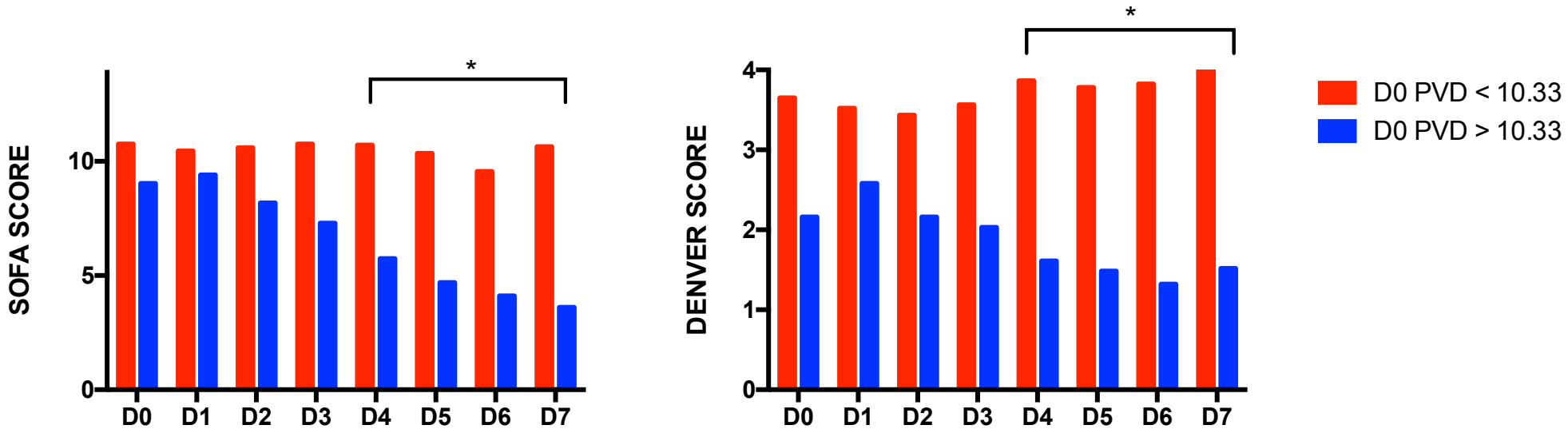


Figure 6.4 Daily mean SOFA and Denver Scores for those patients with D0 PVD values of > 10 and < 10 mm/mm^2. Values represent the mean scores of all patients in the group at that time point. Patients who died were assigned a maximal score for the day of death and subsequent days. * $p < 0.05$ between groups at time points (1 way ANOVA)

Table 6.3 shows the characteristics of patients based on the PVD value recorded at study inclusion (D0). Other reported variables are also recorded at the D0 time point unless otherwise stated. Patients with lower PVD scores received more crystalloid fluid during resuscitation. There were no observed differences in the amount of administered blood products between the groups. The noradrenaline dose in both groups was extremely low in the immediate post resuscitation period. 31% of patients had echocardiographic data recorded at the D0 time point. There were no observed differences in IVC dynamics between the two groups.

Patients with lower microcirculatory perfusion had a significantly higher lactate concentration following resuscitation but there were no differences in either the central venous oxygen saturation or the difference in central venous and arterial carbon dioxide tensions.

	PVD < 10.33 mm/mm^2 n=29	PVD > 10.33 mm/mm^2 n=29	p value
Age	44±20	42±19	0.74
ISS	33±13	26±14	0.09
PRBC prior to D0 (units)	6 (4-11)	6 (4-9)	0.91
FFP prior to D0 (units)	4.5 (1.7-9)	4.5 (2.7-8.2)	0.99
Crystalloid fluid prior to D0 (ml)	3350 (500-5000)	2000 (0-3500)	0.05
Noradrenaline dose (mcg/kg/min)	0.02 (0-0.3)	0 (0-0.07)	0.07
Cardiac Index (L/min/m2)	2.2±0.7	2.6±0.6	0.09
IVC Diameter Max (cm) (n=18)	1.7±0.8	1.7±0.6	0.89
IVC Collapsibility (%) (n=18)	18 (9-31)	22 (3-51)	0.91
Lactate (mmol/l)	3.1 (2.3-5.3)	1.9 (1.5-3.7)	0.02
Base Deficit (MEq/l)	-4.1 (-6.3 to -2.1)	-2.6 (-6.7 to-0.1)	0.2
ScvO2 (%)	71±9	69±4	0.5
PcvCO2-PaCO2 (kPa)	1.36 (0.8-2.0)	1 (0.7-1.3)	0.19

Table 6.3 Characteristics of patients with values of Perfused Vessel Density (PVD) above and below a threshold value of 10.33 mm/mm^2 at the D0 time point. Values given as mean±SD or median (IQR). Differences between groups assessed using 1-way ANOVA or Mann Whitney tests. ISS Injury Severity Score, PRBC Packed Red Blood Cells, FFP Fresh Frozen Plasma, IVC Inferior Vena Cava, ScvO2 central venous oxygen saturation, PcvCO2 central venous CO2 tension, PaCO2 arterial CO2 tension. All values are those at D0 unless otherwise stated

6.4 Discussion

6.4.1 Microcirculatory impairment is associated with persistent organ dysfunction

The principal finding of the present study is that microcirculatory hypo-perfusion in the period immediately following traumatic hemorrhagic shock and resuscitation is associated with an increased risk of multiple organ dysfunction syndrome (MODS) a week after injury. Microcirculatory perfusion indices, such as PVD & MFI, appear to be better predictive indicators for the development of MODS than measures of global haemodynamic function, such as cardiac output, or surrogate markers of perfusion such as lactate concentration. Notably, microcirculatory perfusion indices also appeared superior to the lowest recorded systolic blood pressure (SBP) in predicting MODS. This is important as SBP is still used as a marker of shock severity in many trauma registry studies and is a common resuscitation endpoint.

Persistently high organ dysfunction scores in this cohort of patients predominantly resulted from elevated central nervous system (CNS), cardiovascular and respiratory components with values for the renal, liver and haematology components making little contribution for most patients. This pattern perhaps suggests a general ongoing critical care dependency, with failure to wean quickly from mechanical ventilation and inotropic support rather than overt fulminant multiple organ failure. A potential criticism of the use of the SOFA score in trauma patients is the inclusion of a CNS component and the potential for the score to be confounded by the presence of traumatic brain injury (TBI). However, there was a relatively low incidence of TBI in this cohort and no patient remained ventilated at day 7 as a result of persistent neurological disability. The addition of the Denver score, which removes the CNS

component, was used in the present study to overcome this potential confounder. The results for the changes in Denver score over time support the SOFA score data and suggest that these findings are more than an epiphenomenon caused by excess sedation or neurological obtundation.

6.4.2 Microcirculatory impairment is an early phenomenon following THS

The results of the current study suggest that microcirculatory impairment is an early phenomenon following haemorrhagic shock and resuscitation. Maximal derangement for all measured microcirculatory variables was observed at the D0 time point, reflecting the period immediately following resuscitation. Although patients who subsequently developed MODS exhibited modest improvements in all microcirculatory variables over the subsequent 48 hours the difference in microcirculatory variables between those who did, or did not, go on to develop MODS appeared to be lost by 24 hours after study enrollment. However, caution was must be exercised in drawing these conclusions, given the relatively high drop-out rate of patients from the study. These drops out were not random and almost exclusively resulted from patients recovering and being extubated. Any bias introduced by these drop outs would likely reduce the overall microcirculatory variables of the "non-MODS" group and thus minimize any observed difference between groups at the D1 & D2 time points.

Despite these caveats, the findings of the present study suggest that whilst there may be some persisting microcirculatory abnormalities contributing to prolonged MODS the main impact of microcirculatory impairment is likely to occur early during the resuscitation process. Accordingly, the full impact of early hypo-perfusion may only be clinically apparent

some days later. Although the present study only assessed the microcirculation for the first 48 hours after injury there was no suggestion of a second hit phenomena occurring during this time. Rather, there appears to be an extended impact of an excessive "first perfusion hit" on organ function, even when perfusion is subsequently restored.

There are several possible explanations for these findings. Although the microcirculation is the final pathway of oxygen and substrate delivery the utilization of those substrates relies on intact cellular and sub-cellular apparatus, especially the mitochondria. Although hard to assess *in vivo* several investigators have suggested that mitochondrial failure or even programmed hibernation may be an important mechanism in the pathogenesis of circulatory shock in experimental models. Such a condition which has been termed bio-energtic failure or cytopathic hypoxia has most often been described in septic shock,[263 264] but may potentially be a feature in haemorrhagic shock also. [265]

At a more intrinsic level increasing evidence is becoming available that there is widespread early genomic change occurring as a result of traumatic injury and the degree of genomic change is related to the severity of the injury and to the development of persistent MODS. [36,266-268] However, at the time of writing no study has reported on the relationship between microcirculatory tissue perfusion and the magnitude of genomic change in patients with traumatic haemorrhage. Given the fact that both of these factors have been independently linked to post traumatic organ dysfunction this relationship is worthy of further investigation.

6.4.3 Haemodynamic coherence can be lost during traumatic haemorrhagic shock and resuscitation

Many studies in septic patients have demonstrated a loss of correlation between the systemic "macro" circulation and the microcirculation; the term loss of haemodynamic coherence has been coined to encapsulate this phenomenon.[118] The findings of the current study go some way to suggesting that such a loss of coherence also occurs following traumatic haemorrhage and resuscitation. The findings of the present study do not suggest that differences in systemic variables for either blood flow (cardiac output) or pressure (MAP) are related to the development of persistent organ dysfunction. These findings also seem to suggest that changes in microcirculatory perfusion are determined as much by factors intrinsic to the microcirculation as to changes in the systemic inflow or outflow.

6.4.4 Factors associated with microcirculatory impairment

In the present study patients with a lower PVD at DO appeared to receive significantly more crystalloid fluid during resuscitation. However, there were no observed differences in the amount of blood products administered. There are several potential explanations for these findings. The results of this study suggest that patients with greater degrees of microcirculatory impairment exhibit worse values of tissue perfusion markers, such as lactate concentration, and as a result may receive more resuscitation fluids. Such an explanation presumes that clinicians target resuscitation fluid boluses to perfusion based indices; however, a recent multinational study has suggested that this is not often the case.[269] An alternative explanation is that poor microcirculatory flow may lead to more injury at the level of the endothelium, potentially increasing endovascular leak, rendering

the patient hypovolaemic and requiring the administration of a greater volume of resuscitation fluid. Such an explanation is supported by recently published evidence from the present study cohort [270] which demonstrates a link between microcirculatory flow and expression of glycocalyx degradation products. However, it is not yet clear whether glycocalyx damage *per se* leads to increased endothelial leak. Finally, several experimental studies have indicated that low viscosity crystalloid fluids may not be the most efficacious fluid for resuscitation of the microcirculation. [132,271,272]

There is no evidence from the present study that the observed changes in the microcirculation were a feature of either reduced circulating blood volume or elevated central venous pressure in the initial post resuscitation period. The use of IVC variability to assess preload is somewhat controversial [224] but in this cohort of mostly young, previously fit, patients in whom right ventricular function could reasonably be expected to be normal it is likely to be a valid marker of preload. Although data was only collected from a proportion (31%) of study participants neither the IVC collapsibility index or the absolute size of the IVC at study enrollment was suggestive of either clinically significant hypovolaemia or hypervolaemia in this cohort. Additionally, there was no difference in IVC variables for patients based on PVD at study inclusion (D0).

The use of vasopressors in the immediate post resuscitation period was very low for patients enrolled in the current study. Only 36% were receiving noradrenaline at D0 with a median dose of 0 (0-0.14) mcg/kg/min. This reflects standard UK clinical practice which is to use aggressive early volume replacement in THS, with vasopressors reserved for the short-

term treatment of critical hypotension. There was no discernible effect of vasopressor use on microcirculatory variables in this study cohort.

6.4.5 Comparison with previously published work in this field

The findings of the present study are broadly in agreement with the one previously published study in this area. *Tachon* and colleagues[128] reported a persistent degree of microcirculatory impairment for up to 3 days following THS in a single centre cohort of 18 patients with THS. Mirroring the findings of the current study they found that microcirculatory parameters were better predictors of persistent MODS than either systemic haemodynamic values or surrogate markers of tissue perfusion such as lactate concentration.

The microcirculatory values reported by *Tachon* show a degree of impairment more pronounced than those reported in the present study, which requires comment. Initial (D0) values for MFI were 1.5 (1.2-1.8) in the *Tachon* cohort contrasting with 2.7 (2.6-2.9) in the present study. This degree of severe microcirculatory flow impairment is more pronounced than that reported in many studies of patients with severe sepsis, [120,121,207] a group in whom microcirculatory dysfunction is acknowledged to play a key role in determining outcome. [209] Given the disparity in mortality between patients with severe sepsis and those with THS this is an interesting finding, warranting further exploration.

Patients in the *Tachon* cohort had more anatomical injury compared to the current study cohort ISS 42 (33-50) & 29 (16-41) respectively. Blood transfusion volumes were similar between the two cohorts, although the *Tachon* cohort received a larger volume of crystalloid fluids than patients in the present study 4,400 (3,375-5,500) ml & 2,500 (250-

4,200) ml respectively. Colloid resuscitation fluid was not used for resuscitation in the current study cohort but patients in the *Tachon* cohort received 2,500 ml on average. Perhaps the most striking difference in the results of the two studies relates to the use of vasopressors. All patients in the *Tachon* cohort were receiving noradrenaline at study enrolment compared to 36% of patients in the current study. Furthermore, the median noradrenaline dose at study enrollment was far greater in the *Tachon* cohort than the current study 0.7 (0.2-0.95) & 0 (0-0.14) mcg/kg/min respectively. It is possible that this disparity in vasopressor dose may be, at least partially, responsible for the differences in observed microcirculatory variables between the two study cohorts. However, it is far from clear that vasopressors are associated with a worsening of microcirculatory perfusion. Indeed, some experimental and clinical studies have suggested that this may not be the case,[229,273] whilst others have conflicting findings. [274] An uncontrolled observational study has reported that the use of vasopressors was associated with an increased mortality following THS. [275] However, patients receiving vasopressor were more severely injured and had a greater perfusion deficit during resuscitation. What is cause and what effect is unclear from the results of this study. Further experimental and clinical studies examining vasopressor usage following traumatic haemorrhagic shock are required in order to provide a definitive answer to the important question of the role of vasopressor therapy in trauma shock resuscitation.

Tachon et al reported a 50% incidence of MODS, but this was based on SOFA scores at day 4 post injury; arguably this may not represent true, persistent, organ dysfunction. Choosing a discriminator at day 7 following injury maybe more clinically significant. This approach seems to be supported by recent work by *Shepherd* and colleagues who demonstrated that

only MODS persisting at day 7 after injury was associated with adverse clinical outcomes. [26] The range of SOFA scores for patients in the present study who were defined as having MODS (SOFA 6 or more at D7) is almost identical to that reported in the *Tachon* study, perhaps surprising given the large discrepancy in the reported absolute values of microcirculatory flow and perfusion.

6.4.6 Clinical relevance and therapeutic targeting of the microcirculation

The results of the present study appear to highlight the importance of tissue perfusion in the period during and immediately following trauma shock resuscitation. Current resuscitation paradigms, supported by international consensus guidelines, stress the importance of early blood pressure control and make little mention of restoring tissue flow or perfusion.[78] The present study findings suggest that early impairment of microcirculatory perfusion may have an impact on persistent MODS and the corollary of this may be that early targeting of microcirculatory perfusion could prevent or mitigate this outcome. However, targeting the microcirculation is not without difficulty and relies on the development of robust, reliable and reproducible monitoring tools that can be used at the bedside. Such tools are already in development. [257,258]

An important issue is the timing of any such change in resuscitation strategy with the results of the current study suggesting that the window for restoring tissue perfusion after traumatic haemorrhagic shock may be narrower than previously thought. Current paradigms advocate relative systemic hypotension until bleeding is brought under control. However, the presence of significant traumatic brain injury is taken to be a relative

contraindication to such a strategy. The results of the current study suggest that the brain may not be unique in terms of the risk from prolonged hypo-perfusion and that other organs may also be affected to some degree. Ultimately, a balance needs to be sought between initial haemorrhage control and the later restoration of tissue perfusion. It may be that based on the findings of the current study, that balance needs to shift in the direction of the latter goal.

6.5 Conclusion

Microcirculatory impairment following initial resuscitation from THS is associated with an increased incidence of organ dysfunction at a week post injury. Such microcirculatory abnormalities appear to resolve quickly and are not usually discernible 24 hours after injury, suggesting that it is the initial perfusion insult that is generating the propensity for organ injury rather than a persisting problem at the microcirculatory level. Patients with greater degrees of microcirculatory impairment appeared to receive larger volumes of crystalloid fluid, but not blood products. The absolute values of microcirculatory perfusion were greater in the present study than in the only other published cohort of patients and a possible explanation is the difference in vasopressor usage. Treatment strategies should be developed that target microcirculatory perfusion and tested in clinical trials to confirm whether early goal directed targeting of the microcirculation can improve patient centered outcomes.

Chapter 7

Mechanisms and Consequences of Microcirculatory Impairment following Traumatic Haemorrhagic Shock

7.1 Introduction

Haemorrhagic shock is an important cause of morbidity and mortality following traumatic injury and a proportion of initial survivors go on to develop multiple organ dysfunction[3,22,26]. Data presented in this thesis appear to show that disturbances in microcirculatory blood flow contributes to this phenomenon. If haemodynamic coherence is at least partially preserved then microcirculatory impairment may be caused by synchronous impairment of the macrocirculation. However, impairment of microcirculatory flow can also be caused by factors intrinsic to the microcirculation itself, either related to the vascular endothelium or specific intra-vascular morphotic elements such as leucocytes and erythrocytes. Furthermore, poor microcirculatory flow, aggravated by reperfusion injury, could potentially lead to direct damage to elements of the microcirculation, potentiating a cycle of worsening cellular injury and hypo-perfusion.

This chapter presents an overview of some of the potential mechanisms of microcirculatory impairment following THS and examines some pilot data from a small set of samples collected from patients enrolled in the clinical study presented in Chapter 6. The aim was to look for signals of association that may explain either the mechanisms or consequences of microcirculatory failure in this cohort of injured patients.

7.2 Review of selected biomarkers associated with endothelial function

7.2.1 Angiopoetin 1 &2 – markers of endothelial permeability

There are 4 sub types of Angiopoetin, but the most commonly studied are Angiopoetin 1 (Ang-1) & Angiopoetin 2 (Ang-2) , both being 70kDa glycoprotein molecules. [276] Angiopoetins are responsible for the regulation of endothelial integrity and neo-vascularization and act at the Tie 2 tyrosine kinase receptor where they produce mutually antagonistic effects. Ang-1, produced by pericytes and smooth muscle cells, promotes endothelial integrity and decreases expression of a variety of pro inflammatory cytokines and cellular adhesion molecules. [277] In contrast, Ang-2, rapidly released from Weibel – Palisade bodies of vascular endothelial cells acts to destabilise vascular endothelium by increasing sensitization to pro –inflammatory cytokines and up-regulation of adhesion molecules leading to increased interaction and activation of circulating leucocytes. [278] Because of this mutual antagonism, some investigators have suggested that the ratio of Ang-1 to Ang-2 may provide useful information regarding the state of endothelial activation. [279]

Wada et al studied 57 patients with traumatic injury (ISS > 9) admitted to a Japanese trauma center observing that Ang-2 levels and Ang-2: Ang-1 ratio were higher in those patients who developed trauma induced coagulopathy. [280] Interestingly these differences were maximally apparent at day 5 post injury, whilst tests on immediate post injury samples did not reveal significant differences. High levels of Ang-2 were also strongly associated with the development of organ dysfunction, but again differences were only apparent in samples taken 3-5 days following injury, suggesting that the Angiopoetin system may play a late role in any ongoing endothelial activation process following traumatic injury.

Ganter et al studied 208 consecutively injured patients presenting to a single US major trauma center.[281] Plasma samples were obtained as soon as possible after hospital admission (median 32 minutes following injury). The investigators found that high levels of Ang-2 were associated with a greater degree of injury (ISS), poor perfusion (Base Excess), coagulopathy (PT/ APTT), increased renal failure and increased mortality. There was no similar association with Ang-1 levels. The authors concluded that Ang-2 is released early, in contrast to the findings of *Wada* et al, and may be an important mediator of organ dysfunction rather than merely a marker of endothelial activation.

7.2.2 Cytokines and DAMPs – markers of the inflammatory response

As discussed in Chapter 1, the inflammatory response to injury is complicated and pleotropic. Albeit accepting that a reductionist approach has significant limitations after review of the literature it was decided to examine 3 discrete molecules and to assess the relationship of the expression of these molecules with the state of the microcirculation. The molecules selected were a pro inflammatory cytokine (IL-6), an anti-inflammatory cytokine (IL-10) and a Damage Associated Molecular Pattern (DAMP) molecule (HMGB-1).

IL-6

IL-6 is predominantly a pro-inflammatory cytokine, secreted by T cells and macrophages. Key actions include maturation, differentiation and activation of lymphocytes (B & T cells) granulocyte and macrophages and increased production of acute phase proteins.[45] IL-6 levels rise early following traumatic injury and then typically fall between day 2 and 5 post injury.[43] In a recent study examining serial changes in cytokine levels in 99 patients admitted to a Portuguese trauma ICU IL-6 was the only cytokine where admission levels correlated

with outcome. [37] Several studies have shown that IL-6 levels correlate with the development of post injury organ failure or mortality. *Cushieri* et al reported that an IL-6 level of >350 pg/ml was predictive of MOF development with a likelihood ratio of 3.25 [35] whilst *Svoboda et al* reported that an IL-6 level of >400pg/ml was an independent predictor of mortality.[282] *Maier* et al reported a bimodal distribution of IL-6 which correlated with the development of MOF in a subset of patients[283].

