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Tissue microvascular flow and oxygenation in critically ill patients

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Tissue microvascular flow and oxygenation in critically ill patients

Thesis submitted for PhD

University of London

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To

Elhum, Mischka and Talia

Abstract

The use of fluid resuscitation and vasoactive agents to optimise global haemodynamics has been demonstrated to improve outcomes in patients undergoing major surgery and in early sepsis. Whether changes in global haemodynamics result in similar improvements in the microcirculation in critically ill patients remains unclear. The aim of this thesis was to investigate the changes in tissue microvascular flow and oxygenation that occur in patients undergoing major surgery and in those with sepsis, and specifically how haemodynamic therapies may affect these changes.

The first part of this thesis investigates the treatment pathway of the high risk surgical patient. Analysis of two large health databases was performed and confirmed the existence of a high risk sub-population within the local surgical population. Only about a third of these high-risk patients were admitted to a critical care unit at any stage during their hospital admission.

An observational trial was performed examining the relationship between global oxygen delivery, microvascular flow and tissue oxygenation in 25 surgical patients receiving usual care. Data including global haemodynamics, sublingual and cutaneous microvascular flow, and tissue oxygenation were collected before, and for eight hours after surgery. Abnormalities in sublingual microvascular flow were found to be associated with worse outcomes.

A randomised controlled study investigating the effects of two goal directed haemodynamic therapy (GDHT) algorithms on tissue microvascular flow and oxygenation compared to central venous pressure guided fluid therapy in 135 perioperative patients was performed. For eight hours after surgery, intravenous fluid therapy was guided by measurements of central venous pressure (CVP group) or stroke volume (SV group). In a third group stroke volume guided fluid therapy was combined with dopexamine (SV & DPX group). In the SV & DPX group, increased global oxygen delivery was associated with improved sublingual and cutaneous microvascular flow. Microvascular flow remained constant in the SV group but deteriorated in the CVP group. Cutaneous PtO_2 improved only in the SV & DPX group. There were no differences in complication rates between groups.

The importance of derangements in microvascular flow in patients with established sepsis is well recognized. However, little data is available to describe microvascular changes in early sepsis. Observational data were collected in 16 healthy volunteers and within six hours of presentation in 48 patients with sepsis and severe sepsis. Sublingual microvascular flow was impaired in patients with sepsis and severe sepsis compared to healthy volunteers. Greater alterations in flow were seen with increasing severity of illness.

The dose-related effects of vasopressor therapy on microvascular flow and tissue oxygenation in sepsis have not been previously fully investigated. The effects of increasing doses of noradrenaline, targeted to achieve successively greater mean arterial pressures, on microvascular flow and tissue oxygenation in 16 patients with septic shock were investigated. Increasing doses of noradrenaline were associated with improvements in

global oxygen delivery, cutaneous PtO_2 and cutaneous microvascular red blood cell flux. No changes in sublingual microvascular flow were identified.

This thesis confirms the existence of a large sub-population of high risk surgical patients. It demonstrates that abnormal microvascular flow in the perioperative period may be associated with poor outcomes. The use of flow guided fluid therapy alongside low dose dopexamine infusion is shown to improve global haemodynamics, microvascular flow and tissue oxygenation in perioperative patients. Microvascular abnormalities are shown to occur in the earliest stages of sepsis with increasing severity of disease being associated with greater changes. Increasing doses of noradrenaline were found to improve global haemodynamics, cutaneous microvascular flow and cutaneous tissue oxygenation in septic shock. Further work is required to investigate the effects of haemodynamic therapies on microvascular flow and organ dysfunction in critically ill patients and the use of the microcirculation as a resuscitation endpoint.

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Peer reviewed publications associated with this work

Original research

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Chapter 1
Review of the relevant literature

1.1 The clinical problem

There are approximately 234 million surgical procedures performed worldwide each year.[1] This translates to 4000 procedures per 100,000 population overall which increases to 11,000 procedures per 100,000 population in high income countries. The care of the surgical patient is an important public health issue and in 2007 the World Health Organisation (WHO) launched, as part of their patient safety programme, the Safe Surgery Saves Lives Challenge.[2]

In spite of perceived improvements in perioperative care in the UK, there was little change in the 30 day mortality in surgical patients between 1990 and 2003.[3,4] It is generally accepted that patients of advanced age with co-existing medical disorders undergoing major surgery are those most at risk of post-operative complications and death.[3,4] A recent study of surgical practice in the UK identified a total of 4.1 million non-cardiac surgical procedures performed over a five year period with an overall mortality rate of 0.44%.[5] The authors identified within this, a small subgroup of patients that accounted for only 12.5% of in-patient surgical procedures but over 80% of deaths. This study confirmed that although the overall post-operative mortality rate is low, a large sub-population of patients exists who are at high risk of post-operative complications and death. Another important finding of this study was that only 15% of these high risk patients were admitted to a critical care

area following surgery. The high mortality rates and prolonged hospital stays described for high-risk patients in this study are also consistent with published data from Europe and North America.[6,7] Complications, in particular, infectious complications, are common following surgery and have been associated with poor long term outcomes.[8] There is a clear need to improve identification of patients at high risk of post-operative complications and death and to provide clinical interventions which can reduce this risk.

Sepsis is a major cause of death and disability worldwide. It is estimated that 750,000 people develop sepsis each year in North America alone.[9] In the UK, approximately 35,000 patients are admitted to intensive care each year, of whom 45% do not survive to leave hospital.[10] The number of severe sepsis cases is projected to increase by 1.5% per annum.[9] Often, sepsis related deaths result from multi-organ failure which generally develops in the early stages of the septic process.[11] With the aim of reducing global mortality from severe sepsis, a number of international critical care organisations joined forces in 2002 to develop the Surviving Sepsis Campaign.[12] This campaign aimed to raise awareness of severe sepsis as a major healthcare issue, to create guidelines for the management of sepsis and to translate these guidelines into practice internationally. Despite an apparent improvement in outcomes from severe sepsis, perhaps in part related to these guidelines, there remains a need for further research into clinical interventions which may further improve outcomes.[13]

One of the most important areas of clinical practice is determining the optimal use of intravenous fluid and vasoactive agents in critically ill and high risk surgical patients.[14] For over forty years there has been particular interest in the utility of measuring cardiac output and related variables as haemodynamic goals to better determine the use of these

treatments. Various terms have been coined to describe this approach including optimisation and goal directed haemodynamic therapy (GDHT). In effect, these are treatment algorithms which guide the clinician to select the dose of intravenous fluid or vasoactive drug therapy at any given time point. It has been suggested that GDHT improves tissue microvascular flow and thus tissue oxygenation but this hypothesis remains untested. In fact there is some data suggesting that there is little relationship between changes in global haemodynamics and microvascular flow in patients with severe sepsis.[15] Thus there is a clear need for further investigations of tissue microvascular flow and oxygenation in patients undergoing major surgery and those with severe sepsis, to confirm the importance of these abnormalities and how haemodynamic therapy may modify them.

1.2 Historical perspective

Current concepts of the delivery of oxygen from the atmosphere to the tissues began in the late Renaissance when Andreas Vesalius (1514-1564) began to question the teachings of Galen, the Greek philosopher and physician. Galen's theory regarding the circulation had been accepted for almost 1300 years. Galen believed that the circulation was composed of two entirely separate systems. In the arterial system, blood was produced by the heart which then pumped blood around the body where the blood was then consumed by various organs. In the separate venous system, blood was produced in the liver and again consumed after flowing to other organs. Miguel Serveto (1511-1553) discovered the link between the two circulations (the pulmonary circulation) when looking for a passage between the right and left heart. Jacques Dubois (1475-1555), also known as Jacobus Sylvius, was the first person to suggest that venous blood flowed from the

periphery to the heart. In the early 1600's, William Harvey (1578-1657) produced a series of elegant experiments including animal dissection, some simple mathematical work and observations of the flow of venous blood after tying a ligature around a patient's upper arm. These led to the publication of '*An anatomical study of the motion of the heart and of the blood in animals*' in which he proposed the presence of a single intact circulation with blood being pumped from the left side of the heart to the tissues and then returning via the pulmonary circulation. It was Marcello Malphigi (1628-1694) who found the link between the arteries and veins that eluded William Harvey. Unlike Harvey, Malphigi had access to a microscope and on inspection of a partially inflated frog's lung he discovered multiple small blood vessels between the arteries and veins. He coined the term capillary from the Latin word *capillus* meaning hair. It was a century later before oxygen was discovered by Joseph Priestley (1733-1804) and Antoine- Laurent Lavoisier (1743-1794) conducted his seminal animal experiments which linked energy production to the consumption of oxygen. The next steps in the understanding of oxygen delivery to the tissues were the discovery of haemoglobin by Felix Hoppe-Seyler in the 19th century and then the demonstration of its oxygen-carrying capacity by George Gabriel Stokes. In 1919 Krogh published his seminal work exploring the relationship of capillary geometry with the diffusion of oxygen into the tissues with a geometrical model of the microcirculation.[16] The optimal method for improving microcirculatory blood flow, how this varies in different disease states and its effect on prognosis remains unclear.

The administration of intravenous fluid as a therapy dates back to 1492 when Pope Innocent VIII received a blood transfusion from three youths. This was not a successful experiment however as, unfortunately, all involved died. In 1831, William O'Shaughnessy published a series of fascinating observations concerning blood drawn from sufferers of

cholera. He described a reduction in the water content of blood, uraemia and low bicarbonate concentrations. He also noted that all the patients had the symptom of 'depressed urine' and a failure of arterialisation [oxygenation] of the blood. He reported his findings to the Lancet but did not attempt to give fuid therapy to any of his patients.[17] A few months later, Thomas Latta infused saline and sodium bicarbonate to some moribund victims of cholera in Sunderland with some success.[18] Despite this, however, venesection remained the most common treatment for cholera. The use of intravenous fluids for the treatment of shock was described further in the late 19th Century but it was not until the 2nd World War that the role of fluid therapy in the treatment of haemorrhagic shock was widely acknowledged. The United States Army led the world in this respect and later published work reporting improved outcomes with the use of fluid therapy during surgery in combat casualties from the Korean War. The optimal dose of fluid for the treatment of shock, however, remains unclear.

Fick's method for calculating cardiac output was first described in 1870.[19] Its use for calculating cardiac output in man was not possible, however, until Werner Forsmann devised a method of sampling mixed venous blood in 1929.[20] Forssman passed a ureteric catheter through his own cephalic vein and into his right ventricle, before walking to the X-ray department to confirm its position, an experiment that led both to his dismissal and the award of the Nobel prize. The following year Otto Klein became the first person to draw mixed venous blood and calculate cardiac output in man using the Fick principle.[21] This technique was then perfected by Cournand and colleagues during the 1940's.[22] Use of the Fick principle to calculate cardiac output was, however, difficult at the bedside. Cardiac output monitoring only became routine practice with the development of indicator dilution

techniques,[23-28] in particular thermodilution,[29,30] and the introduction of the flow directed balloon tipped pulmonary artery catheter.[31]

1.2.1 Early investigations of cardiac output measurement in surgical patients

Some of the first data linking surgical outcome to cardiac output was provided by Clowes and colleagues.[32] These investigators found that the survivors of thoracic surgery and abdominal surgery for peritonitis tended to achieve a higher cardiac output after surgery than those who died. There were no differences in blood pressure between the groups. William Shoemaker repeated this work in a larger group of patients in a series of observational studies and produced a list of haemodynamic variables that correlated with survival after major surgery.[33-35] These variables were a Cardiac Index (CI) > 4.5 L min^{-1} m^{-2}, oxygen delivery index (DO_2I) > 600 ml min^{-1} m^{-2} and oxygen consumption (VO_2I) > 170 ml min^{-1} m^{-2}. It is important to note that although these values are often termed 'supra-normal goals', they were in fact the median values achieved by surviving patients and were also achieved by a smaller proportion of those patients who subsequently died. One of these studies suggested that changes early in the post-operative period were perhaps most important.[36] This paper demonstrated that differences between survivors and non-survivors were greatest in the first twelve hours following surgery. Some point to the heterogeneity of the surgical population in Shoemaker's work which included patients undergoing surgery for major trauma, as a weakness of the data. However there have been similar findings in a homogenous group of patients undergoing elective oesophageal surgery.[37] In this observational study, 115 consecutive patients undergoing oesophagectomy and reconstruction via a laparotomy and right thoracotomy were

recruited. Significant reductions in stroke volume, CI and DO_2I were identified in non-survivors six hours after surgery. These changes were no longer apparent 24 hours after surgery. Reductions were also apparent in those patients who developed anastomotic leak. The median DO_2I in survivors was similar to previous work at 600ml min^{-1} m^{-2} six hours after surgery. Similar to Shoemaker's work, this study suggested that changes early in the post-operative period were important to clinical outcomes.

1.2.2 Goal Directed Haemodynamic Therapy (GDHT)

Many terms have been coined in attempt to describe algorithms which utilise cardiac output related treatment end-points. These include 'optimisation', 'flow-guided therapy' and 'goal-directed haemodynamic therapy' or 'GDHT'. These descriptive terms continue to evolve with the algorithms they are used to describe. For convenience, the term GDHT will be used here to describe this treatment approach. GDHT involves the use of intravenous fluids and vasoactive agents to achieve pre-defined cardiac output or related parameters. GDHT has been successfully utilised in the care of peri-operative patients, trauma and in early sepsis.[38-45] Its use, however, in other groups of patients has shown no benefit or even harm.[46,47] GDHT has been associated with reductions in mortality,[39,43] complications [41,44,48] and more recently the post-operative incidence of acute kidney injury.[49]

GDHT was, initially, dependent on the pulmonary artery catheter for cardiac output and related data and this resulted in controversy due to concerns regarding the safety of this measuring device.[50,51] Early work in this area commenced GDHT protocols in critical care areas prior to surgery. However, routine pre-operative admission to critical care in the UK is often not possible due to limited resources. GDHT has still to become part of routine

perioperative care despite potential solutions to these issues. Less invasive cardiac output monitors are now available and recent evidence of improved patient outcomes utilising GDHT commenced in the immediate post-operative period has less resource implications.[38] One explanation for the lack of enthusiasm for the use of GDHT is the absence of a physiological explanation for its apparent benefit. Originally, it was thought that the beneficial effects of GDHT were related to improvements in DO_2I, VO_2I and reversal of an 'oxygen debt'. It was believed that, in critical illness, a phenomenon termed 'supply dependency' occurred whereby oxygen delivery is insufficient even at high levels. However, it now seems likely that this observation was largely explained by mathematical coupling of the variables used to calculate both oxygen delivery and oxygen consumption.[52] The most likely explanation is that GDHT improves tissue perfusion and thus oxygen delivery to the tissues. However, the theory that optimising global haemodynamic parameters leads to an improvement in tissue oxygenation and microvascular flow remains unproven. A detailed evaluation of the effects of GDHT on tissue perfusion and oxygenation would significantly improve our understanding of the effects of GDHT and might encourage more widespread use of the technique. As such, it is a necessary step towards reducing the disability and death which often follows common high-risk surgical procedures.

A number of investigators have explored the effect of different doses of peri-operative intravenous fluid prescribed according to body mass. There have been a number of clinical trials comparing 'liberal' and 'restrictive' protocols for peri-operative intravenous fluid prescription according to body mass, with varying results.[53-56] The findings of these trials have led to a certain amount of confusion regarding optimal fluid management during the peri-operative period. Given that the stress response to surgery will induce fluid and salt

retention, it seems logical to prescribe maintenance fluids at a reduced rate in the post-operative period.[57] However, it should be noted firstly that whilst maintenance fluid can and should be prescribed according to body mass, this is not a rational method of estimating fluid losses and the need for resuscitation of the circulation. Furthermore, in many of these trials, the protocol termed 'restrictive' most closely reflects routine clinical care whilst 'liberal' protocols risk excessive fluid administration. Nonetheless, the findings of these trials have proved inconsistent. It is clear that those patients who develop oedema are more likely to develop post-operative complications.[58] However, this may relate as much to the patient and the nature of the surgical procedure as to the fluid strategy.[58] Intravenous fluid resuscitation should be prescribed according to relevant physiological end-points. The administration of excessive maintenance fluids is no more or less preferable than the failure to adequately resuscitate a hypovolaemic patient.

1.3 Microcirculation

The microcirculation is the major site of gas exchange, delivery of nutrients and removal of cellular waste products. Until recently microvascular function has remained somewhat of a mystery due to limitations in monitoring. Thus clinicians have concentrated on investigations of the more easily accessible macrocirculation.

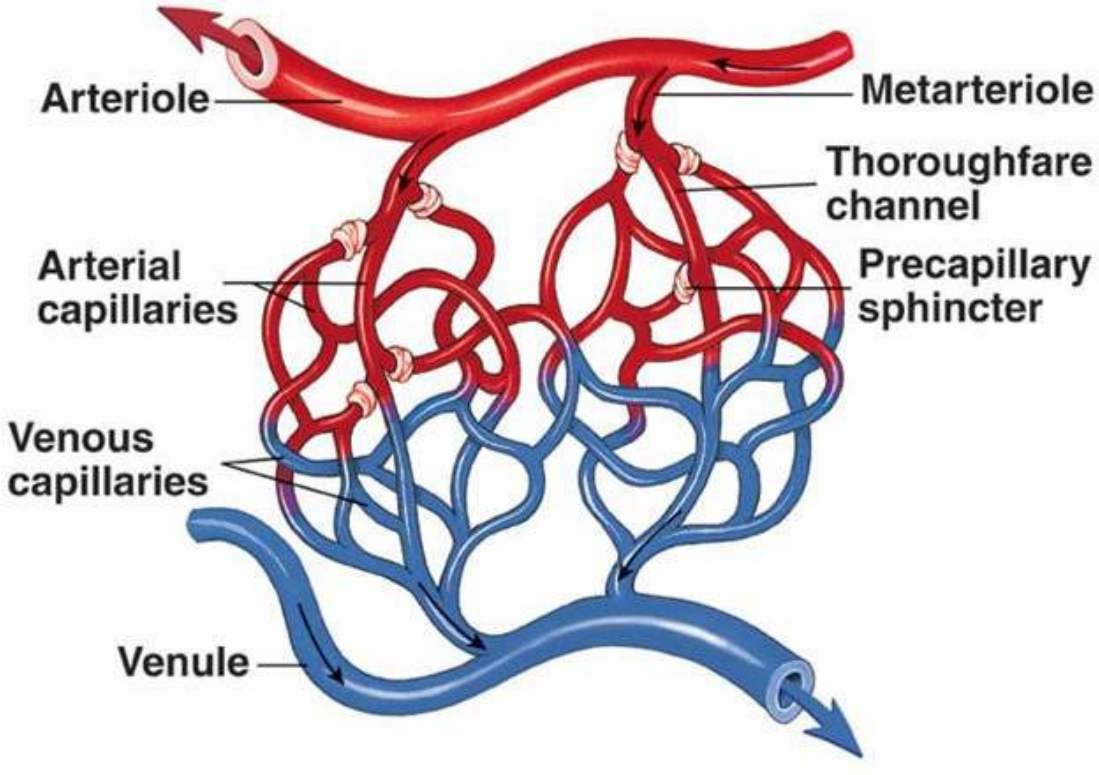


Figure 1.1 Structure of the microcirculation

The microvascular unit consists of the smallest blood vessels (<100 µm diameter) including arterioles, capillaries and post-capillary venules. The microcirculation is also considered to include the blood flowing within these vessels including red and white blood

cells and coagulation factors. Numerous cytokines play important roles in microvascular function. In health, microvascular flow is controlled by myogenic, metabolic and neurohumoral mechanisms.[59] Autocrine and paracrine interactions ensure distribution of blood flow to meet tissue metabolic demands. Cell-to-cell signalling between endothelial cells allows upstream microvessel recruitment based on conditions downstream in the capillary bed.[60] The endothelium is fundamental to this process sensing flow and metabolic substances to regulate smooth muscle tone and thus capillary recruitment.[61] Other functions of the endothelium include control of coagulation, leucocyte adhesion / migration and immune function.

The central nervous system exerts significant control over the microcirculation, integrating it with baroreceptor reflexes. Through sympathetic innervation of arterioles and venules, afterload and venous return to the heart is controlled through neuronal release of noradrenaline. Circulating vasoactive hormones also act on endothelium and vascular smooth muscle to mediate changes in vessel tone. Arterioles with low tone will be patent, allowing perfusion of capillaries, whereas arteriolar constriction will reduce the number of perfused downstream capillaries. This is important as perfused capillary density may be a key determinant of tissue oxygenation through effects on the average inter-capillary distance, blood capillary transit time and the surface area available for nutrient and gas exchange.[62] Other factors which also influence blood flow include the myogenic response to flow and endothelial nitric oxide (NO) production.[62,63] Microvascular blood flow is strongly influenced by vascular topology, blood viscosity and the interaction of cellular constituents of blood and endothelium.[63] Recent research suggests that red blood cells can regulate perfusion by releasing vasodilators including nitric oxide and ATP when exposed to a hypoxic environment.[64,65]

1.3.1 Sepsis and the microcirculation

Abnormalities of microvascular function are central to the pathophysiology of the systemic inflammatory response to severe sepsis. During sepsis, there is disruption of autoregulatory mechanisms leading to microvascular dysfunction characterised by a decrease in the number of perfused capillaries and heterogeneous flow allowing shunting of blood through dilated microvessels.[66] This is likely to result from a decreased response to vasoconstrictor agents,[62,67] dysfunction of endothelial signalling,[62] changes in NO production and loss of the glycocalyx.[68]

Endothelial activation results from inflammatory stimuli leading to reduced vasomotor tone due to loss of signal transduction pathways,[61,69] a procoagulant and proadhesive cell surface and compromised barrier function.[62,67] Activated endothelial cells mediate leucocyte trafficking through a multistep process of rolling, firm adhesion and transmigration.[70,71] Red blood cells become less deformable and have an increased tendency for aggregation.[72,73] They also may lose their ability to release nitric oxide in the presence of hypoxia so decreasing local vasodilation.[74,75]

Nitric oxide is thought to play a pivotal role in microvascular homeostasis in health by regulating microvascular tone, leucocyte adhesion, microthrombi formation and microvacular permeability.[76-79] The systemic inflammatory response associated with sepsis can lead to localised areas of NO deficiency despite a total body excess of NO. This leads to the classical pattern of heterogeneity of microvascular flow.[66,80,81] Functionally vulnerable microvascular units are rendered hypoxic, in part explaining the oxygen extraction deficit associated with sepsis.[80,82-84] This effect can result in a decrease in capillary pO_2 below the venous pO_2 and is an indicator of microvascular shunting of arteriolar blood into the venous system.[59,82,83,85]

The glycocalyx is a gel-like structure that forms the interface between the capillary lumen and endothelial cells. Its integrity is highly sensitive to the oxidative stress that may accompany sepsis, hypovolaemia or trauma.[68] It is likely that the endothelial dysfunction and changes in microvascular flow are contributed to by the loss of the glycocalyx.[86] Leucocyte adhesion follows and a loss of barrier function, which can lead to changes in Starling forces and oedema formation.[87,88] It is possible that intravenous starch solutions may beneficially modulate some of these changes in the glycocalyx though this remains poorly understood.[89]

With the advent of new in vivo microscopy techniques,[90] it is now possible to visualise the microcirculation in human subjects. The techniques of orthogonal spectral imaging (OPS) and sidestream darkfield imaging have been validated in experimental models and human subjects.[90-93] The first clinical study demonstrating sublingual microvascular changes associated with sepsis was performed by De Backer and colleagues.[66] They found that microvascular density and flow was reduced in patients with established severe sepsis compared to non-septic intensive care unit patients and healthy volunteers. Other workers have also identified the prognostic significance of abnormalities of microvascular flow.[94] Similar findings have been reported in early sepsis.[45,95]

1.3.2 Surgery and the microcirculation

The effects of surgery and the associated inflammatory response on microvascular flow remain unclear. It is conceivable that one may see similar changes to the classical inflammation induced abnormalities associated with sepsis, including reduced capillary

density and heterogeneity of flow but this has yet to be characterised fully. The effects of haemodynamic therapies on microvascular flow in surgical patients has also yet to be investigated and may shed some light on the biological mechanisms behind the apparent clinical benefit of GDHT in this patient group.