The relationship between pro and anti-inflammatory cytokines may play an important role in determining the host response to injury. [53] Excessive production of either can cause consequences, either through excessive acute inflammation and organ injury or immune paresis and impaired host defence to subsequent insults, especially sepsis. Whilst IL-6 is an important example of a predominantly pro-inflammatory cytokine, IL-10 has mostly anti-inflammatory activity.

IL-10

IL-10 has been shown to attenuate both the innate and the adaptive immune response following injury; gene pathways regulating IL-10 production are one of the most up regulated immediately following trauma. [36] IL-10 acts partly by suppressing pro-inflammatory cytokine production; administration of IL-10 reducing TNF levels in an animal model of hemorrhagic shock [284]. Although initially thought to be associated with the development of the so called CARS syndrome, and a late phenomenon, most studies have shown that IL-10 levels peak very early following traumatic injury and fall, in line with pro-inflammatory cytokines over time[34,37,283]. However, *Neidhardt* et al demonstrated that IL-10 remained persistently elevated in trauma patients who developed MODS or sepsis.[285]

The ratio of pro-inflammatory and anti-inflammatory cytokines may be a marker of injury severity and physiological derangement. *Taniguchi* et al described an association between the IL-6:IL-10 ratio, injury severity and APACHE II in a cohort of 20 patients with chest and abdominal trauma, those with the highest ratios having the worst outcome.[34]

HMGB-1

High mobility group box 1 (HMGB-1) is a ubiquitous nuclear protein. It is the most important example of a group of molecules termed Alarmins or Damage Associated Molecular Patterns (DAMPS) that are released from necrotic cells and cause a series of pro-inflammatory downstream effects.[39,41] HMGB-1 is also released actively from certain cells involved in the innate immune response, particularly macrophages.[286] HMGB-1 acts via Toll Like Receptor 4 (TLR-4) and Receptor for Advanced Glycation End products (RAGE) to produce up regulation of cellular innate immunity and promote release of cytokines.

HMGB-1 is released very early following injury and is detectable in the blood of patients following major trauma within 60 minutes. [32] Levels of HMGB-1 immediately following injury correlate with injury severity, tissue perfusion and also the level of post injury organ dysfunction.[33]

7.2.3 Plasma free haemoglobin - marker of red cell fragility

Patients with traumatic hemorrhagic shock are invariably transfused with PRBCs. In the most severe cases this can result in an effective exchange transfusion with donor erythrocytes. Numerous publications and a meta-analysis[287] suggest the potential for harm

associated with older banked blood but this assertion was not supported in a large multinational prospective randomized study which used mortality as the primary endpoint.[288] One of the potential issues with stored erythrocytes is that they may be less deformable and more prone to lysis when exposed to shear stress. *Harm* et al demonstrated that washing of erythrocytes, but not irradiation or leucodepletion, as part of the storage process increased the mechanical fragility index.[289] This index is calculated by measuring the amount of plasma free haemoglobin (PFHb) in samples of blood exposed to standardized mechanical agitation. The amount of PFHb is important as free haemoglobin is known to act as an avid scavenger of nitric oxide, which has the potential to cause potent vasoconstriction.

Impairment of the microcirculation following THS could potentially act as a cause of increased red cell fragility, as luminal sizes are reduced and red cells which may be rigid and brittle are introduced to the circulation. Additionally, lysed erythrocytes could cause vasoconstriction in the microcirculation through a process of nitric oxide scavenging. There are no studies in the published literature reporting the degree of red cell lysis in the immediate post trauma period. *Alves* et al reported in abstract form findings from a small cohort of stable resuscitated trauma patients who required further blood transfusion.[290] They showed a statistically significant increase in PFHb following transfusion of 1 unit of PRBC but the absolute values were very low (<0.2 g/dl) making the clinical significance doubtful. Although the same study also reports that aged red cells (>14 days) produced a more pronounced increase in PFHb, again the absolute values and differences reported were very small (<0.01 g/dl) casting the clinical significance into doubt.

7.2.4 Syndecan 1 - marker of endothelial glycocalyx injury

The endothelial glycocalyx (EG) is a layer of proteoglycans and glycosaminoglycans that lines the vascular endothelium and performs several key functions relating to barrier integrity. These include maintenance of osmotic pressure through retention of plasma proteins, [291] inhibition of coagulation by anticoagulant factors such as antithrombin III and thrombomodulin[250] and prevention of leucocyte endothelial reactions through caging of adhesion molecules in the glycocalyceal layer.[107]

There are four Syndecans in the glycocalyx but Syndecan-1 is the most commonly studied and referenced. Damage and disruption to the EG causes release, or shedding, of Syndecan 1 into the circulation. Glycocalyx shedding appears to occur early following any insult to the EG with levels then falling from a peak following resuscitation as the shed Syndecan is cleared from the circulation.

Following THS peak Syndecan-1 levels have been correlated with a variety of clinical outcome measures including mortality, [108] coagulopathy, [108] acute kidney injury [292] and nosocomial infections.[292]

The precise mechanism behind EG degradation following THS remains unclear and is the subject of ongoing studies. One hypothesis is that the EG is directly damaged by the high circulating levels of complement, cytokines and Damage Associated Molecular Proteins (DAMPS) that are found in the circulation following THS. Such a theory maybe supported by evidence from *Haywood – Watson* et al who reported a negative correlation between

Syndecan 1 levels and three pro-inflammatory cytokines.[256] These investigators hypothesized that this correlation was a result of cytokine binding to shed Syndecan-1, effectively mopping it up and reducing the levels in the circulation. However, a converse view may be that such removal of cytokines exerts a protective effect and is not in itself evidence that the prime mover in the process is cytokine inflicted EG damage.

Another explanation may be related to the dynamics of microcirculatory flow following THS. Animal studies using electron microscopy have demonstrated that the degree of loss of the EG barrier appears related to the degree of blood flow reduction.[109] Recently published work from our group demonstrated that the degree of glycocalyx shedding was correlated with microcirculatory flow during THS. [252]

7.3 Aim

The aim of this study was to examine a series of selected biomarkers, in samples obtained from patients with traumatic hemorrhagic shock, in order to investigate potential mechanisms and consequences of microcirculatory impairment.

7.4 Methods

Blood samples were drawn from patients enrolled into the Microshock study at King's College Hospital; inclusion criteria for the study are outlined in Chapter 6. Blood samples were drawn at the D0 time point (ICU admission) into EDTA coated tubes. Due to restrictions in processing, samples were only taken during normal working hours. Samples were spun at 1620G for 10 minutes after which aliquots of serum were stored at -80^{0}C for later analysis. Serum samples were obtained from 5 healthy volunteers and were processed using the same method.

All assays, except that for plasma free haemoglobin, were performed using a sandwich ELISA method, the general principles of which are outlined in Chapter 2. Specific manufacturers instructions were followed. The following assays were performed: sCD138 (Syndecan-1), IL-6, IL-10 (Diaclone SAS, Besancon, France); Angiopoetin 2,HMGB-1 (E labscience Biotechnology Ltd, Wuhan, China); Angiopoetin 1 (Abcam plc, Cambridge, UK).

Plasma free haemoglobin (PFHb) assays were conducted using a point of care test (HaemoCue, California, USA). 10 mls of blood was obtained in a sodium heparin coated tube and spun at 1000G for 5 minutes. Estimation of PFHb was performed on the resultant plasma in accordance with the manufacturers instructions.

Microcirculatory assessment was conducted at the D0 time point as described in Chapter 2. Patients were divided into two groups based on the PVD value at the D0 study time point and using the same methodology described in Chapter 6. Patients were classified as having

either High PVD (> 10.33 mm/mm^2) or Low PVD (< 10.33 mm/mm^2). SOFA scores were calculated for all patients at Day 7 following injury as described in Chapter 6. Details of the SOFA score are provided in the appendices.

As the majority of the data was not normally distributed differences between control samples and patients with High and Low PVD were assessed using Kruskal-Wallis test with Dunn's correction for multiple comparisons. A p value of < 0.05 was taken to indicate a significant difference.

7.5 Results

Blood samples were analysed from the first 12 patients enrolled in the clinical study reported in Chapter 6. Patient characteristics are shown in Table 7.1. 7 patients had Low PVD and 5 High PVD at D0. 6 patients had a day 7 SOFA score > 6, 6 patients had a day 7 SOFA score < 6.

There was a significant delay between drawing of blood samples and processing (Median freeze delay time 1380 (450-4320) min.

Age (years)	48±21
Gender	8 male, 4 female
ISS	31.7±16.4
APACHE 2	16.3±10.6
Blunt Mechanism of Injury (%)	12/13 (92)
PRBC in 24 hours (units)	10 (4.5-13)
Lowest recorded pre ICU SBP (mmHg)	63 (52-73)
Highest recorded pre ICU lactate (mmol/l)	8.0±5.4
SOFA score at Day 7 post injury	4.2±3.2
Time interval between injury and D0 time point (hours)	10 (4.2-11.7)

Table 7.1 Characteristics of Microshock study patients in whom samples were obtained for analysis. Data presented as median (IQR) or mean±SD

7.5.1 Relationship between microcirculatory perfusion at D0, organ failure at Day 7 and subject biomarkers

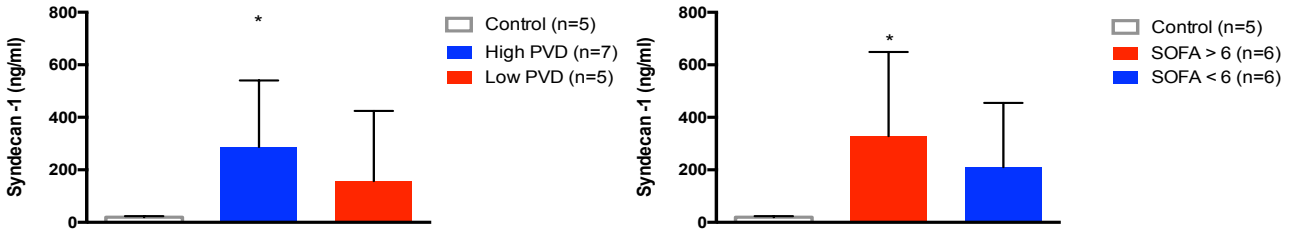


Figure 7.1 Syndecan-1 levels in patients with High & Low PVD at D0 & SOFA score >6/<6 at Day 7. *p < 0.05 versus controls (Kruskal-Wallis test)

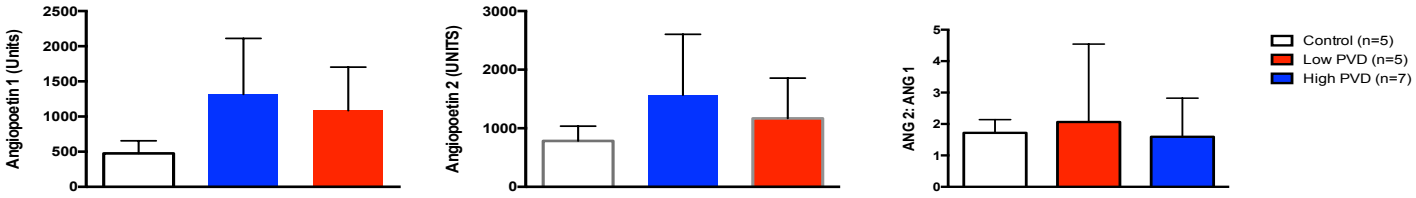


Figure 7.2 Angiopoetin 1 (ANG1), Angiopoetin 2 (ANG2) & ANG2:ANG1 ratio in patients with High & Low PVD at D0. No significant differences between groups (Kruskal-Wallis test)

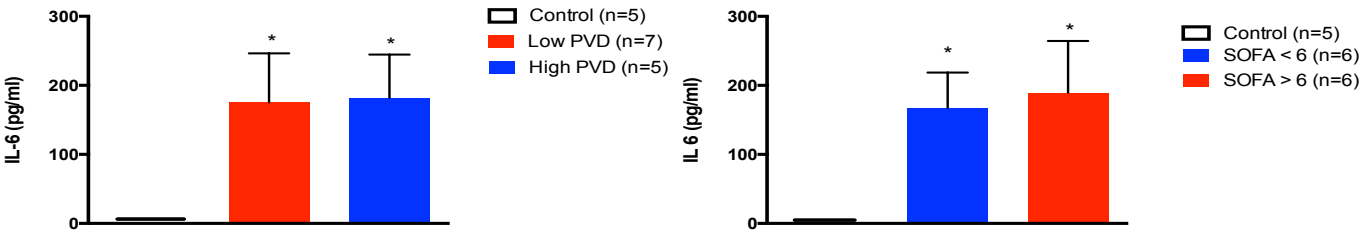


Figure 7.3 IL-6 levels in patients with High & Low PVD at D0 & SOFA score >6/<6 at Day 7. *p < 0.05 versus controls (Kruskal-Wallis test)

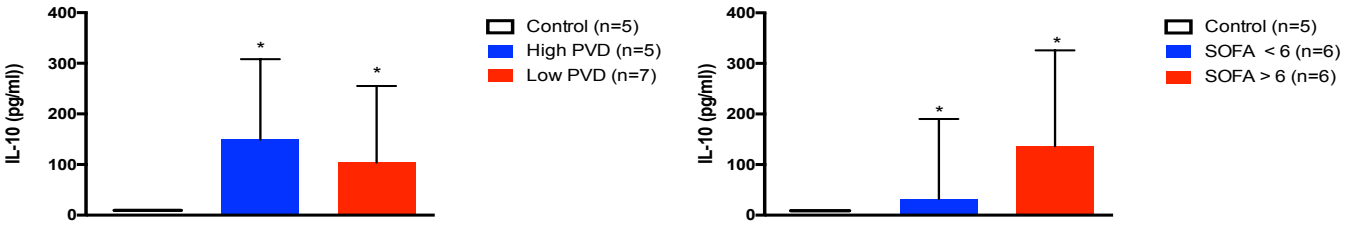


Figure 7.4 IL-10 levels in patients with High & Low PVD at D0 and SOFA score >6/<6 (Day 7). *p < 0.05 for all groups versus controls (Kruskal-Wallis test)

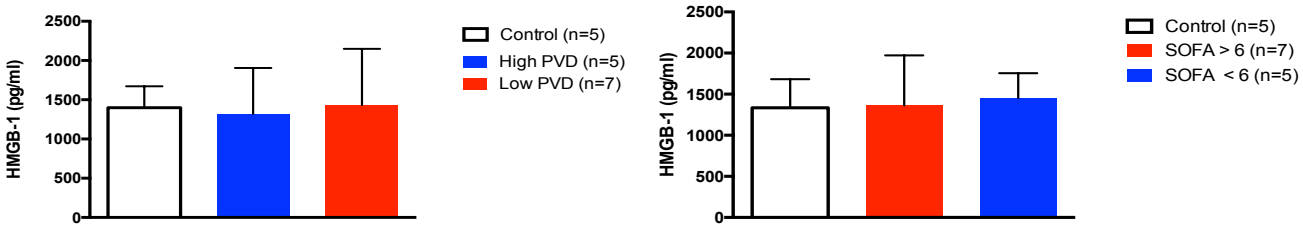


Figure 7.5 HMGB-1 levels in patients with with High & Low PVD at D0 and SOFA score >6/<6 (Day 7). No significant differences between groups (Kruskal-Wallis test)

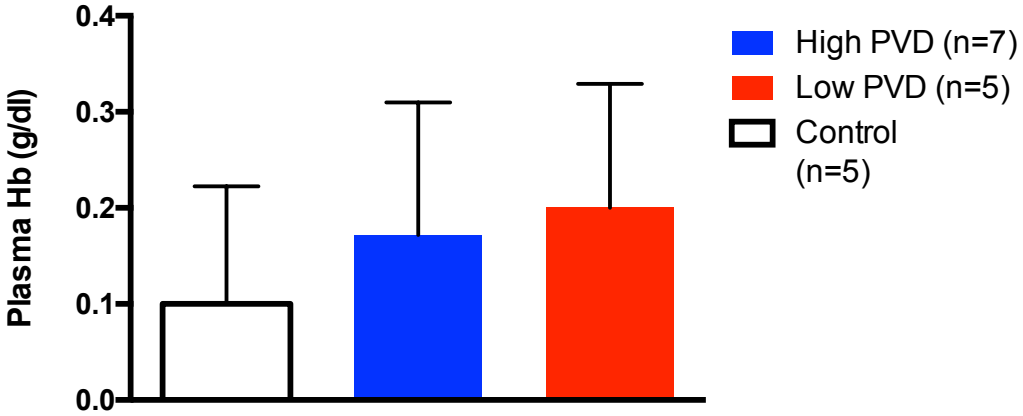


Figure 7.6 Free Plasma Haemoglobin (FPHb) in patients with above and below average PVD (D0). No significant differences between groups

7.6 Discussion

Microcirculatory impairment following traumatic hemorrhagic shock is a complex phenomenon involving a plethora of interconnecting pathophysiological processes. Several studies have shown that there is a substantial endothelial injury following traumatic haemorrhage and this "endotheliopathy" may be related to the state of microcirculatory perfusion.[252,293,294]

It is tempting to draw a distinction between factors causing microcirculatory impairment and those that stem from such impairment. However, in reality the two are likely to be interdependent. As discussed in Chapter 1 poor microcirculatory flow can lead to a plethora of squealae including endothelial cell injury and swelling, leucocyte activation, margination and adhesion and loss of erythrocyte deformability. Any of these factors can contribute to a heightened inflammatory state and potentially be a cause of organ dysfunction. Additionally, these same factors can themselves be a cause of impaired microcirculatory blood flow.

Aside from the effect of disordered systemic haemodynamic function, the intrinsic causes and consequences of microcirculatory impairment can be considered in three broad areas namely: (i) endothelial activation and injury, including damage to the endothelial glycocalyx, (ii) an acute inflammatory response which can involve up and down regulation of the immune response and (iii) changes to circulating erythrocytes. In order to assess the impact of THS across these three areas the present study measured specific biomarkers directed at quantifying the relative importance of these factors.

Overall the number of patients in whom samples were obtained was small (n=12) and this effectively precluded meaningful analysis once the patients were dichotomized into groups with high and low microcirculatory perfusion. No statistically significant differences were observed between patients with above or below average microcirculatory perfusion for any of the studied biomarkers. Furthermore, there were methodological issues, including very long delays between sample acquisition and freezing which limit the value of the reported findings.

The findings of the present study do not suggest that microcirculatory hypoperfusion was associated with raised markers of glycocalyx shedding (in this instance Syndecan-1). This finding stands in apparent contrast to the results of another study, conducted by our group and using samples from patients in the same study cohort but collected at a different study site[252]. These findings suggest that Syndecan-1 levels are elevated in patients with below average microcirculatory perfusion, but only when samples were taken within 10 hours of injury. The median time from injury to sample collection was 10 hours in the present study and it may be that this influenced the results seen. Although there was a signal in these results that elevated Syndecan 1 levels were correlated with later organ dysfunction, as previously reported in other studies, the results did not reach significance.

As expected there was increased expression of both pro and anti-inflammatory cytokines in this patient cohort. Expression of DAMPs in the form of HMGB-1 appeared similarly elevated but the results must be viewed with suspicion given the very high levels of HMGB-1 in the healthy control assays. It is difficult to explain this and these results suggest that the

assay or method itself may have been at fault. There were no discernible differences in any of the measured inflammatory mediators based on the state of the microcirculation at the D0 time point. The inflammatory and immune response in the post trauma shock period is complex and with this small sample size it is perhaps optimistic to expect a signal from these biomarkers. However, one hypothesis for the linkage between microcirculatory hypoperfusion and the development of MODS is that poor tissue perfusion generates an exaggerated response. Again, repeating this work with a larger sample size would be of interest. There were no discernible differences in either absolute Angiopoetin-1 or Angiopoetin-2 levels, or the Ang-1: Ang-2 ratio, based on the state of microcirculatory perfusion.

The present study showed very low levels of plasma free Hb in patients resuscitated from THS. These were patients who had received a significant amount of transfused blood products (median PRBC of 10 units in 24 hours) and it may have been expected that this could have led to an increase in red cell fragility. Given the very low level of red cell lysis observed in these samples this does not seem to be the case. It is possible that the transfused red cells may have been less deformable than healthy native erythrocytes but this was not tested for in the current study. Previous studies suggest that any lack of deformability is associated with a degree of increased lysis. The fact that this was not seen in this small cohort is not definitive but suggests that erythrocyte factors may not play a major role in microcirculatory dysfunction in this setting.

In summary, the data from the present study are not clear enough to be more than hypothesis generating. The lack of clear signals from this data, alongside previously

discussed findings suggesting a rapid restoration of microcirculatory dynamics after resuscitation may suggest that persisting endothelial injury is not a major feature of THS in many patients. It is possible that many of these changes occur early and that by collecting samples relatively late in the resuscitation process some findings may have been overlooked; future work should perhaps focus on the early resuscitation period. Given the key role that genomic expression appears to play in the response to traumatic injury it would also be fascinating to explore the role that reduced tissue perfusion plays in altering the genome in the immediate aftermath of THS.

Chapter 8

Conclusions and further work

8.1 Summary of findings

The development of hand held video microscopes has revolutionized the in vivo study of the microcirculation. Data presented in this thesis show that a new device for assessing the microcirculation, the Cytocam, produces results comparable to a precursor device but with significant improvements in image quality. The findings presented in this thesis represent the first experimental investigation of the new device under both baseline conditions and during shock and subsequent resuscitation. These findings are important for investigators wishing to transition to the new device.

In a porcine model of experimental traumatic hemorrhagic shock, these results demonstrate that microcirculatory perfusion plays a key role in determining the speed of reversal of the shock state. Wide inter-individual variation was observed in all microcirculatory variables relating to both flow and vessel density. Animals with relatively preserved perfusion during shock and initial resuscitation were able to clear lactate at a significantly faster rate than those with more deranged microcirculatory parameters. Although when the group of animals was considered *en bloc* haemodynamic coherence between the macrocirculation and microcirculation was broadly preserved, it was this wide inter individual variation that was the key finding of the experimental study presented in Chapter 4. The cause of this significant variation is unclear, but it appears to occur very early in the shock process. One

possible explanation is an excessive degree of arteriolar vasoconstriction in the most affected animals.

Importantly, the state of the microcirculation was difficult to predict from an assessment of macro haemodynamic indices, especially blood pressure. This finding is important for the management of patients with hemorrhagic shock, which is currently conducted almost exclusively with reference to blood pressure, at least in the early stages. The findings presented in this thesis suggest that if a strategy that specifically targeted flow and tissue perfusion were adopted early in the resuscitation process then clinical outcomes may be improved.

The use of blood products early in the resuscitation of traumatic hemorrhagic shock has gained considerable ground in recent years, partly as a result of the experience of the military in recent conflicts. Despite this, there is no clear evidence of benefit in terms of tissue perfusion. The initial hypothesis for this work stated that blood products would resuscitate the microcirculation more effectively than a crystalloid fluid such as 0.9% saline, but the findings did not convincingly support this hypothesis. There were some methodological reasons why this may have been the case, but it is possible that the key factor is the individual response to shock rather than the type of fluid used for resuscitation.

The quest for effective haemoglobin based oxygen carriers has been fraught with difficulty but the product examined in this work, MP4OX, has several important differences to earlier agents that suggest it should be more efficacious with a lower incidence of side effects. The data presented in this thesis demonstrates that MP4OX is effective at restoring

microcirculatory variables during shock resuscitation with no evidence of associated vasoconstriction. Unfortunately, further development of this fascinating and potentially clinically useful molecule is unlikely at the time of writing due to the commercial failure of the manufacturer.

Trauma induced coagulopathy is an important issue in patients with severe traumatic injury and hemorrhagic shock. It is clear that patients with poor perfusion and extensive tissue injury are particularly prone to developing TIC but data presented for the first time in this thesis establishes a clear link between poor microcirculatory perfusion and the development of TIC. Furthermore, it demonstrates that the use of blood products appears to be protective against the development of coagulopathy irrespective of the state of the microcirculation. In contrast, the combination of severe microcirculatory dysfunction during shock and the use of 0.9% saline as an initial resuscitation fluid was associated with the rapid development of TIC. If these findings are replicated in a clinical study it has important ramifications for clinical practice, potentially allowing the targeting of scarce blood products in austere environments to those patients in whom they would be of most value.

Post-traumatic organ dysfunction remains a significant clinical problem and most previous studies have suggested that the degree of tissue hypoperfusion plays an important role in predicting its development. The findings presented in this thesis confirm that this is the case and show that the post resuscitation state of the microcirculation is a key predictor for late or persisting organ dysfunction. The microcirculatory changes appeared to occur early and were relatively non-persistent. Despite this, early microcirculatory impairment appeared to predict organ dysfunction implying perhaps that there is a critical window to restore cellular

perfusion in order to mitigate these risks. The striking difference in the absolute microcirculatory variables reported in the present study and those of the only previously published work raises important questions. The disparity in vasopressor usage between the two patient cohorts offers one possible answer.

Precisely how microcirculatory impairment is implicated in organ dysfunction remains to be established. The data presented in this thesis supports the view that such changes are predicated early and not a result of days of sustained microcirculatory impairment and chronically poor perfusion. But if microcirculatory indices are rapidly restored then why is there a signal suggesting that the microcirculation is a key determinant of post traumatic organ failure? One possible explanation is that initial tissue hypo-perfusion followed by reperfusion sets in motion a sequence of inflammatory / immunological consequences that lead to persisting organ dysfunction. We attempted to find a signal for those elements of that process of particular significance but the findings were limited and inconclusive.

Overall this thesis has addressed the set hypotheses and found that restoration of microcirculatory perfusion following traumatic hemorrhagic shock is a key element in rapidly resolving the shock state, preventing trauma induced coagulopathy and reducing the incidence of post traumatic organ dysfunction.

8.2 Strengths and limitations

An important feature of this work is the combination of findings from an animal experimental model and a clinical observational study. The animal model allowed an examination of the microcirculation under conditions of controlled induced shock and targeted resuscitation with measurements obtained immediately after injury and throughout resuscitation. This allowed an examination of the immediate response of the microcirculation to haemorrhage and reperfusion. In contrast to the heterogeneous nature of the clinical population, animals received standardized injuries and treatments and this allowed more confidence in the conclusion that where wide differences in micirocirculatory indices were observed these were due to factors intrinsic to the animal subjects. By contrast the clinical study was relatively uncontrolled but still signaled the importance of microcirculatory perfusion in this clinical setting, providing an important translational element to the work. Although less data was obtained during the very early stages of the clinical study, in contrast to the animal work, patients were studied for days rather than hours and this allowed important observations to be made regarding the long term response of the microcirculation.

The animal experimental model used in the studies presented in this thesis was explicitly designed to mirror battlefield injury and resuscitation. It included a proportion of animals with blast injury, a simulated pre-hospital phase, where animals received varying fluids, pressure targeted resuscitation and outcome measures focused on the development of coagulopathy. The strengths of such an approach are the potential translation of findings into the clinical sphere. The limitations are the wider ranges of potential treatments and

injury patterns which created large numbers of groups. This had the impact of limiting some the findings, particularly with regard to the effect of differing fluids on the microcirculation. An experimental model aimed solely at assessing perfusion and with more homogeneity of treatment may have added value to the results.

A significant limitation of this work is the fact that there was only one main method of assessing the microcirculation. Sublingual Dark Field video microscopy is an effective microcirculatory monitoring tool with extensive data demonstrating its predictive value. However, it is only able to provide information on vessel flow and density, not on tissue oxygenation. Although NIRS was utilised in the animal experimental model it did not prove to be a particularly useful monitoring modality in this setting. Having a means of reliably assessing tissue oxygenation would have added benefit to both the animal and clinical studies, particularly in assessing the impact of MP4OX during resuscitation.

8.3 Suggestions for further work

The main finding of this work is that microcirculatory blood flow is an important determinant of outcome following traumatic hemorrhagic shock. All of the studies conducted as part of this thesis were observational and it is now important to determine whether interventions targeted to restoring microcirculatory perfusion can improve outcome, both in experimental models of traumatic shock and also in clinical trials. This requires the development of more rapid point of care measures of the microcirculation in order to generate clinically useful information within a meaningful time frame. Our research group have already started work in this area.

A novel finding of the present work was the fact that pigs with poor microcirculatory perfusion, treated with 0.9% saline, were prone to develop trauma induced coagulopathy. It would be useful to confirm these findings in a clinical study. Such a finding would potentially open up new therapeutic approaches in the use of blood products, especially in remote or austere environments.

We reported a wide divergence in microcirculatory indices between the findings presented in this thesis and the only previous study that examined the microcirculation following THS. This difference is potentially related to the extensive use of vasopressor agents in the other study cohort. There is variance in practice across the world with regard to the use of vasopressor therapy in this setting and further work comparing cohorts of patients treated in different trauma systems would be of value.

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Appendices

SOFA & Denver Organ Failure Scores

		Score Allocation				
	Units	**0**	**1**	**2**	**3**	**4**
DENVER						
Respiratory	PaO_2/FiO_2 (mmHg)	>250	250-175	175-100	<100	
Renal	Creatinine (μmol/l)	<159	160-210	211-420	>420	
Hepatic	Bilirubin (μmol/l)	<34	34-68	69-137	>137	
Cardiac	Inotropes	None	1 – small dose	1 – moderate dose, >1 small dose	1 – large dose, > 2 moderate dose	
SOFA						
Respiratory	PaO_2/FiO_2 (mmHg)	>400	<400	<300	<200 & IPPV	< 100 & IPPV
Renal	Creatinine (μmol/l)	<110	110-170	171-299	300-440	>440
Hepatic	Bilirubin (μmol/l)	<20	20-32	33-101	102-204	>204
Cardiac	Inotropes (μg/kg/min)	MAP >70	MAP < 70	DOP <5 OR DOB (any dose)	DOP>5 OR EPI <0.1 OR NA < 0.1	DOP >15 OR epi >0.1 OR NA > 0.1
Coagulation	Platelets ($x10^3/mm^3$)	>150	<150	<100	<50	<20
CNS	GCS	15	13-14	10-12	6-9	<6

MAP mean arterial pressure; GCS Glasgow Coma Score; DOP Dopamine; DOB Dobutamine; EPI epinephrine; NA norepinephrine

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The Cytocam video microscope. A new method for visualising the microcirculation using Incident Dark Field technology

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Abstract.
We report a new microcirculatory assessment device, the Braedius Cytocam, an Incident Dark Field (IDF) video microscope, and compare it with a precursor device utilising side stream dark field (SDF) imaging.
METHODS: Time matched measurements were made with both devices from the sublingual microcirculation of pigs subjected to traumatic injury and hemorrhagic shock at baseline and during a shock phase. Images were analysed for vessel density, microcirculatory flow and image quality.
RESULTS: There were no differences in density or flow data recorded from the two devices at baseline [TVD IDF 14.2 ± 2.4/TVD SDF 13.2 ± 2.0, p 0.17] [MFI IDF 3 (2.8–3.0)/MFI SDF 3 (2.9–3.0), p 0.36] or during the shock state [TVD IDF 11.64 ± 3.3/TVD SDF 11.4 ± 4.0 $p = 0.98$] [MFI IDF 1.9 (0.6–2.7)/MFI SDF 1.7 (0.3–2.6) p 0.55]. Bland and Altman analysis showed no evidence of significant bias. Vessel contrast was significantly better with the IDF device for both capillaries [17.1 ± 3.9 (IDF) v 3.4 ± 3.6 (SDF), $p = 0.0006$] and venules [36.1 ± 11.4 (IDF) v 26.4 ± 7.1 (SDF) p 0.014]
CONCLUSION: The Braedius Cytocam showed comparable vessel detection to a precursor device during both baseline and low flow (shock) states.

Keywords: Microcirculation, sidestream dark field imaging, incident dark field imaging, haemorrhagic shock

1. Introduction

The microcirculation plays a critical role in the delivery of oxygen and substrates at a cellular level. Microcirculatory impairment can lead to significantly increased morbidity and mortality across a range of shock states [9, 13, 14] and significant impairment to microcirculatory flow may be present in around a fifth of unselected patients within critical care units [15]. Assessment of the microcirculation can occur through two broad and separate approaches; the first approach involves assessment of tissue substrate delivery and uptake and the second relies on direct visualisation of microcirculatory vessels in order to determine flow and density characteristics. Examples of the former approach include Near Infra Red Spectroscopy (NIRS) [10] and fluorescence quench photometry [6] which provide an estimation of tissue oxygenation and sublingual capnometry which, by assessing the degree of carbon dioxide production in an individual tissue bed, provides a surrogate marker of perfusion [2, 16]. These methods produce an aggregated overview of microcirculatory performance but may be influenced by the heterogeneity that is often found in microcirculatory vessel beds. The second broad method of microcirculatory assessment relies on the direct visualisation of small vessels (arterioles, venules and capillaries) at various sites throughout the body. Historically this required the use of large microscopes

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and tissue dyes, so called intra vital microscopy, and was therefore mainly limited to experimental work in small animal models. The development of handheld microscopes has revolutionised this area of research. The first generation handheld devices used Orthogonal Polarised Spectroscopy (OPS) imaging [5] which produced a somewhat limited field of view, whilst the devices were relatively bulky. The next generation devices, used a Sidestream Dark Field (SDF) technique [4]. Similar to OPS in theory, SDF technology involves illuminating the edges of the examined field with visible green light in an 530 nm wavelength from a circular array of diodes. The light illuminates the target tissue from the edges, whilst the field itself is excluded from external light. Haemoglobin (both oxygenated and deoxygenated) absorbs the green light and thus appears black. Thus perfused blood vessels appear as black lines on a white/greyscale background. Lighter gaps within blood vessels are caused by plasma or by leucocytes. Producing qualitative data from SDF images requires editing and processing into discrete sequences followed by off line analysis. Such analysis produces data on vessel density and flow. At its most basic this analysis can be entirely manual consisting of a subjective assessment of flow using a qualitative scale. More commonly semi automated software is used to assist in the process, but a significant amount of user interaction is still required and each ten to twenty second video sequence can take up to thirty minutes to process, even with an experienced analyst. Given the widespread heterogeneity seen within the microcirculation during shock states it is important to obtain a large number of video sequences, ideally five, at each time point being examined, thus increasing the amount of video sequences that need to be analysed. This prolonged off line analysis has, thus far, limited the use of this technique to research rather than as a clinical point of care perfusion test.

The latest generation of optical device for assessing the microcirculation is the Braedius Cytocam (Fig. 1), which utilises an Incident Dark Field (IDF) technique. This technique, originally described by Sherman and colleagues in the 1970s [12] is similar to Sidestream dark field illumination, in that it uses a ring of circumferential LEDs to illuminate the target tissue tangentially, the illuminating light being excluded from the central column of the microscope. However, the LED strobe speed is significantly slower in the Cytocam device (2 ms versus 16 ms); this theoretically produces less distortion of the erythrocyte image potentially allowing more accurate automated flow analysis. The Cytocam is a small handheld camera, which weighs considerably less than the previous generation Microscan SDF device (Fig. 2) (115 versus 350 grams). It is also less bulky than the SDF device and this has the effect of making it easier for the operator to manipulate (Fig. 3). In turn this has the potential to reduce pressure artifacts caused by the tip of the device occluding flow in microcirculatory vessels. The Cytocam has a higher optical resolution than the Microscan (3.12 versus 4.54 micrometers) and a wider field of view (1.79 mm^2 versus 0.84 mm^2). This larger field of view enables the user to more quickly identify suitable areas of microcirculation for analysis. The Cytocam has an electronically driven focusing motor as opposed to the analogue focusing mechanism of the Microscan making it easier to carry out fine focusing actions. Additionally the device returns to the same focus depth for subsequent measurements, speeding up the time taken to acquire images. The Cytocam is linked to a dedicated computer with integral software which controls image acquisition, editing and potentially on line analysis. This is in contrast to the previous generation devices which required analogue to digital signal conversion through a signal adapter as well as bulky separate batteries and a non proprietary laptop computer with adapted software.

In this paper we report a series of experiments designed to assess the performance of the Cytocam IDF device and to compare and contrast it to it's immediate predecessor, the Microscan SDF device. In the first part of the study, the Cytocam is directly compared with the Microscan in an existing large animal model of haemorrhagic shock and complex injury. The aim is to compare the performance of the devices with a particular focus on vessel identification. The second part of study is a direct comparison of image quality between the two devices, using matched sublingual microcirculatory images, obtained from one of the study investigators.

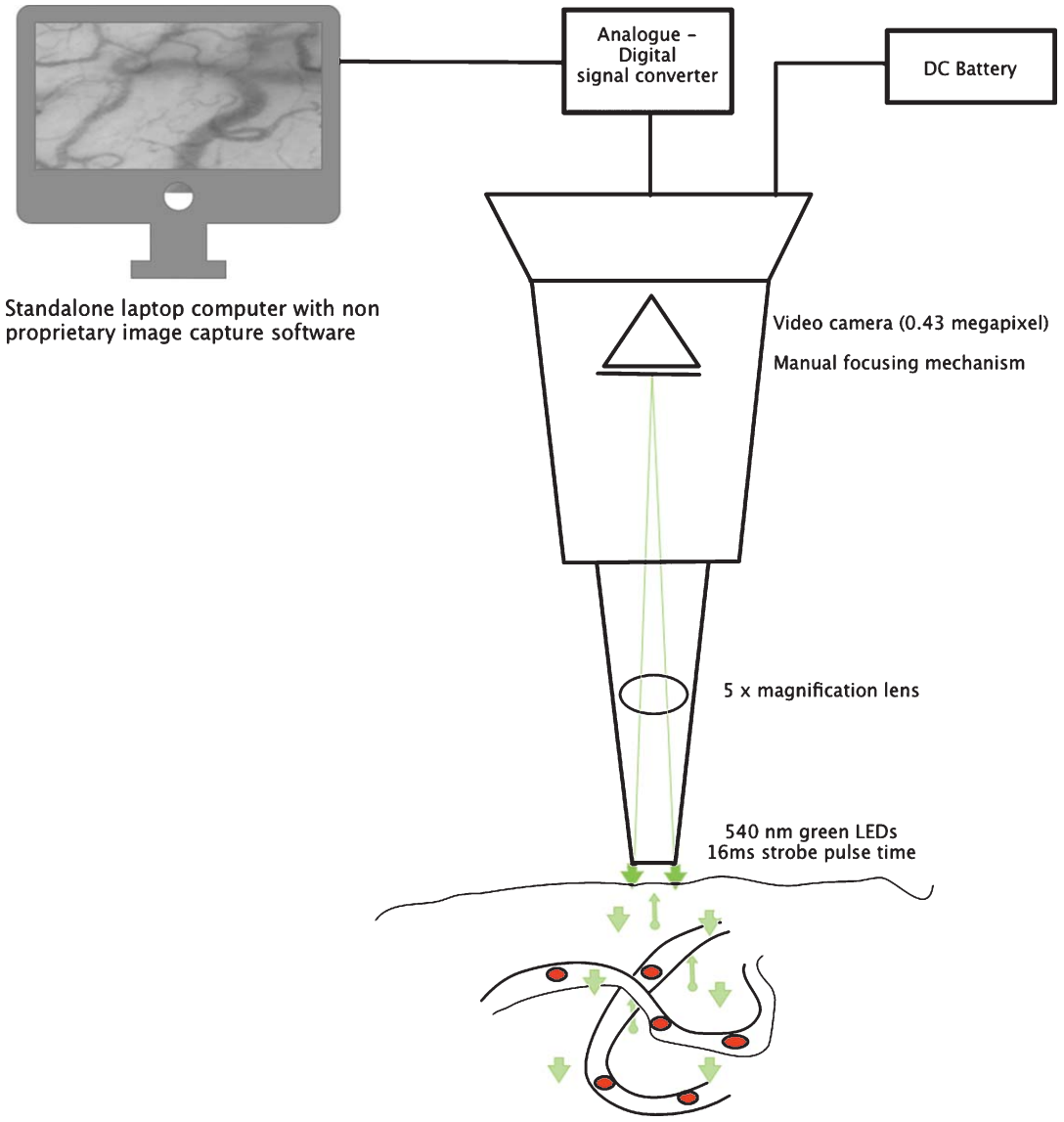

Fig. 1. Microscan SDF video microscope. Green polarized light, produced from a ring of circumferential LEDs is transmitted to the tissue and absorbed by haemoglobin. Residual light returns to the video camera via a magnifying lens and focus mechanism. Analogue images are converted to digital and viewed on a stand alone laptop computer.

2. Methods

Conduct of this research was approved under license from the United Kingdom Home Office: Animals (Scientific Procedures Act) 1986.

2.1. Subjects

Briefly, six terminally anaesthetised and surgically prepared large white pigs, were subjected to a controlled haemorrhage and reproducible extremity injury and, in some instances, blast wave exposure.

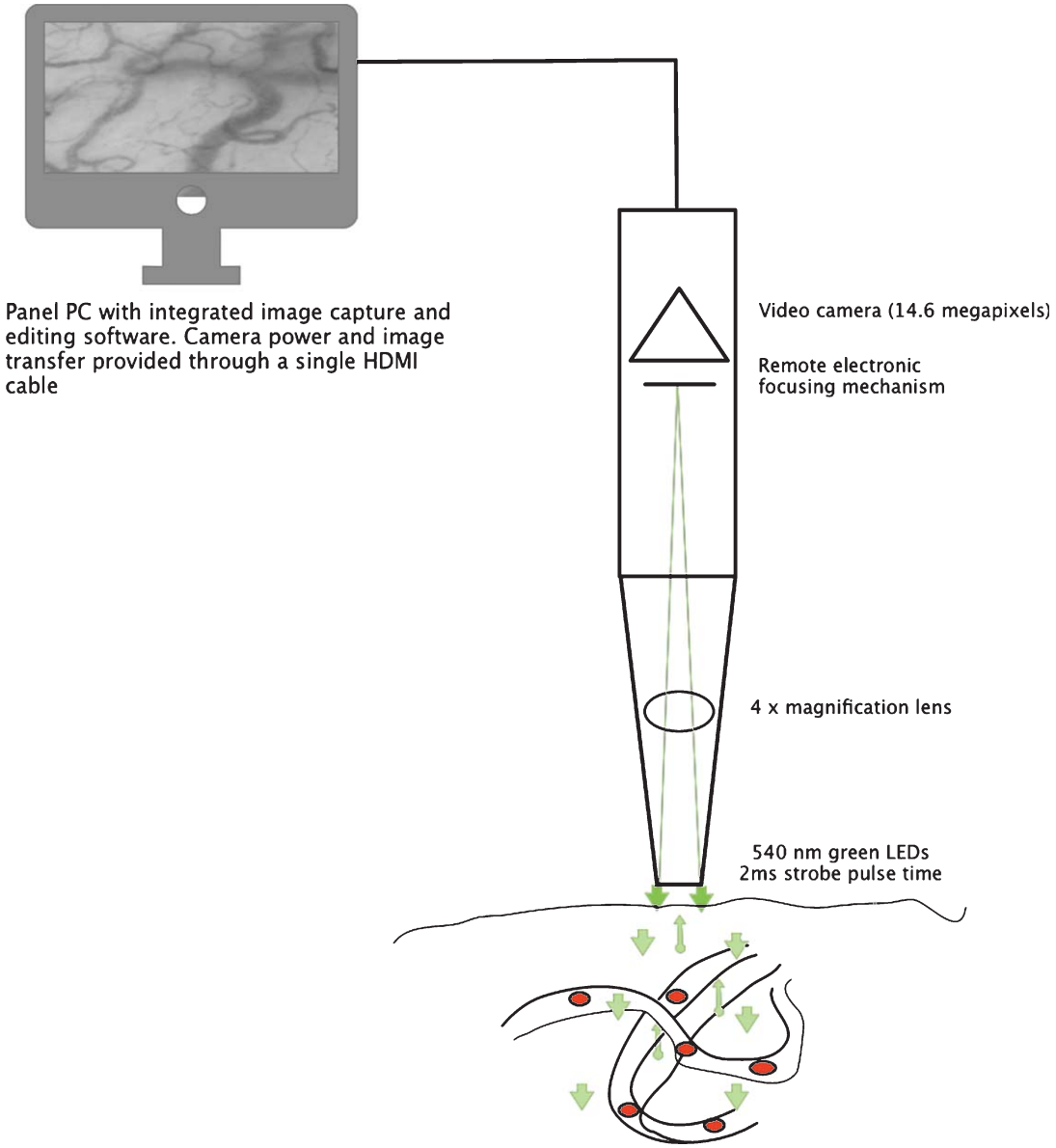

Fig. 2. Cytocam IDF video microscope. Green light is produced from circumferential LEDs in a similar way to the SDF device. However image quality is improved by a higher resolution camera. The device is also simpler with fewer connections and accessories required.