1.3.2.1 Abdominal surgery

A number of studies have investigated microvascular flow in abdominal surgery though there are few studies to date using direct visualisation of the microcirculation. Vignali and colleagues used laser Doppler flowmetry to investigate microvascular changes in 55 patients following colonic surgery.[96] Transmural colonic flow was assessed before bowel manipulation and after anastomotic formation. 76% of patients showed a reduction in blood flow after anastomotic formation. 15% of patients suffered anastomotic leak. The mean rectal stump flow reduction was 6% in patients without anastomotic leak and 16% in those with anastomotic breakdown ($p<0.01$). A later study using a similar group of patients investigated the use of gastric and colorectal anastomotic tonometry.[97] These authors investigated 90 patients undergoing colorectal surgery measuring intramucosal pH at these two sites 48 hours post-operatively. 11% of patients developed an anastomotic leak. After multivariate analysis, only anastomotic pH was found to be an independent predictor of leak ($p=0.001$). A pilot study investigating the use of near infrared spectroscopy (NIRS) for investigation of colorectal anastomotic tissue oxygen saturation (StO_2) found NIRS to be a safe and reliable technique.[98] In this study of 20 patients, only two suffered anastomotic complications. StO_2 was reduced in these 2 patients when compared to those without complications. Further work is needed to investigate the utility of NIRS to predict postoperative complications. In the only study to date to directly visualise the microcirculation using OPS imaging following non-cardiac surgery, Boerma found no

difference in intestinal stoma flow flow within 24 hours of construction compared to stoma more than three months after surgery.[99]

Anastomotic complications are an important cause of morbidity following oesophageal surgery. Pierie and colleagues investigated 30 patients undergoing transhiatal oesophagectomy with gastric tube reconstruction and cervical oesophagogastric anastomosis.[100] Pefusion of the anastomosis (assessed by laser Doppler flowmetry) of less than 70% of pre-construction levels was a predictor of anastomotic stricture ($p=0.02$) but not leakage. In a study of 39 patients undergoing thoracic oesophagectomy, blood flow within the gastric tube was assessed with laser Doppler flowmetry alongside gastric and rectal tonometry.[101] These authors found that gastric pHi values correlated significantly with measurements of blood flow at the anastomotic site ($p<0.01$). A post-hoc analysis of those patients who suffered anastomotic leak demonstrated a failure to improve gastric pHi from the nadir two hours following surgery. In those patients who did not have a leak, gastric pHi improved to day 2 post-operatively. Microvascular changes have also been found to be associated with abdominal aortic aneurysm surgery. Nakatsura and colleagues investigated in eight patients the effect of infrarenal aortic cross clamping on gastric tonometry and colonic serosal perfusion using laser Doppler.[102] These investigators found small reductions in gastric mucosal pHi and colonic perfusion associated with cross-clamping that returned to pre-cross-clamp levels 60 minutes after release of the cross-clamp.

1.3.2.2 Cardiac surgery

A number of studies have investigated changes in microvascular flow during cardiopulmonary bypass (CPB). There are a number of factors that may influence

microvascular flow including haemodilution, hypothermia, non-pulsatile flow, and vasoactive drugs. More recent studies have used OPS or SDF imaging to directly visualise microvascular flow, identifying derangements during CPB which subsequently resolve.[103-105] Similar to sepsis, no obvious correlation between global and microvascular flow was identified suggesting a distributive nature of microvascular dysfunction. The aetiology of this distributive shock is unclear but may relate to the effects of CPB on endothelial function.[106] The use of vasoactive drugs during CPB is routine to control mean arterial pressure (MAP). Ketanserin was not associated with any change in microvascular flow,[107] whilst GTN initially improved flow with an associated fall in MAP but at higher doses was associated with reduced sublingual flow.[108] Phenylephrine use during CPB resulted in significant reduction of sublingual microvascular flow and a simultaneous increase in microcirculatory haemoglobin saturations suggesting shunt across the microcirculatory units.[109]

1.3.2.3 Effects of anaesthetic agents

In almost all patients undergoing major surgery some type of anaesthesia is required which has the potential to alter normal autoregulation and vasoreactivity of the microcirculation. In contrast to ketamine's sympathomimetic actions, both propofol and thiopental inhibit central sympathetic nervous tone.[110-112] Thiopental, propofol and ketamine all have vasodilatory properties.[113-116] Sublingual microvascular flow has been shown to fall during short term administration of propofol.[117] A more recent study showed no effect of propofol on CO_2 vasoreactivity of the human cerebral microcirculation assessed by laser Doppler flowmetry.[118] Propofol may affect microvascular flow by its effects on NO production, increasing constitutive NOS and reducing inducible NOS.[119] The effect of volatile anaesthetic agents on splanchnic blood flow is unclear but in general it

appears that organ perfusion remains sufficient for oxygen demand.[120,121] A number of experimental studies suggest an increase in gastrointestinal perfusion with thoracic epidural anaesthesia / analgesia.[122,123] Clinical studies, however, have produced conflicting results. Intraoperative laser Doppler flowmetry during bowel surgery demonstrated an increase in colonic blood flow in patients receiving thoracic epidural analgesia in one study.[124] However, a subsequent study using similar techniques showed a decrease in colonic serosal flow.[125]

1.3.3 Fluid therapy and the microcirculation in critical illness

Findings of worse outcomes associated with impaired microvascular flow in sepsis have prompted investigations of therapeutic interventions to counteract these changes. Almost invariably, the first step in the treatment of a shocked patient is the administration of intravenous fluid. Traditionally fluid resuscitation has been targeted to macrocirculatory variables but the optimal target for fluid resuscitation remains unclear and the impact of fluids on the microcirculation is poorly understood. It is likely that fluids influence microvascular flow in a number of ways. The most obvious is enhanced filling of the vasculature leading to greater forcing pressures resulting in enhanced microvascular flow. Fluids will also change the haemorheology of blood with effects varying according to the type of fluid.[126] It has also been shown that excessive haemodilution can reduce regional tissue oxygenation by causing shunting in the microcirculation.[127] Fluid resuscitation has been shown to improve sublingual microvascular blood flow in two clinical studies of patients with septic shock.[128,129] In the first of these studies,[128] improvements in perfused vessel density were found in those patients with ‘early’ sepsis (less than 24 hours after diagnosis of severe sepsis) but not in those with ‘late’ sepsis (more than 48 hours after

diagnosis). There were no associated changes in global haemodynamics. The second study investigated the effects of passive leg raise and fluid boluses on macrohameodynamics and microvascular flow in patients within 24 hours of their ICU admission for sepsis.[129] These investigators found increases in cardiac ouput and improvements in microvascular flow associated with both passive leg raise and volume expansion.

The choice of fluid for resuscitation will also influence acid-base status, with solutions containing high chloride concentrations, such as 0.9% saline, resulting in a reduction of strong ion difference and metabolic acidosis. 0.9% saline appears also to have deleterious effects on the microcirculation and organ function.[14,130] Choice of resuscitation fluid may also modulate the inflammatory process in critical illness. Saline has been implicated in worsening the pro-inflammatory insult, whereas there is limited data suggesting that certain colloids dissolved in balanced solutions may help to ameliorate the pro-inflammatory response.[131,132] Colloids have also shown improved microvascular flow compared to saline in a variety of experimental models using intravital microscopy to directly observe leucocyte – endothelial interaction.[133,134] Some reports suggest that the use of crystalloids for resuscitation may impair microvascular flow and cause fluid shifts into the interstitium.[135,136] Increased diffusion path length alongside poor oxygen solubility in aqueous solutions may then result in a reduction in oxygen availability to the cells. In general, clinical trials have shown improved microvascular flow associated with colloids (in particular starches) compared to crystalloids. Two prospective randomised studies comparing crystalloid and colloid (hydroxyethyl starches) in perioperative patients using gastric tonometry found improved gastric perfusion associated with starches.[137,138] A recent study by Dubin and colleagues, found that fluid resuscitation with 6% HES 130/0.4

caused a greater improvement in sublingual microvascular flow and perfused vessel density assessed using SDF imaging compared to saline resuscitation in a group of patients with early sepsis.[139] Comparing gelatins to starches has given mixed results, with some investigators finding improvements in gastric perfusion [140,141] whilst others found no difference.[142] A prospective randomised trial of a 'balanced' (plasma adapted) starch solution versus starch suspended in 0.9% saline found improved gastric perfusion using the 'balanced' starch.[143] Hypertonic saline used for the treatment of hypovolaemic shock has been shown to increase microvascular blood flow and perhaps reduce endothelial cell oedema.[144] Despite these findings, a recent large pragmatic multicentre trial of colloid (albumin) versus crystalloid (0.9% saline) as fluid resuscitation in a hetergeneous group of critically ill patients demonstrated no difference in outcome.[145] It appears that choice of fluid type amongst clinicians will continue to vary ('colloid vs crystalloid debate').

There is little data describing the effects of fluid resuscitiation aimed at macrohaemodynamic goals on microvascular flow. Hiltebrand, Kimberger and colleagues published two papers in March 2009 investigating the effects of goal directed fluid therapy in a porcine model of surgery. In the first of these papers, 27 pigs underwent open laparotomy.[146] They were randomly assigned to one of three treatment groups. All groups received Ringer's lactate (RL) at 3ml/kg/hour. The 'restrictive' group (R-RL) received no further fluid, the goal directed RL group (GD-RL) received 250ml boluses of RL, and the goal directed colloid group (GD-C) received 250ml boluses of 6% hydroxyethyl starch (130/0.4). The boluses were given when mixed venous saturations fell to below 60%, although there was a 'lockout' period of 30 minutes. This group found improvements in global haemodynamics and regional blood flow assessed by mesenteric artery flow in both the GD-RL and GD-C groups compared to the R-RL group. Microcirculatory flow in the

intestinal mucosa assessed by laser Doppler increased in the GD-C group but was unchanged in the other groups. Similarly, tissue oxygen tension assessed by intramural Clark type electrodes increased in the GD-C group, remained unchanged in the GD-RL group and fell in the R-RL group. The authors concluded that GDHT using colloid improved microcirculatory blood flow in the small intestine. A further study that included the addition of a hand sewn colonic anastomosis investigated the changes in microvascular flow and oxygenation in healthy and anastomotic colon.[147] With exactly the same treatment groups, they found a significant increase in tissue oxygenation and microvascular flow in the healthy and injured (anastomotic) colon in the colloid goal directed group compared to the crystalloid goal directed and restrictive groups.

1.3.4 Microcirculation and vasoactive medications

Cellular oxygen delivery is dependant on three main factors. Microcirculatory perfusion is dependant on the net effect of precapillary inflow pressure minus venular outflow pressure. As previously described, at times of venular hypoxia, cell-to-cell signalling between endothelial cells allows upstream microvessel recruitment based on conditions downstream in the capillary bed.[61] The second factor is the diffusion distance from capillaries to cells.[148] The third factor affecting microcirculatory oxygen delivery is changes in capillary haematocrit. The characteristics of blood flow within blood vessels leads to a plasma layer next to the vessel wall and increased haematocrit in the centre. Blood flow velocity also has a parabolic shape with greater flow in the centre of the vessel and low flow at the vessel walls. This leads to a decrease in red cell transit time and thus dynamic lowering of capillary haematocrit compared to arteriole and venular haematocrit. This is

known as the Fahraeus effect.[149] Capillary haematocrit is also affected by a phenomenon described by Krogh as 'plasma skimming'.[150] This describes the distribution of red blood cells at diverging branches of the capillary network. The greater the diameter of the the daughter vessel, the greater the haematocrit found in that vessel. The use of exogenous vasopressors is conventionally thought to worsen microvascular flow due to all three of these factors. Vasopressors may increase pre- and post-capillary pressures leading to a reduction in perfusion pressure across the capillary bed and may also prevent endothelial derived vasodilatation aimed at preventing local hypoxia.[151] Diffusion distances may increase due to the closure of capillaries in order to maintain perfusion pressure.[148] During in vitro experiments, capillary haematocrit ranged from 6.8% during vasoconstriction, to 38% during vasodilatation when systemic haematocrit was 50%.[152]

An ideal drug to recruit the microcirculation would stabilise the endothelial dysfunction associated with inflammation and vasodilate low flow areas. Despite abundant experimental data, the effects of vasoactive compounds on microvascular flow in critically ill patients remains unclear. Early studies used agents with vasodilatory actions (prostacyclin) or combined inotropic / vasodilator properties (dobutamine).[153,154] These studies found an increase in systemic oxygen uptake (VO_2) with the authors concluding that that this was due to successful microvascular recruitment. The use of regional or systemic surrogates of microvascular flow are unable to demonstrate the heterogeneity of capillary flow which is a hallmark of distributive shock due to sepsis / inflammation. Thus recent studies have utilised in vivo microscopy to directly visualise the changes in the sublingual microcirculation. De Backer and colleagues have reported improvements in sublingual microvascular function with dobutamine,[15] and drotrecogin alfa activated.[155] Of interest, the changes with dobutamine were independent of changes in global

haemodynamics. The microvascular effects of glyceryl trinitrate (GTN) have also been investigated. An initial study by Spronk and colleagues found that in a group of eight patients with septic shock, GTN resulted in a marked improvement in sublingual microvascular flow.[156] This has been followed by a recently reported randomised clinical trial in 70 patients with septic shock performed by the same group.[157] Patients were randomised to GTN or placebo alongside protocol driven resuscitation. These authors found that protocolised resuscitation resulted in recruitment of sublingual microvascular flow over 24 hours but there was no additional benefit of GTN. There were no differences in global haemodynamics between groups. Interestingly this study revealed an insignificant but substantial difference in hospital mortality of 31.4% (n=11) in the GTN group and 11.4% (n=4) in the placebo group (p=0.09). The authors hypothesised that this may have been due to enhanced production of peroxynitrite and other reactive oxygen species that might aggravate endothelial dysfunction, alter mitochondrial pores or interference with the adaptive mechanisms that close microcirculatory beds as a result of mitochondrial failure. Levosimendan is a calcium channel sensitiser that improves myocardial contractility and exhibits vasodilatory properties through activation of ATP-dependent potassium channels.[158] Levosimendan also exerts anti-inflammatory, anti-ischaemic and anti-apoptotic properties that may be important in critically ill patients.[159] Morelli and colleagues recently randomised 40 patients with fluid resuscitated septic shock to receive either levosimendan or dobutamine for 24 hours.[160] They found no differences in systemic haemodynamic variables at 24 hours apart from an increase in cardiac index in the levosimendan group. Levosimendan was associated with an improvement in microvascular flow index and a reduction in the heterogeneity of flow at 24 hours. There were also relative increases in the proportion of perfused vessels and perfused vessel density in those patients who received levosimendan. There was no correlation between

systemic and microcirculatory parameters. Trzeciak and colleagues investigated the changes in microvascular flow associated with GDHT in a series of patients in the early stages of severe sepsis.[161] This group used a protocol including fluid resuscitation, blood transfusion and dobutamine using central venous oxygen saturations as an endpoint. They found significant improvements in sublingual microvascular flow assessed by SDF imaging in those patients in whom there was an improvement in Sequential Organ Failure assessment (SOFA score) in the first 24 hours of hospital admission. Interestingly, they found no significant improvement in global haemodynamics in these patients. They concluded that microvascular alterations in sepsis are caused by intrinsic microcirculatory abnormalities as opposed to being a by-product of global haemodynamic changes. Microvascular changes may yield physiological and prognostic information that global haemodynamic monitoring is unable to provide. Further work is needed to investigate the benefits of therapies aimed at recruiting the microcirculation on organ failure in sepsis and possibly the use of microvascular flow as an endpoint for haemodynamic therapies.

A number of investigators have also looked at the microvascular effects of therapies aimed at improving haemodynamics during the perioperative period although there is little data describing direct visualisation of the microcirculation in this group. Miyakazi and colleagues investigated a group of 44 patients undergoing oesophagectomy.[162] Serosal tissue blood flow assessed using laser Doppler was lower in five patients who developed an anastomotic leak. This group also randomised half of the patients to receive the intravenous vasodilator prostaglandin E_1 (PGE_1). There was no significant improvement in blood flow with PGE_1. Buise and colleagues looked at a series of 14 patients undergoing oesophagectomy with gastric tube formation and cervical anastomosis.[163] Their aim was to determine whether the decrease in anastomotic blood flow was the result of arterial

insufficiency or venous congestion. In an elegant study they measured microvascular blood flow using laser Doppler, microvascular haemoglobin concentration and microvascular oxygen saturations using reflectance spectrophotometry. This group found that although blood flow did not change at the pylorus, it decreased progressively at the fundus during surgery. There were no changes in microvascular oxygen saturations or haemoglobin concentration. After completion of the anastomosis, glyceryl trinitrate applied topically for two minutes was associated with a significant increase in microvascular flow. The authors concluded that the decrease in microvascular blood flow at the anastomosis during oesophagectomy may be the result of venous congestion. The authors had hypothesised that if venous congestion resulted in poor microvascular flow they would also see an increase in the microvascular haemoglobin concentration and decrease in microvascular oxygen saturation. However, they point to the short interval of measurements as a possible explanation for their findings. The same group then performed a single-centre prospective double blind randomised controlled trial of intravenous glyceryl trinitrate versus placebo intraoperatively in a group of 32 patients undergoing oesophagectomy.[164] They found a similar reduction in microvascular blood flow and microvascular haemoglobin oxygen saturations in both groups during surgery. Post-operative anastomotic leak was identified in 13% in the treatment group and 31% in the control group (p=0.19).

1.4 Tissue oxygenation

For obvious reasons, maintenance of adequate tissue oxygenation is believed to be a key goal in the care of peri-operative and critically ill patients. In the peri-operative period tissue oxygenation may be impaired for a variety of reasons including smoking history,

anaesthesia, use of vasoconstrictor drugs, mechanical effects of surgery, tissue injury related to surgery and post-operative pain. A number of studies have explored the effects of tissue hypoxia and treatments which may prevent its occurence.

1.4.1 Supplemental oxygen therapy

Oxygen is an important substrate for oxidative killing by neutrophils and thus resistance to infection may relate to the partial pressure of oxygen in the wound. Oxygen is also fundamental for collagen synthesis and for cell signalling pathways for growth factors involved in wound healing.[165] Any surgical wound will disrupt the local vasculature and thus may result in wound hypoxia. Wound infections are a common and serious complication following surgery and may occur in 10-30% of patients undergoing colon surgery.[54,166,167] Wound infections prolong hospital stay, may result in intensive care unit admission and thus increase healthcare related costs.[168-170]

There are some concerns associated with oxygen therapy. High concentrations of oxygen can cause direct pulmonary toxicity, although this usually only develops after several days of exposure and it is unlikely that oxygen induced lung injury will develop within the timeframe of a surgical procedure.[171] It is possible, however, to develop pulmonary atelectasis rapidly when breathing high fractional concentrations of oxygen.[172] Clinical trials in surgical patients, however, have not demonstrated clinically or radiologically evident atelectasis associated with high intraoperative oxygen concentrations.[173,174]

A number of experimental and clinical studies have examined the physiological effects of supplemental oxygen. In a porcine model, supplemental oxygen was demonstrated to

increase both limb tissue oxygen tension and intestinal intramural oxygen tension.[175] In a study of 45 patients undergoing colorectal surgery, patients were randomised to 30% or 80% perioperative oxygen therapy.[176] Intragastric and colonic anastomotic catheters were placed in each patient. Significant differences in gastric and anastomotic pHi, and pCO_2 gap were found both during surgery and for six hours following surgery. A further group investigated 30 patients randomised to 30 or 80% oxygen perioperatively.[177] These investigators found an increase in markers of oxidative stress in those patients receiving the lower concentration of oxygen. The authors hypothesized that in the high inspired fractional concentration of oxygen (FiO_2) group, intestinal hypoxia was prevented, thereby limiting the effects of ischaemia-reperfusion injury. Hopf and colleagues found that tissue oxygenation in a surrogate wound of the upper arm was inversely correlated with post-operative wound infection rate in a group of general surgical patients thought to be at high risk of wound infections ($r=0.91$, $p<0.01$).[178]

In a landmark study, Greif and colleagues investigated the effects of intra- and post operative hyperoxia on the incidence of wound infections.[170] A group of 500 patients undergoing colorectal resection were randomised to receive an FiO_2 of 0.3 or 0.8 during and for two hours following surgery. The group assigned to receive more oxygen were found to have significantly higher arterial and subcutaneous partial pressures of oxygen. Associated with improved oxygenation was a lower incidence of surgical wound infection (5.2% vs 11.2% $p=0.01$). Patients who suffered wound infections, stayed in hospital a week longer than uninfected patients.

In a subsequent smaller study by Pryor and colleagues,[167] however, findings differed with an increase in the incidence of surgical site infections with higher perioperative oxygen

concentrations. However, there are a number of factors that may have contributed to these findings, including a lack of guidance on peri-operative care such as anaesthesia, fluid, temperature and pain management. Two further randomised controlled trials have produced conflicting results with Belda and colleagues finding that the incidence of surgical site infection was lower with higher inspired oxygen concentrations[179] and Meyhoff and colleagues, in the largest study to date (1400 patients), finding no difference.[173] Thus at present, the use of high concentrations of supplemental oxygen perioperatively is not standard practice.

1.4.2 Intra-venous fluid therapy and tissue oxygenation

A number of factors have been identified as influencing tissue oxygen tension. Smoking, uncontrolled surgical pain and epinephrine administration all reduce tissue oxygen tension.[180-182] All of these factors suggest a pathophysiological process relating to impaired tissue blood flow as one cause for the impairment of oxygenation. Clearly supplemental oxygen cannot improve oxygenation in nonperfused tissues.[183] Thus a number of investigators have explored the effect of perioperative intravenous fluids on tissue oxygenation. One of the first studies compared 'conservative' ($8ml.kg^{-1}.hour^{-1}$) or 'aggressive' ($16ml.\ kg^{-1}.\ hour^{-1}$) fluid management using crystalloids.[184] Subcutaneous tissue oxygenation in a surrogate wound in the upper limb was found to improve in the 'aggressive' fluid group. However, subsequent studies did not confirm these findings in porcine models of major surgery.[185,186] In one small study, the effects of crystalloids and colloids on tissue oxygenation were compared. Lang and colleagues compared lactated Ringer's solution and 6% hydroxyethyl starch (HES) in a group of 42 patients undergoing elective major abdominal surgery. [187] Ringer's lactate was associated with a decrease in

tissue oxygenation whilst 6% hydroxyethyl starch was associated with an increase. Further work is needed to investigate the effects of fluid resuscitation and other measures aimed at improving tissue perfusion in perioperative patients.

1.4.3 Other therapies and tissue oxygenation

Adequate pain control has been shown to ameliorate the fall in tissue oxygenation post-operatively.[181] A number of investigators have looked specifically at the role of epidural anaesthesia and analgesia on tissue oxygenation perioperatively. The findings of a number of studies suggest that regional analgesia and anaesthesia results in an increase in tissue oxygenation, both in healthy volunteers,[188] and surgical patients.[189] Given the vasodilator effects of regional analgesia through a reduction in sympathetic tone, the most plausible mechanism for such changes would relate to improved tissue blood flow in turn suggesting that impaired microvascular flow may play an important role in abnormalities of tissue oxygenation.

Some studies have demonstrated an improvement in subcutaneous oxygenation associated with mild hypercapnia.[190,191] This may be due to increased cardiac output or to a right shift in the oxy-haemoglobin dissociation curve. Hypercapnia has also been demonstrated to improve intramural intestinal oxygenation in a porcine model.[175] These findings were confirmed in a clinical trial of normocapnia (end-tidal carbon dioxide tension 35 mmHg) versus mild hypercapnia (end tidal carbon dioxide tension 50 mmHg) in patients undergoing elective bowel resection.[192] An interesting study by Akca and colleagues investigated the effects of hypercapnia on tissue oxygenation in patients undergoing cardiac surgery using cardiopulmonary bypass (fixed blood flow).[193] They

found no improvement in tissue oxygenation and concluded that hypercapnia results in improvements in tissue oxygenation through changes in cardiac output rather than vasodilation.

1.5 Discussion / summary

Major surgery and severe sepsis are important causes of death and disability. With the ageing population and the increasing use of invasive procedures, the number of high risk surgical procedures and incidence of severe sepsis are likely to increase.[1,9] This will place an increasing burden on healthcare resources. Many previous investigators have used surrogate global markers to diagnose organ hypoperfusion in these patient groups such as global oxygen delivery, mixed venous oxygen saturations and blood lactate concentrations. The introduction of direct in-vivo microscopy has allowed the investigation of microcirculatory changes at the bedside. Abnormalities of microvascular flow have been identified in established sepsis and interestingly, these changes are not necessarily obvious using routine global haemodynamic parameters. There is little data, however, describing changes in the earliest phases of sepsis or following major surgery and the relationship of these changes to outcome. We hypothesised that microvascular flow would deteriorate in the early phase of sepsis, as well as during the inflammatory response to surgery and that these changes would be related to outcome.