Following a 30 minute shock phase the animals were resuscitated using differing resuscitation strategies that are outside the scope of this paper, but which have been reported elsewhere [7, 8]. The experiment ended at four hours after the commencement of resuscitation at which point the animals were euthanised.

The aspect of the study examining image contrast required two closely matched microcirculatory images, obtained from the subject devices. In practice, this was not possible within the constraints of our existing experimental setup. We therefore obtained these images from the sublingual region of one of the investigators (SDH).

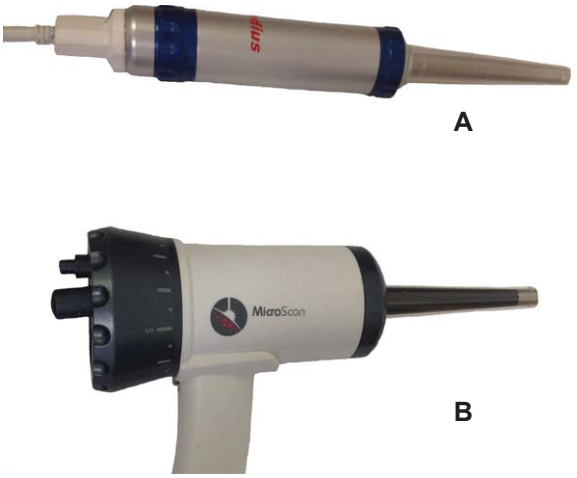

Fig. 3. Cytocam IDF (A) and Microscan SDF (B) devices.

2.2. *Vessel identification and flow assessment*

Two devices were used to record video images of the sub lingual microcirculation, utilising Incident Dark Field (IDF) technology (Cytocam, Braedius Medical, Amsterdam, NL) or Sidestream Dark Field (SDF) technology (Microscan, Microvision Medical, Amsterdam, NL).

Serial video images of the sublingual microcirculation were recorded at the following experimental time points: Baseline (a steady state time point prior to injury or blood loss) and Shock (15 minutes after extremity trauma, controlled blood loss and blast wave injury). The shock time point represents a low flow state, with a degree of dynamic response within the microcirculation in contrast to the predictable steady state seen at baseline. At each time point at least three and ideally five ten second sequences were recorded using each device, in accordance with accepted consensus opinion on assessment of the microcirculation [3]. Images were recorded using the SDF device (Microscan), immediately followed by the IDF device (Cytocam). Images were either directly recorded onto a dedicated integral panel PC using integral software (Cytocam Tools v. 7.1, Braedius Medical) or via an analogue to digital signal converter (Canopus, ADVC 110) onto a stand alone laptop computer (Microscan). Microscan acquired images were saved as digital DV-AVI files, whilst those acquired by the Cytocam were converted to this format during export using integral software. As previously mentioned the field of view from the Cytocam is considerably wider than that obtained using the Microscan necessitating a reduction in the field of view during export to DV-AVI format. Video captures were all performed by a single operator. All images were recorded from the same part of the sublingual area, to the left side of the midline. Care was taken to minimise pressure artefact, by applying the probe tip and pulling back until contact was just lost and then reapplying with the minimum amount of pressure required to produce an image. Focus was adjusted by the operator in order to produce the best possible vessel definition, either manually (Microscan) or via the integral software linked to the electronic focusing mechanism (Cytocam). Videos were assigned a five digit random number prior to archiving in order to reduce bias during interpretation. The videos were linked to study time point and device by reference to a separate database. Videos were exported in DV-AVI format and with standardised characteristics. Video files were saved on an external hard drive prior to off line analysis.

Analysis of video sequences was carried out by a single operator using dedicated software (AVA 3.0, Automated Vascular Analysis, Microvision Medical, NL). The operator was blinded to both the device used for recording and the time point of the video sequence. Only video sequences that conformed

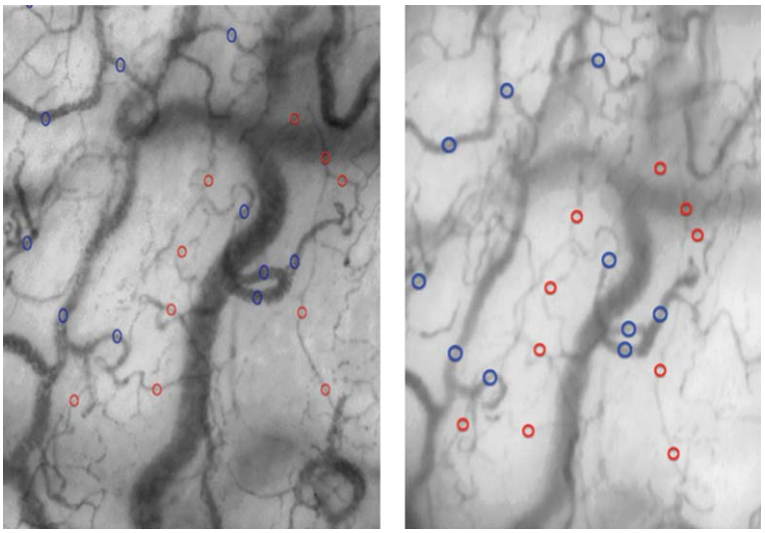

Fig. 4. Matched images from sublingual microcirculation used for quality comparison. Points used for image analysis circled (blue = venules, red = capillaries).

to pre determined standards of stability, focus, illumination, length and absence of pressure artefacts were included in the analysis. These standards were detailed by Massey et al. in a previous study [11]. All visible vessels were manually traced by the operator and flow characteristics were assigned to each individual vessel segment, using a four point scale originally described by Boerma et al. [1]. The mean flow across all segments was calculated in order to produce an overall Microcirculatory Flow Index (MFI) for each video sequence. Analysis of each video sequence produced data for the total number of vessel segments compared to the size of the analysis area (Total Vessel Density, TVD).

2.3. Vessel contrast assessment

Vessel contrast and sharpness was analysed using a technique originally described by Goedhart etl al. [4] To compare vessel contrast between the two devices a single operator obtained two near identical images from their own sublingual microcirculation, using the two subject devices. Optimal image characteristics were obtained with respect to focus, illumination and avoidance of pressure. Video sequences were examined and one video frame, demonstrating the highest quality of focus and illumination, was selected for each device. This video frame was saved as a Portable Network Graphic (PNG) file. In each video frame image, ten venule segments and ten capillary segments were selected for quality analysis. (Fig. 4) To determine vessel contrast and sharpness, cross sectional grey scale profiles (greyscale value 0 corresponding to black and 255 to white) were obtained using Image J software (freeware developed by the US National Institute for Health). The contrast was defined as the absolute difference between the minimum value within the vessel and the maximum value on either side of the vessel wall (average of the two sides of the vessel).

2.4. Statistical analysis

Statistical Analysis was performed using GraphPad Prism version 6. Data was tested for normality using D'Agostino & Pearson omnibus normality test. Students paired t tests were used to compare

Table 1
Total Vessel Density (TVD) values recorded from each device at baseline and shock time points. Units are mm/mm^2 and values are expressed as mean ± standard deviation

		IDF Cytocam	SDF Microscan	p value
Animal 1	BASELINE	13.1 ± 0.2	12.2 ± 0.9	0.40
	SHOCK	11.1 ± 2.6	7.2 ± 2.5	0.33
Animal 2	BASELINE	13.3 ± 1.0	13.1 ± 1.0	0.88
	SHOCK	9.2 ± 1.0	9.8 ± 1.6	0.79
Animal 3	BASELINE	15.6 ± 1.4	15.1 ± 1.3	0.80
	SHOCK	13.1 ± 1.0	13.3 ± 1.6	0.96
Animal 4	BASELINE	15.7 ± 1.0	14.2 ± 0.3	0.31
	SHOCK	14.2 ± 1.8	14.9 ± 0.2	0.73
Animal 5	BASELINE	Not analysed – insufficient quality video sequences		
	SHOCK	8.7 ± 0.7	10.5 ± 1.2	0.22
Animal 6	BASELINE	12.9 ± 1.0	11.2 ± 0.8	0.19
	SHOCK	12.8 ± 1.0	14.0 ± 1.9	0.60

vessel density data which was normally distributed. Mann Whitney U tests were used to compare vessel flow data, which did not have a normal distribution. Bland – Altman distribution analysis was performed on the TVD results from the two devices. Contrast values for the two devices were analysed using paired t tests after confirming normal distribution. A p value of < 0.05 was considered to be statistically significant.

3. Results

3.1. Vessel identification and flow assessment

Results from six animals were included in the study. Forty four video sequences were obtained with each device, giving a total of eighty eight matched video sequences. In order to assess for possible variance at different flow states video sequences were subdivided into Baseline ($n = 40$) and Shock ($n = 48$) time points. As expected there was a wider spread of results during the shock time point as the microcirculation demonstrated increased heterogeneity. By contrast the Baseline time point was more homogenous representing a relatively steady state at this stage of the experiment. One experimental time point was excluded as at least three video sequences of sufficient quality could not be obtained.

There were no significant differences in vessel detection, expressed as the Total Vessel Density, recorded by the two devices at either the baseline or shock time points (Table 1).

There was also no difference in the observed Microvascular Flow Index, recorded by the two devices at either experimental time point. These findings are consistent when the data is aggregated (Fig. 5) or examined for each individual animal (Table 2).

Bland Altman analysis confirms the comparability of data collection from each device and shows no evidence of bias (Fig. 6).

3.2. Vessel contrast and sharpness

Vessel contrast was significantly higher in the image obtained from the Cytocam IDF device. This applied to both capillary and venules (Table 3, Fig. 4).

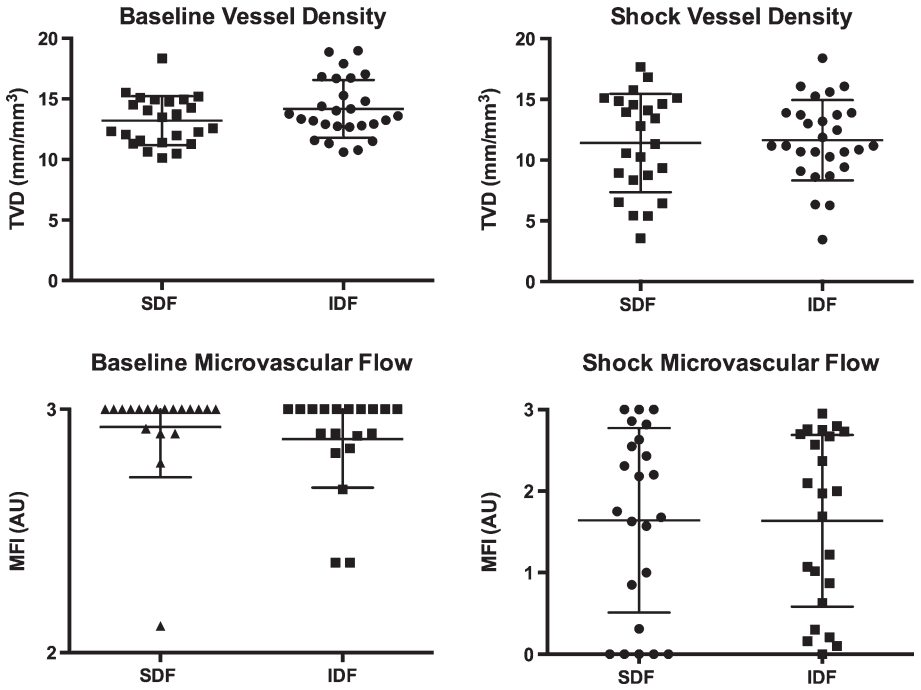

Fig. 5. Aggregated IDF and SDF data for flow and density parameters.

Table 2
Microvascular Flow Index (MFI) values recorded from each device at baseline and shock time points. Units are arbitrary units of flow ranging from 0 (no flow) to 3 (continuous flow). The average value of flow in all vessel segments is presented. Values are expressed as median with 95% confidence interval

		IDF Cytocam	SDF Microscan	*p* value
Animal 1	BASELINE	2.9 (2.9–3.0)	3.0 (3.0)	0.40
	SHOCK	0.6 (0–1.7)	0.5 (0–2.0)	0.74
Animal 2	BASELINE	2.9 (2.4–3.0)	2.9 (2.1–3.0)	0.41
	SHOCK	0.2 (0.1–0.6)	0.8 (0–1.7)	0.86
Animal 3	BASELINE	2.9 (2.7–3.0)	3.0 (3.0)	0.99
	SHOCK	2.7 (2–2.9)	3.0 (3.0)	0.63
Animal 4	BASELINE	3.0 (2.9–3.0)	2.9 (2.8–3.0)	0.99
	SHOCK	2.7 (2.6–2.7)	2.3 (2.2–2.4)	0.10
Animal 5	BASELINE	Not analysed – insufficient quality video sequences		
	SHOCK	1.1 (0.9–2.0)	0.6 (0–2.2)	0.69
Animal 6	BASELINE	2.6 (2.4–2.9)	2.9 (2.9–3.0)	0.99
	SHOCK	2.7 (2.4–2.8)	3 (2-3)	0.70

4. Discussion

We have described a series of experiments designed to assess the performance of a new device for assessing the microcirculation, the Braedius Cytocam. To our knowledge this is the first such evaluation of the Cytocam IDF device performed over a range of flow states, in an experimental model of shock. Our experimental setup was designed to replicate as closely as possible conditions found within a

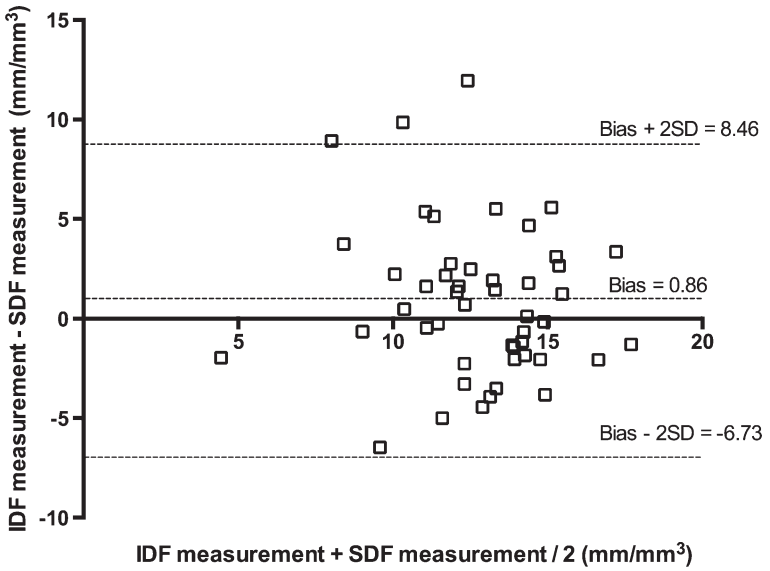

Fig. 6. Bland Altman plot showing density data produced from IDF and SDF devices across all experimental time points.

Table 3

Contrast values from two matched microcirculation images recorded from the two devices. 10 individual points were assessed for both capillaries and venules (see Fig. 4). Units are arbitrary units of pixel intensity. Higher values indicate a greater contrast between vessels and vessel borders

	IDF Cytocam	SDF Microscan	*p* value
Contrast (capillaries)	17.1 ± 3.9	9.4 ± 3.6	0.0006
Contrast (venules)	36.1 ± 11.4	26.4 ± 7.1	0.014

clinical environment. We feel that this adds considerable value to this study as it enables a comparison of the two imaging devices under conditions that closely replicate that which will be encountered in clinical practice. The inclusion of imaging comparisons at low flow shock states is often omitted from validation studies, which are usually conducted on healthy volunteers under controlled conditions. By including these low flow images in this study we have produced a comparison between the two devices over the full operating range in which they would be used in clinical practice.

In the first part of the study we showed that, in the pragmatic setting of an existing trial, the two devices performed in a comparable fashion and the data obtained, with respect to both vessel density and flow, was virtually indistinguishable. By selecting a baseline steady state timing point we attempted to reduce any changes in the microcirculation seen between the recording of the SDF and the IDF acquired images. The addition of the shock timing point is important because it allows a comparison of the two devices at a low flow state, where vessel identification may be predicted to be more difficult. However, at this time point the microcirculation was in a state of flux, induced by haemorrhage and injury, and this can be seen in the much wider spread of data. Despite this dynamism and heterogeneity there is still a remarkable similarity between the results collected from the two devices. As far as possible we attempted to reduce the time between image acquisition, which in practice was achieved within five minutes from the start of SDF collection to the end of IDF collection.

We acknowledge that this approach has limitations and that it would be optimal to compare identical images derived from the two devices. However, in practical terms it is extremely difficult to find even two such images, given the magnification and field of view constraints as well as the instability inherent in the use of handheld cameras. In reality it is impossible to obtain matched images in a dynamic study that includes animals or patients with shocked microcirculations. The first part of our study therefore represents an attempt to answer the question of comparability of data generated by the two devices, rather than a completely objective assessment of image identification under perfect operating conditions.

In the second part of this study we examined whether the image quality produced by the IDF device was superior to that of the SDF device. Subjectively, we felt that the IDF images were sharper and had greater definition than the precursor SDF images and were able to confirm this objectively on one matched image using pixel analysis software. Further studies are needed to replicate this finding which is of potential importance as sharper images and improved contrast between blood vessels and background tissue potentially make the automated analysis of such images by computer software more achievable. Automated analysis programs cannot yet reliably measure vessel density in recorded video-microscopy images and improvements in image quality are a necessary prerequisite for the development of this technology.

In summary the Braedius Cytocam is a new device for imaging the microcirculation which we have validated against a common precursor device. Microcirculatory data produced by the two devices is comparable and this should allow researchers to confidently transition to the new device whilst maintaining the integrity of data previously recorded with precursor technology.

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Conflicts of interest

None declared.

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Microcirculatory perfusion shows wide inter-individual variation and is important in determining shock reversal during resuscitation in a porcine experimental model of complex traumatic hemorrhagic shock

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Abstract

Background: Traumatic hemorrhagic shock (THS) is a leading cause of preventable death following severe traumatic injury. Resuscitation of THS is typically targeted at blood pressure, but the effects of such a strategy on systemic and microcirculatory flow remains unclear. Failure to restore microcirculatory perfusion has been shown to lead to poor outcomes in experimental and clinical studies. Systemic and microcirculatory variables were examined in a porcine model of complex THS, in order to investigate inter-individual variations in flow and the effect of microcirculatory perfusion on reversal of the shock state.

Methods: Baseline standard microcirculatory variables were obtained for 22 large white pigs using sublingual incident dark field (IDF) video-microscopy. All animals were subjected to a standardised hind-limb injury followed by a controlled haemorrhage of approximately 35 % of blood volume (shock phase). This was followed by 60 min of fluid resuscitation with either 0.9 % saline or component blood products and a target SBP of 80 mmHg (early resuscitation phase). All animals were then given blood products to a target SBP of 110 mmHg for 120 min (mid-resuscitation phase), and a further 100 min (late resuscitation phase). IDF readings were obtained at the midpoint of each of these phases. Cardiac output was measured using a pulmonary artery catheter. Animals were divided into above average (A) and below average (B) perfused vessel density (PVD) groups based on the lowest recorded PVD measurement taken during the shock and early resuscitation phases.

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Results: There was minimal inter-individual variation in blood pressure but wide variation of both systemic and microcirculatory flow variables during resuscitation. During shock and early resuscitation, group A ($n = 10$) had a mean PVD of 10.5 (SD ± 2.5) mm/mm^2 and group B ($n = 12$) 5.5 (SD ± 4.1) mm/mm^2. During the later resuscitation phases, group A maintained a significantly higher PVD than group B. Group A initially had a higher cardiac output, but the difference between the groups narrowed as resuscitation progressed. At the end of resuscitation, group A had significantly lower plasma lactate, higher lactate clearance, lower standard base deficit and smaller mixed venous-arterial CO_2 gradient. There was no significant difference in blood pressure between the two groups at any stage.