The use of intravenous fluids and vasoactive medications are a routine and fundamental aspect of care for critically ill patients. Their optimal use, however, is poorly understood and remains hotly debated. Whether the use of fluid resuscitation and / or vasoactive agents to optimise global haemodynamics results in similar improvements in the microcirculation in critically ill patients remains unclear.[15,160,161] Thus there is a clear need for further investigations of tissue microvascular flow and oxygenation in patients undergoing major surgery and in those with sepsis, to confirm the importance of these abnormalities and how they may be modified by haemodynamic therapies. GDHT has

been demonstrated in a number of perioperative studies to improve outcome. It is believed that this is due to improvements in tissue perfusion and oxygenation, although this hypothesis has yet to be confirmed. Dopexamine, an inodilator, has been used in a many of these perioperative GDHT trials. We hypothesised that a fixed dose infusion of dopexamine alongside stroke volume guided fluid therapy would improve microvascular flow and tissue oxygenation in a group of high risk perioperative patients. Noradrenaline is recommended to maintain mean arterial pressure greater than 65 mmHg after fluid resuscitation in patients with septic shock. Some clinicians advocate targeting higher arterial pressures, but the effects of such as approach on the microcirculation and tissue oxygenation are unknown. We aimed to investigate the effect of escalating doses of noradrenaline titrated to achieve successively higher mean arterial pressures in a group of patients with septic shock.

1.6 Aims of thesis

1) To confirm the existence of a high risk sub-population within the local surgical population and characterise this population in more detail (in particular with regard to treatment pathways)
2) To investigate the changes in tissue microvascular flow and oxygenation in patients receiving usual care following major abdominal surgery and their relationship to outcome
3) To investigate the effects on tissue microvascular flow and oxygenation of two commonly used GDHT algorithms compared to central venous pressure guided fluid therapy in perioperative patients
4) To investigate the effects of GDHT on renal outcomes in a group of perioperative patients
5) To describe the changes in microvascular flow in patients with early sepsis and its association with outcome
6) To investigate the effects of increasing doses of noradrenaline on tissue oxygenation and microvascular flow in patients with septic shock

Chapter 2
Methods

2.1 Cardiac output monitoring

One of the earliest attempts to take physiological measurements of the circulation was made in 1733 by the Reverend Stephen Hales who measured arterial pressure by attaching a manometer to the carotid artery of a horse. However, the measurement of blood flow has proved to be much less straightforward than the measurement of blood pressure. As a consequence, an undue emphasis has been placed on the value of arterial pressure measurements which often give a poor indication of tissue blood flow.[194,195] It was not until the early 1970's that the introduction of the balloon tipped pulmonary artery catheter finally allowed the routine measurement of cardiac output at the bedside.[30,31] As use of the pulmonary artery catheter became more widespread, a number of commentators voiced concerns that this technique might be associated with an increase in mortality.[50,51,196] This debate was in particular fuelled by the publication of a retrospective analysis of data collected prospectively on 5735 patients for a study on decision making in ICU. The outcome of patients who received a pulmonary artery catheter in the first 24 hours of ICU admission was compared to that of those who did not. The groups were adjusted with the use of a severity of illness propensity score and the authors found that mortality was higher among patients who received a pulmonary artery catheter. There are a number of problems with this retrospective analysis. Firstly the database was not originally compiled for this purpose. Secondly patients in whom a pulmonary artery catheter was inserted after the first 24 hours of ICU admission were analysed as not having received one at all. Lastly the relevance of outcome data adjusted using a

retrospective propensity score is questionable. It is possible that there were a number of co-variables which could account for the difference found. Although the belief that the use of PA catheters worsens outcome has now been refuted by three large multi-centre trials, which showed no difference in outcomes[197-199] there has, in the meantime, been a shift towards the use of less invasive technology. A number of different cardiac output monitoring devices are now commercially available. The requirements for the ideal monitoring technique to be used in the post-operative surgical patients are that it should be readily tolerated by conscious patients, convenient to use and well validated.

2.1.1 Fick Principle

In 1870, Adolf Fick suggested that blood flow through an individual organ might be calculated by measuring the arterio-venous concentration gradient of an indicator, a known mass of which had previously been added to the arterial circulation.[19] Fick originally suggested the calculation of pulmonary blood flow and therefore total cardiac output by taking measurements of oxygen consumption and carbon dioxide production along with the pulmonary arterial and venous concentrations of these gases (equation 1 and figure 1). Fick's principle was subsequently used to measure cardiac output in animals,[200,201] but it was not until 1929, when Werner Forssman devised a method of sampling mixed venous blood that the application of the Fick principle became possible in man. Forssman passed a ureteric catheter through his own cephalic vein and into his right ventricle, before walking to the X-ray department to confirm its position, an experiment that led both to his dismissal and the award of the Nobel prize.[20] The following year Otto Klein became the first person to draw mixed venous blood and calculate cardiac output in man using the Fick principle.[202] This technique was then perfected by Cournand and colleagues during the

1940's.[203,204] The Fick technique is still regarded by many as the most accurate method of cardiac output measurement currently available; however, it is rarely used clinically due to the availability of more practical alternatives.

$$Q = \frac{VO_2}{CaO_2 - C\bar{v}O_2}$$

Figure 2.1 Fick equation. Blood flow (Q), or in this example cardiac output, is equal to oxygen uptake (VO_2) divided by the arteriovenous oxygen difference. The arteriovenous oxygen difference is calculated by subtracting the mixed venous oxygen content (CvO_2) from the arterial oxygen content (CaO_2). This equation can be adapted to look at blood flow across individual organs.

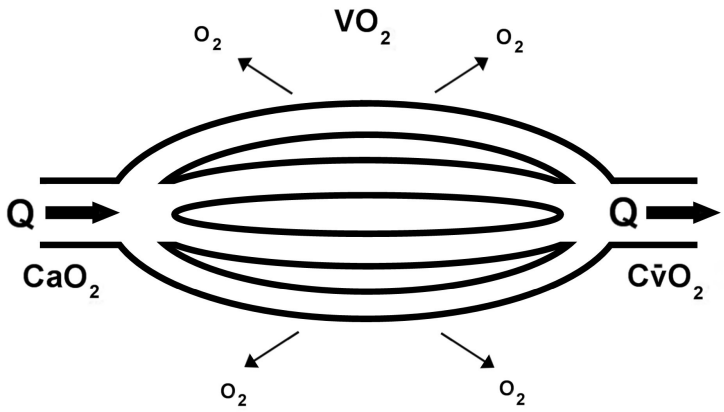


Figure 2.2 Fick principle. Blood flow (Q) across the tissue bed is equal to the oxygen consumption divided by arteriovenous oxygen difference (arterial oxygen content [CaO2} minus the venous oxygen content [CvO2]).

2.1.2 Indicator dilution technique

The use of exogenous indicators to determine circulation time was first reported as early as 1761, when Haller described the measurement of pulmonary circulation time in an animal model using coloured dye.[205] During the 1890's, George Stewart further developed the concept of indicator dilution in a series of papers on circulation time.[206-208] By using a hypertonic saline indicator, Stewart was able to detect a signal in circulating blood by measuring changes in electrical conductance. William Hamilton then developed Stewart's work to allow the measurement of cardiac output using an indicator dilution technique.[28] Hamilton used phenolphthalein as an indicator and, with the help of an automated system of blood sampling, was able to plot the systemic arterial concentration of dye against time. This now familiar curve was characterised by a brief delay following injection during which dye transits the pulmonary circulation followed by a rapid rise to a peak value before an exponential decline with a second much smaller peak due to indicator recirculation (figure 2). Cardiac output was shown to be inversely proportional to the area under the curve as described by the Stewart-Hamilton equation (equation 2).

$$M = Q \int C(t)\,dt$$

Figure 2.3 Stewart-Hamilton equation. If an indicator is injected rapidly into the right atrium, it will appear downstream in the pulmonary artery in a concentration that varies with time, C(t). As all the injected indicator (M) must leave the system, M is equal to the sum of the concentrations at each interval (t) multiplied by flow (Q), which is assumed to be constant.

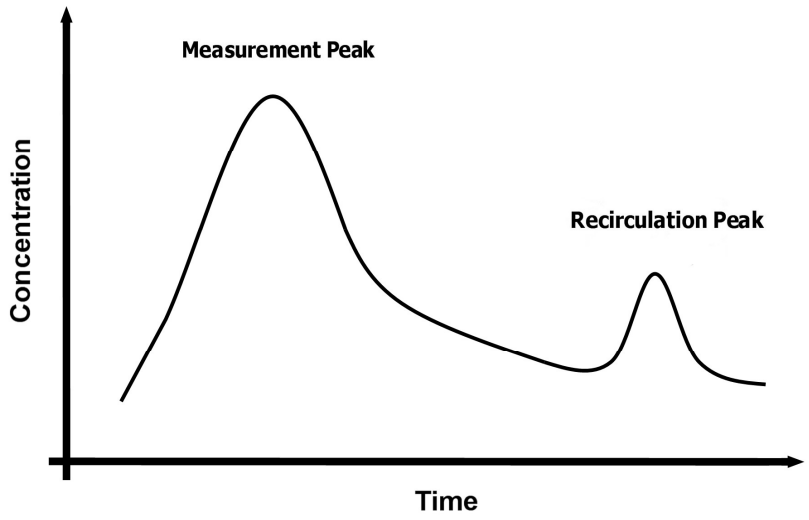


Figure 2.4 Indicator-dilution curve. Changing dye concentration with respect to time demonstrating initial peak followed by exponential decline and second peak due to recirculation.

Various dyes have been used for indicator dilution measurements, most commonly indocyanine green. However, few indicators conform closely to the ideal indicator which is stable, non-toxic, easily measured, uniformly distributed within the fluid compartment of interest, not lost from the circulation during the first transit and yet rapidly dissipated to avoid recirculation. The most common difficulties with indicator dilution techniques have been lack of indicator stability, inaccurate concentration measurement and indicator accumulation. The use of thermal indicators (thermodilution) avoids some of these difficulties, an approach which was first described by Fegler in 1954.[29] However, temperature equilibration may still result in significant indicator loss during transit. In 1967, Branthwaite and Bradley described the measurement of cardiac output by thermal indicator dilution using a thermistor tipped pulmonary artery catheter.[30] Indicator loss was

minimised because of the short path between indicator injection in the right atrium and measurement in the pulmonary artery. Swan and Ganz made a further adaptation in 1970,[31] adding a small inflatable balloon to the catheter tip. Flow directed placement of the catheter without fluoroscopic imaging allowed the routine clinical use of the pulmonary artery catheter. Temporary occlusion of the pulmonary artery during balloon inflation also allowed measurement of pulmonary artery occlusion pressure, an index of left ventricular preload.

Traditionally, five successive measurements were made at end-expiration following manual injection of a cold saline bolus. An average was then taken of the three most closely related values. However, many centres now use an automated system; a filament incorporated into the body of the catheter intermittently warms aliquots of blood passing through the right ventricle, the output signal being an increase in temperature rather than a decrease. Measurements are repeated automatically every few seconds and averaged over ten readings. This method is as accurate as manual measurement but more convenient.[209,210] Measurements of cardiac output by pulmonary artery catheter thermodilution correlate well with alternative methods including dye dilution and the Fick technique.[30,211-214] Important sources of error include indicator loss,[30,215,216] and structural cardiac abnormalities.[217] Cardiac output varies with positive pressure ventilation, particularly in hypovolaemic patients.[218,219] However, provided intermittent measurements are taken at the same point in the respiratory cycle, wide fluctuations in the measured value for cardiac output can be avoided.

An important limitation of the pulmonary artery catheter is the risk of damage to cardiac valves with prolonged use. Ideally, they should be removed after 48 hours, or at least after

a maximum of 72 hours. Other complications include catheter knotting, pulmonary artery rupture and pulmonary embolism.[220] It is important to note however, that several multi-centre trials have shown that significant complications are unusual and that pulmonary artery catheter use is not associated with excess mortality.[197-199] Use of this device is now declining in favour of less invasive technology, although the technique will continue to have a niche role in management of pulmonary hypertension and patients receiving intra-aortic balloon counterpulsation.

Trans-pulmonary indicator dilution techniques in which indicator is injected into the superior vena cava and measured in a systemic artery are also well described. [221,222] This approach is less invasive and may provide additional physiological data related to the pulmonary circulation.[221,222] Two of the most commonly used techniques for measuring cardiac output at present utilise trans-pulmonary indicator dilution to calibrate continuous arterial pressure waveform analysis (one using a lithium indicator, the other a thermal indicator).

2.1.3 Arterial waveform analysis

Otto Frank first suggested calculating cardiac output by analysis of the arterial pressure waveform in 1899.[223] Frank realised that total peripheral resistance can be calculated from the time constant of diastolic aortic pressure decay and arterial compliance, estimated by measuring aortic pulse wave velocity. Cardiac output can then be calculated from total peripheral resistance and mean arterial pressure (equation 3).

$$CO = \frac{MAP}{TPR}$$

Figure 2.5 Relationship between cardiac output (CO), mean arterial pressure (MAP) and total peripheral resistance (TPR)

In 1904, Erlanger and Hooker suggested that the principal determinant of aortic pulse pressure was the volume of blood ejected during each cardiac cycle. Measurement of pulse pressure should therefore allow the calculation of stroke volume and hence cardiac output.[224] In 1970, Kochoukos described a more accurate method of stroke volume estimation involving measurement of the area under the systolic portion of the arterial pressure waveform (figure 3).[225]

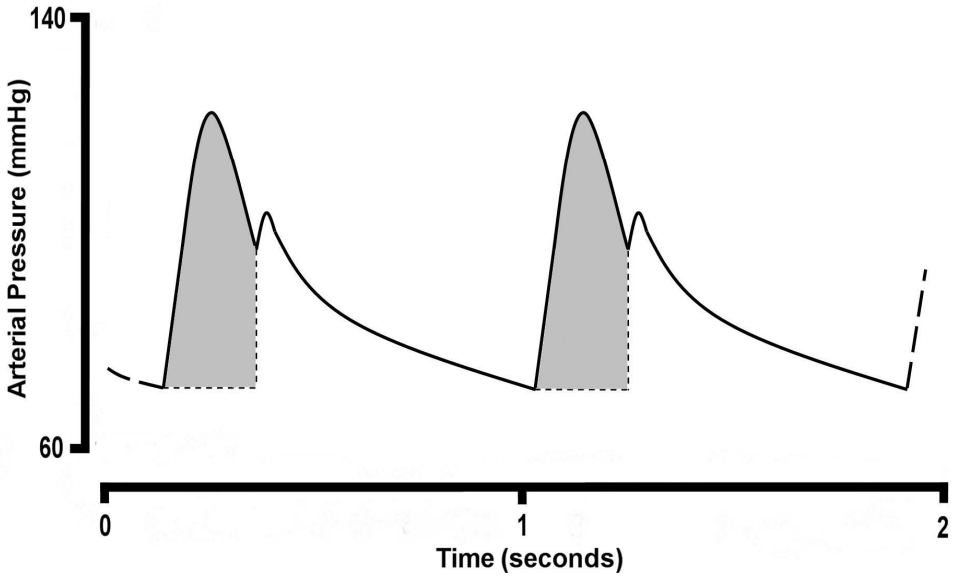


Figure 2.6. Stroke volume estimated from systolic portion (shaded) of arterial waveform.

This work was developed by Wesseling and colleagues who devised an algorithm for the calculation of stroke volume from aortic impedance and the change in arterial pressure during systole (equation 4).[226,227] However, calculation of stroke volume and cardiac output through analysis of the arterial pressure waveform is far from straightforward. This complex waveform comprises both an incident pressure wave which is proportional to stroke volume and a reflected pressure wave created as the incident wave reflects back from the peripheries. Changes in arterial compliance affect both the velocity and amplitude of the pressure waves and will in turn be affected by changes in arterial wall tension. As a consequence, the arterial waveform may vary considerably, depending on physiological circumstances and anatomical location. Although the method of pulse contour analysis suggested by Wesseling may provide a reliable estimate of changes in cardiac output,[228-230] accuracy may be influenced by changes in total peripheral resistance.[231] Perhaps the most important difficulty with arterial waveform analysis is the fact that aortic impedance is dependent both on cardiac output and aortic compliance. Consequently, it is only possible to reliably estimate changes in stroke volume rather than absolute values. Such systems must therefore be calibrated before use.

$$SV = \frac{\int dP/dt}{Z}$$

Figure 2.7 Calculation of stroke volume using pulse contour analysis. Stroke volume (SV) is estimated from the integral of the change in pressure (P) from end diastole to end systole (t) i.e. the systolic portion of the curve until aortic valve closure. This estimate of stroke volume is also dependent on the impedance of the aorta (Z).

An alternative method of arterial waveform analysis is to apply the physical principle of conservation of mass to calculate changes in pulse power. The net power change in the aorta is determined by the difference between the input and removal of mass. The change in power during a single cardiac cycle should therefore be determined by stroke volume (input of mass) and the distribution of blood from the aorta into the peripheral circulation (removal of mass). Ejection of blood into the aorta during systole causes fluctuations in blood pressure around a mean value. Using a mathematical technique termed autocorrelation, analysis of these fluctuations allows determination of changes in stroke volume with each cardiac cycle.[232] This algorithm is not morphology based and takes account of changes in the arterial waveform throughout the cardiac cycle rather than systole alone. This approach may be more accurate because the effects of the reflected arterial pressure wave are taken into consideration. Once again, calibration is required to correct for arterial wall compliance damping and other sources of inter-individual variability.

2.1.4 Trans-pulmonary thermodilution and arterial waveform analysis

In this technique, trans-pulmonary thermodilution is performed using a cold saline indicator injected via a central venous catheter. The measurement of the temperature of arterial blood is performed using a thermistor tipped catheter sited in the femoral or brachial artery. Cardiac output is then calculated using a modified Stewart-Hamilton equation allowing calibration of continuous arterial waveform analysis software which provides continuous cardiac output data by a pulse contour analysis method.

This technology can be safely used in both conscious and unconscious patients for prolonged periods and correlates well with measurements made by pulmonary artery catheter thermodilution,[233,234] and the direct Fick technique.[235,236] An important limitation is the requirement for a specific thermistor tipped arterial catheter sited in either the femoral or brachial artery to allow thermodilution measurements. In most cases this necessitates the insertion of a new arterial catheter. Changes in compliance of the arterial tree or damping of the arterial pressure transducer system may cause measurement error, necessitating repeated calibration. This system was not appropriate for our purposes as the requirement for insertion of a thermistor tipped arterial cannula would have nececessitated arterial cannulation following surgery.

2.1.5 Trans-pulmonary lithium indicator dilution and arterial waveform analysis

This monitor utilises the lithium indicator dilution technique to calibrate software which performs continuous arterial waveform analysis by a pulse power method to provide

updated cardiac output data for each cardiac cycle. Lithium indicator dilution is a novel concept which was first described in 1993.[221] Lithium satisfies many of the criteria for the ideal indicator; whilst there is minimal indicator loss during the first circulation, rapid redistribution allows repeated measurements.[237,238] In the doses used for calibration, lithium chloride is considered safe, even in a 40 kg patient with non-functioning kidneys.[239] Following intravenous injection, lithium is detected by an external lithium ion sensitive electrode attached to a standard arterial catheter. Cardiac output is calculated using a modified Stewart-Hamilton equation (equation 5). A correction is applied for plasma sodium concentration which, in the absence of lithium, is the chief determinant of potential difference across the electrode. As lithium is only distributed in plasma, a correction for haematocrit is also required.[240]

$$CO = \frac{Li\ x\ 60}{AUC\ x\ (1 - PCV)}$$

Figure 2.8 Modified Stewart-Hamilton equation for the measurement of cardiac output by lithium indicator dilution. CO cardiac output; Li lithium dose; AUC area under lithium concentration / time curve; PCV packed cell volume.

Lithium indicator dilution measurements for calibration are generally performed every eight hours but may be required more frequently where there is evidence of significant changes in arterial compliance or damping of the arterial waveform.[241] In an animal study, lithium indicator dilution compared well with measurements taken using an electromagnetic aortic flow probe.[242] Studies in humans suggest a good correlation between lithium indicator

dilution and other methods.[221,243,244] Similarly, comparisons of pulse power analysis to intermittent determinations of cardiac output by thermodilution and lithium dilution suggest this method of continuous cardiac output measurement is reliable.[241,245,246]

This combined technology has a number of advantages for the series of investigations reported here when compared to pulmonary artery catheter thermodilution or other cardiac output monitors. The method is less invasive than pulmonary artery catheterisation allowing use for longer periods of time in a wider range of patients. Due to the nature of the patients studied in, central venous catheters and arterial lines were inserted by the treating team as part of ususal care. There was therefore no need for insertion of additional arterial or venous catheters. Another advantage is that this technique can be employed in both conscious and unconscious patients There are, however, some limitations to this combined technology. The use of non-depolarising muscle relaxants may interfere with the lithium ion sensitive electrode resulting in difficulties with calibration due to baseline drift. Irregular cardiac rhythms may occasionally result in an irregular data output from the pulse power analysis software and, as with all arterial waveform analysis techniques, damping of the arterial waveform will result in measurement error.

2.1.6 Aortic velocimetry

Cardiac output may be calculated from measurements of aortic blood velocity and cross-sectional area. Such techniques include the measurement of Doppler frequency shift of ultrasound waves and electromagnetic methods. The simplicity of these methods of velocity measurement is a major advantage. However, in most situations, the diameter of

the aorta cannot be measured directly, introducing a possible source of error. If velocity measurements are made on blood flowing through the descending aorta, a correction factor must also be applied to account for distribution of an unknown proportion of the total cardiac output to the upper body, introducing another potential source of error.

The oesophageal Doppler technique involves the measurement of blood velocity in the descending thoracic aorta using an ultrasound probe placed in the lower oesophagus. The probe emits an ultrasound beam at a 45° angle to its long axis which is aimed towards the aorta by the operator. A continuous visual velocity versus time display helps to ensure correct probe placement (figure 2.9). Measurement of the Doppler frequency shift of the reflected ultrasound waves allows calculation of blood velocity. Cardiac output may then be calculated by one of two methods. The first involves measurement of the aortic cross sectional area, measured using M mode ultrasound visualisation of the aorta and then multiplying this value by blood velocity to calculate flow. A correction factor is then applied to take account of distribution of part of the total cardiac output to the upper body, assuming that this ratio is constant. A simpler and seemingly equally reliable method, is simply to derive a value of total cardiac output from a nomogram using aortic blood velocity, height, weight and age.[247]

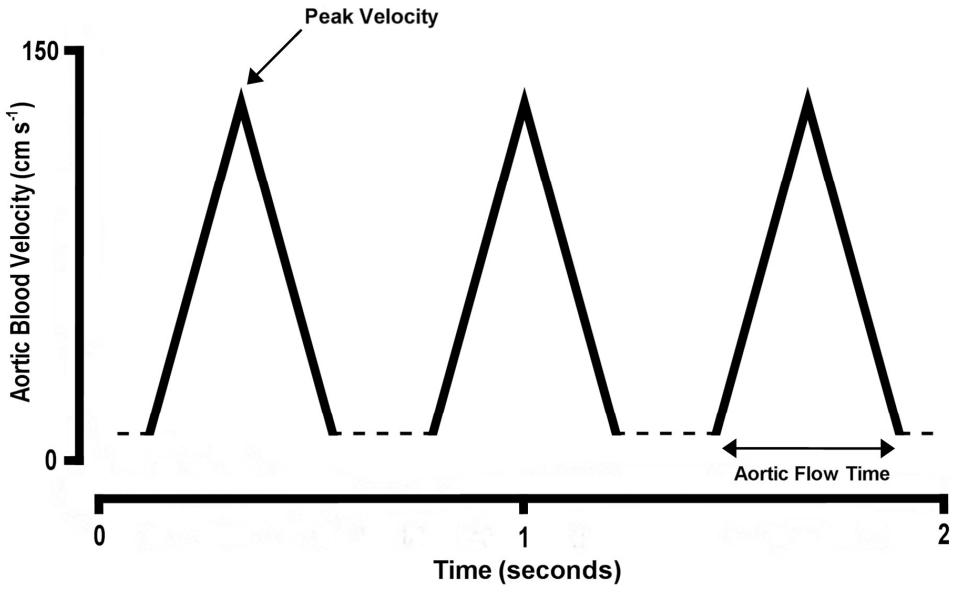


Figure 2.9 Velocity-Time trace from oesophageal Doppler probe demonstrating aortic flow time and peak velocity.

A systematic review of eleven studies in which the nomogram based oesophageal Doppler technique was compared to pulmonary artery catheter thermodilution suggested minimal bias and good limits of agreement between the two techniques.[248] The principal advantage of this technique is speed and ease of use and the method has proved ideal for intra-operative use. The most important contra indication to use is severe oesophageal pathology, in particular varices or recent surgery. The major limitation of this technique is that the probe is not well tolerated by conscious patients and its use is therefore generally confined to anaesthetised or sedated patients. The inability to use this method in non-sedated post-operative patients prevented the use of this technology in the series of experiments reported here.

2.1.7 Supra-sternal Doppler

Using a non-invasive ultrasound probe positioned in the jugular notch (figure 2.10), it is possible to measure blood velocity in the ascending aorta. This is essentially a non-invasive alternative to the oesophageal Doppler technique. Stroke volume and cardiac output are calculated using a measurement of cross sectional area of the aortic outflow tract. Because these measurements are taken from the aortic root, the technique is not affected by changes in distribution of cardiac output between the upper and lower body. Cardiac output measurements taken using the supra-sternal Doppler method were similar to those taken with an electromagnetic aortic flow probe in an animal study,[249] and with pulmonary artery catheter thermodilution in clinical studies.[250-252] The portable and non-invasive nature of this technology is a major advantage, allowing use in any clinical setting. However, it may be very difficult to identify the aortic root in some subjects. Although the supra-sternal probe should allow ultrasound waves to be orientated at 0° to the direction of blood flow, in practice this alignment is affected by operator skill as well as the anatomy and position of the subject. Consequently, this technique may have greater inter-observer variability than other methods. Where identification of the aortic root proves difficult, the pulmonary valve may be used instead. Because measurements are taken in the supine position, they may be poorly tolerated by breathless patients. The main advantages of this technique, however, are its ease of use and portability. This was necessary for our study investigating the microcirculation in early sepsis as we were aiming to recruit these patients as soon as possible after hospital admission and often prior to the insertion of arterial and central venous catheters.

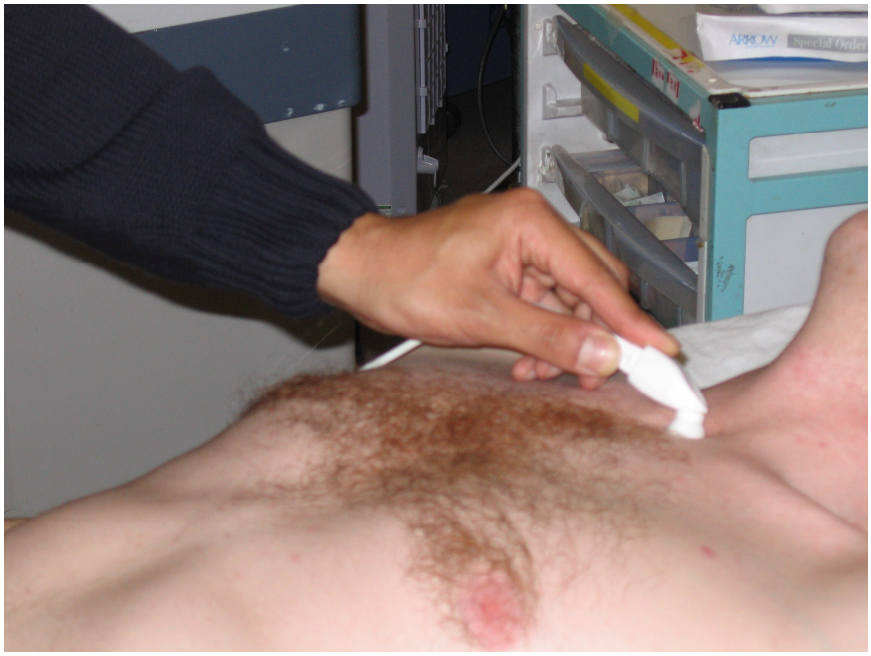


Figure 2.10 Suprasternal Doppler applied to jugular notch in supine subject for measurement of cardiac output.

2.1.8 Bioimpedance

Bioimpedance is a non-invasive technique which involves the application of a small alternating current across the chest via topical electrodes. This current is thought to distribute primarily to blood because of its high electrical conductivity compared with muscle, fat and air. Pulsatile changes in thoracic blood volume result in changes in electrical impedance. The rate of change of impedance during systole is measured allowing a value of cardiac output to be derived. A number of studies have compared bioimpedance to alternative methods of cardiac output measurement with inconsistent findings.

2.2 Transcutaneous tissue oxygenation

Transcutaneous tissue oxygenation was assessed using a heated Clark type electrode. Continuous non-invasive measurement of oxygen tension is possible because oxygen can diffuse through the skin. Although normally the skin is not very permeable to gases, at higher temperatures skin blood flow and, hence diffusion of oxygen, is increased. Oxygen sensors for transcutaneous electrochemical measurements are based on polarography. The sensors contain a conventional amperometric transducer in which the rate of a chemical reaction is detected by the current drained through an electrode. The sensor heats the skin to 43–45 degrees Celsius (°C). The skin surface oxygen tension is increased as a result of three effects. Firstly, heating the stratum corneum beyond 40°C changes its structure, allowing oxygen to diffuse more rapidly. Secondly, the local oxygen tension is increased by shifting the oxygen dissociation curve to the right in the heated dermal capillary blood. Finally heating causes dermal capillary hyperemia. In neonates, this technology is often used to estimate arterial oxygenation as the dermal layer is very thin so that transcutaneous oxygenation can accurately track changes in arterial oxygenation.[253] In adults the dermal layer is thicker; consequently measured transcutaneous oxygen tension is lower than arterial oxygen tension. Importantly the correlation between transcutaneous oxygenation and arterial oxygen tension depends on blood flow; reductions in local blood flow during shock states can therefore result in low transcutaneous oxygen tension measurements. For this reason reductions in transcutaneous oxygenation have been used as a marker of poor cutaneous blood flow during shock.[254-258] Yu and colleagues, for example, prospectively measured transcutaneous oxygen and its response to an oxygen challenge alongside global haemodynamics in a group of 38 patients with septic shock.[259] They found the ability to

increase transcutaneous oxygen in response to an oxygen challenge, as a marker of tissue blood flow, separated survivors from non-survivors. The same group went on to perform a randomised controlled trial using transcutaneous oxygenation targets in a group of patients with septic shock.[260] Interestingly, they again found that the ability to improve transcutaneous oxygenation was a predictor of outcome and showed reduced mortality in the group randomised to using transcutaneous oxygenation as a resuscitation goal.

2.3 Laser Doppler Flowmetry

Laser Doppler is a non-invasive technique used for determining blood flow in the microcirculation. The method is based on the reflection of a beam of laser light that undergoes a change in wavelength when it encounters moving red blood cells. The technique depends on the Doppler principle whereby low power light from a monochromatic stable laser is transmitted via an optic fibre and scattered on contact with tissue and moving blood cells. As a consequence its frequency distribution broadens (Doppler shift). This light is transmitted via a second optical cable in the same fibre to a photodetector along with the laser light scattered by the static tissue. The fibres within the head are separated by a few tenths of a millimetre and consequently blood flow is assessed in a tissue volume of 1mm^3 or less.

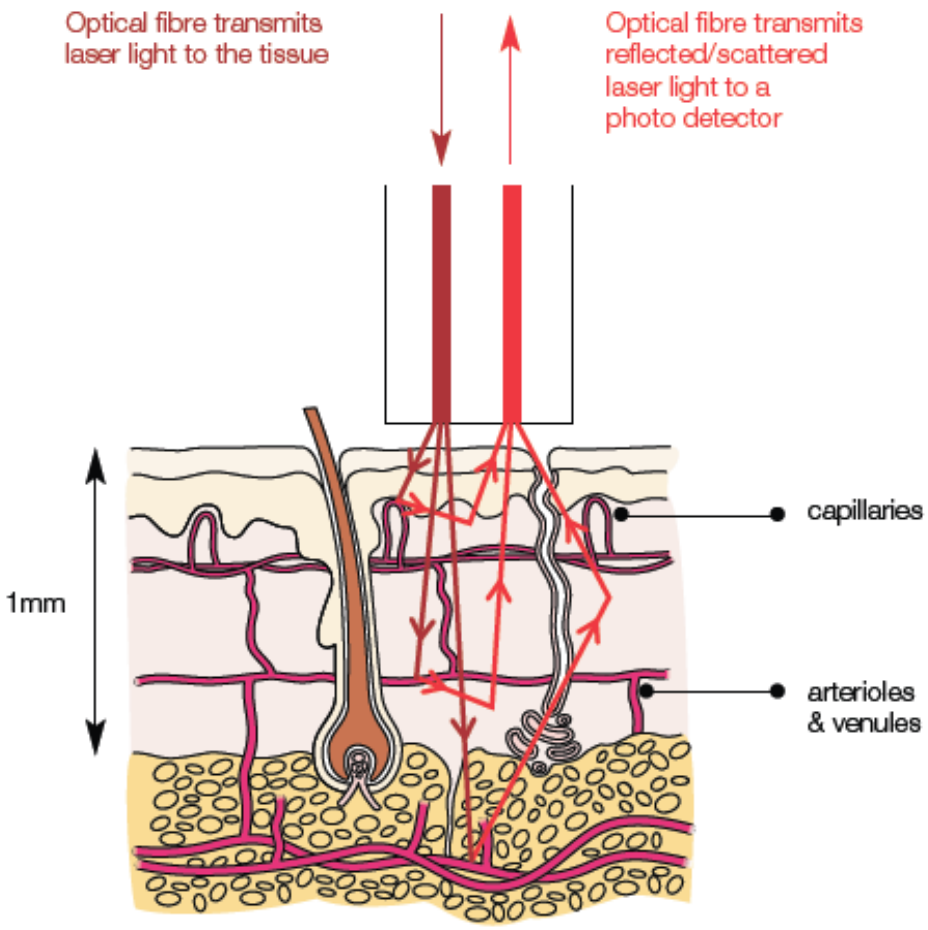


Figure 2.11. Laser Doppler flowmetry. Low power laser light is transmitted via an optical fibre to the tissues. The light is scattered by tissue; some of this scattered light is collected by the optical fibres and transmitted to a photodetector. The resulting photo current is electronically processed to produce a laser Doppler flux signal. Reproduced with permission of Moor Instruments Ltd, UK.

Laser Doppler flowmetry, cannot determine absolute perfusion values but instead flow is quantified using arbitary perfusion units. The units are given as a quantity proportional to the product of the average speed of the blood cells and their concentration. It is therefore not possible to compare absolute flux values across different studies that use different probes / machines or sites of measurements. Also flux does not reach the value of zero when perfusion is absent because Brownian motion of molecules within the interstitial

space contributes to the signal when flow of red cells is absent. For measurement of flux, probes were simply placed on the area of interest (abdominal wall or upper limb) and measurements taken at set intervals (Chapters 4 and 9).

Post-occlusive reactive hyperaemia is the increase in skin blood flow above baseline levels following the release of a brief arterial occlusion. It can be characterised by an initial peak in flux within a few seconds of removal of the occlusion, followed by a sustained hyperaemia.[261] The test was performed by placing a cuff on the upper arm and inflating the pressure to 20-30mmHg above systolic pressure. The commonly used ischaemic times are three and five minutes.[261] A period of three minutes was used in these trials as longer periods were deemed likely to be poorly tolerated in the post-operative patient. Laser Doppler probes were placed on the forearm, distal to the cuff, and measurements were taken before, during and after cuff inflation. A number of parameters can be assessed from the flux response. The most commonly used parameter is peak hyperaemia, although there is no consensus as to the most informative end point. Studies have examined the peak hyperaemia minus baseline flow,[262] peak as a percentage of baseline [263] and area under the curve of the postischaemia blood flow.[264] We decided to look at the relationship between peak hyperaemic flow and baseline flow for two main reasons. Firstly, this derivative appears to be the most commonly used. Secondly we felt we would obtain more robust data by looking at these two parameters, given that patient movement artefact would limit area under the curve calculations (movement artefact was common in the early stages post-operatively due to residual sedation) (Chapter 5).

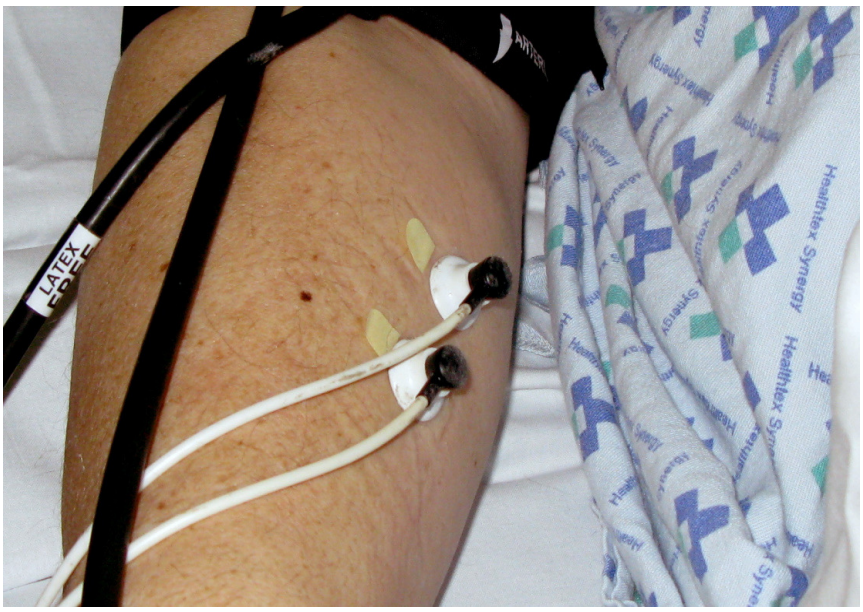


Figure 2.12. Laser Doppler assessment of post-occlusive hyperaemic response

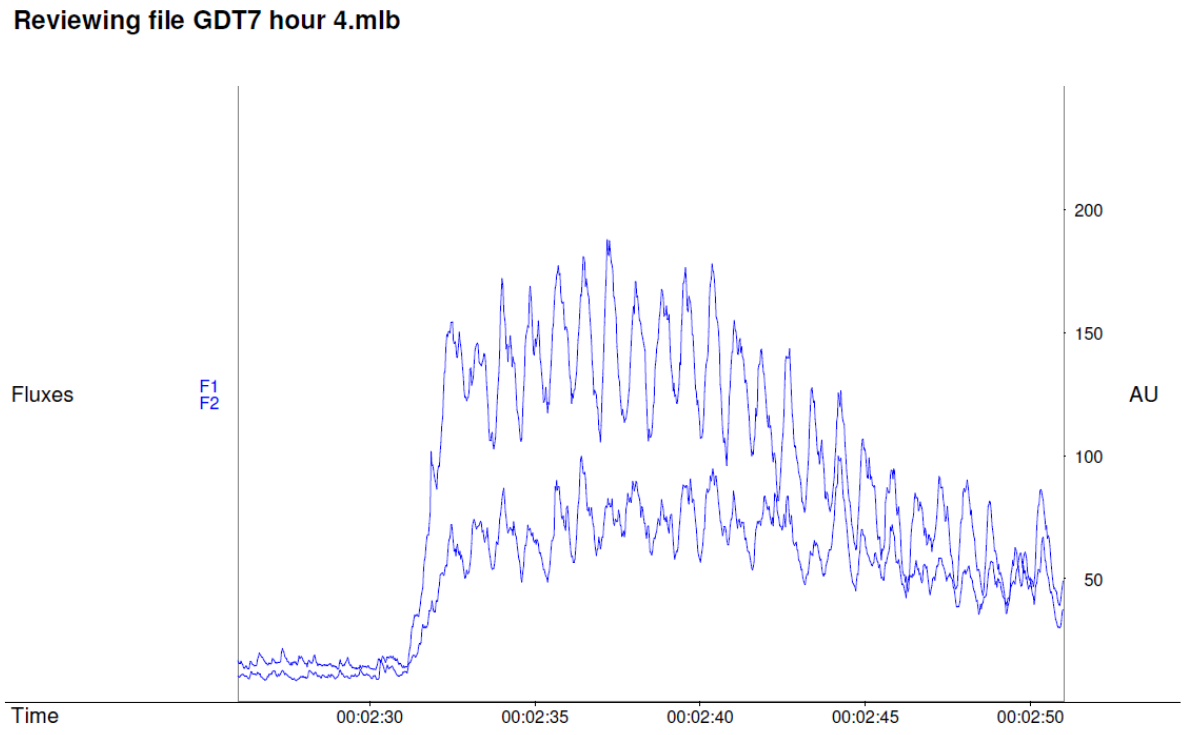


Fig 2.13. Hyperaemic response measured using Laser Doppler

2.4 Sublingual microscopy

Until 1999, direct visualisation of the microcirculation in humans was limited to the use of bulky stage microscopes, mainly used to observe the nailfold capillary bed. This constrained the applicability of clinical studies especially since there is uncertainty as to the relevance of changes in nailfold capillary flow to other microvascular beds. The introduction of Orthogonal Polarization Spectral (OPS) imaging by Slaaf and colleagues and its incorporation into a hand-held microscope allowed the study of the human microcirculation in exposed tissue surfaces at the bedside.[265] With this technique, the object is illuminated by light that has been linearly polarised in one plane. The OPS camera then images the remitted light through a second polariser (analyser) that is orientated in a plane precisely orthogonal to that of the illumination. A diagram of this set-up is shown below.

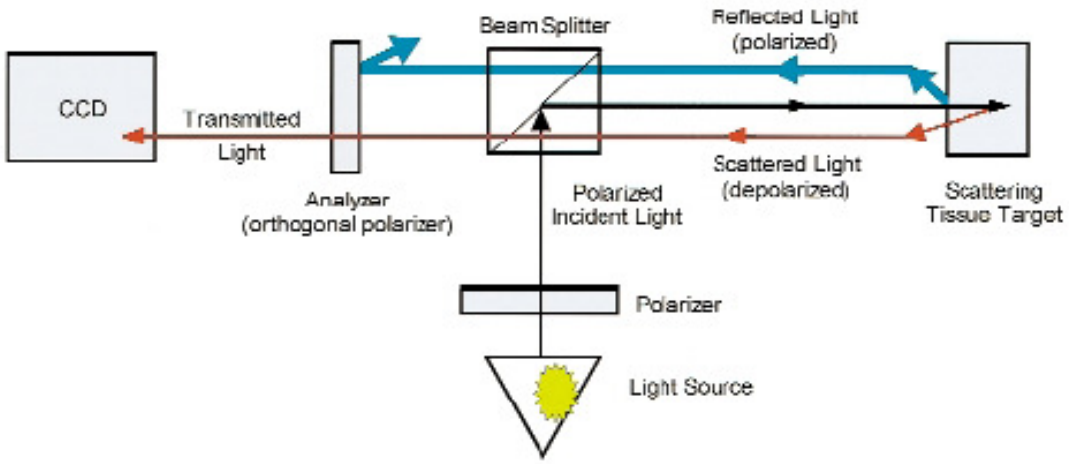


Figure 2.14 Schematic of OPS imaging. Reproduced from [90] with permission from Nature publishing group.

Light emitted from the light source is passed therough a spectral filter to isolate the chosen wavelength and to polarise the light. The beam of light is then reflected towards the target tissue by a beam splitter. The light is focussed onto an area of approximately 1mm in diameter by the objective lens. The light that is reflected from the target is then collected by the same objective lens. The light passes through a polarisation analyser placed orthogonally to the original polariser. This allows depolarised light but not polarised light to form an image on the video camera. Once light has been polarised, this state remains even after reflection or single scatterings on contact with the target tissue. (More than ten scattering events are required to depolarise light.[266,267]) Therefore using OPS imaging, the only light that can pass through the analyser is light that has undergone multiple scattering (thus now depolarised) deep within the imaged medium. This scattered light therefore back illuminates any absorbing material in the foreground. In OPS, polarised green light is used to illuminate the tissue containing the microcirculatory area of interest.[90,268,269] Green light (wavelength 548nm) is used as this corresponds to one of the isobestic points of oxy- and deoxy-haemoglobin to ensure optimal imaging of the blood vessels of the peripheral microcirculation. This isobestic point balanced the requirements of haemoglobin absorption and scattering more effectively than is the case for other isobestic points.[90] This highlights the two fundamental features of OPS imaging, forming an image in reflected light requires both scattering for illumination and absorption for contrast. Despite this major advance in microvascular research, there are still some technical drawbacks associated with OPS imaging. Importantly imaging of the capillaries can be compromised by movement of the OPS device, the tissues and/or flowing red blood cells. During continuous flow, it is sometimes difficult to appreciate the granular nature of flowing blood

cells due to blurring. This means that computer software designed to measure flow would be unable to capture individual erythrocytes, so making calculation of flow very difficult.

A related technique termed Sidestream Darkfield Imaging (SDF) attempts to address some of these problems. In SDF imaging, illumination is provided by a series of concentrically placed light emitting diodes (LED) surrounding a central light guide, thus providing sidestream darkfield illumination. The core of the light guide contains the lens system and is thus optically isolated from the illuminating outer ring in order to prevent contamination of the image with tissue surface reflections. The light emitted is also at a wavelength of 530nm to ensure optimal absorption by haemoglobin containing red cells. To improve the imaging of moving structures, however, the LED's provide pulsed light that is in synchrony with the the video camera frame rate, thereby performing intra-vital stroboscopy. This helps to partially prevent the smearing of moving objects such as flowing red cells and the motion induced blurring of capillaries thanks to the shorter illumination time. In a recent comparison of the two techniques, validation of the new technique was confirmed by quantatitve measures of nailfold capillary diameters and red cell velocities using OPS and SDF imaging.[93] This study also developed an image quality system to quantitatively compare the quality of sublingual OPS and SDF images. Venular contrast, sharpness and quality were similar with both techniques but capillary imaging was superior with SDF imaging.[93]

Any mucosal surface can be assessed using these techniques, but the sublingual site is now preferred for assessment in the critically ill for two reasons. Firstly, the sublingual space is readily accessible. Secondly, the sublingual mucosa has the same embryological origin as the splanchnic mucosa. A number of investigators have shown that

derangements in sublingual perfusion can track dysfunctional splanchnic flow which is thought to be one of the earliest indicators of systemic hypoperfusion.[270-274] Creuteur and colleagues examined changes in sublingual and gastric CO_2 alongside sublingual microscopy in a group of patients with septic shock.[274] They found that sublingual microvascular flow tracked changes in gastric and sublingual CO_2. An interesting study by Boerma and colleagues investigated the relationship between sublingual and intestinal (newly constructed stoma) microvascular flow in a group of patients with abdominal sepsis using OPS.[99] They found on day 1 that there was no correlation between flow in the two vascular beds. By day 3, a correlation between the two vascular beds had returned (mainly due to normalisation of values). The authors concluded that this dispersion of flow was consistent with the distributive nature of septic shock.[275] A recently reported study investigating the relationship between sublingual and intestinal mucosal microcirculatory perfusion used a porcine model of abdominal sepsis specifically designed to avoid the confounder of raised intra-abdominal pressure.[276] In contrast to the previous study, these investigators found that the severity and time course of microcirculatory changes were similar in the sublingual and intestinal regions.

Various scoring systems for video analysis have been developed by different investigators. In addition several software packages are under development. Given the high variability in image analysis, a round table conference involving many of the key researchers in the field was held in 2007. The various aspects of video acquisition and analysis were discussed and Delphi methodology was used to formulate concensus statements.[277] We obtained and analysed all of our videos in accordance with these recommendations.

2.4.1 Image aquistion

Sublingual microvascular flow was evaluated using sidestream darkfield imaging with a x5 objective lens (Microscan, Microvision Medical, Amsterdam, Netherlands). Given the intrinsic variability of microvascular flow, it is important to average results from a number of sites in the organ of interest. Thus in each patient, microcirculatory videos were obtained from between three and five sublingual sites. Videos were recorded directly from the SDF imager onto a laptop computer (large LCD screen) via an analogue to digital convertor (Canopus ADVC 110). Below are the steps taken for image aquistion.