Conclusion: There was a wide variation in both macro- and microcirculatory flow variables in this pressure-targeted experimental model of THS resuscitation. Early changes in microvascular perfusion appear to be key determinants in the reversal of the shock state during resuscitation. Microcirculatory flow parameters may be more reliable markers of physiological insult than pressure-based parameters and are potential targets for goal-directed resuscitation.

Keywords: Haemorrhagic shock, Microcirculation, Traumatic injury, Sublingual video-microscopy, Lactate clearance, CO_2 gap, Blood products, Perfused vessel density

Background

Traumatic hemorrhagic shock (THS) is a leading preventable cause of death following severe traumatic injury [1–3], and timely reversal of the shock state is essential in reducing post-injury morbidity and mortality.

Resuscitation from THS is invariably conducted with reference to a pressure-based paradigm that currently emphasises so-called "permissive hypotension". This strategy is based on the hypothesis that excessive blood and fluid administration, given with the goal of achieving normotension, may lead to clot disruption and increased bleeding. This is seemingly well supported by experimental [4] and clinical [5] evidence and recommended by expert authorities [6, 7]. Crucially, however, the effects of pressure-targeted resuscitation upon blood flow, especially microcirculatory blood flow, are less well defined.

The microcirculation is a collective term for the network of small blood vessels that perform the final role in the delivery of cellular oxygen and substrate and is therefore of paramount importance during shock and resuscitation. Small animal studies have shown that the ability to maintain microcirculatory perfusion is critical to outcome following THS [8]. However, these studies utilised models of simple haemorrhage and may have limited translation into clinical practice. To date, only one published clinical study has examined the effects of microcirculatory impairment on end-organ function following THS [9], finding that there was an association between microcirculatory impairment and organ failure. However, this study examined the microcirculation in the period after haemodynamic stability had been restored, not during the shock phase itself.

If it could be demonstrated that individuals with THS who were able to maintain higher systemic and microcirculatory blood flows during resuscitation had improved outcomes, then this could potentially identify avenues for the development of novel end points of resuscitation based on flow-based parameters.

The current study presents data on macro- and microcirculatory blood flow collected from a porcine experimental model of THS. The aims of the study are to investigate (i) inter-individual variations in systemic and microcirculatory blood flow, (ii) the relationship between systemic and microcirculatory blood flow and (iii) to examine whether animals which maintained a higher degree of microcirculatory perfusion during shock and resuscitation had more effective and timely reversal of the shock state.

Methods

Study design

This is an opportunistic prospective observational study that investigates the haemodynamic and microcirculatory variables for some of the subjects of a previously published porcine experimental study. The original experimental study was undertaken to address the effects of different initial resuscitation fluids on the development of trauma-induced coagulopathy, the results of which have been published elsewhere [10]. Data were obtained from experiments conducted with the presence of an investigator trained in the acquisition of microcirculatory images.

Animal preparation

The whole experiment was approved and conducted under license from the Home Office Animals (Scientific Procedures) Act 1986.

The full animal preparation is described in detail elsewhere [10]. In summary, female large white pigs (43–56 kg) were terminally anaesthetised and allowed spontaneous respiration following tracheal intubation. The left femoral artery and left common carotid arteries were cannulated to allow the withdrawal of blood and infusion of resuscitative fluid, respectively. Cardiac output monitoring was performed using a pulmonary artery catheter (Vigilance Volumetrics CEDV; Edwards Lifesciences Ltd). The bladder was catheterised. A midline laparotomy and splenectomy was performed, and the abdomen was closed.

Experimental protocol

The full experimental protocol is detailed previously [10, 11] and was designed to replicate battlefield injury, shock and subsequent resuscitation. The experiment was designed to keep all animals alive until the end of resuscitation. A proportion of animals received a controlled blast injury, and all animals received a controlled hindlimb injury from a captive bolt gun as described elsewhere [10]. Following the haemorrhagic shock phase, animals were assigned to receive initial resuscitation with either (a) 0.9 % saline or (b) component blood therapy consisting of leucodepleted porcine red blood cells and plasma (delivered in a 1:1 ratio). The sampling, storage and preparation of these blood products are described in detail elsewhere [10].

Data was collected at five time points as follows:

(a) *Baseline* (pre-injury)
(b) *Shock* (30 min after controlled haemorrhage and injury)
(c) *Early (hypotensive) resuscitation* (30 min after commencement of resuscitation)
(d) *Mid-resuscitation* (90 min after commencement of resuscitation)

(e) *Late resuscitation* (170 min after commencement of resuscitation)

In accordance with currently accepted trauma shock resuscitation protocols, the target systolic blood pressure was set at 80 mmHg for the early resuscitation phase and 110 mmHg during subsequent resuscitation phases. Animals were given boluses of fluid to achieve these pressure targets. During early resuscitation, animals were treated with either 0.9 % saline or blood products. During mid- and late resuscitation, all animals exclusively received blood products.

Microcirculatory monitoring

The microcirculation was assessed using incident dark field (IDF) video-microscopy (Cytocam, Braedius Medical B.V), which has been recently validated against a precursor device in this setting [12]. Video clips of the microcirculation were acquired from the sublingual area at each of the five time points. Images were acquired by three operators but reviewed for quality by a single experienced operator. At least three (and ideally five) video sequences, of at least five seconds duration, were recorded at each time point, in accordance with the current consensus for assessing the microcirculation [13]. Quality assessment criteria were used to optimise image quality according to standard practice [14]. Each video sequence was assigned a five-digit random number when saved to digital media. Images were therefore de-identified with respect to animal, treatment and time point.

Analysis of microcirculation video clips

Analysis was conducted offline by a single operator (S.H.) using semi-automated software (Automated Vascular Analysis v. 3.0, Microvision Medical, NL). All vessels were identified and traced by hand. Only small vessels (< 20 μm diameter) were included in the analysis. Results were obtained for the following variables:

(i) Total vessel density (TVD); the total numbers of vessels in the visual field.
(ii) Microcirculatory flow index (MFI), originally described by Boerma et al. (27) rates each quadrant of the image in terms of the most appropriate flow rating (0 = no flow; 1 = intermittent flow; 2 = sluggish flow; 3 = continuous flow). To improve the accuracy of this measurement, we ascribed MFI values to each individual vessel segment and the mean value for each video sequence was calculated.
(iii) Perfused vessel density (PVD); the total number of vessels in the visual field with either sluggish or continuous flow.

Microcirculatory perfusion

Animals were divided ex priori into two groups classed as having above average (group A) and below average (group B) microcirculatory perfusion based on their lowest PVD values. PVD was selected as the discriminator as it is a composite marker composed of both vessel density and flow data. Firstly, the lowest PVD during the shock and early resuscitation phases was calculated for each of the 22 animals. Then the mean of all these PVD values (for all animals) was selected as the reference point above and below which groups A and B were, respectively, divided.

End points of resuscitation

Since the experimental protocol was designed for all animals to survive, we chose endpoints reflective of the degree of shock reversal. The primary measure of shock reversal was lactate clearance (% change between early and late resuscitation time points). Lactate concentration, standard base excess and the gradient between mixed venous and arterial PCO_2 (CO_2 gap) at the late resuscitation time point were selected as secondary endpoints. Urine output was used as a surrogate marker of renal perfusion.

Statistical analysis

Data analysis was performed using Prism version 6.0 (GraphPad). Data was assessed for normality using D'Agostino-Pearson omnibus normality test. Repeated *t* tests or Mann-Whitney *U* tests with Holm-Sidak correction for multiple comparisons were used to assess for differences between groups. A p value of < 0.05 was considered significant.

Results

Data was collected on twenty-two animals which survived to the end of resuscitation. During initial resuscitation, 12 animals received blood products and ten 0.9 % saline. Ten animals were exposed to blast.

Inter-individual variation in macro- and micro-haemodynamic variables

Macro-circulatory flow and pressure variables and microcirculatory perfusion variables showed a similar trend during shock and resuscitation (Fig. 1a–c). However, although blood pressure, as the targeted parameter, showed little variation between animals (Fig. 1a), there was a wider variation in both cardiac output (Fig. 1b) and PVD (Fig. 1c).

During the shock and early resuscitation phases, the mean of the lowest recorded PVD for all animals was 6.2 mm/mm^2 and this value was used to divide animals into groups with above average (group A) and below average (group B) microcirculatory perfusion. Illustrative IDF video sequences from these two groups are provided in the supplementary electronic information (Additional file 1: Video 1 and Additional file 2: Video 2).

Expectedly, there was no difference in blood pressure between animals in groups A and B (Fig. 2a). PVD in animals with above average perfusion during shock and early resuscitation returned to baseline by the end of the experiment in contrast to those in the below average perfusion group in whom it remained depressed (Fig. 2b). The cardiac output fell sharply following haemorrhage in both groups, but the difference between the groups was less pronounced and became non-significant as resuscitation progressed (Fig. 2c). Animals in group A had significantly lower systemic vascular resistance during the shock and early resuscitation phases than those in group B (group A 1086 ± 296, group B 1503 ± 319 dyn s cm^{-5} $p = 0.002$). By the end of the experiment, there was no difference in cardiac output between the two groups but a persisting gap in microcirculatory perfusion.

Effect of microcirculatory perfusion on shock reversal

Data on shock reversal variables are presented in Table 1. Group A had a significantly higher lactate clearance than group B. At the end of the experiment, group A also had

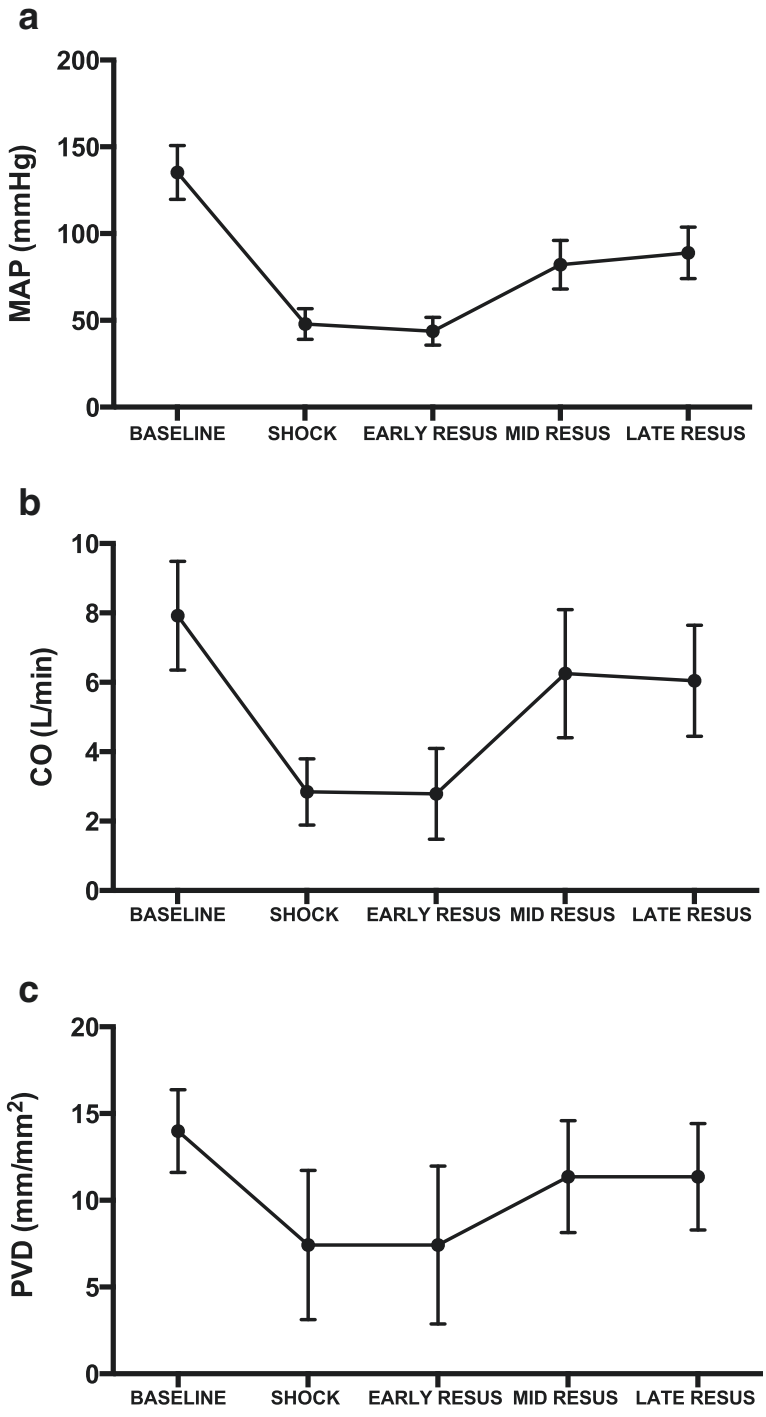

Fig. 1 Changes in mean arterial pressure (MAP) (**a**), cardiac output (CO) (**b**) and perfused vessel density (PVD) (**c**), during shock and resuscitation. Values shown as mean (SD) of all animals at each time point

a lower standard base deficit, lower final lactate concentration and a reduced CO_2 gap compared to group B. There was a trend to higher urine output in group A, but this did not reach significance.

We further analysed each group of animals based on the type of initial resuscitation fluid and the state of microcirculatory perfusion. Although the numbers in each of

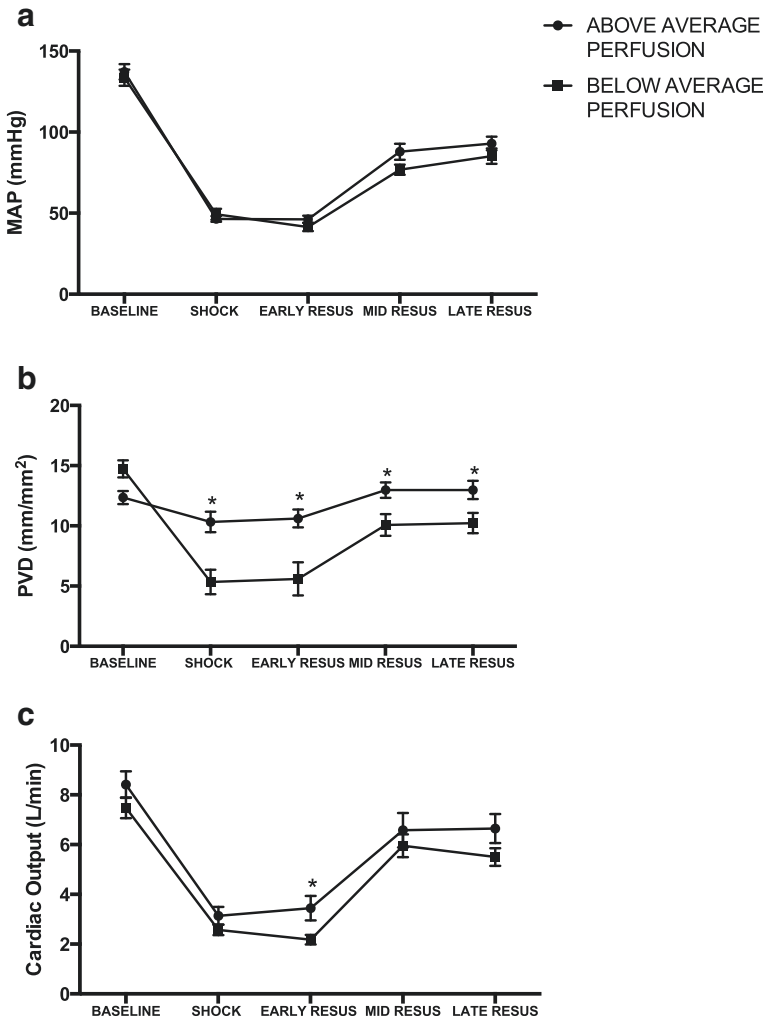

Fig. 2 Differences in mean arterial pressure (MAP) (**a**), perfused vessel density (PVD) (**b**) and cardiac output (CO) (**c**), between animals with above average and below average perfusion recorded during shock and early resuscitation

Table 1 Markers of shock reversal at the end of resuscitation in animals with above and below average perfused vessel density (PVD) recorded during shock and early resuscitation

	Group A (Above average PVD) $n = 10$	Group B (Below average PVD) $n = 12$	p value
Tissue perfusion variables, mean (SD)			
Lactate at late resuscitation (mmol/l)	4.9 (2.5)	8.6 (3.7)	0.02*
Lactate clearance during resuscitation (%)	73.1 (11.9)	40.7 (32.3)	< 0.001*
Standard base deficit at late resuscitation	1.3 (4.3)	-4.8 (5.7)	0.01*
($PvCO_2$–$PaCO_2$) at late resuscitation (kPa)	1.3 (0.6)	2.1 (0.7)	0.03*
Urine output (ml)[a]	260 (163)	168 (152)	0.25

*Significant using two-tailed *t* test
[a]Cumulative between shock phase and end of experiment

these four groups was too small for statistical significance, the impact of impaired microcirculatory perfusion can be observed within each group of identically treated animals (Fig. 3).

Effect of initial resuscitation fluid and blast exposure

Animals treated with 0.9 % saline as the initial resuscitation fluid had significantly lower PVD prior to treatment than those that received blood products. Although there was a suggestion that animals initially treated with 0.9 % saline continued to have a lower PVD throughout subsequent resuscitation (Fig. 4), further meaningful analysis was precluded by this lack of homogeneity prior to treatment. Examining the effects of administration of initial resuscitation fluid on PVD in each individual animal also failed to reveal a uniform trend (Fig. 5). There were no discernable differences in lactate clearance or final lactate concentration based purely on the type of initial resuscitation fluid used (Additional file 3).

Ten out of twenty-two animals were exposed to blast injury. There were no apparent differences in either macro- or microcirculatory variables between animals attributable to blast exposure, nor in the lactate profile, although further analysis was confounded by the heterogeneity of fluid treatments applied (Additional file 4).

Discussion

The main finding of this study is that in a large animal model of complex traumatic injury and haemorrhagic shock, with resuscitation targeted at systolic blood pressure, there is wide inter-individual variations in both macro- and microcirculatory flow variables. Furthermore, animals which were able to maintain flow, both at the systemic and microcirculatory level, had more timely reversal of the shock state during resuscitation.

Our study adds to the previous work conducted by Dubin and co-workers [15] who showed that microcirculatory flow fell in line with cardiac output during the early phases of haemorrhage in an ovine model. Our study supports these findings but further shows that there is a group of animals in whom the reduction in microcirculatory perfusion appears less marked and that these animals exhibit a more rapid reversal

Fig. 3 Differences in lactate concentration at the end of resuscitation between animals with above and below average microcirculatory perfusion recorded during shock and early resuscitation. Animals are grouped by initial resuscitation fluid

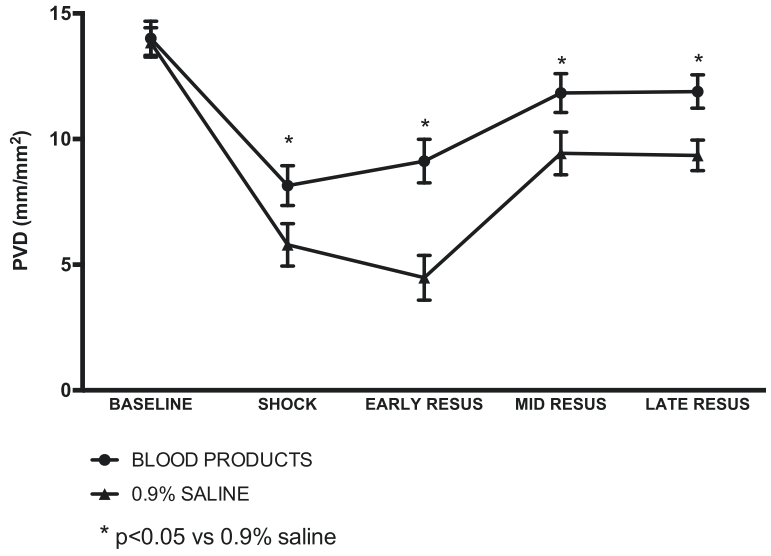

Fig. 4 Changes in perfused vessel density (PVD) in animals initially resuscitated with 0.9 % saline ($n = 10$) or blood products ($n = 12$). Values shown as mean (SEM) of all animals at each time point

of the shock state during subsequent resuscitation. Although microcirculatory flow did initially follow cardiac output in our experimental model, the coherence between macro- and microcirculatory flow was, to some extent, lost during late resuscitation.

A feasible physiological explanation of our study findings is that those animals with below average microcirculatory perfusion during shock and early resuscitation have responded to a fall in cardiac output with excessive vasoconstriction compared to animals with above average perfusion. Such excessive vasoconstriction, above that required to maintain organ perfusion pressure, may lead to microcirculatory impairment and consequent tissue hypo-perfusion, leading to the reduced lactate clearance observed in this group. Precisely why some animals responded to an equivalent insult with differing degrees of vasoconstriction remains unexplained, and further investigative work is required to elucidate the exact mechanistic processes involved.

In the clinical setting, the identification of patients who have poor microcirculatory flow during shock and early resuscitation may potentially lead to important changes in management. Such an approach could see the introduction of more individualised therapy, and this would have a better physiological rationale than a blanket 'one size fits all' pressure-targeted resuscitation strategy. Xu and co-workers elegantly demonstrated that targeting microcirculatory perfusion rather than blood pressure leads to less fluid administration with no detriment to outcome in a porcine model of THS [16]. The use of hand-held video microscopes combined with a point-of-care assessment tool for bedside video analysis may allow the introduction of such a strategy into clinical practice. There are early indications that this may be feasible [17]. Adoption of such systems into clinical practice may allow the development of new resuscitation protocols for THS based around flow rather than pressure targets.