1) Place new sterile disposable cap on the SDF imager
2) Remove secretions from the sublingual mucosa using a suction catheter and saline soaked gauze
3) Position probe on the sublingual mucosal surface – once image obtained look for evidence of pressure arterfact (altered large venular blood flow). Gently withdraw camera until image about to be lost to ensure no pressure artefact.
4) Re-focus image
5) Aim to record minimum 20 second video clip

This was repeated three to five times at each timepoint and images stored on the hard drive of a laptop computer and backed up on DVD's / external hard drive.

Figure 2.15 Sidestream darkfiled imaging camera (Microscan, Microvision Medical)

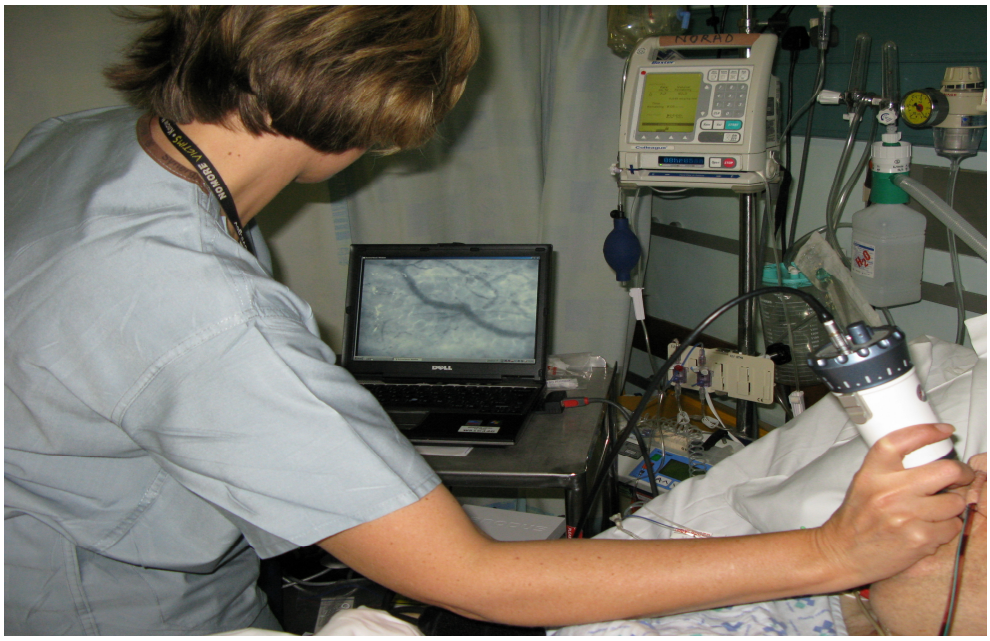


Figure 2.16 Sublingual microcirculation video assessment at the bedside

2.4.2 Image analysis

According to concensus conference guidelines,[277] it is important to differentiate capillaries from venules because the former contribute predominantly to organ perfusion and are therefore the vessel of interest. It is in practice difficult to separate venules from capillaries, but guidelines suggest an arbitary cut-off value of 20 µm diameter, recognising that vessel size can vary. The main interest of the larger vessels is as a quality control measure to ensure that excessive pressure was not used in obtaining the videos. In larger venules, rolling and adherent leucocytes can be visualised but, higher magnifications are needed, which often results in movement artefact.

A number of semi-quantative scoring sytems exist to measure capillary density and flow. All the described measures were used in all studies to evaluate the sublingual microcirculation.

1) Microvascular Flow Index (MFI) was calculated after dividing each image into four equal quadrants (figure 2.17). Quantification of flow was achieved using an ordinal scale (0: no flow, 1: intermittent flow, 2: sluggish flow, 3: normal flow) for small (<20µm) and large (>20µm) vessels. MFI is the average score of all quadrants for a given category of vessel size at a given time point. Images were recorded at a minimum of three sites at each time point giving a total of at least twelve quadrants for analysis.

2) Vessel density is calculated by inserting a grid of three equidistant horizontal and three equidistant vertical lines over the image (Figure 2.18). Vessel density is equal to the number of vessels crossing these lines divided by their total length.

3) Flow is categorised as present, intermittent or absent to calculate the proportion of perfused vessels and thus the perfused vessel density.

4) The heterogeneity index was introduced by Trzeciak and colleagues.[95] This calculation involves evaluating the MFI in three to five sites and taking the difference between the highest MFI and lowest MFI in any quadrant and dividing by the mean MFI of all sublingual sites at the same timepoint.

In each study, two observers evaluated the microcirculation videos. Inter-observer variability for analysis of the SDF images was assessed by calculating the Kappa coefficient (κ).[278]

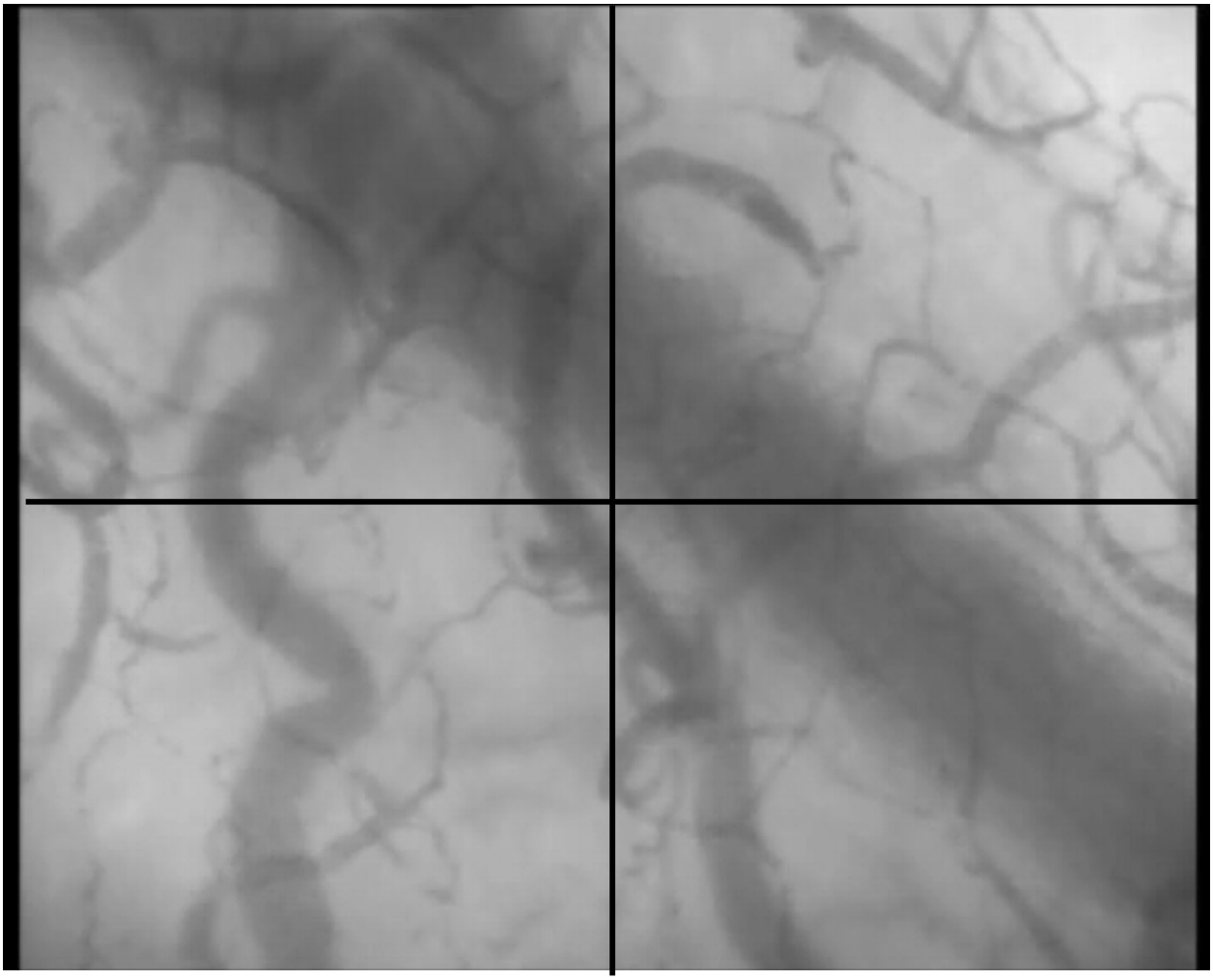


Figure 2.17 Calculating Microvascular Flow Index (MFI). **Video in quadrants for analysis of MFI**

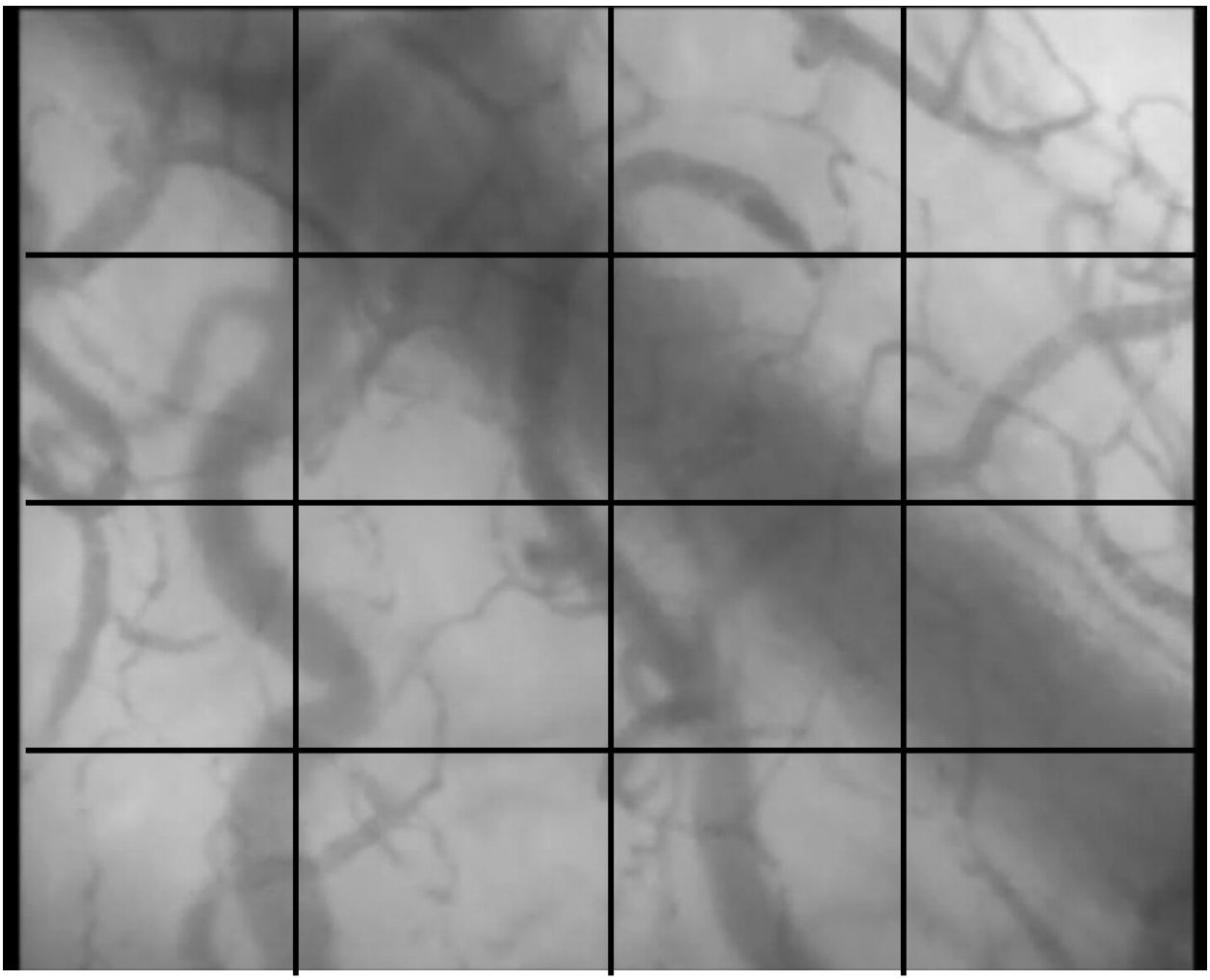


Figure 2.18 Calculating vessel density. Three equidistant horizontal and vertical lines overlying video for calculation of vessel density, proportion of perfused vessels and perfused vessel density.

2.5 Serum and urine inflammatory markers

Serum samples were taken at four timepoints in the randomised controlled trial of GDHT (see chapter 5). 4 ml of blood was collected from the patients arterial catheter after induction of anaesthesia but before surgery, at the end of surgery (hour 0), at the end of the eight hour intervention period (hour 8) and 24 hours following the end of surgery (hour 24). Samples were collected into serum gel separator tubes (BD Biosciences) and left for

30-45 minutes at room temperature to ensure adequate clotting. The samples were subsequently centrifuged (Clinispin Horizon 853VES [Woodley Equipment Co Ltd]) at 3000g for 10 minutes. The serum was then aliquoted into four eppendorf tubes (approx 0.5ml) and immediately frozen at -80℃ until required for analysis.

The measurements of Interleukin (Il) 1-beta, Il-6, Il-8, Tumour Necrosis Factor-alpha (TNF-α) and intercellular adhesion molecule -1 (ICAM-1) were performed using the Mesoscale Discovery Platform by SJ. This Multi-array ® and Multi-spot ® technology allows the detection of biomarkers in single and multiplex formats. The technique uses electrochemiluminescence detection using a trademarked molecule they term Sulfo-Tag ™ (Ruthenium [II] tris-bipyridine-[4-mehylsulphonate] NHS ester). This label emits light upon electrochemical stimulation at the the electrode surfaces of multi-array ® microplates. Advantages of this electrochemiluminescence technique are :

1) minimal background signals as the stimulation mechanism (electricity) is decoupled from the signal (light) in contrast to more traditional ELISA techniques
2) emission at 620nm eliminates problems with colour quenching
3) multiple excitation cycles of the label result in signal amplification and thus enhance light levels and improve sensitivity

A 4-spot multiplex kit was used to measure Il-1beta, Il-6, Il-8 and TNFalpha. A single kit was used to measure changes in soluble ICAM-1. In brief, both protocols involved the following steps with variable incubation periods:

1) Add Diluent / blocker to plate to prevent binding of analyte to any part of plate except capture antibody

2) Add calibrator (after serial dilution to produce normal curve) and sample in duplicate to wells according to pre-designed 'map' of 96 well plate
3) Wash and add Sulfo-Taf Antibody to plates
4) Wash and add read buffer
5) Plates can be read on Sector 2400 (Mesoscale Discovery)

The use of multiplex kits has been investigated with initial reports suggesting acceptable accuracy and precision.[279]

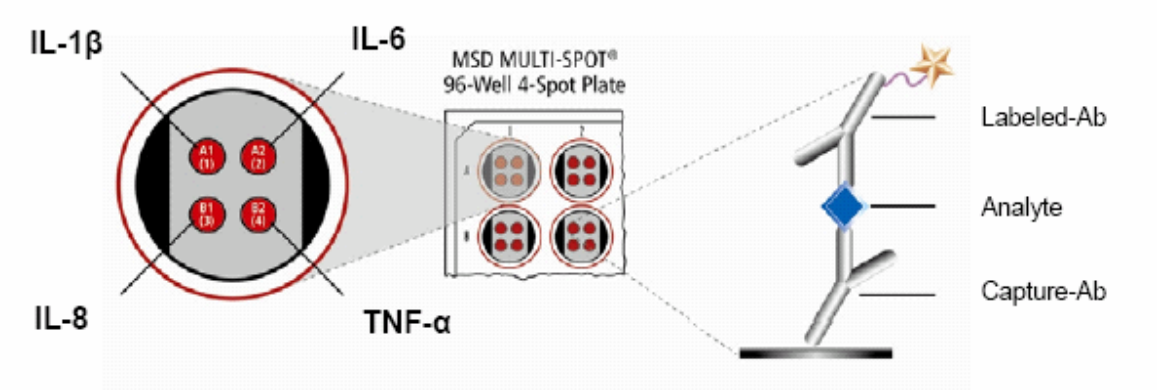


Figure 2.19 Multiplex kit for cytokine analysis. Reproduced with permission from Meso Scale Discovery.

2.6 Microalbuminuria

Urine was collected aseptically into polypropylene test tubes from the urinary catheter of patients recruited to the GDHT trial. Urine samples were taken at four timepoints. T0 was after induction of anaesthesia but before surgery commenced. T1 was following surgery on admission to the critical care unit before the intervention commenced. T2 was at the end of

the eight hour intervention period. T3 was 24 hours following the end of surgery. Samples were placed in a -80 freezer within 15 minutes. Microalbuminuria was measured using immunoturbidimetric analysis with a Cobas tina-quant albumin reagent on the Roche / Hitachi modular P system.

Chapter 3
Mortality and utilisation of critical care resources amongst high-risk surgical patients in a large NHS trust

3.1 Introduction

In a recent UK study of 4.1 million selected non-cardiac surgical procedures, a high-risk surgical population which accounted for only 12.5% of in-patient surgical procedures but more than 80% of post-operative deaths was identified.[5] This study confirmed the suspicion that low overall post-operative mortality rates conceal the existence of a large sub-population of patients at much greater risk of post-operative complications and death. Another important finding of this analysis was that fewer than 15% of high-risk surgical patients were admitted to a critical care unit at any time following surgery. The small number of patients who did receive this level of care were discharged after a median of only 24 hours and subsequently lingered for many days on standard surgical wards (median [IQR] stay 16 [10-30] days). However, in this study more detailed analysis of critical care utilisation was not possible because not every critical care unit in the participating centres contributed data to the Intensive Care National Audit & Research Centre (ICNARC) database.

Recent clinical trials suggest that substantial improvements in outcome may be achieved for high-risk surgical patients through the use of protocolised cardio-respiratory therapy delivered in a critical care setting.[48,280] However, continued debate over both the size of the high-risk surgical population and the potential benefit of admitting such patients to

critical care represent important obstacles to the introduction of such approaches into routine practice.

This study was designed to examine in more detail the role of critical care in the management of high-risk surgical patients in the United Kingdom (UK). We used methods similar to those employed in the previous National audit, but conducted the present study in a single institution, so that individual patient data could be cross-referenced using additional local databases and, where necessary, hospital notes. This analysis also provided an opportunity to confirm or refute the existence of a definable population of high-risk surgical patients within the local surgical population prior to further work investigating the microvascular changes associated with surgery. The aim of this study was to identify patients undergoing selected high-risk non-cardiac surgical procedures within a large NHS Trust and describe critical care resource provision and utilisation for this population in relation to outcome.

3.2 Methods

The study was performed in a large NHS Trust incorporating two teaching hospitals which between them provide all major secondary and tertiary surgical services. This service evaluation was conducted with the approval of the local audit committee. One hospital has 114 surgical beds with a thirteen bed intensive care unit that provides post-operative care for cardiac and other surgical patients as well as medical intensive care for oncology and HIV positive patients. The other hospital has 214 surgical beds with a sixteen bed intensive care unit that provides care for medical, non-cardiac surgical and neurosurgical patients and a six bed high dependency unit providing care for non-cardiac surgical

patients including a large number of patients admitted following traumatic injury. Using an analogous design to the previous national study [5], data was extracted from two databases. The local Hospital Episodes Statistics (HES) database is maintained by clerical coding staff and includes data on all clinical activity within the Trust.[281] Validation is performed locally by Trust information managers. The intensive care audit database is maintained locally by dedicated intensive care audit staff and contributes data to the ICNARC case mix programme. These data are subject to local and central internal error checks.[282]

Data were extracted on all adult (age ≥18 years) surgical admissions to hospital and to critical care between April 1st 2002 and March 31st 2005. All data relating to length of stay were calculated by subtracting the discharge date from the admission date. Surgical specialities with highly developed treatment pathways (often including critical care) such as cardiothoracic surgery and neurosurgery were excluded. Surgical specialities analysed included general and vascular surgery, orthopaedics, gynaecology, ear nose and throat, plastic surgery, urology, maxillofacial and oral surgery. Admissions involving endoscopy, day case surgery, cardio-thoracic surgery, neurosurgery, organ transplantation, obstetrics or the surgical management of burns were excluded. There are 6,920 surgical procedure codes in the Office of Population, Censuses and Surveys (OPCS) classification.[283] Surgical admissions to hospital were identified in the HES database by the presence of one of 4,910 codes that satisfied the inclusion criteria. Where more than one surgical procedure was performed during the same hospital admission, only the first procedure was included in the analysis. Several alternative OPCS codes may exist for any given procedure. In order to reduce bias arising from discrepancies in the coding process, procedures were categorised into elective (includes elective and scheduled cases according to ICNARC definition) and non-elective (includes urgent and emergency cases

according to ICNARC definition). Healthcare Resource Groups (HRG) are based on clinical similarity and resource homogeneity.[283] The codes may specify the presence of a complicating medical condition, complexity of surgery or a particular age group. HRGs were then ranked according to mortality rates. High-risk surgical procedures were prospectively defined as those procedures included in a HRG with a mortality rate of 5% or more. The remaining procedures were classified as standard risk. Surgical admissions to critical care units were identified in the ICNARC database by source of admission, and were only included if the primary reason for admission was not an excluded surgical procedure. Critical care admissions were prospectively divided into admissions directly from the operating theatre and admissions to critical care following a period of post-operative care on a standard ward. Data describing critical care resource use was verified through a manual check of individual patient details using the ICNARC database.

Data are presented as median [IQR]. Categorical data were tested with Fisher's exact test and continuous data with the unpaired t test with Welch's correction. Analysis was performed using GraphPad Prism version 4.0 (GraphPad Software, San Diego, USA). Significance was set at $p < 0.05$.

3.3 Results

3.3.1 Hospital admissions

During the 36 months of the study, there were 26,051 hospital admissions involving one of the selected non-cardiac surgical procedures, with 390 (1.5%) deaths. The median age was 46 [32-63] years and 13,169 (50.6%) patients were male. There were 16,294 elective surgical admissions with 65 (0.4%) deaths and 9,757 non-elective surgical admissions with 325 (3.3%) deaths in hospital. The duration of hospital stay was shorter for elective admissions when compared to non-elective admission (3 [2-7] days vs. 4 [2-12] days; $p<0.0001$).

Out of 427 HRGs, 69 were associated with a mortality rate of 5% or greater. From these, 2,414 (approximately 800 per annum) individual high-risk surgical procedures were identified with a total of 294 deaths (12.2%) (Figures 3.1 and 3.2). The high-risk surgical population accounted for 75.4% of deaths in hospital but only 9.3% of admissions. Complex or major surgery, advanced age, the presence of a complicating medical condition or a combination of these was specified by 40 (58%) of the 69 high-risk HRG codes compared to 119 (33%) of 358 standard-risk HRG codes. Although less than 10% of patients were classified as high-risk, this population utilised 46,138 in-patient bed days (23% of total in-patient bed days).

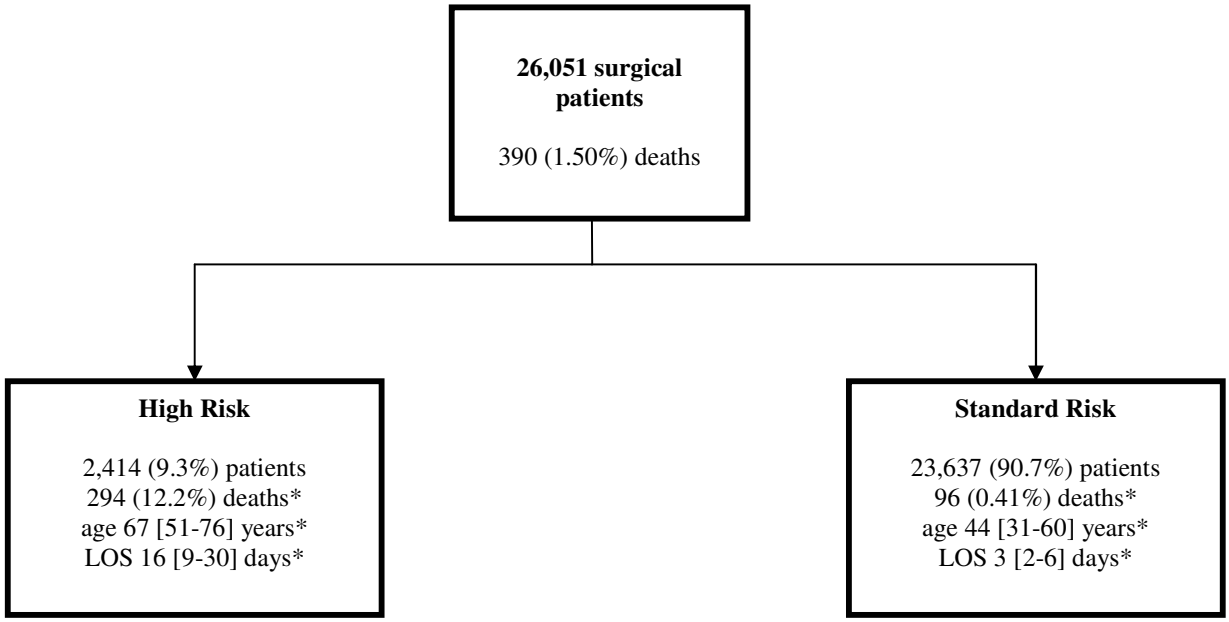


Figure 3.1 Standard and high risk populations of patients undergoing selected non-cardiac surgical procedures. Data presented as median [IQR] or absolute values (%). LOS: length of hospital stay. * denotes p<0.0001 between standard and high risk groups

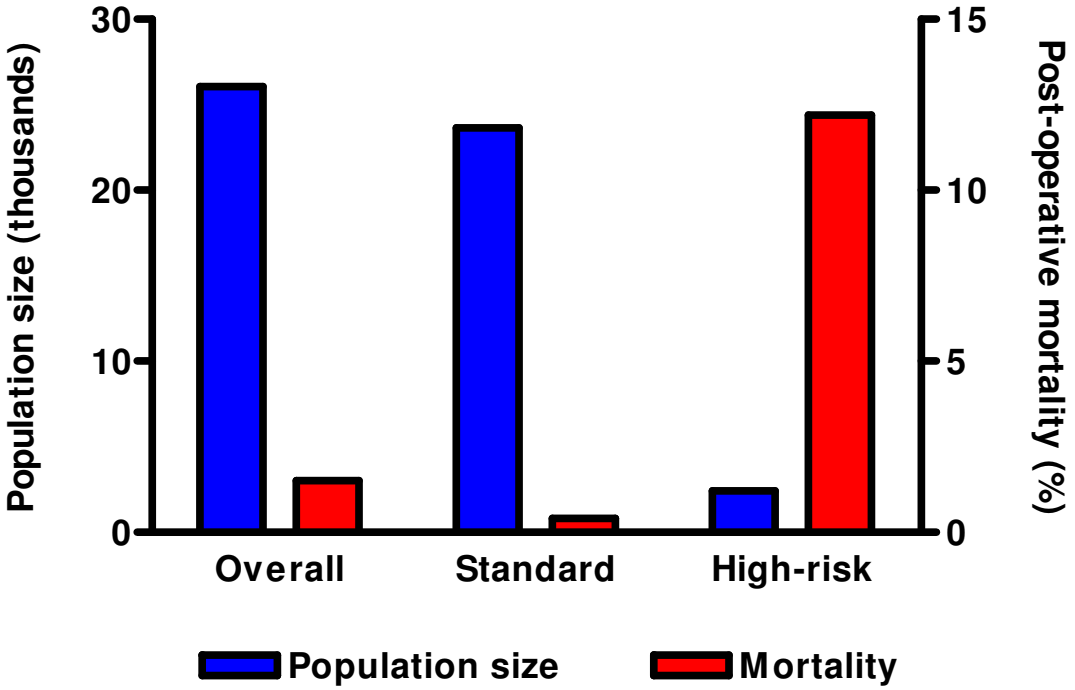


Figure 3.2 Population size and mortality rates for standard and high-risk general surgical patients. **Standard-risk: patients undergoing a procedure with an overall mortality rate of less than 5%. High-risk: patients undergoing a procedure with an overall mortality rate of 5% or more.**

3.3.2 Critical Care admissions

There were 1,627 surgical admissions to critical care which met the inclusion criteria with 297 deaths (18.3%). Of these patients, 1,470 (90.4%) were admitted directly to critical care from the operating theatre. 214 patients were discharged prematurely from critical care because of bed shortages (defined according to ICNARC criteria) with 32 deaths (15.0%). Of the 297 patients who were admitted to critical care (either immediately postoperatively or subsequently) and later died, 188 (63.3%) did so after initial discharge from critical care. 61 patients who were discharged from critical care were subsequently readmitted, of whom 23 (37.7%) died. 157 patients were admitted to critical care following an initial period of post-operative care on a standard ward, of whom 47 patients died (29.9%). The median duration of standard ward care for these patients was 2 [1-3] days for elective patients and 2 [1-7] days for emergency patients. 775 surgical patients classified by our criteria as standard risk were admitted to a critical care facility postoperatively (Figure 3.3). The overall mortality rate for these patients was 4.5%. Within this was a sub-group who were admitted to critical care after a period on a standard ward of whom 29 (21.2%) died; the duration of standard ward care for this sub-group was 2 [1-5] days.

3.3.2.1 Critical Care resource utilisation by the high-risk surgical population

Patients were classified as high-risk on the basis that they underwent a procedure with an overall mortality rate of 5% or greater. Only 852 of these high-risk patients were admitted to a critical care unit at any stage during their hospital admission (35.3% of the overall high-risk population) (Figure 3.4 and 3.5). Of the 294 high-risk patients who died, the median time from hospital admission to the first surgical intervention was 2 [1-3] days for elective patients and 3 [1-11] days for non-elective patients. The median time between

hospital admission and death was 16 [6-32] days for elective patients and 22 [7-43] days for non-elective patients. For those admitted to critical care, the median time between hospital admission and death was 28 [12-48] days compared to 15 [3-34] days for those not admitted to critical care. Only 144 (49.0%) high-risk patients who died were admitted to a critical care area at any stage during their admission and only 75 (25.6%) of these deaths occurred in a critical care area.

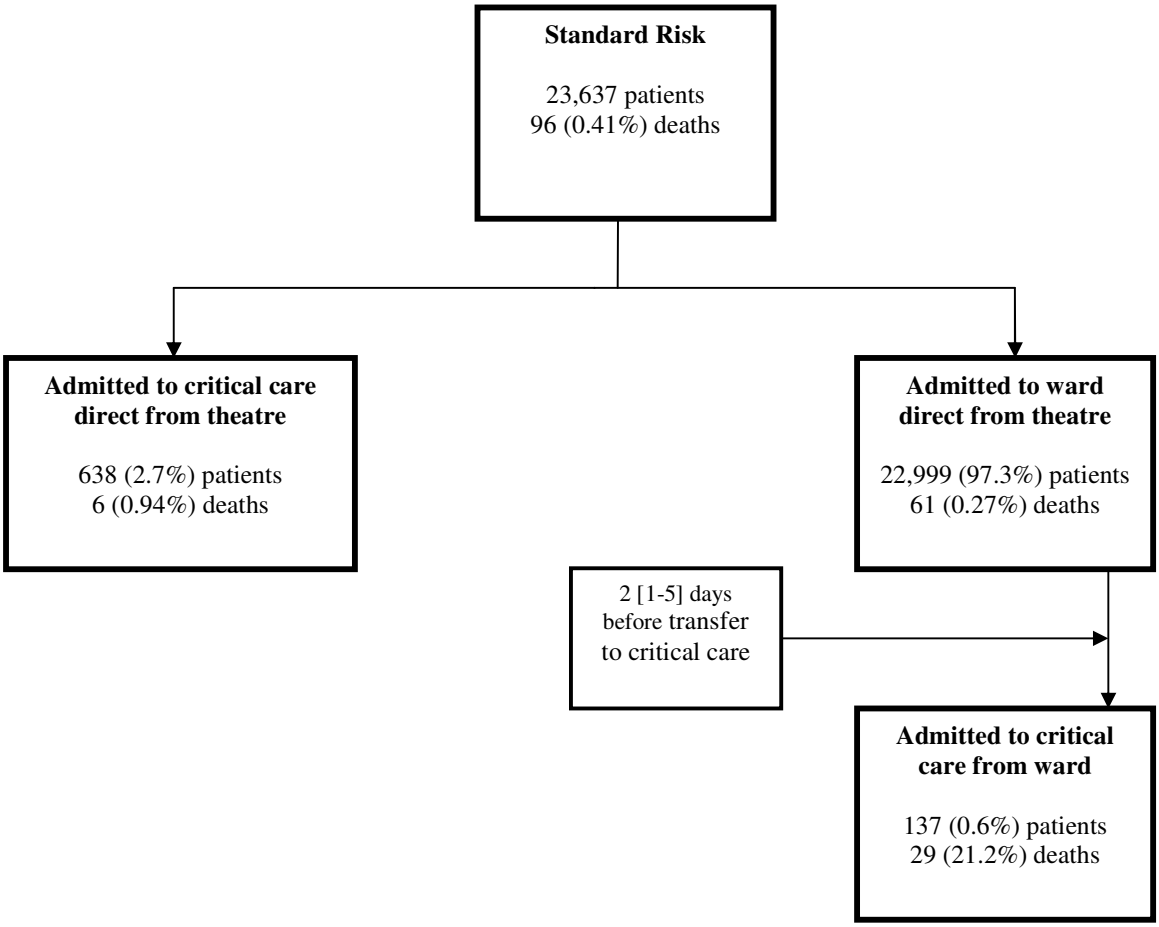


Figure 3.3. Sub-groups of the standard risk population of surgical patients. **Data presented as median [IQR] or absolute values (%).**

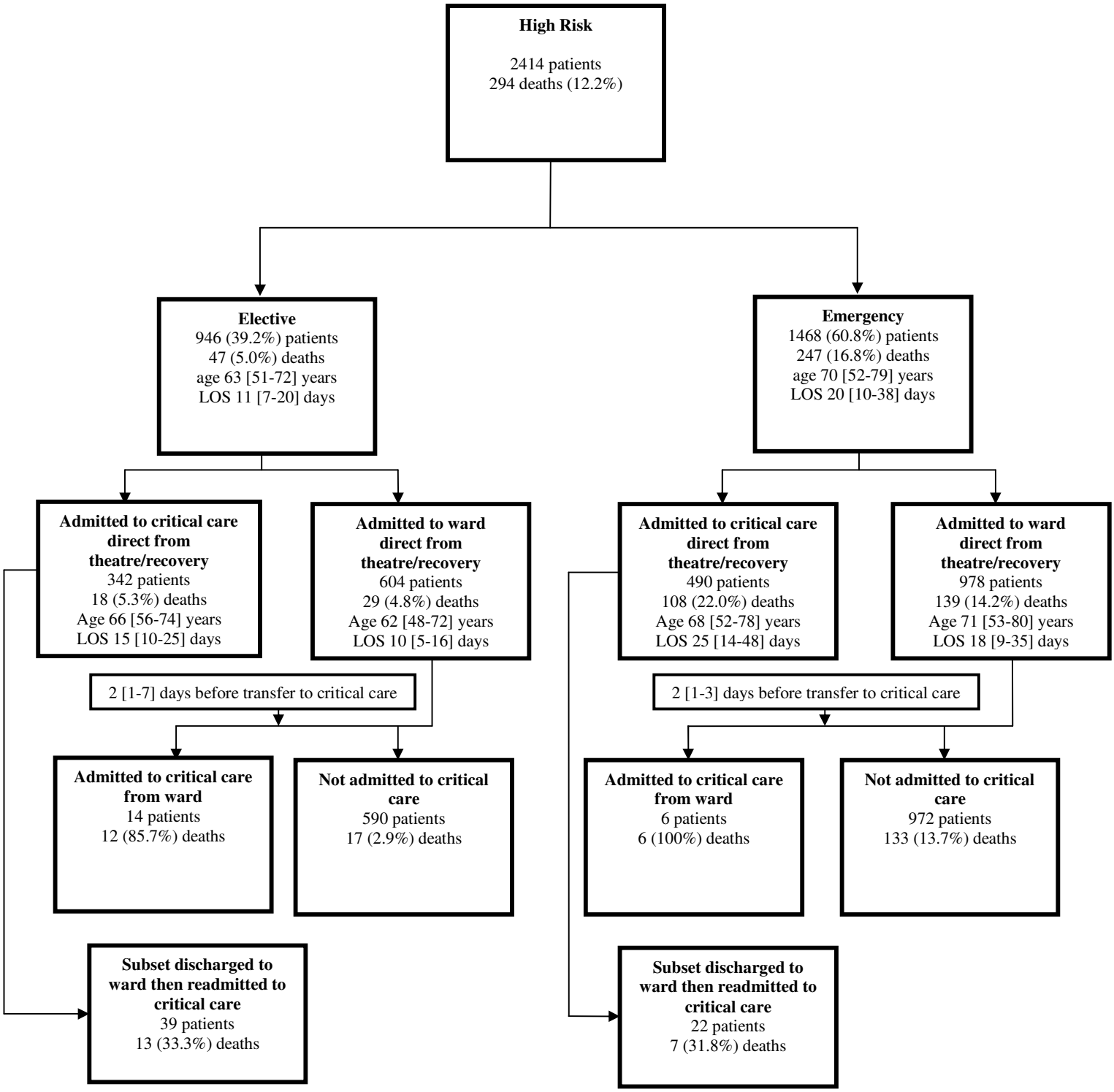


Figure 3.4 Sub-groups of the high-risk population of surgical patients. Data presented as median [IQR] or absolute values (%). LOS; length of hospital stay.

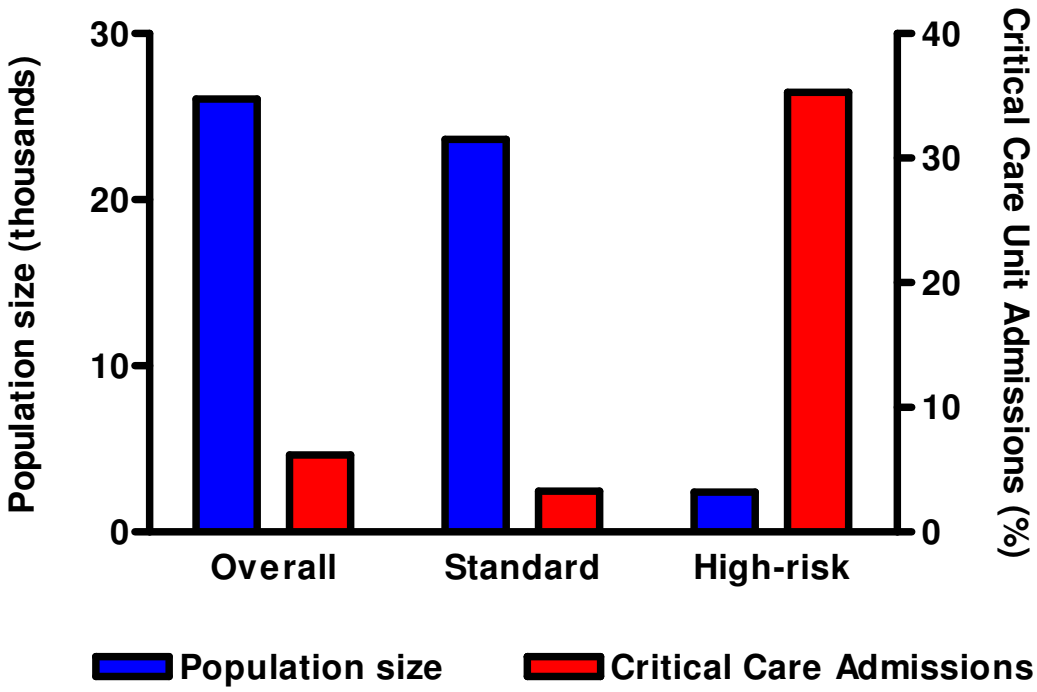


Figure 3.5 Number of patients from each population who were admitted to a critical care unit at any point following surgery. Standard-risk: patients undergoing a procedure with an overall mortality rate of less than 5%. High-risk: patients undergoing a procedure with an overall mortality rate of 5% or more.

3.4 Discussion

The principal finding of this study is that, despite having an overall mortality of 12% and accounting for over 70% of postoperative deaths, only one third of high-risk surgical patients were admitted to critical care at any stage following surgery. Our findings suggest that the failure to admit such patients to critical care immediately after surgery contributes to the poor outcomes for the high-risk group as a whole. Premature discharge from, and the need to re-admit patients to critical care were both factors associated with a particularly high mortality rate. Similarly, mortality was high amongst the small number of patients admitted to intensive care following an initial period of postoperative care on a standard ward (30%). The median duration between surgery and critical care admission for this group was only two days. It seems likely that outcomes would be improved for these patients if they were admitted to critical care immediately after surgery to receive protocolised cardio-respiratory care. This approach seems to be very effective following cardiac surgery and provides the opportunity for use of additional interventions which may improve survival still further.[48,280] Finally only 49% of the total population of high risk surgical patients who died were admitted to a critical care facility at any stage and only 26% died in a critical care area. These observations indicate a failure to provide an appropriate level of care even to the highest risk patients.

Whilst the number of high-risk patients admitted to a critical care unit in our institution (35%) compares favourably with the national study (15%),[5] the findings of both studies clearly indicate that this resource is underprovided and/or underutilised. This conclusion is supported by the observation that postoperative critical care admission is routine for cardiac surgical patients, who have a high incidence of co-morbid disease and yet

undergo major surgery with an overall mortality rate in the UK of only 3.5%.[284] Although in many instances limited resources prevent admission of high-risk surgical patients to critical care, it is unclear whether the lack of critical care facilities is compounded by clinicians' underestimation of risk or doubts over the potential benefits of this facility. Whilst the mortality rate for standard risk patients admitted to critical care was significant (4.5%), suggesting clinicians are able to identify patients at greater risk, it is interesting to note that most such patients underwent elective surgery whilst our data suggest that it is the emergency surgical population that is in greatest need of critical care admission. This raises the possibility that involvement of a more senior clinician may be a key factor in the decision to admit a patient to critical care following surgery.

The findings of this single centre study are consistent with published data describing the high-risk surgical population.[7,8,285] The size and nature of the high-risk population in our institution is similar to that identified in the national UK study and although it might appear that a greater proportion of high risk surgical patients are admitted to a critical care area in our Trust, this observation may also be explained by an underestimate of critical care admissions in the national study.[5] In keeping with the current study, reports from the National Confidential Enquiry into Peri-operative Deaths (NCEPOD) also describe advanced age, co-morbid disease, major and urgent surgery as the key characteristics of the high-risk patient.[3,4] The high mortality rates and prolonged hospital stays described for high-risk patients in this study are also consistent with published data from Europe and North America.[6,8]

The use of healthcare databases to test clinical hypotheses is associated with a number of important limitations. In particular, HES data may underestimate mortality rates because

the coding process is not designed to capture detailed mortality data.[4,286] In the current analysis cross-referencing data describing individual patients between the two databases has allowed us to minimise this source of error. Ideally, prospective studies should be performed to evaluate this issue in more detail. Such a study would allow identification of the reasons underlying decisions regarding the allocation of critical care resources to individual patients.

This study confirms that the high-risk surgical population accounts for the majority of post-operative complications and deaths. Only around one third of such patients are admitted to a critical care unit at any stage after surgery. Where critical care admission does occur it is often for short periods, whilst the duration of hospital stay is prolonged. The allocation of additional critical care facilities to this population is likely to be a cost effective means of improving both short and long-term outcomes.

Chapter 4
Microvascular flow and tissue oxygenation after major abdominal surgery: association with post-operative complications

4.1 Introduction

It is estimated that 234 million major surgical procedures are performed worldwide each year.[1] Complications following major surgery are a leading cause of morbidity and mortality.[3,4] High-risk surgical patients account for over 80% of deaths but only 12.5% of in-patient surgical procedures.[5] In the UK, around 170,000 patients undergo high-risk surgical procedures each year, of whom 25,000 patients die and as many as 100,000 develop complications.[5] Even for those patients who survive to leave hospital, post-operative complications remain an important determinant of long-term survival.[8] It is essential, therefore, that efforts are made to improve outcomes following major surgery.

The aetiology of post-operative complications is complex. Risk factors include advanced age, co-morbid disease and major and urgent surgery.[5] A number of reports describe an association between derangements in global oxygen delivery (DO_2I) or related parameters and poor outcome following major surgery.[32,33,287] In several small studies, the use of fluid and inotropic therapy to enhance global oxygen delivery has led to a reduction in post-operative complications.[39,43,48] It has been suggested that the benefits of this approach are a result of improved tissue perfusion and oxygenation. Whilst unconfirmed, this hypothesis is consistent with the findings of a number of previous investigations. Surgical wound

infections are associated with reductions in tissue PO_2 [178] and may be prevented by the early use of supplemental inspired oxygen.[170,179] The use of warming blankets to prevent peri-operative hypothermia may also reduce the incidence of wound infection, suggesting a pathophysiological process related to tissue perfusion.[169,288] The findings of several studies suggest that reductions in microvascular blood flow during gastro-intestinal surgery may be associated with impaired anastomotic healing.[96,100,162] However, a more detailed knowledge of the relationship between changes in global determinants of oxygen delivery and regional measures of tissue perfusion and oxygenation is required. The objective of this study was to describe the relationship between global oxygen delivery, tissue microvascular flow and oxygenation in high-risk patients receiving usual care who did and did not develop complications following major abdominal surgery.

4.2 Methods

The study was approved by the Local Research Ethics Committee. Written informed consent was obtained prior to surgery. Patients admitted to a critical care unit following major elective abdominal surgery were eligible for recruitment. Exclusion criteria were refusal of consent, concurrent lithium therapy, acute myocardial ischaemia, acute arrhythmias, pregnancy, patients receiving palliative treatment only and weight less than 40 kg. Patients were enrolled when a member of the research team was available. General anaesthesia was standardised to include intravenous fentanyl, propofol and atracurium for induction of anaesthesia and maintenance with inhaled isoflurane in oxygen enriched air alongside epidural analgesia. Clinical staff administered intravenous fluids, blood products and, if required, vasoactive drugs in order to maintain pulse rate, arterial pressure, central venous pressure, urine output, haematological and biochemical parameters within the normal range. Appropriate measures were taken to maintain $SpO_2 \geq$ 94%, haemoglobin above 8 g dl^{-1} and temperature at 37 °C. Following surgery, all patients were managed in a critical care unit. Additional fluid challenges and inotropic therapy were prescribed by clinical staff. Cardiac output data were made available when specifically requested by clinical staff. Patients did not, however, receive specific flow guided haemodynamic therapy in order to attain pre-determined values for stroke volume, cardiac index or global oxygen delivery.

4.2.1 Measurements

In addition to heart rate, mean arterial pressure and central venous pressure, cardiac output and DO_2I were measured by lithium indicator dilution and arterial waveform analysis (LiDCOplus, LiDCO Ltd, Cambridge, UK).[239] Arterial and central venous blood samples

were taken for analysis of blood gases, lactate and haemoglobin concentration (ABL 600 and OSM3, Radiometer, Copenhagen, Denmark). Cutaneous tissue PtO_2 was measured at two sites on the abdominal wall using a Clark electrode (TCM400, Radiometer, Copenhagen, Denmark). These probes are designed to warm the skin to 44 ℃ in order to minimise artefact due to local vasoconstriction. Cutaneous red blood cell flux was measured at two sites on the abdominal wall by laser Doppler flowmetry (Moorlab, Moor Instruments, Axminster, UK). Sublingual microvascular flow was evaluated using sidestream darkfield imaging with a x5 objective lens (Microscan, Microvision Medical, Amsterdam, Netherlands).[59] Microvascular and tissue oxygenation data were obtained on the ward prior to surgery and all data were collected at two hour intervals after surgery for eight hours. Hour 0 refers to the time of admission to the critical care unit. Image acquisition and subsequent analysis was performed as described in Chapter 2. Analysis of the videos was performed by two observers (SJ and CL). The observers were blinded to all outcome and physiological data. Patients were followed up for 28 day in-hospital post-operative complications (supplementary file), mortality, duration of hospital stay and the number of days patients were alive and free from critical in the first 28 days following admission. The major categories of complications were infectious, respiratory, cardiovascular, renal, neurological and gastrointestinal.