Failure to normalise the microcirculation following resuscitation has significant effects on organ function [18]. It is common for critically ill septic patients to have a disjunction between macro- and microcirculatory function, and this has also been

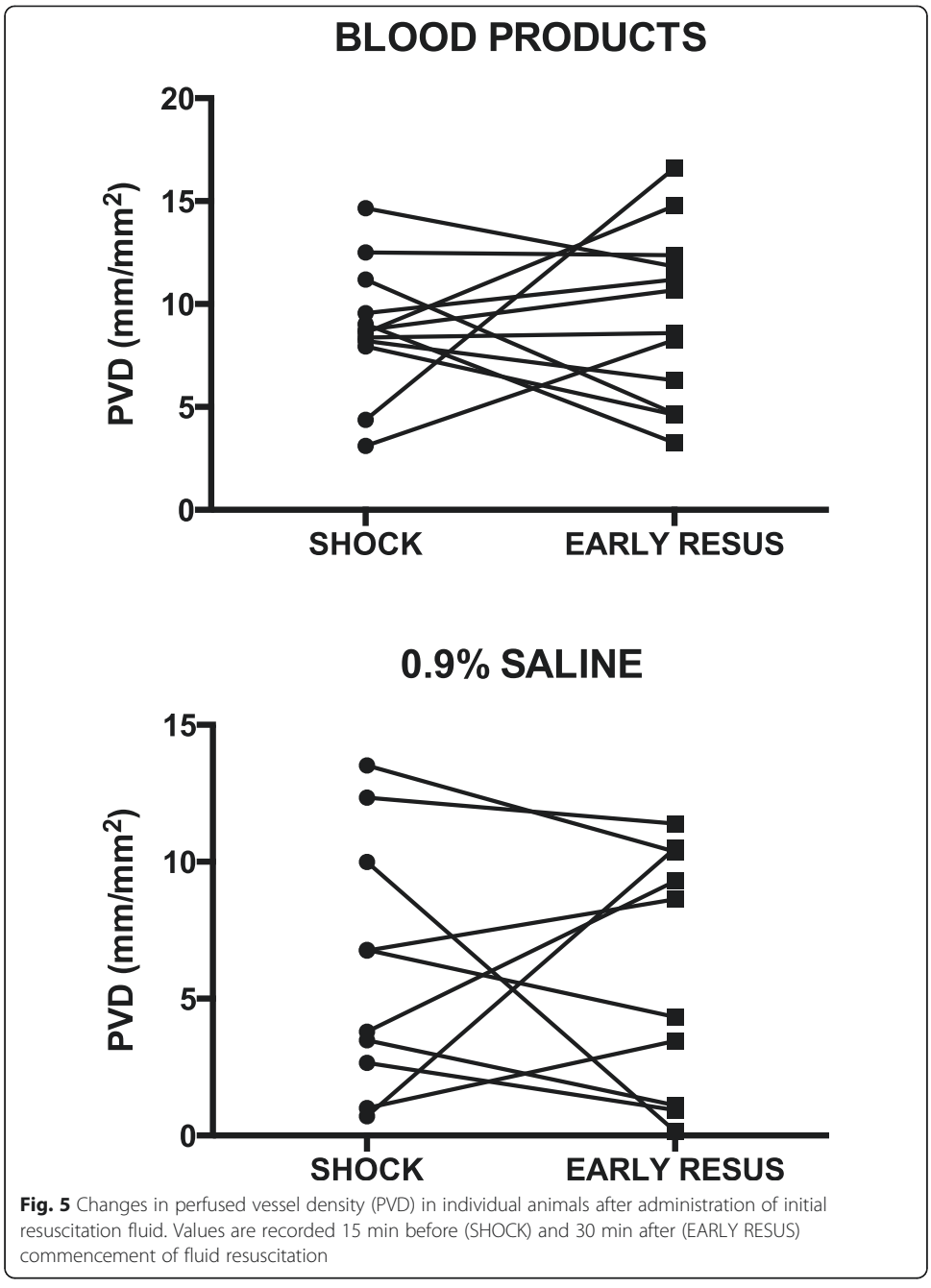

Fig. 5 Changes in perfused vessel density (PVD) in individual animals after administration of initial resuscitation fluid. Values are recorded 15 min before (SHOCK) and 30 min after (EARLY RESUS) commencement of fluid resuscitation

recently demonstrated in patients following THS [9]. Previous preclinical studies of experimental haemorrhagic shock have variably demonstrated either recovery [19] or persistence [20, 21] of microcirculatory dysfunction after systemic haemodynamic variables have been restored. Whilst our results show that microcirculatory perfusion broadly follows systemic blood flow in returning to normal levels following resuscitation, there were a group of animals in whom the microcirculation continued to be disproportionately impaired. Our results suggest that those animals which show very significant falls in microcirculatory impairment during the shock period never return to baseline values of perfusion, in contrast to those with more modest initial falls in

perfused vessel density. Possible explanations for persisting microcirculatory failure after restoration of systemic blood flow include dysfunction of any or all of the individual elements that make up the microcirculation, for example, endothelial cell swelling caused by ischaemia and re-perfusion injury [22], clogging of small vessels with activated leucocytes [23] and dysfunction of transfused stored erythrocytes leading to vessel occlusion [24]. Our study was only designed to assess the initial response of the microcirculation to shock and resuscitation, and further studies are required to answer these interesting questions relating to the precise mechanisms behind persistent microcirculatory dysfunction.

Our study was opportunistic in nature and although we prospectively collected data, we not unsurprisingly ended up with groups of animals which had received a variety of heterogeneous injuries and treatments. We did examine the data to determine if there was any discernible effect of differing fluid administration on microcirculatory perfusion in animals which received saline and blood products but failed to find a unifying pattern. In part, this was because animals which received saline had a significantly lower PVD during the shock phase prior to initial treatment, precluding subsequent analysis. Other studies have appeared to show enhanced microcirculatory perfusion following the administration of fluids with higher viscosities [25, 26], but many of these were conducted in small animals with limited translatability into the clinical setting. Furthermore, of the small number of large animal studies conducted in this field [27, 28], none examined complex injury as opposed to simple blood loss, again limiting the potential for translation. The interesting question of which fluid more effectively resuscitates the microcirculation is still not fully answered, and further studies specifically designed to address this question in a model of complex THS would be of value.

Our study has several limitations, the most obvious being the heterogeneity of the applied treatments. In order to reduce the use of animals, we collected data from an existing experimental model, whose primary aim was to detect differences in trauma coagulopathy based on the choice of initial resuscitation fluid. Furthermore, in order to replicate recent military combat conditions, some animals were exposed to blast injury. We were unable to control for these variations in our small sample size.

Despite the pre-allocation of animals into 0.9 % saline and blood product groups, these groups were statistically different, in terms of PVD, even before the start of resuscitation. This made further detailed comparison of animals based on the type of fluid difficult.

The authors also recognise the limitation of not having survival as an outcome measure in this observational study; ideally, this would be a useful translatable outcome for clinical relevance. However, the experiment was designed for all animals to survive until the end of the protocol. Therefore, surrogate markers of shock reversal such as lactate clearance, base excess, and the "CO_2 gap" [29] were used as measures of resuscitative 'success'.

Conclusions

In a large animal model of complex hemorrhagic shock and pressure-targeted resuscitation, there was a wide variation in both macrocirculatory flow and microcirculatory perfusion. Pressure-based monitoring did not discriminate between animals with

poor and preserved perfusion. Animals with above average microcirculatory perfusion had more timely reversal of the shock state during subsequent resuscitation. If translated to the clinical setting, microcirculatory perfusion may represent a useful endpoint of resuscitation and a potential therapeutic target.

Approvals

The whole experiment was approved and conducted under license from the UK Home Office Animals (Scientific Procedures) Act 1986.

Additional files

Additional file 1: Video 1. IDF video sequence taken from animal with above average microcirculatory perfusion during early resuscitation phase. (MP4 8730 kb)

Additional file 2: Video 2. IDF video sequence taken from animal with below average microcirculatory perfusion during early resuscitation phase. (MP4 18187 kb)

Additional file 3: Further information relating to the effects of initial resuscitation fluid on systemic haemodynamic and microcirculatory variables. (PDF 39 kb)

Additional file 4: Effect of blast injury on systemic haemodynamic and microcirculatory variables. (PDF 35 kb)

Abbreviations

CO, cardiac output; IDF, incident dark field; IQR, inter-quartile range; MAP, mean arterial pressure; MFI, microcirculatory flow index; PAC, pulmonary artery catheter; PaCO2, arterial carbon dioxide tension; PvCO2, mixed venous carbon dioxide tension; PVD, perfused vessel density; SBP, systolic blood pressure; SBE, standard base excess; SEM, standard error of the mean; SD, standard deviation; SVR, systemic vascular resistance; TVD, total vessel density

Competing interests

The authors declare that they have no competing interests. All authors except JW are employed by the UK Ministry of Defence.

Authors' contributions

SH conceptualised the study, assisted with design of the protocol, collected and analysed data and wrote the first draft and revision of the manuscript. DN revised the manuscript and assisted with the literature review. JW advised on the concept and design of the study and critically appraised the manuscript. CB and CW collected the data and critically reviewed the manuscript. SW and EK designed the protocol, collected the data and critically appraised the manuscript. All authors read and approved the final manuscript.

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BMJ Open

Observational study of the effects of traumatic injury, haemorrhagic shock and resuscitation on the microcirculation: a protocol for the MICROSHOCK study

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ABSTRACT

Introduction: The microcirculation is the physiological site of oxygen and substrate exchange. Its effectiveness during circulatory shock is vital for the perfusion of tissues, and has a bearing on subsequent organ function and prognosis. Microcirculatory dysfunction following traumatic haemorrhagic shock (THS) has been understudied compared with other pathologies such as sepsis. The aim of the MICROSHOCK study is to investigate changes seen in the microcirculation of patients following THS, and to assess its response to resuscitation. A greater understanding of the behaviour and mechanisms of microcirculatory dysfunction in this context may direct future avenues of goal-directed resuscitation for these patients.

Methods and analysis: This multicentre prospective longitudinal observational study includes patients who present as an emergency with THS. Microcirculatory parameters are recorded using sublingual incident dark field microscopy alongside measurements of global flow (oesophageal Doppler and transthoracic echocardiography). Patients are enrolled into the study as soon as feasible after they arrive in hospital, and then at subsequent daily time points. Blood samples are taken for investigation into the mechanisms of microcirculatory dysfunction. Sequential Organ Failure Assessment scores will be analysed with microcirculatory parameters to determine whether they correlate with greater fidelity than more conventional, global circulatory parameters.

Ethics and dissemination: Research Ethics Committee approval has been granted for this study (Reference: 14/YH/0078). Owing to the nature of THS, capacity for informed consent will be absent on patient enrolment. This will be addressed according to the Mental Health Capacity Act 2005. The physician in charge of the patient's care (nominated consultee) may consent on behalf of the patient. Consent will also be sought from a personal consultee (close relative or friend). After capacity is regained, the participant will be asked for their consent. Results will be submitted for publication in peer-reviewed journal format and presented at relevant academic meetings.

Trial registration number: NCT02111109; Pre-results.

Strengths and limitations of this study

- This study's main strength is that it will be the largest clinical investigation into the microcirculatory response to traumatic injury and haemorrhagic shock to date.
- The MICROSHOCK study will recruit from major trauma centres in London and Birmingham, the two largest cities in the UK.
- The study is limited by its observational nature. However, the key research question 'Does the microcirculatory behaviour predict outcome better than global haemodynamic parameters?' can be addressed using observational data.
- A further limitation is the heterogeneous nature of injury patterns in the UK trauma patient population. Such differences will be addressed during data analysis, and reported during the dissemination of the results. These differences will be much greater than those in animal studies. However, a clinical study allows us to translate what has been learnt from animal research into useful and clinically relevant knowledge.

BACKGROUND

Massive haemorrhage and associated shock is a leading cause of preventable death among casualties with severe traumatic injury, accounting for around 40–50% of all deaths.[1 2] Despite significant improvements in treatment and mortality within this patient group, systemic inflammatory response, coagulopathy and subsequent multiple organ failure remain common.[3] These issues can lead to prolonged periods of morbidity, critical care dependency and often death. Therapeutic interventions that attenuate these responses may lead to significant benefit for patients who have suffered traumatic haemorrhagic shock (THS).[3]

The microcirculation is of particular clinical importance during circulatory shock

since it is this network of capillaries and other small vessels that perform the essential functions of delivering oxygen and substrates to cells. Traditionally, resuscitative strategies are guided by measurement of global haemodynamic parameters such as blood pressure and cardiac output. However, there may be a poor correlation between such global parameters and the appearance of the microcirculation.[4 5] Microcirculatory parameters may also predict outcome better than more conventional global circulatory measurements in sepsis.[6] Observational data suggest patients who can improve the state of the microcirculation in response to resuscitative fluid therapy have a better outcome than those who cannot.[7 8]

Incident dark field (IDF) video microscopy is a method of assessing the microcirculation that has been developed relatively recently for clinical and research.[9] A light source is applied to the tissue, which illuminates the deep tissues within the target field. The reflected light from the deep tissues is captured and magnified before being received on a viewer. The selective wavelength of transmitted light is completely absorbed by both oxygenated and deoxygenated haemoglobin, and blood vessels therefore appear black. The technique is limited to organs that have a thin epithelial covering and that are accessible to a handheld probe, which in clinical practice has usually been the sublingual microcirculation. The device is small, portable and the technique non-invasive. Obtaining images with the device requires between 2 and 5 min and involves the use of a small, non-traumatic sublingual probe. The images produced by IDF microscopy require offline analysis and the ascription of objective values before they can be used. De Backer *et al*[10] have standardised this approach.

Most clinical studies that examine the microcirculation have focused on sepsis,[7 11 12] whereas studies relating to haemorrhagic shock are mostly limited to animal experiments, and have usually looked at controlled haemorrhagic shock rather than a complex traumatic injury model.[13–15] One clinical study has examined the microcirculation after THS once the patients had been admitted to the intensive care unit (ICU), but did not gather data during the early phases of resuscitation.[16] Although they share similarities, septic and haemorrhagic shock must be examined separately in a clinical context, so that their respective differences can be delineated, and unanswered questions can be addressed.

Primary objective

The primary objective of the MICROSHOCK study is to examine the microcirculatory function in patients following THS, and to determine whether these parameters are superior to global haemodynamic parameters in the prediction of clinical outcomes.

METHODS

Study design

This is a multicentre prospective longitudinal observational study that involves serial assessments of the sublingual microcirculation alongside measurements of global flow, volume status and blood pressure, determined from existing monitoring devices and using focused transthoracic echocardiography. In conjunction, data will be gathered relating to the patients physiology and resuscitation.

Patient screening

All patients with a history of traumatic injury presenting to the emergency department (ED) at the study site hospitals will be screened by research staff. These sites are all UK Major Trauma Centres, and include (1) Kings College Hospital, London, UK; (2) The Royal London Hospital, London, UK; and (3) Queen Elizabeth Hospital Birmingham, Birmingham, UK. Patient details will be recorded in a screening log.

Number of patients

A single pilot study demonstrated feasibility in the examination of the microcirculation following THS, and reported data for 18 patients.[16] The current study will be the first to examine the microcirculation of patients with THS as soon as they have arrived in the ED. The MICROSHOCK study will enrol 60 patients in order to further investigate the microcirculation following THS, and form hypotheses that might direct future goal-directed interventions.

Inclusion criteria

Adult patients with evidence of haemorrhagic shock exhibiting all of the following features:

1. Mechanism of injury consistent with blood loss;
2. Intubated and ventilated;
3. Serum lactate concentration >2 mmol/L recorded at any stage prior to admission to the ICU;
4. Have received any blood products (eg, packed red blood cells (PRBC), fresh frozen plasma (FFP), cryoprecipitate, platelets) during the initial period of resuscitation, prior to admission to ICU, or are predicted to receive blood products during this timeframe in the opinion of the trauma team leader.

Exclusion criteria

Patients with facial injuries, where access to the sublingual area would be problematic will be excluded. Patients with injuries deemed unsurvivable in whom the focus of care is palliation rather than active treatment are also ineligible for inclusion.

Time points and techniques for data collection

Figure 1 outlines the techniques that will be utilised, samples taken and data collected at each time point. There are five main time points:

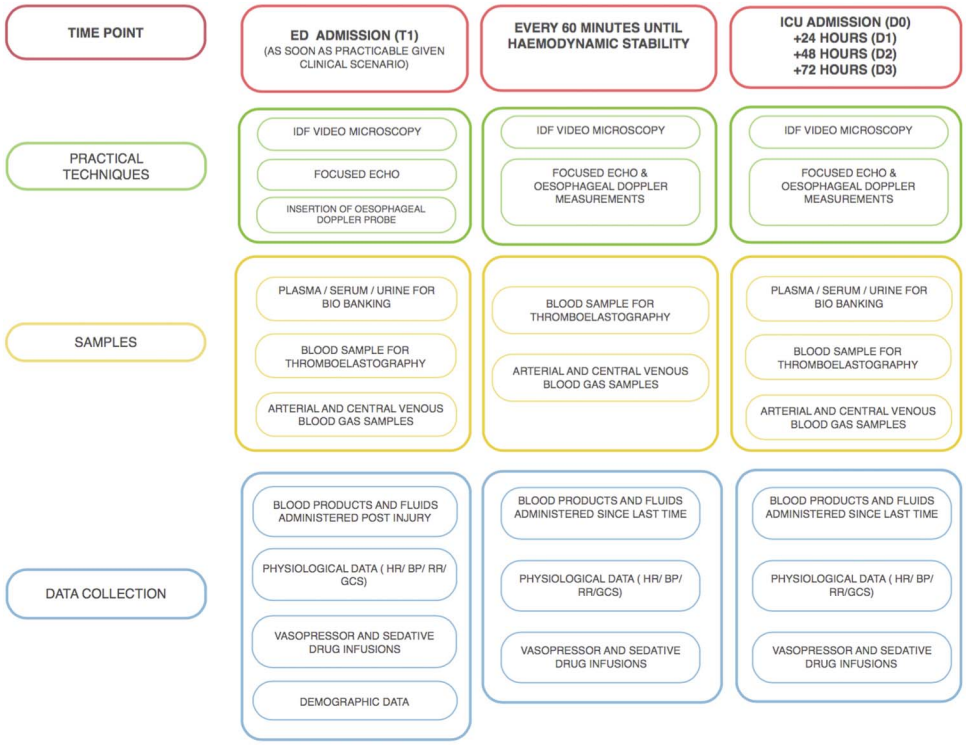

Figure 1 Sampling and techniques. BP, blood pressure; ED, emergency department; GCS, Glasgow Comas Scale; ICU, intensive care unit; IDF, incident dark field; HR, heart rate; RR, respiratory rate.

1. As soon as feasible after arrival in hospital (T1), and up to every 60 min until haemodynamically stable;
2. On admission to ICU (D0);
3. 24 h after D0;
4. 48 h after D0;
5. 72 h after D0.

Where feasible, and appropriate, further readings will be taken between the first and second time points. Data collection is stopped once the patient has been extubated.

Demographic and resuscitation data

The following baseline (hospital admission) demographic and physiological data will be collected from study participants and recorded in an electronic database on a secure encrypted computer used only for research purposes. All data in this log will be anonymised and assigned a sequential study number.

- Demographic details (age, gender, ethnicity);
- Mechanism of injury;
- Injury Severity Score (ISS);
- Admission systolic blood pressure (SBP), respiratory rate and Glasgow Comas Scale (GCS; to calculate Trauma and Injury Severity (TRISS) score);
- Administered prestudy blood products and intravenous fluids;
- Timings from injury to start of prehospital resuscitation, ED resuscitation and operating theatre-based resuscitation;
- Nature of initial surgical procedures and treatments.

Acquisition of IDF images

The investigator will obtain video clips of the sublingual microcirculation using an IDF videomicroscope (Cytocam, Braedius Medical B.V., Huizen, The Netherlands). In accordance with international consensus criteria for assessing the microcirculation, enough video will be recorded to enable at least three (and preferably five) ×10 s video clips to be obtained at each time point. Because the microcirculation can demonstrate considerable heterogeneity, particularly in shock states, images will be acquired from different sites within the sublingual area. When acquiring images, investigators should actively seek to minimise pressure artefact by observing flow in medium and large vessels. The image should be stabilised with minimal movement artefact. Light intensity and focus should be optimised. Individual video sequences will be exported from the image acquisition software as DV-AVI files.

Acquisition of transthoracic echo images

Subcostal and apical five-chamber windows will be obtained. The inferior vena cava will be visualised in M mode, and the minimum and maximum diameters across the respiratory cycle recorded. The left ventricular outflow tract (LVOT) will be visualised and a pulsed wave Doppler signal acquired. The LVOT velocity time integer (VTi) and the degree of variation across the respiratory cycle will both be recorded.

Oesophageal Doppler

An oral oesophageal Doppler probe (Deltex Medical, Chichester, UK) will be inserted at the same time as acquisition of the first IDF images. The probe will remain in situ continuously for 72 h or until the patient is ready for extubation. The following data will be recorded: corrected flow time, stroke volume, cardiac output. Data obtained from this monitoring device will also be available for use to the patients' attending clinicians if they wish.

Thromboelastography (ROTEM)

A 5 ml sample of blood will be withdrawn from existing vascular access devices and used to perform point-of-care analysis of coagulation status using the technique of thromboelastography (ROTEM). The following values will be recorded: EXTEM/FIBTEM CFT, A5, A10, MCF, LY 30.

Biological sampling

Samples of blood and urine will be withdrawn from existing vascular access devices and stored for later assessment of the mechanisms of microcirculatory dysfunction.