4.2.2 Statistical Analysis

For evaluating changes over time between groups, two-way repeated measures analysis of variance (ANOVA) was used. Post hoc t-tests were performed at the different time points using the Bonferroni correction for multiple testing. Analysis was performed using GraphPad Prism version 4.0 (GraphPad Software, San Diego, USA). Significance was set

at $p < 0.05$. The normality of data was evaulated using the D'Agostino-Pearson test. Data are presented as mean (SD) where normally distributed or median (IQR) where not normally distributed. Inter-observer variability for analysis of the SDF images was assessed by calculating the Kappa coefficient (κ).[278] We did not perform a sample size calculation based on any single variable and instead elected prospectively to recruit 25 patients.

4.3 Results

Twenty five patients were recruited with a median age of 69 years (63-72). Patient characteristics, peri-operative haemodynamic management and outcome data are summarised in Tables 4.1 to 4.3. Two patients were mechanically ventilated after surgery. All other patients were extubated prior to critical care admission. Two patients died (8%) and 14 patients (56%) developed post-operative complications. The median time to first complication was 3 (2-6) days. Ten patients (40%) developed infectious complications (Table 4.4). Critical care ($p<0.01$) and hospital stay ($p<0.001$) were significantly longer in those patients who developed complications. There were no differences in PtO_2, DO_2I, $ScvO_2$, cardiac index, heart rate, mean arterial pressure, serum lactate or cutaneous red cell flux between patients who did and did not develop complications. There was a small statistically significant difference in base deficit (Figure 4.1, 4.2 and 4.4 and Tables 4.5 and 4.6). The inspired fractional concentration of oxygen after surgery at all time points was 0.4 (0.4-0.4).

There was a significant difference in sublingual microvascular flow index (MFI) in small vessels (<20µm) after surgery between those patients who subsequently developed complications and those who did not (Figure 4.3 and Table 4.6). MFI for large vessels (>20µm) remained normal in all patients throughout the study period. Neither the total vessel density nor the small vessel (<20µm) density was significantly different between these groups. The proportion of perfused small vessels (<20µm) was significantly lower after surgery in those patients who developed complications. Perfused vessel density, a measure of functional capillary density, was also lower after surgery in patients who developed complications (Table 4.6). The density and proportion of perfused large vessels

(>20µm) were not significantly different between groups. We were only able to collect sublingual microvascular images prior to surgery in 14 patients. Despite this, MFI for small vessels (<20µm) was reduced in those patients who subsequently developed complications compared to those who did not (2.6 [2.5-2.9] vs 3.0 [3.0-3.0]; $p<0.05$). Large vessel MFI (>20µm) was 3.0 (3.0-3.0) in both groups. The kappa coefficient for inter-observer reliability was 0.81 (95%CI 0.72-0.89). In one patient, it was not possible to obtain any SDF images due to painful oral mucositis related to chemotherapy.

	No complications (n=11)	Complications (n=14)
Age (years)	66 (63-72)	69 (64-73)
Gender (%)	5 males (36%)	6 males (43%)
P-POSSUM score	35 (31-37)	37.5 (33-43)
History of ischaemic heart disease	7 (64%)	8 (57%)
History of diabetes mellitus	4 (36%)	4 (29%)
Upper gastrointestinal surgery (%)	4 (36%)	4 (29%)
Pancreatic surgery (%)	3 (27%)	5 (36%)
Lower gastrointestinal surgery (%)	3 (27%)	5 (36%)
Open abdominal aneurysm repair (%)	1 (9%)	0 (0%)

Table 4.1 Demographic characteristics of patients who did and did not develop complications after surgery. Data presented as median (IQR) or absolute values (%). P-POSSUM: Physiological and operative severity score for the enumeration of mortality score.

	No complications (n=11)	Complications (n=14)
Intra-venous crystalloid during surgery (ml)	3750 (2125-4750)	4000 (2625-5375)
Intravenous collod during surgery	500 (0-1000)	1000 (1000-1000)
Total intravenous fluid during surgery (ml)	4500 (3250-5000)	5375 (3500-6500)
Intravenous crystalloid during study period (ml)	1000 (1000-1150)	1050 (844-1206)
Intravenous colloid during study period (ml)	0 (0-500)	500 (0-1000)
Total intravenous fluid during study period (ml)	1250 (1000-1650)	1250 (1100-2200)
Number of patients receiving vasopressor therapy (%)	2 (18%)	4 (29%)
Number of patients receiving epidural analgesia (%)	10 (91%)	13 (93%)

Table 4.2 Data describing intra- and post-operative management for patients who did and did not develop complications after surgery. Data presented as median (IQR) or absolute values (%).

	No complications (n=11)	Complications (n=14)
28 day alive and critical care free days	27 (26-27)	24 (22-26)*
Duration of hospital stay (days)	8 (8-9.5)	20 (14-26)**
Mortality (%)	0 (0%)	2 (14.3%)

Table 4.3 Outcome data for patients who did and did not develop complications after surgery. Data presented as median (IQR) or absolute values (%). *p<0.01, **p<0.001.

Primary Complication	Number of patients
Infectious	10
Cardiovascular	1
Respiratory	1
Gastrointestinal	2
Neurological	0

Table 4.4. Frequency of complications following surgery. Fourteen patients developed complications in total.

		Hour 0	Hour 2	Hour 4	Hour 6	Hour 8
Heart rate (bpm)	**Complications**	70 (15)	75 (19)	73 (15)	73 (15)	66 (5)
	No complications	78 (17)	76 (17)	73 (16)	74 (15)	75 (15)
MAP (mmHg)	**Complications**	77 (13)	79 (13)	76 (17)	74 (10)	75 (12)
	No complications	89 (15)	78 (14)	79 (17)	78 (17)	75 (16)
CVP (mmHg)	**Complications**	7 (4)	7 (4)	6 (2)	8 (7)	9 (6)
	No complications	7 (3)	6 (4)	7 (5)	6 (4)	6 (3)
Cardiac index ($l\ min^{-1}\ m^{-2}$)	**Complications**	2.9 (0.9)	3.0 (0.9)	2.9 (0.9)	2.9 (0.8)	2.7 (0.7)
	No complications	2.8 (0.7)	2.9 (0.8)	2.8 (0.8)	2.7 (0.9)	2.8 (0.9)
DO_2I ($ml\ min^{-1}\ m^{-2}$)	**Complications**	373 (102)	394 (103)	379 (98)	384 (124)	345 (87)
	No complications	361 (55)	379 (106)	361 (104)	356 (115)	369 (111)
$ScvO_2$ (%)	**Complications**	71 (11)	73 (9)	73 (9)	72 (9)	68 (7)
	No complications	68 (9)	70 (8)	68 (6)	69 (8)	72 (7)
Serum lactate ($mmol\ l^{-1}$)	**Complications**	1.2 (0.9-2.0)	1.2 (0.9-2.0)	1.2 (0.8-2.1)	1.3 (0.9-2.5)	1.3 (0.8-1.4)
	No complications	1.4 (1.2-1.7)	1.0 (0.9-1.2)	1.1 (0.9-1.2)	1.0 (0.8-1.6)	1.1 (1.0-1.7)
Base deficit ($mmol\ l^{-1}$)	**Complications***	2.6 (2.6)	3.3 (3.1)	3.0 (3.3)	3.8 (4.6)	2.3 (1.5)
	No complications	2.1 (2.7)	1.6 (1.9)	1.0 (2.1)	1.1 (2.1)	0.9 (2.3)

Table 4.5 Cardiovascular physiology during eight hour study period. **Data presented as mean (SD) or median (IQR). MAP: mean arterial pressure; CVP: central venous pressure; DO_2I: oxygen delivery index; $ScvO_2$: central venous oxygen saturation. * denotes $p<0.01$ between groups over time (two-way repeated measures ANOVA).**

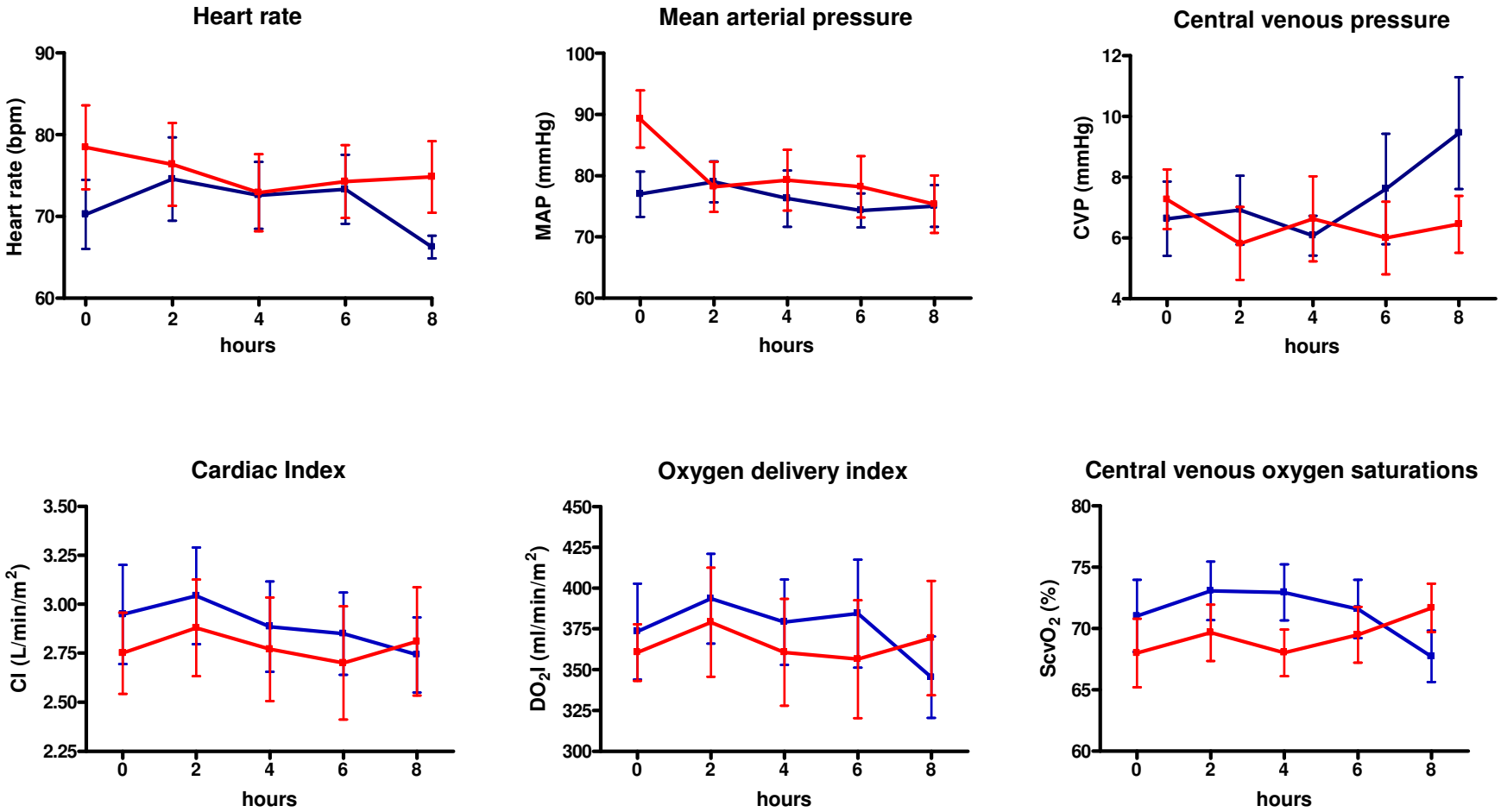


Figure 4.1. Global haemodynamics during eight hour study period. Data presented as mean (SEM). MAP: mean arterial pressure; CVP: central venous pressure. No significant difference between groups (two way repeated measures ANOVA).
Blue : complications, **Red** : no complications.

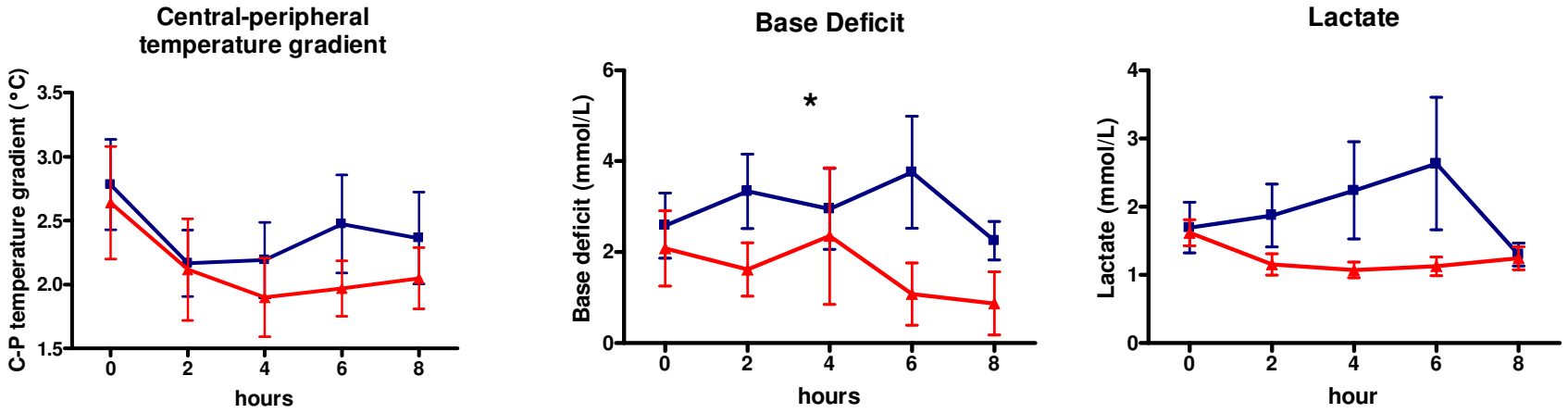


Figure 4.2. Markers of tissue perfusion during eight hour study period. Data presented as mean (SEM) or median (IQR).
* denotes p<0.01 between groups over time (two way repeated measures ANOVA).
Blue : complications, Red : no complications.

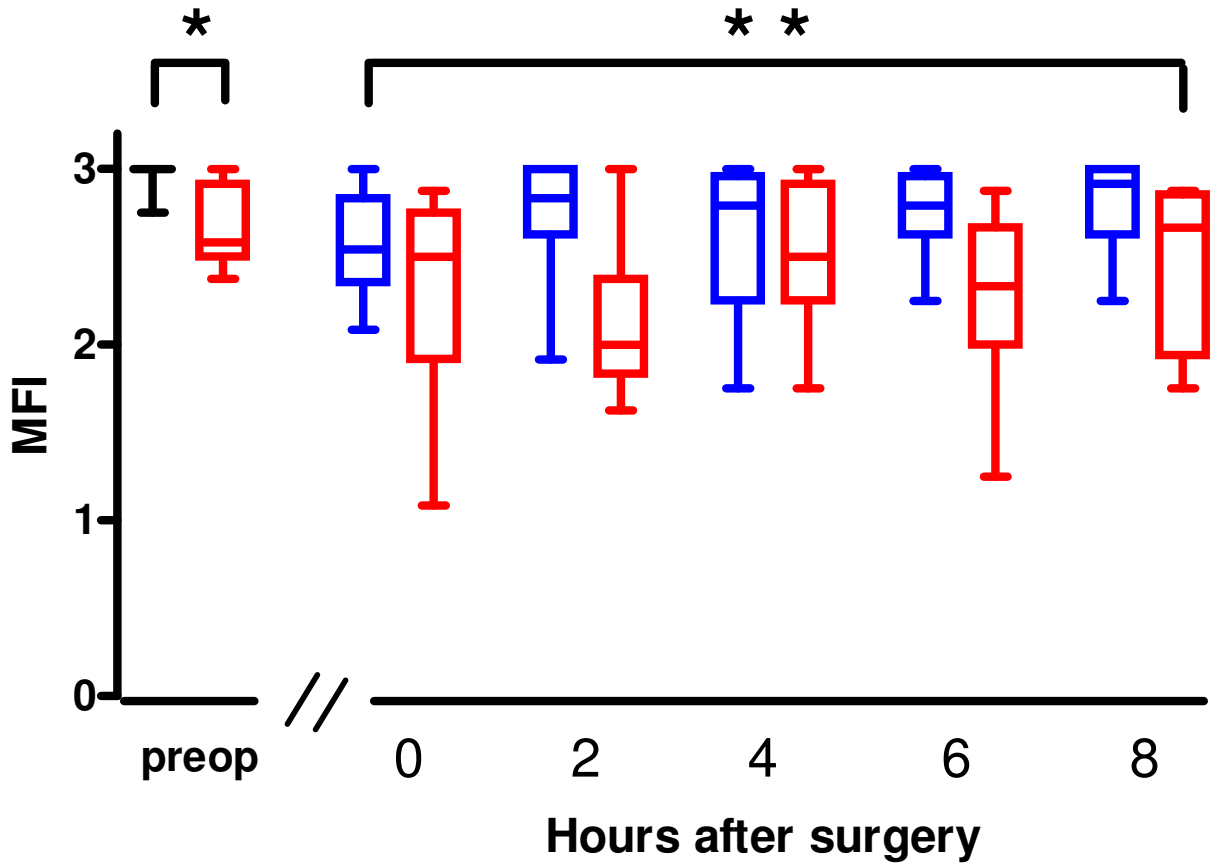


Figure 4.3 Sublingual microvascular flow index (MFI) of small vessels (<20µm) in patients who did and did not develop complications. Data presented as median (IQR). p<0.0001 between groups over time (two-way repeated measures ANOVA). * p<0.01 between groups at this timepoint (post-hoc t-test with Bonferroni correction)

Blue : complications, Red : no complications.

		Pre-op	Hour 0	Hour 2	Hour 4	Hour 6	Hour 8
Microvascular Flow Index	**Complications****	2.6 (2.5-2.9)	2.5 (1.9-2.8)	2.0 † (1.8-2.4)	2.5 (2.3-2.9)	2.3 (2.0-2.7)	2.7 (1.9-2.9)
	No complications	3.0 (3.0-3.0)	2.5 (2.4-2.8)	2.8 (2.6-3.0)	2.8 (2.3-3.0)	2.8 (2.6-3.0)	2.6 (2.3-2.9)
Small vessel density mm^{-1}	**Complications**	6.8 (5.5-7.8)	6.5 (5.8-7.4)	6.1 (4.6-7.3)	6.9 (6.2-7.4)	6.3 (4.6-7.0)	6.8 (5.1-7.5)
	No complications	5.9 (5.7-6.5)	6.3 (5.3-8.2)	6.7 (6.0-7.4)	6.2 (5.3-8.0)	6.6 (5.9-7.6)	7.0 (6.5-7.5)
Proportion of perfused small vessels (%)	**Complications***	79 (73-92)	74 (61-90)	64 (45-80)	67 (58-85)	75 (60-83)	87 (78-100)
	No complications	89 (83-95)	80 (61-93)	86 (72-99)	86 (57-93)	90 (78-97)	95 (83-97)
Perfused small vessel density mm^{-1}	**Complications***	5.7 (4.1-6.9)	4.5 (3.8-6.2)	3.8 (3.0-4.4)	4.9 (4.0-5.5)	4.1 (3.1-6.2)	6.3 (3.7-6.8)
	No complications	5.6 (4.8-5.8)	4.9 (3.6-6.3)	5.8 (4.3-6.6)	5.1 (3.5-6.3)	5.7 (5.1-7.2)	6.6 (5.2-7.3)
Heterogeneity Index	**Complications**	0.09 (0.0-0.50)	0.13 (0.0-0.3)	0.25 (0.07-0.47)	0.21 (0.0-0.44)	0.10 (0.0-0.54)	0.32 (0.09-0.35)
	No complications	0.0 (0.0-0.0)	0.25 (0.09-0.25)	0.11 (0.0-0.28)	0.18 (0.0-0.32)	0.18 (0.04-0.25)	0.09 (0.0-0.26)
Red cell flux (arbitrary units)	**Complications**	30 (19-40)	38 (28-43)	28 (21-38)	37 (26-44)	34 (25-46)	31 (24-55)
	No complications	33 (22-41)	34 (26-64)	32 (19-55)	35 (30-60)	31 (19-40)	41 (27-66)

Table 4.6 Sublingual small (<20μm) vessel density and red cell flux. Data presented as mean (SD) or median (IQR).p<0.001 and *p<0.01 between groups over time (two-way repeated measures ANOVA). † p<0.01 between groups at this time point (post-hoc t-test with Bonferroni correction).**

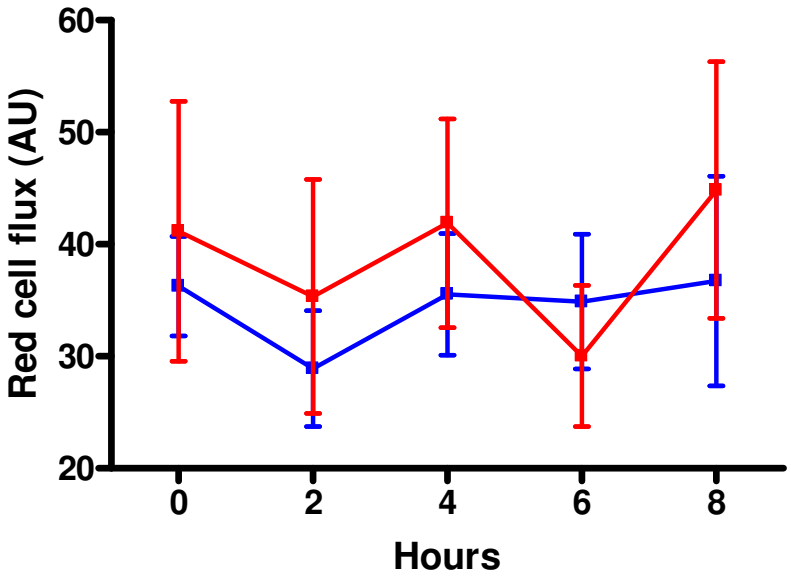


Figure 4.4. Red cell flux of the abdominal wall assessed by laser Doppler flowmetry in those patients who did and did not develop complications.

Blue : complications, Red : no complications.

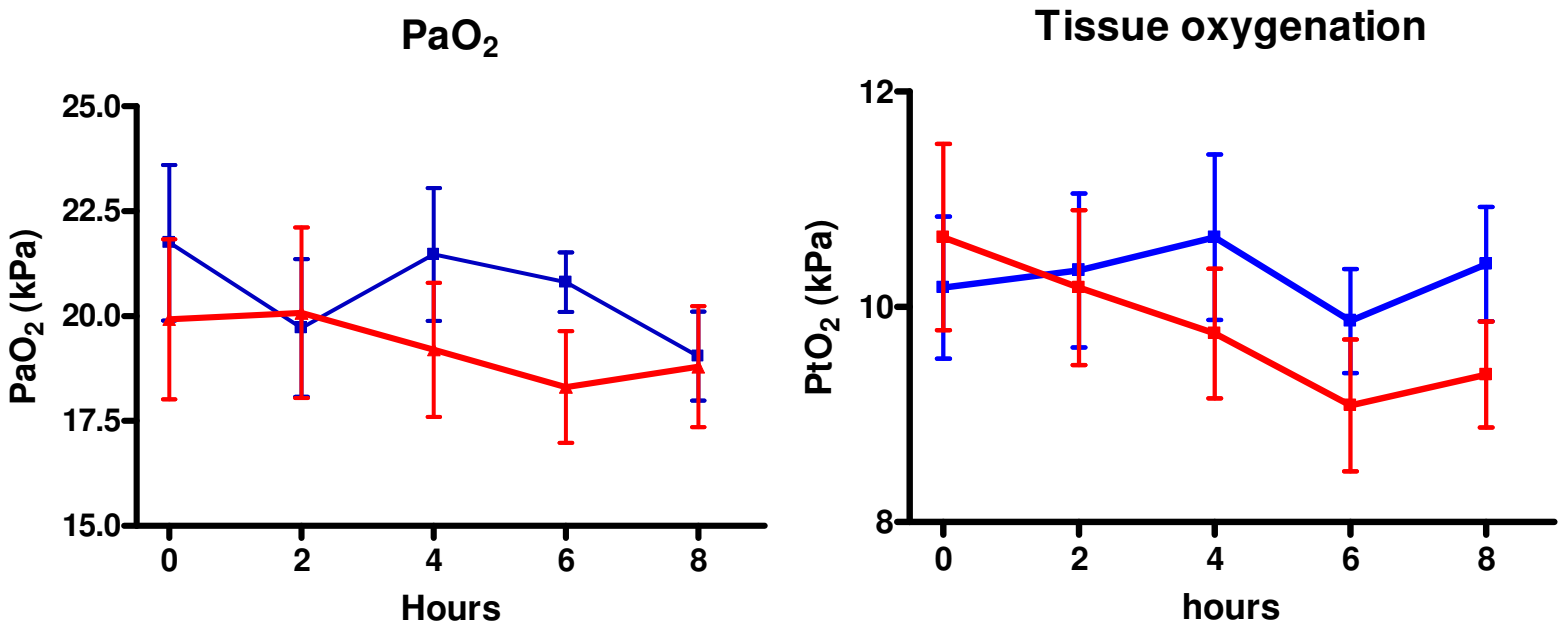


Figure 4.5. Arterial and tissue oxygenation during eight hour study period. Data presented as mean (SD). PaO_2: partial pressure of oxygen in arterial blood; PtO_2: partial pressure of oxygen in tissues. No significant difference between groups (two way repeated measures ANOVA).
Blue : complications, Red : no complications.