Physiological and pharmacological data

The following parameters will also be recorded at every time point where available:

- SBP, diastolic blood pressure and mean arterial blood pressure;
- Central venous pressure;
- Heart rate;
- Arterial Blood Gas Data (including lactate, base deficit, arterial oxygen saturation, arterial carbon dioxide saturation)
- Haemoglobin concentration;
- Central Venous Blood Gas Data (central venous oxygen saturation ($ScvO_2$), central venous carbon dioxide saturation ($ScvCO_2$));
- Plasma (free) haemoglobin;
- Administration of blood, blood products and bolus challenges of other intravenous fluids since the previous time point;
- Type of sedative drug infusions currently being administered;
- Type and dose of inotropic and vasopressor drugs currently being administered.

Primary outcome

The primary outcome is the Sequential Organ Failure Assessment (SOFA) score at the D3 time point (72 h after admission to ICU), and will be recorded using information from the patients' charts and laboratory results.

Secondary outcomes

Secondary outcomes include SOFA scores and multiorgan dysfunction score on days 1, 7 and 28 of hospital admission (if the patient is still an inpatient), mortality at 28 days, length of stay (LOS) in hospital, LOS in ICU, number of days of mechanical ventilation, thromboelastography parameters, arterial and venous blood gas parameters, and blood product requirements.

Analysis of IDF video sequences

Prior to analysis, the recorded video sequences will be edited into at least three (and no more than five) video clips. All clips will be rated for quality using a five-point scale.[17] Substandard images will be rejected. All IDF images will be analysed using a dedicated software tool (Automated Vascular Analysis V.3.02, Microvision Medical, The Netherlands). This is a semiautomated process which produces a number of data points from the video sequence. Analysis of the video clips will produce the following data:

- Microvascular flow index;
- Total vessel density;
- Perfused vessel density;
- Proportion of perfused vessels
- Microcirculatory heterogeneity index.

This process is in accordance with recommendations made by an international consensus conference on assessing the microcirculation.[10] Interobserver variation will be tested at regular intervals in order to minimise heterogeneity of data analysis between investigators. At the time of analysis, the investigators will be blinded with respect to the clinical time point of the study, the patients' details, and the other outcome variables (SOFA score and haemodynamic data).

The following relationships will be examined:

- Between microcirculatory parameters during the post-resuscitation period and the development of SOFA scores;
- Between microcirculatory parameters and systemic haemodynamic data (stroke volume, cardiac output, blood pressure);
- Between microcirculatory parameters and plasma lactate and $ScvO_2$ over the resuscitation period;
- Between microcirculatory parameters and coagulation parameters as assessed by ROTEM.

Capacity and consent

This study complies with the Declaration of Helsinki, and will be conducted in accordance with the principals of Good Clinical Research Practice (GCP). In order to assess the response of the microcirculation to haemorrhagic shock and resuscitation, intubated and ventilated patients will be recruited to the study at an early stage following their admission to hospital, when capacity for informed consent will be absent.

Lack of patient capacity will be addressed by referring to the Mental Health Capacity Act (2005). In this situation, the physician in charge of the care of the patient (nominated consultee) will be consulted in order to consent on behalf of the patient. These physicians will have received prior briefing on the study protocol, and will not be a study investigator. If possible consent will also be sought from a personal consultee (the next of kin, relative or friend of the patient). When the patient regains capacity, the study will be explained and the

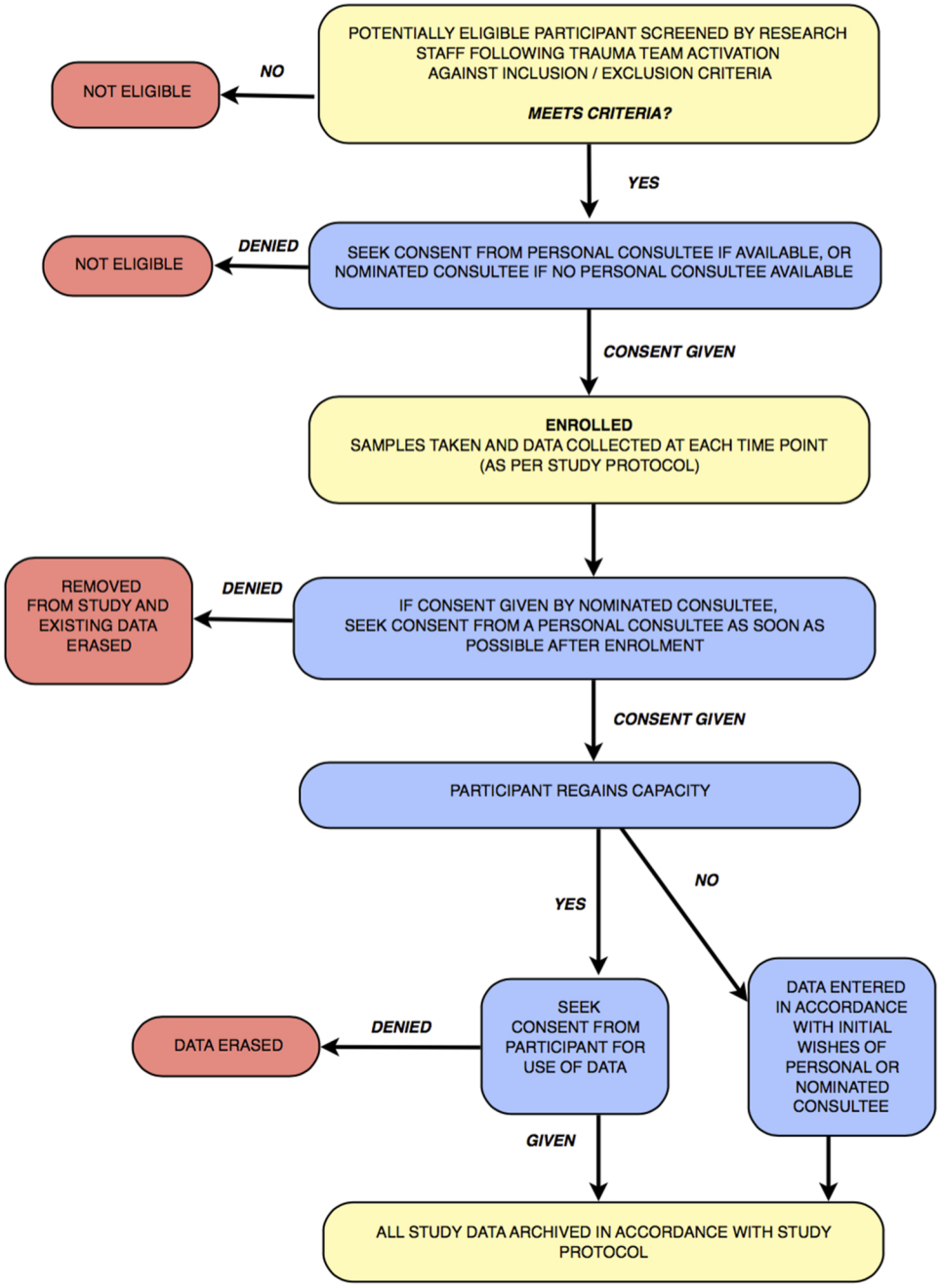

Figure 2 Process flow chart.

participant asked for their consent to use the data collected so far, as well as any further samples required by the protocol. If the participant declines consent and wishes to be removed from the study, their data will be erased and no further samples will be collected.

Patients who die or who do not regain capacity within 28 days of injury will have their data entered into the study in accordance with the wishes of their personal or nominated consultee. This process is summarised in figure 2.

Confidentiality, data storage and security

All data relating to study participants will be treated in accordance with the Data Protection Act, 1998. All study data will be stored on a secure encrypted laptop used exclusively by the study investigators. Patients will be assigned an alphanumeric sequential study number that will be used to identify all clinical data. These numbers will be specific to each site. The patients' demographic details will be linked to the study number on a separate screening database held on the secure computer at each site. Individual IDF image sequences will be assigned a random five-digit number. Identification of these video sequences will be achieved by cross-reference with an electronic database. On completion of the study, all participant identifying information and other study data will be securely archived in accordance with the policy of the sponsoring institution. All biological samples will be

identifiable only by study number, and once analysis is complete the samples will be disposed off.

Safety

The chief investigator has overall responsibility for the conduct of the study including responsibility for safety. Individual investigators will be responsible for reporting all serious adverse events (SAEs) and adverse events (AEs) to the chief investigator. There are no reported AEs associated with the use of IDF imaging in the published literature, and the risk of harm occurring to a study participant as a direct result of undergoing IDF imaging is close to zero. An example of an attributable AE would be a sublingual haematoma following IDF microscopy. SAEs will include death, any event which poses a threat to life, prolongs hospital stay or produces significant disability. Given the nature of the study population, it is anticipated that such features will be relatively common as part to the pathological process of trauma, but not as a result of participation in the study. The chief investigator will review all events to decide if there is any causal link. If this is the case action will be taken in accordance with the principals of ICH GCP.

Statistical analysis

Normally distributed data will be presented as mean and SD, and non-normally distributed data will be presented as median and IQR. For comparison, patients will be dichotomised according to day 3 SOFA score into those with a score ≥6 versus those with SOFA score <6. The score of 6 has been chosen due to its prognostic relevance[18] and previous utilisation as an end point for haemorrhagic trauma patients.[16] Further analysis will be conducted according to the change in SOFA scores between days 1 and 3, so that patients are dichotomised into SOFA score 'improvers' and 'non-improvers'. This approach has also been used as an end point for haemorrhagic trauma patients.[19] Groups will be compared using a two-tailed t test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. A p value of <0.05 will be considered statistically significant.

DISCUSSION

Bedside point-of-care technology that enables direct visualisation of the microcirculation offers an opportunity to gain insight into the real-time behaviour of the site of oxygen and nutrition exchange during shock. Although this has been primarily been a research tool in the past, advances in microcirculatory imaging may pave the way for a future clinical role.[20] In particular because real-time assessment of images by clinicians may be as reliable as detailed offline computer analysis.[21]

Further research into bedside microcirculatory imaging, and the assessment of microcirculatory parameters as end points for resuscitation have been recommended by other investigators.[22] The current study aims to address important questions with regard to the microcirculation during THS. Of particular interest is to investigate any immediate microcirculatory derangement on arrival in hospital following traumatic haemorrhagic injury, and whether there is a relationship between these changes and clinical outcomes. It is also not fully known whether there is a persistent microcirculatory derangement following initial resuscitation, and whether there is a relationship between this and subsequent organ dysfunction. Only one clinical study has reported such a finding,[16] but this was a relatively small study, with high usage of vasopressors, which is a relatively uncommon practice in the UK. Further data are required in order to fully answer this research question. If there is a true lack of coherence between macro haemodynamic parameters (eg, cardiac output and blood pressure) when compared with microcirculatory parameters, then this may strengthen the argument to improve techniques and technology to enable bedside microcirculatory monitoring. The results of this study will be examined with these clinical questions in mind.

Trauma patients are heterogeneous, with multiple confounding variables that make interpretation of data more difficult than an animal model may offer. Nevertheless, a clinical study is both timely and necessary in order to start the process of translating what has been learnt from animal research into useful and clinically relevant knowledge. The Microshock study will be the first to enrol patients with THS as soon as they arrive in hospital. Measuring microcirculatory parameters this soon after injury offers an opportunity to gain a greater insight into the early processes and mechanisms of microcirculatory dysfunction in this context. If clinically relevant details are discovered, then this may enable future investigators to determine the priorities and processes that may be put in place to move the technology towards clinical utilisation.

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Contributors SH designed and developed the Microshock study, and is the chief investigator. The first approved REC application was made by SH. DNN assisted in protocol amendments, study design, manuscript preparation and REC significant amendment applications. TH provided intellectual input into the design of the study, and is the principal investigator for the Royal London Hospital. MJM and JW have provided intellectual input, protocol and study design revision, mentorship, academic and clinical supervision.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval NRES Committee Yorkshire & The Humber—Leeds West and Research Ethics Committee (REC) approval has been granted (REC reference: 14/YH/0078).

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Microcirculatory Impairment Is Associated With Multiple Organ Dysfunction Following Traumatic Hemorrhagic Shock: The MICROSHOCK Study

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Objectives: To assess the relationship between microcirculatory perfusion and multiple organ dysfunction syndrome in patients following traumatic hemorrhagic shock.

Design: Multicenter prospective longitudinal observational study.

Setting: Three U.K. major trauma centers.

Patients: Fifty-eight intubated and ventilated patients with traumatic hemorrhagic shock.

Interventions: Sublingual incident dark field microscopy was performed within 12 hours of ICU admission (D0) and repeated 24 and 48 hours later. Cardiac output was assessed using oesophageal Doppler. Multiple organ dysfunction syndrome was defined as Serial Organ Failure Assessment score greater than or equal to 6 at day 7 post injury.

Measurements and Main Results: Data from 58 patients were analyzed. Patients had a mean age of 43 ± 19 years, Injury Severity Score of 29 ± 14, and initial lactate of 7.3 ± 6.1 mmol/L and received 6 U (interquartile range, 4–11 U) of packed RBCs during initial resuscitation. Compared with patients without multiple organ dysfunction syndrome at day 7, patients with multiple organ dysfunction syndrome had lower D0 perfused vessel density (11.2 ± 1.8 and 8.6 ± 1.8 mm/mm^2; $p < 0.01$) and microcirculatory flow index (2.8 [2.6–2.9] and 2.6 [2.2–2.8]; $p < 0.01$) but similar cardiac index (2.5 [± 0.6] and 2.1 [± 0.7] L/min//m^2; $p = 0.11$). Perfused vessel density demonstrated the best discrimination for predicting subsequent multiple organ dysfunction syndrome (area under curve 0.87 [0.76–0.99]) compared with highest recorded lactate (area under curve 0.69 [0.53–0.84]), cardiac index (area under curve 0.66 [0.49–0.83]) and lowest recorded systolic blood pressure (area under curve 0.54 [0.39–0.70]).

Conclusions: Microcirculatory hypoperfusion immediately following traumatic hemorrhagic shock and resuscitation is associated with increased multiple organ dysfunction syndrome. Microcirculatory variables are better prognostic indicators for the development of multiple organ dysfunction syndrome than more traditional indices. Microcirculatory perfusion is a potential endpoint of resuscitation following traumatic hemorrhagic shock. (*Crit Care Med* 2018; XX:00–00)

Key Words: critical care; hemorrhage; microcirculation; shock; trauma

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Hemorrhage remains the leading cause of preventable mortality following major trauma (1–3). Although some of these deaths result from uncontrolled blood loss and overt exsanguination, others occur later as a result of multiple organ dysfunction syndrome (MODS)—the prevalence of which has been reported as between 7% and 29% (4–7). The mechanisms that lead to MODS after traumatic hemorrhage are yet to be clearly elucidated, but several observational cohort studies have shown that the degree of shock, assessed using surrogate markers such as lactate concentration or base deficit, is associated with the magnitude of subsequent organ dysfunction (4, 6, 8).

A key element of effective tissue perfusion after traumatic hemorrhagic shock (THS) and resuscitation is the maintenance of blood flow through the network of small microvessels, which represent the final pathway for cellular oxygen and substrate delivery. The state of microcirculatory perfusion has been shown to influence outcome in experimental models of THS (9, 10) and has also been implicated in the development of early posttraumatic organ failure in patients in a single-center study involving 18 patients (11). The development of hand-held point of care video microscopes has allowed real-time in vivo assessment of the microcirculation in clinical practice (12).

The aim of the present study was to examine the association between microcirculatory impairment and MODS in a larger cohort of patients admitted to three major trauma centers in the United Kingdom and to investigate whether there is a threshold of microcirculatory perfusion that might be predictive of MODS.

METHODS

Study Design

A multicenter prospective longitudinal observational study was undertaken at three U.K. major trauma centers (King's College Hospital London, The Royal London Hospital, and Queen Elizabeth Hospital Birmingham). Data were collected on the state of the microcirculation in patients with traumatic hemorrhage at three time points: D0, following bleeding control procedures, but less than 12 hours after admission to the ICU, D0 + 24 hours (D1), and D0 + 48 hours (D2). Data were also collected at these time points relating to the state of the systemic circulation. Daily organ failure scores were calculated for the first 7 days of hospital admission.

The study received ethical approval from a Research Ethics Committee (REC) (Yorkshire and the Humber—Leeds West; Ref 14/YH/0078) and was registered at Clinical Trials.gov (NCT02111109). The full study protocol was published (13).

Inclusion and Exclusion Criteria

Patients were eligible for inclusion if they had been injured, had required blood product transfusion during resuscitation, had a lactate concentration of greater than 2 mmol/L at any stage prior to enrollment, and were intubated and ventilated. Study enrollment took place as early as was feasible following arrival in hospital and up to 12 hours after admission to ICU. Patients were excluded from the study if they had unsurvivable injuries with a palliative focus of care or if they had facial injuries that precluded hand-held videomicroscopy. Due to the emergency nature of the admission, a system of deferred informed consent was approved by the REC.

Microcirculatory Measurements

An assessment was made of microcirculatory perfusion using incident dark field (IDF) videomicroscopy (Cytocam; Braedius Medical, Huizen, the Netherlands) (14). Briefly, the video microscope is gently placed under the tongue until a view of the sublingual microcirculation is acquired. At least four video clips of 5 seconds (100 frames) duration are recorded, with due attention to quality factors, especially the absence of pressure artefact, in accordance with the accepted consensus for assessing the microcirculation (15, 16). Clips were deidentified and stored until a later date, at which point en masse blinded analysis was undertaken away from the patient. IDF videomicroscopy was performed by study investigators after comprehensive training by the chief investigator (S.D.H.), who was responsible for ensuring consistent, high-quality imaging across study sites. Microcirculatory videos were analyzed by a single operator using automated vascular analysis (AVA) v.3.02 (Microvision Medical, Amsterdam, The Netherlands) and manually tracing all vessels by hand. These video analyses give values for total vessel density (TVD), perfused vessel density (PVD), proportion of perfused vessels (PPV), microcirculatory flow index (MFI), and microcirculatory heterogeneity index (MHI), according to the current best practice guidelines for reporting microcirculatory variables (17). It should be noted that the calibration settings used in AVA software are of vital importance when calculating the density-related variables (TVD, PVD), and changes in these settings will produce significant variation in density values between studies. The calibration settings used in the present study are provided in the **supplementary material** (Supplemental Digital Content 1, http://links.lww.com/CCM/D691). An example of a sublingual dark field video image is provided in **Video 1** (Supplemental Digital Content 2, http://links.lww.com/CCM/D692).

Macrocirculatory and Physiologic Measurements

Cardiac output was concurrently assessed using an oesophageal Doppler monitor (Deltex Medical, Chichester, United Kingdom). Blood was drawn from arterial and central venous catheters (when placed) and used to determine lactate, base deficit, hemoglobin oxygen saturation, central venous CO_2 tension ($Pcv\overline{CO}_2$), and $Pa\overline{CO}_2$. Standard physiologic variables were recorded using existing monitoring devices. We decided a priori to discontinue hemodynamic and microcirculatory measurements following extubation for two reasons. First, oesophageal Doppler monitoring is not feasible in awake patients. Second, although sublingual videomicroscopy is possible in extubated patients, it is our experience and that of others (18) that the quality of sublingual videomicroscopy is substantially reduced in conscious, noncooperative patients, a typical circumstance in the recently extubated critically ill patient.

Outcomes

Organ failure was assessed using Sequential Organ Failure Assessment (SOFA) (19, 20) and Denver (21) scores every day for the first 7 days following injury. Full details of the SOFA and Denver scoring systems are provided in the supplemental material (Supplemental Digital Content 1, http://links.lww.com/CCM/D691). The primary outcome was the presence or absence of MODS at day 7 following injury; MODS was defined as a SOFA score of 6 or higher. Patients who had died by day 7 following injury were assigned maximum SOFA and Denver scores (24 and 12, respectively) for the day of death

and each subsequent day until day 7. Twenty-eight–day mortality and length of stay in ICU were also recorded as secondary outcomes.

Statistical Analysis

Statistical analysis was conducted using Prism v 6.0 (GraphPad Software, San Diego, CA). Data were assessed for normality using the D'Agostino and Pearson omnibus normality test; values are reported as reported as mean (SD) or median (interquartile range).

In order to compare patients who developed MODS with those who did not, patients were dichotomized into two groups based on the development of MODS at day 7. Differences in variables at the initial (D0) time point for these patients were assessed using unpaired *t* tests or Mann-Whitney *U* tests. Differences in daily organ failure rates over time between patients based on initial (D0) microcirculatory perfusion were assessed using repeated measures two-way analysis of variance with Sidak's multiple comparison test.

In order to determine the predictive value of selected variables, receiver operator characteristic (ROC) curves were constructed to predict the incidence of MODS by plotting sensitivity versus (1–specificity) for different threshold values of PVD, MFI, highest lactate, lowest systolic blood pressure (SBP), and cardiac index.

A further analysis was planned in order to determine the best fit predictive value for the microcirculatory variable that exhibited the largest area under the ROC curve. This was performed using the Youden index (22) for that microcirculatory variable, calculated by determining the J value (sensitivity + specificity–1) for each threshold measurement. The threshold measurement with the largest J value was selected as the best fit or cutoff value for determining MODS.

A p value of less than 0.05 was taken to indicate significance.

RESULTS

Patient Characteristics

There were 60 patients enrolled from July 2014 to April 2017. Data from two patients were excluded due to insufficient quality of microcirculatory video clips, so that all further analysis was performed for 58 study patients. Patients were generally young (43 ± 19 yr) and male (81%) with severe injuries (Injury Severity Score 29 ± 14). Patients were enrolled in the study on average 9 hours (3–13 hr) following injury and 1.5 hours (0–6 hr) after admission to ICU. The mechanism of injury in the majority of patients was blunt (75%) with transport-related accidents most common (53%), followed by falls (17%) and crush injuries (7%). Those with a penetrating mechanism of injury included one with a gun-shot wound, whereas the remainder sustained knife injuries. The median number of packed RBCs (PRBC) transfused during initial resuscitation (from injury until D0) was 6 U (4–11 U) , and 29% of patients received a massive transfusion (defined as > 10 U PRBC in the first 24 hr). Enrolled patients exhibited signs of shock and hemodynamic compromise; the mean highest recorded lactate concentration prior to ICU admission was 7.3 ± 6.1 mmol/L, and mean lowest recorded SBP was 69 ± 27 mm Hg. Eight patients (13%) suffered a traumatic brain injury (TBI) in addition to their other injuries. The majority of patients had surgical control of hemorrhage (81%), 10% had combined surgical and interventional radiologic (IR) control of bleeding, 2% IR alone, and 7% were managed conservatively. The vast majority of resuscitative fluid (blood products and crystalloid/colloid) was given prior to study enrollment, and the D0 time point therefore represents a period of relative hemodynamic stability following resuscitation. Full details of fluid and vasopressor administration are provided in **Table S.1** (Supplemental Digital Content 1, http://links.lww.com/CCM/D691).

Outcomes

Patient characteristics according to development of MODS at day 7 are shown in **Table 1**.

Microcirculatory variables at all time points according to the presence of MODS at day 7 are shown in **Figure 1**. Although all enrolled patients had complete data recorded at study inclusion (D0), there were a proportion of patients in whom data were not obtainable at the D1 and D2 time points; this was predominantly due to early patient extubation and mostly affected the group without MODS at day 7. Full details of these exclusions are contained in **Table S.2** (Supplemental Digital Content 1, http://links.lww.com/CCM/D691).

There are significant differences between TVD, PVD, MFI, and MHI, at the D0 time point, for patients according to subsequent MODS. Conversely, there were no significant differences in systemic hemodynamic variables (cardiac index, mean arterial pressure) between those who developed MODS and those who did not. Similarly, there were no differences in noradrenaline dose at D0 between those with and without MODS (median dose 0 µg/kg/min [0.25–0.53 µg/kg/min] and 0 µg/kg/min [0.05–0.38 µg/kg/min], respectively; $p = 0.10$).

Comparison of Variables Used for the Prediction of MODS

ROCs curves were constructed for several variables to predict the development of MODS (**Fig. 2**). The microcirculatory variables of PVD and MFI had area under curve (AUC) values of 0.87 (0.76–0.99) and 0.83 (0.71–0.95), respectively. These values provided a more accurate prediction of MODS at day 7 than cardiac index (AUC, 0.66 [0.49–0.83]) or highest recorded lactate prior to ICU admission (AUC, 0.69 [0.53–0.84]). The lowest recorded SBP appeared to be the least predictive variable for the development of MODS (AUC, 0.54 [0.39–0.70).

Factors Associated With Postresuscitation Microcirculatory Impairment

Based on the ROC curve analysis, PVD was selected as the microcirculatory variable with the best discrimination at predicting MODS. The threshold value for PVD which represented the best fit for MODS prediction was 10.33 mm/mm^2. **Figure 3** shows mean daily organ failure scores for patients with D0 PVD scores

TABLE 1. Characteristics of Study Patients With or Without Multiple Organ Dysfunction Syndrome at Day 7 After Injury

Patient Characteristics	SOFA ≥ 6 (*n* = 23)	SOFA < 6 (*n* = 35)	*p*
Age, median (IQR)	49 (28–74)	36 (27–46)	0.06
Injury Severity Score, mean ± SD	35 (± 12)	25 (± 14)	0.01[a]
Resuscitation fluids prior to bleeding control procedures, but < 12 hours after admission to the ICU			
Packed RBC, units, median (IQR)	6 (4–13)	6 (3–8)	0.20
Fresh frozen plasma, units, median (IQR)	5 (4–10)	4 (2–8)	0.39
Crystalloids, mL, median (IQR)	2,900 (225–6,125)	2,000 (0–3,500)	0.19
Noradrenaline dose, μg/kg/min, median (IQR)	0 (0–0.25)	0 (0–0.05)	0.10
Microcirculatory variables			
Total vessel density, mm/mm^2, mean ± SD	10.6 (± 1.6)	11.8 (± 1.6)	< 0.01[a]
Perfused vessel density, mm/mm^2, mean ± SD	8.6 (± 1.8)	11.2 (± 1.8)	< 0.01[a]
Microcirculatory flow index, AU, median (IQR)	2.6 (2.2–2.8)	2.8 (2.6–2.9)	< 0.01[b]
Proportion of perfused vessels, %, median (IQR)	88 (76–96)	96 (92–99)	< 0.01[b]
Microcirculatory heterogeneity index, AU, median (IQR)	0.9 (0.5–1.2)	0.35 (0–0.39)	< 0.01[b]
Systemic hemodynamic variables, mean ± SD			
Cardiac index, L/min/m^2, mean ± SD	2.1 (± 0.7)	2.5 (± 0.6)	0.11
Mean arterial pressure, mm Hg, mean ± SD	76 (± 18)	81 (± 15)	0.26
Lowest systolic blood pressure pre-ICU, mm Hg, mean ± SD	66 (± 24)	71 (± 28)	0.53
Hemoglobin (g/dL), mean ± SD	10.8 (± 1.9)	11.9 (± 1.9)	0.053
Tissue perfusion variables			
Lactate, mmol/L, median (IQR)	4.1 (2.1–6.9)	2.2 (1.4–3.8)	< 0.01[b]
Base deficit, mmol/L, median (IQR)	−5.6 (−8.9 to −2.1)	−2.6 (−5.6 to −0.2)	0.05
Central venous O_2 saturation, %, mean ± SD	71.6 (± 5.7)	68.1 (± 7.8)	0.20
Central venous CO_2 tension–$Paco_2$, kPa, mean ± SD	1.27 (± 0.7)	1.1 (± 0.5)	0.51
Highest lactate pre-ICU, mmol/L, median (IQR)	8.5 (4.5–13.6)	4 (2.8–5.1)	< 0.01[b]
Clinical outcomes			
ICU length of stay, d, mean ± SD	28 (± 14)	12 (± 11)	< 0.01[a]
28-d mortality, %, *n* (%)	6 (25)	0 (0)	< 0.01[c]

IQR = interquartile range, SOFA = Sequential Organ Failure Assessment.
[a]Significant according to *t* test.
[b]Significant according to Mann-Whitney *U* test.
[c]Significant according to χ^2.
All variables are those recorded at following bleeding control procedures, but < 12 hours after admission to the ICU, unless otherwise stated.

of greater or less than 10.33 mm/mm^2. At each day from day 4 to day 7 after injury, patients with initial (D0) PVD values of less than 10.33 mm/mm^2 had significantly higher organ failure scores than those with PVD values of greater than 10.33 mm/mm^2. For those patients with a SOFA score of greater than 6 at day 7, the greatest contributor to the score was the CNS component (28 total points), followed by the cardiovascular and respiratory components (19 and 17 points, respectively). The renal, liver, and hematology components were relatively small contributors to an elevated SOFA score (scores of 8, 4, and 4, respectively).

Patient characteristics were then compared between patients according to their PVD value recorded at study inclusion (D0), again dichotomized to above or below a PVD of 10.33 mm/mm^2 (**Table 2**). Other reported variables are also recorded at the D0 time point unless otherwise stated. Patients in the lower PVD group received more crystalloid fluid during resuscitation, but there were no observed differences in the amount of administered blood products between the groups. The noradrenaline dose in both groups was extremely low in the immediate postresuscitation period. Patients with lower PVD had a significantly higher

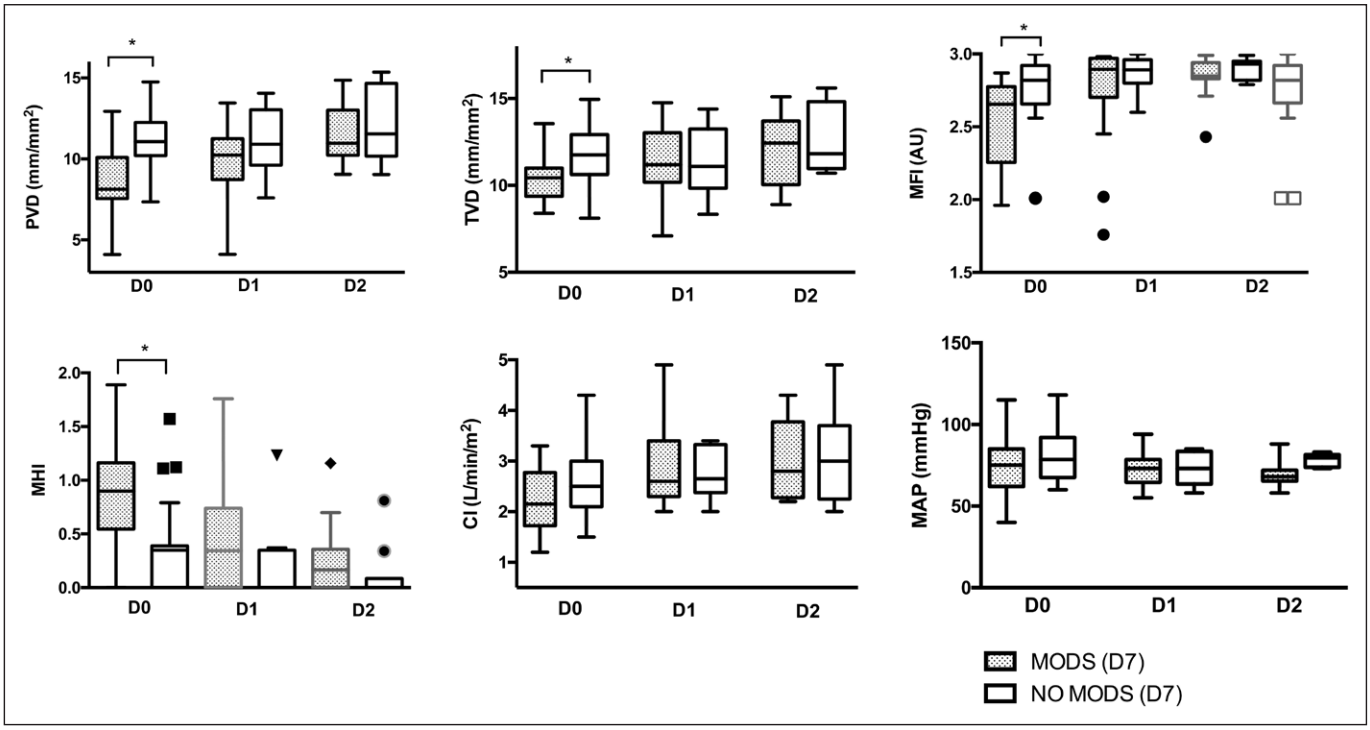

Figure 1. Microcirculatory and macrocirculatory variables at all study time points according to multiple organ dysfunction syndrome (MODS) at day 7. Patients are dichotomized into those who did or did not develop MODS by day 7 after injury. *$p < 0.05$ between groups (*t* test/Mann-Whitney *U*). NB comparative analysis only performed at following bleeding control procedures, but < 12 hours after admission to the ICU (D0) time point due to patient drop out at D0 + 24 hours (D1) and D0 + 48 hours (D2). AU = arbitrary units, CI = cardiac index, MAP = mean arterial pressure, MFI = microcirculatory flow index, MHI = microcirculatory heterogeneity index, PVD = perfused vessel density, TVD = total vessel density.

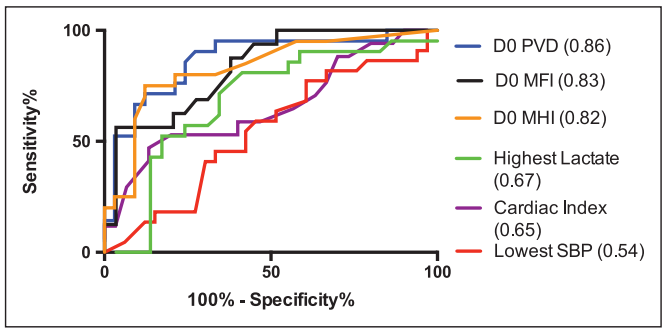

Figure 2. Receiver operator characteristic curves constructed to predict the development of multiple organ dysfunction syndrome at day 7 post injury. Values are area under curve for variable. The value for lactate represents the highest recorded pre-ICU measurement and that for blood pressure the lowest recorded pre-ICU measurement. D0 = following bleeding control procedures, but < 12 hours after admission to the ICU, MFI = microcirculatory flow index, MHI = microcirculation heterogeneity index, PVD = perfused vessel density, SBP = systolic blood pressure.

lactate concentration following resuscitation, but there were no differences in either the central venous oxygen saturation or the difference in $Pcvco_2$ and $Paco_2$.

DISCUSSION

The main finding of the present study is that early microcirculatory hypoperfusion in the period immediately following THS and resuscitation is associated with an increased risk of MODS a week after injury. Microcirculatory perfusion indices appear to be better predictive indicators for the development of MODS than other more commonly used markers of posttraumatic shock such as lowest SBP or highest plasma lactate concentration.

The results of the current study suggest that microcirculatory impairment is an early phenomenon following THS. However, our results suggest that the impact of early hypoperfusion persists for up to a week after the initial insult. One possible explanation for these findings may relate to cellular and genomic changes that are known to occur very early after injury (23, 24). It is conceivable that the magnitude of such change may be influenced by the degree of early tissue and cellular hypoperfusion. Furthermore, given that the measurements in our study were made after reperfusion, ischemia-reperfusion injury may also have had an impact on the state of the microcirculation (25). Whatever the mechanism, our findings suggest the potential importance of the rapid restoration of tissue perfusion once bleeding has been brought under control. Treatment targeted at microcirculatory flow is not yet in main stream clinical practice but may be moving closer with the development of novel point of care measures of microcirculatory perfusion (26, 27).

The findings of the present study are broadly in keeping with a smaller single-center study conducted by Tachon et al (11). A comparison of data reported in the current study cohort and that of Tachon et al (11) is provided in **Table S.3** (Supplemental Digital Content 1, http://links.lww.com/CCM/D691). There are many similarities between the two cohorts, although the cohort by Tachon et al (11) appeared to have a greater burden of injury, received more blood products and other fluids, and had higher lactate concentrations

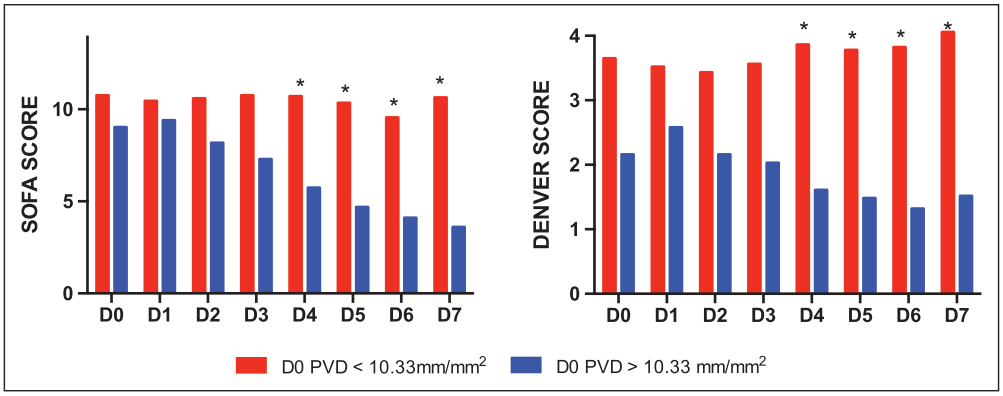

Figure 3. Daily Sequential Organ Failure Assessment (SOFA) and Denver scores for those patients with following bleeding control procedures, but < 12 hours after admission to the ICU (D0) perfused vessel density (PVD) values of greater or less than 10.33 mm/mm^2. Values represent the mean scores of all patients in the group at that time point. Patients who died were assigned a maximal score for the day of death and subsequent days. *$p < 0.05$ between groups at time point (two-way analysis of variance). D1 = D0 + 24 hr, D2 = D0 + 48 hr, D3 = D0 + 72 hr, D4 = D0 + 96 hr, D5 = D0 + 120 hr, D6 = D0 + 144 hr, D7 = D0 + 168 hr.

following resuscitation. Patients in the cohort by Tachon et al (11) were explicitly stated to be hemodynamically stable, although it is not clear how this was defined. Patients in the current study received the vast majority of resuscitative fluids prior to study enrollment and required minimal further volume administration after this time point (data presented in Table S.1, Supplemental Digital Content 1, http://links.lww.com/CCM/D691). It is therefore reasonable to presume that initial measurements in both study cohorts were made at similar time points after hemorrhage control and resuscitation.

Although both the current study and that reported by Tachon et al (11) demonstrate a relationship between microcirculatory impairment and organ failure that is independent of changes in systemic hemodynamic variables, there are important differences in the reported microcirculatory indices for both density (functional capillary density/PVD) and flow (MFI/PPV). Although direct comparison of density variables is difficult, given the difference in video microscopes and without knowledge of the calibration settings used in the respective AVA software packages, flow variables (MFI/PPV) should be directly comparable and appear markedly worse in the cohort by Tachon et al (11). One possible explanation may be the striking difference in the use of vasopressors; patients in the cohort by Tachon et al (11) receiving a substantial higher amount of norepinephrine than patients in the current study. Whether vasopressor use results in impaired microcirculatory flow is controversial and unsettled (28–31), and further experimental and prospective clinical studies are required.

Persistently high organ dysfunction scores in this cohort of patients predominantly resulted from elevated CNS, cardiovascular and respiratory components with values for the renal, liver, and hematology components making little contribution for most patients. This pattern perhaps suggests a general ongoing critical care dependency, with failure to wean

TABLE 2. Characteristics of Patients With Values of Perfused Vessel Density Above and Below a Threshold Value of 10.33 mm/mm^2 at the D0 Time Point

Patient Characteristics	PVD < 10.33	PVD > 10.33	*p*
Age, mean ± SD	44 (± 20)	42 (± 19)	0.74
Injury Severity Score, mean ± SD	33 (± 13)	26 (± 14)	0.09
Resuscitation fluids prior to D0, median (IQR)			
Packed RBC, units	6 (4–11)	6 (4–9)	0.91
Fresh frozen plasma, units	4.5 (1.7–9.0)	4.5 (2.7–8.2)	0.99
Crystalloids, mL	3,350 (500–5,000)	2,000 (0–3,500)	0.05
Noradrenaline (μg/kg/min)	0.02 (0.0–0.3)	0 (0–0.07)	0.07
Cardiac index (L/min/m_2), mean ± SD	2.2 (± 0.7)	2.6 (± 0.6)	0.09
Tissue perfusion variables			
Lactate, mmol/L, median (IQR)	3.1 (2.3–5.3)	1.9 (1.5–3.7)	0.02[a]
Base deficit, mmol/L, median (IQR)	−4.1 (−6.3 to −2.1)	−2.6 (−6.7 to −0.1)	0.20
Central venous O_2 saturation, %, mean ± SD	71 (± 9)	69 (± 4)	0.50
Central venous CO_2 tension–$Paco_2$, median (IQR)	1.36 (0.8–2.0)	1 (0.7–1.3)	0.19

IQR = interquartile range, PVD = perfused vessel density.
[a]Significant according to Mann-Whitney *U* test.

quickly from mechanical ventilation and inotropic support, rather than overt fulminant multiple organ failure. A potential criticism of the use of the SOFA score in trauma patients is the inclusion of a CNS component and the potential for the score to be confounded by the presence of TBI. However, there was a relatively low prevalence of TBI in this cohort, and no patient remained ventilated at day 7 as a result of persistent neurologic disability. The addition of the Denver score, which removes the CNS component, was used in the present study to overcome this potential confounder. Changes in Denver score over time are in keeping with the SOFA score, suggesting that these findings are more than an epiphenomenon caused by excess sedation or neurologic obtundation.

In the present study, patients with a lower PVD at D0 received significantly more crystalloid fluid during resuscitation than those with a PVD above this value. However, there were no observed differences in the amount of blood products administered. There are several potential explanations for these findings. The results of this study suggest that patients with greater degrees of microcirculatory impairment exhibit higher lactate concentration and may, as a result, receive more resuscitation fluids. Such an explanation presumes that clinicians target resuscitation fluid boluses to perfusion-based indices; however, a recent multinational study has suggested that this is not often the case (32). An alternative explanation is that poor microcirculatory flow may lead to more injury at the level of the endothelium, potentially increasing endovascular leak, rendering the patient hypovolemic, with a resultant increase in the volume of resuscitation fluid required. Such an explanation is supported by recently published evidence from the present study cohort (33) demonstrating a link between microcirculatory flow and endotheliopathy.

Our study has limitations. Most significant is the partial loss of patients from the study over time. These losses were not random but mostly due to patient recovery and extubation which made further measurements problematic. Although we decided a priori to discontinue measurements under these circumstances, resultant bias precluded comparative analysis at the D1 and D2 time points. This should not detract from the fact that the development of organ failure at day 7 post injury is solely based on analysis of data from the D0 time point for which a full dataset was available.

A further limitation is the short length (5 s) of individual video sequences used. Although in keeping with the recent consensus guidelines suggesting between 5 and 20 s for each sequence (17), opting for the shorter end of this range may not have allowed for the full assessment of intermittent flow in small vessels.

CONCLUSIONS

In conclusion, microcirculatory impairment following THS is associated with an increased incidence of organ dysfunction at 1 week following injury. Evidence from this study points to the initial time point after resuscitation being the point of maximal divergence in microcirculatory variables, and future studies should focus on early optimization of tissue perfusion in an attempt to ameliorate late organ dysfunction following traumatic hemorrhagic shock. Treatment strategies that target microcirculatory perfusion may be useful in perfusion-based resuscitation but are yet to be realized.

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