4.4 Discussion

The principle finding of this study is that, in a group of patients, receiving usual care, with low global oxygen delivery following major abdominal surgery, impairment of microvascular flow was more marked in those patients who developed complications. Importantly, this difference in microvascular flow was present even before surgery. Although the incidence of complications was high, there were no associated differences in global oxygen delivery, cutaneous red cell flux or tissue oxygenation between patients who did and did not develop complications. These data suggest that impairment of microvascular flow may play an important role in the evolution of complications after abdominal surgery. Further research is required to confirm these findings and to investigate this area in more detail.

A number of investigators have described abnormalities in gastro-intestinal total red blood cell flux (laser Doppler flowmetry) in patients who develop post-operative complications.[96,100,162] This reduction in mucosal blood flow has been associated with an increased incidence of anastomotic breakdown following both oesophagectomy and colorectal surgery.[96,162] Although oesophageal anastomotic flow has been shown to improve following the topical application of nitroglycerin,[163] this effect was not observed with intravenous infusions of either prostaglandin E_1 or nitroglycerin.[162,164] We are not, however, aware of any previous reports of cutaneous red cell flux following gastro-intestinal surgery. In a recent investigation, Boerma and colleagues did not identify any heterogeneity of microvascular flow in colonic stoma within 24 hours of elective colo-rectal surgery.[99] Sublingual microvascular flow was not reported, although it seems likely that the findings would have been similar. There are several reasons why these previous findings

in non-septic patients contrast with those of the current study. We identified abnormalities of microvascular flow that were most evident in the early post-operative phase and appeared to be returning to normal by eight hours. In the previous study, stomal microvascular flow was evaluated only once in the first 24 hours after surgery and possibly at variable time points. Early changes in microvascular flow may have therefore been missed. Perhaps more importantly, we specifically recruited a high-risk group of patients undergoing complex surgery who were deemed to require post-operative critical care admission, whereas patients in the previous study were all admitted to a standard surgical ward. The finding of impaired sublingual microvascular flow prior to surgery in those patients who subsequently developed complications was unexpected. This observation may have related to mild hypovolaemia or to the underlying diagnosis (cancer in almost all cases) but does not appear to have resulted from diabetes or other co-morbid disease. Importantly, microvascular indices continued to deteriorate during and after surgery and appeared to be returning to normal by eight hours after surgery. Further work in a larger group of patients is required to confirm these findings and to explore the underlying explanation in more detail.

Reductions in PtO_2 have previously been associated with an increased incidence of post-operative wound infection.[178] Furthermore, peri-operative supplemental inspired oxygen,[170,179] and therapies to increase global oxygen delivery seem to decrease the incidence of wound infections.[31,32] Supplemental oxygen may also improve colonic anastomotic PtO_2 in pigs.[289] It is not clear why we did not identify any differences, in either cutaneous tissue oxygenation or DO_2I between patients who did and did not develop complications. In the current study, cutaneous PtO_2 was considerably greater than previously reported (10 ±3 kPa *vs* 6.5 ±1.5 kPa),[178] perhaps reflecting the routine use of

supplemental oxygen in the current investigation whereas in the previous study patients only received supplemental oxygen if SpO_2 decreased to less than 90%. In addition, in the current study every patient received epidural analgesia which may also improve abdominal wall cutaneous oxygenation.[188,290] These differences may also relate to the use of a cutaneous Clark electrode which warms the skin in order to minimise artefact due to local vasoconstriction as opposed to a subcutaneous catheter. However, PtO_2 values in the current study were similar to those previously measured using a subcutaneous catheter in patients receiving 40% oxygen after abdominal surgery.[291]

Sublingual sidestream darkfield imaging is a valuable technique which allows real time imaging of the intact microcirculation in the clinical environment. Whilst not as versatile as laser Doppler flowmetry, the capacity to explore heterogeneity of flow in vessels of different sizes has allowed important advances in our understanding of the microcirculation in critical illness.[59] However, this technique remains semi-quantitative and data reliability may be affected by technical expertise and inter-observer bias. It is uncertain to what extent sublingual microvascular flow correlates with that in other vascular beds.[99,274] We used the two-way repeated measures ANOVA test to evaluate changes over the entire course of the study rather than at individual time points. Whilst significant differences were identified in three measures of microvascular flow (MFI, proportion of perfused vessels and perfused vessel density), more stringent post-hoc testing identified a difference at only one time point. It seems likely that this relates to the small sample size. A larger study is therefore required to confirm these important preliminary findings and to evaluate this subject in more detail.

In summary, we have identified an impairment of sublingual microvascular flow in patients who developed complications both prior to and following major abdominal surgery. However, there were no related differences in cutaneous red cell flux, PtO_2 or global oxygen delivery. The findings of previous studies suggest that vasoactive agents may improve sublingual microvascular flow in septic patients.[15,156,274] Further investigations are indicated to confirm our findings and to evaluate the effects of haemodynamic interventions and vasoactive agents on tissue microvascular flow and oxygenation following major surgery.

Chapter 5
Haemodynamic optimisation improves tissue microvascular flow and oxygenation after major surgery: a randomised controlled trial

5.1 Introduction

Complications are common following major non-cardiac surgery and represent an important cause of avoidable morbidity and mortality.[4,5] Estimates suggest that as many as 234 million major surgical procedures are performed worldwide each year, around 15% of which fall into a high-risk sub-group.[1,5] With mortality rates of up to 12%, this high-risk surgical population accounts for over 80% of early post-operative deaths.[5] Long-term survival is also significantly reduced following surgery, in particular for those patients who develop complications.[8,292,293] Importantly, survival amongst patients who develop post-operative complications varies widely between hospitals, confirming both the potential and the need to improve clinical outcomes in this population.[7]

The association between low cardiac output, inadequate global oxygen delivery (DO_2), reduced venous oxygen saturation (SvO_2 and $ScvO_2$) and poor outcomes following major surgery is well recognised.[32,33,287] In several small studies, the use of these variables as treatment end-points for intra-venous fluid and inotropic therapy has been associated with improved clinical outcomes.[39,40,294,295] It has long been suggested that these beneficial effects relate to improved tissue perfusion and oxygenation. This may prevent the evolution of a tissue 'oxygen debt' and hence reduce the incidence of complications and

organ dysfunction.[296] This theory is consistent with the findings of a number of studies demonstrating that impaired tissue microvascular flow and oxygenation are associated with subsequent post-operative complications.[96,100,162,178] In patients with severe sepsis, there is some evidence to suggest that abnormalities of microvascular flow may cause tissue hypoxia,[84,297] whilst the use of vasoactive drug therapy has been shown to improve tissue microvascular flow in this group.[15] Importantly, dopexamine, the agent most often used in trials of perioperative cardiac output guided therapies, has a combination of vasodilator and mild inotropic actions which may enhance microvascular flow and improve outcomes.[294] The findings of recent systematic reviews suggest that cardiac output guided haemodynamic therapy may have particular beneficial effects on splanchnic perfusion and renal function.[49,298] It is also possible that perioperative haemodynamic optimisation could favourably influence the systemic inflammatory response to tissue injury associated with surgery, thereby reducing the incidence and severity of complications and organ dysfunction.

Clearly, the hypothesis that peri-operative cardiac output guided haemodynamic therapies result in improved tissue microvascular flow and oxygenation is plausible but, after many years, still remains untested. It is also uncertain whether low dose inotropic therapy offers incremental benefit over the use of fluid alone to achieve cardiac output related end-points. A detailed understanding of the physiological effects of haemodynamic therapies is therefore necessary to provide a rational basis from which to adapt and refine their use in clinical practice. The aim of this investigation was to evaluate the effects of stroke volume guided intra-venous fluid therapy with and without low dose dopexamine on tissue microvascular flow and oxygenation and systemic markers of inflammation in patients admitted to critical care following major gastrointestinal surgery.

5.2 Methods

Patients scheduled for admission to critical care following major elective gastrointestinal surgery were eligible for recruitment. Exclusion criteria were refusal of consent, pregnancy, patients receiving palliative treatment only and acute arrhythmias or myocardial ischaemia prior to enrolment. In addition, patients receiving lithium therapy or those with a body mass less than 40 kg were excluded because lithium indicator dilution measurement of cardiac output is not licensed in such patients. The study was approved by the Research Ethics Committee and Medical and Healthcare products Regulatory Agency. Written informed consent was obtained from all patients prior to surgery. Participants were randomly allocated to one of three treatment groups by computer generated random sequence in blocks of nine. Blocking was used to ensure that treatment groups were generated of equal size. Type of surgery is likely to be a confounder and therefore, groups were stratified according to surgical procedure (upper gastrointestinal surgery, lower gastrointestinal surgery and pancreatic surgery involving the gut). Study group allocations were placed in serially numbered opaque envelopes.

5.2.1 Clinical management

General anaesthesia was standardised and included intra-venous fentanyl, propofol and atracurium for induction of anaesthesia and maintenance with inhaled isoflurane in oxygen enriched air and epidural analgesia. Clinical staff administered intra-venous fluids, blood products and, if required, vasoactive drugs in order to maintain routine physiological, haematological and biochemical parameters within normal ranges as follows: pulse rate (60-100 bpm), mean arterial pressure (60-100 mmHg), central venous pressure (CVP) (6-12 mmHg), urine output (>25 ml/hr), haemoglobin (> 8 g dl^{-1}), SpO_2 (>94%), temperature

(36-37 °C), serum base excess (-2 - +2 mmol/l) and $PaCO_2$ (35-45 mmHg). Cardiac output monitoring was not used during surgery. Following surgery, all patients were admitted to critical care. For the eight hour intervention period, either a doctor (SJ) or nurse (AVS, SLA – see acknowledgements) administered one of three allocated haemodynamic protocols as described below. These protocols are similar to those used in a previous trial.[48]

CVP group: Intra-venous lactated Ringer's solution was administered at 1 ml/kg/hr for maintenance requirements. Patients received additional 250 ml fluid challenges with intra-venous colloid solution (Gelofusine, BBraun, Germany) to achieve an optimal value of CVP. Colloid solution was administered in one or more rapid boluses to achieve a sustained rise in CVP of at least 2 mmHg for 20 minutes or more. If CVP decreased, fluid challenges were repeated to establish whether the patient was fluid responsive.

SV group: Intra-venous lactated Ringer's solution was administered at 1 ml/kg/hr. Patients received additional 250 ml fluid challenges with intra-venous colloid solution to achieve an optimal value of stroke volume. Colloid solution was administered in one or more rapid boluses to identify whether the patient was fluid responsive. A stroke volume response to fluid was defined as a sustained rise in stroke volume of at least 10% for 20 minutes or more. Where a patient was identified as stroke volume responsive to fluid, further 250 ml boluses of fluid were administered until a plateau value was achieved. If stroke volume decreased, fluid challenges were repeated to establish whether the patient was fluid responsive.

SV & DPX group: Intra-venous lactated Ringer's solution was administered at 1 ml/kg/hr. Patients received additional fluid challenges with colloid solution to achieve an optimal value of stroke volume in an identical fashion to patients in the SV group. In addition, a continuous intra-venous infusion of dopexamine was administered at 0.5 $\mu g\ kg^{-1}\ min^{-1}$ (Cephalon Inc, USA). This infusion rate was not adjusted to achieve a specific value for

cardiac output or DO_2I but was decreased or discontinued in patients with evidence of myocardial ischaemia or tachycardia (>100bpm or increase >20% from baseline value, whichever was greater).

Only the member of the research team who delivered the intervention was aware of study group allocation. Cardiac output data were made available to clinical staff only on specific request. The reasons for this and any subsequent changes in treatment were documented by research staff. Dummy infusions were used in patients not allocated to receive dopexamine. All other management decisions were taken by clinical staff.

5.2.2 Sublingual microvascular flow

Sublingual microvascular flow was evaluated before surgery and at 0, 2, 4, 6 and 8 hours immediately after surgery using sidestream darkfield (SDF) imaging with a x5 objective lens (Microscan, Microvision Medical, Amsterdam, Netherlands).[299] Image acquisition and subsequent analysis was performed as described in Chapter 2. Analysis of the videos was performed by two observers (AVS and SLA). The Kappa coefficient (κ) for inter-observer variability in SDF image analysis was 0.74 (95%CI 0.61-0.81).

5.2.3 Cutaneous microvascular flow and PtO_2

Cutaneous red blood cell flux was measured before surgery and at 0, 4 and 8 hours after surgery at two sites on the forearm by laser Doppler flowmetry (Moorlab, Moor Instruments, Axminster, UK). Baseline red cell flux on the forearm was measured and following this, the post-occlusive hyperaemic response was examined by inflating a cuff around the upper arm to 20 mmHg above systolic pressure for three minutes and

measuring the changes in red cell flux on releasing the pressure in the cuff. The difference between baseline flux and peak hyperaemia was then evaluated at each time point. Cutaneous tissue PtO_2 was measured before surgery and at 0, 2, 4, 6 and 8 hours after surgery at two sites on the abdominal wall using a Clark electrode (TCM400, Radiometer, Copenhagen, Denmark).

5.2.4 Arterial and venous blood gas analysis

Arterial and central venous blood samples were taken at 0, 2, 4, 6 and 8 hours after surgery from indwelling catheters for analysis of SaO_2, $ScvO_2$, base deficit and serum lactate (ABL600, Radiometer, Copenhagen, Denmark).

5.2.5 Serum inflammatory markers

Serum samples were obtained from all patients following induction of anaesthesia but prior to surgery. Further serum samples were obtained immediately following surgery, at the end of the intervention period and 24 hours after the end of surgery. These samples were centrifuged at 3000g for 10 minutes and stored at -80℃. Subsequent analysis of interleukin 1β (Il-1β), Il-6, Il-8, and tumour necrosis factor α (TNF-α) was performed using a multi-array electro-chemiluminescence technique (SECTOR Imager 2400, Mesoscale Discovery, USA) by SJ. Levels of soluble inter-cellular adhesion molecule 1 (ICAM-1) were quantified using a similar technique.

5.2.6 Clinical follow-up

Clinical outcomes data for each patient were collected by a member of the research team who was unaware of study group allocation and then verified by the senior investigator

who was also unaware of the study group allocation. Patients were prospectively followed for 28 days for pre-defined in-hospital complications, mortality and duration of hospital stay.

5.2.7 Statistical Analysis

Assuming a 5% type I error rate and an 80% type II error rate for the sample size calculation, it was calculated that 45 patients would be required in each group to detect a 10 mmHg difference in PtO_2 between each of the intervention groups and the control group.

Trends in physiological variables over time within groups were tested using one way repeated measures analysis of variance (ANOVA) or Friedmann test. Differences in physiological variables between groups were tested using two way repeated measures ANOVA, the t-test and one way ANOVA with post hoc t-test with Bonferroni correction. Categorical variables were tested with the Chi squared or Fisher's exact tests. Statistical analysis was performed using GraphPad Prism version 4.0 (GraphPad Software, USA). Analysis was performed on an intention-to-treat basis including all randomised patients. Significance was set at $p<0.05$. Data are presented as mean (SD) where normally distributed or median (IQR) where not normally distributed.

5.3 Results

One hundred and thirty five patients were recruited between December 2007 and February 2009 (Figure 1). Baseline patient characteristics are presented in table 5.1. Despite the different haemodynamic treatment algorithms, patients in the three groups received similar volumes of fluid during and after surgery and there were no differences in vasopressor requirements (table 5.2). There was no difference in the timing of fluid given. The number of patients who received transfused blood during and after surgery was similar between the groups as was the volume of blood transfused (CVP group: 19 patients, 870 (580-1408) ml; SV group: 12 patients, 561 (398-580) ml; SV & DPX group: 15 patients, 580 (300-877) ml; p=0.11). One patient randomised to the SV & DPX group developed myocardial ischemia during surgery and, in accordance with the study protocol, did not receive dopexamine. In five patients the dose of dopexamine was reduced because of an increase in heart rate and in one patient, dopexamine was subsequently discontinued. On only one occasion, a clinician asked to view a patient's cardiac output data because of concern that poor cardiac function might have been complicated by pulmonary oedema. This information did not prompt any changes in treatment. No patients received additional inotropic therapy during the intervention period.

Stroke volume guided fluid therapy with dopexamine infusion was associated with significant increases in heart rate, cardiac index, DO_2 and $ScvO_2$. Stroke volume guided fluid therapy alone was associated with much smaller increases in cardiac index and DO_2 and no change in heart rate or $ScvO_2$ (figure 5.2 and table 5.3). Sublingual large vessel MFI (>20µm) was 3.0 (3.0-3.0) in all groups suggesting good quality image capture unaffected by pressure artefact. In all three groups, microvascular flow was impaired at baseline (table 5.4). In the SV & DPX group, sublingual microvascular flow significantly

improved during the eight hour study period (figure 5.3 and table 5.4). Sublingual microvascular flow remained constant in the SV group but deteriorated in the control group (figure 5.3). Similarly, there was a significant improvement in the cutaneous hyperaemic response in the SV & DPX group, whereas this variable remained unchanged in the SV group and deteriorated in the control group (figure 5.3). In all three groups, cutaneous PtO_2 initially increased after surgery. This improvement was sustained in the SV & DPX group but decreased towards baseline in the CVP and SV groups (figure 5.4).

There were no significant differences in overall complication rates, critical care free days or duration of hospital stay, although the pattern of mortality was consistent with a beneficial effect of stroke volume guided haemodynamic therapy (table 5.5). Despite improvements in tissue microvascular flow and oxygenation in the SV and SV & DPX groups, there were no differences between the groups in terms of the serum inflammatory markers Il-1β, Il-6, Il-8, TNFα and ICAM-1 within 24 hours of surgery (figure 5.5).

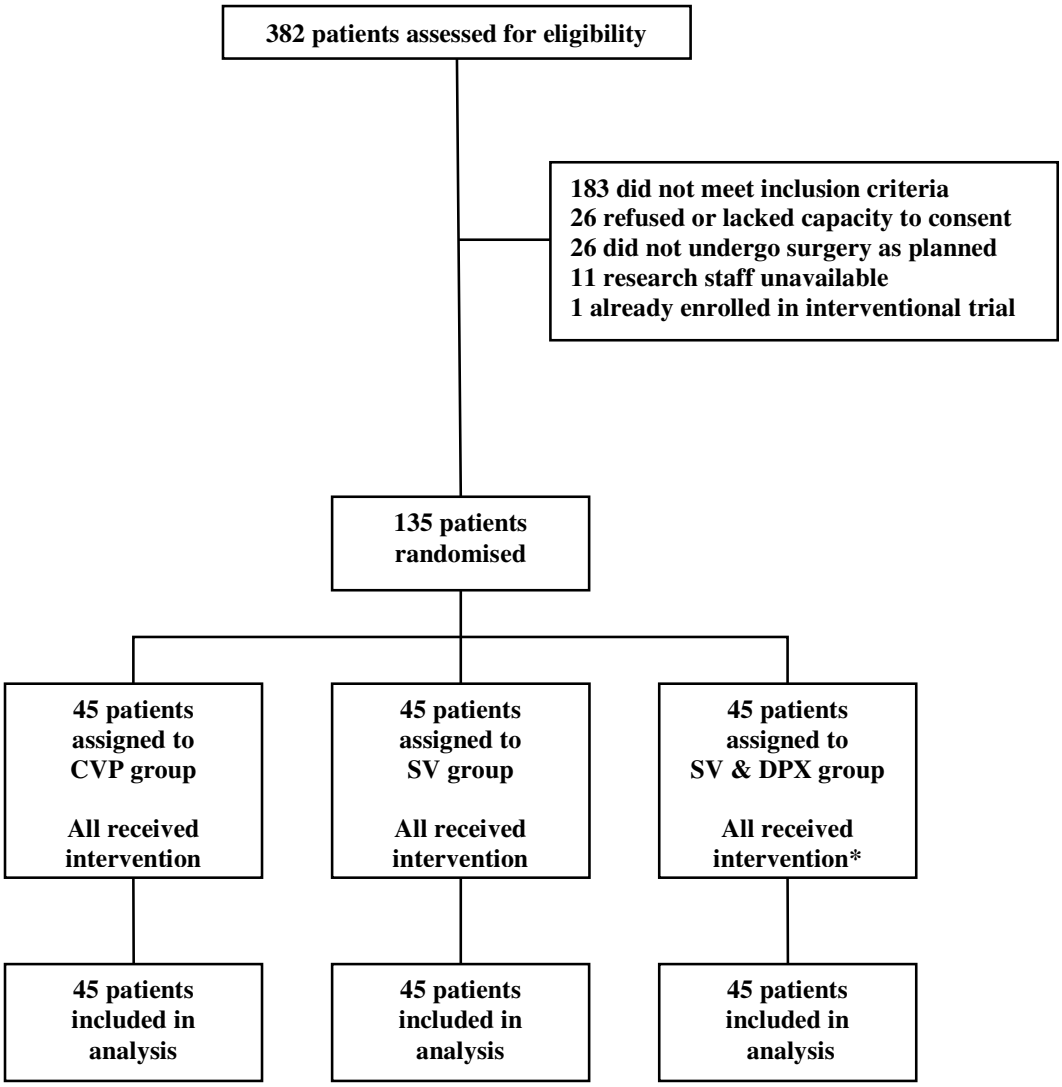


Figure 5.1 CONSORT diagram; flow of patients through trial. *One patient randomised to the SV & DPX group developed myocardial ischemia during surgery (before the trial intervention commenced) and in accordance with the protocol, did not receive dopexamine.

	CVP group n=45	SV group n=45	SV & DPX group n=45
Age (years)	70 (64-78)	68 (59-77)	65 (59-74)
Male (%)	30 (67%)	31 (69%)	28 (62%)
ASA score	2 (2-3)	2 (2-3)	2 (2-3)
Upper gastrointestinal surgery (%)	18 (40%)	18 (40%)	18 (40%)
Pancreatic surgery involving gut (%)	18 (40%)	18 (40%)	18 (40%)
Lower gastrointestinal surgery (%)	9 (20%)	9 (20%)	9 (20%)

Table 5.1. Patient characteristics at baseline. Data presented as median (IQR) or absolute values (%). ASA score : American Society of anesthesiologists physical status classification system

	CVP group n=45	SV group n=45	SV & DPX group n=45	p
Intra-operative period				
Intra-venous crystalloid during surgery (ml)	3595 (1354)	4057 (1495)	4159 (1393)	0.15
Intra-venous colloid during surgery (ml)	756 (815)	835 (688)	709 (559)	0.69
Intervention period				
Intra-venous crystalloid during study period (ml)	639 (281)	652 (237)	626 (250)	0.98
Intra-venous colloid during study period (ml)	1104 (553)	1227 (555)	1307 (549)	0.22
Patients receiving vasopressor therapy (%)	7 (16%)	8 (18%)	5 (11%)	0.82

Table 5.2. Volume of intra-venous fluid administered and use of vasopressor therapy in the three groups. Data presented as mean (SD) or absolute values (%).

	Group	Hour 0	Hour 2	Hour 4	Hour 6	Hour 8
Heart rate (bpm)	**CVP**	74 (13)	76 (14)	76 (14)	76 (15)	76 (15)
	SV	77 (19)	76 (17)	80 (19)	79 (17)	78 (17)
	§ SV & DPX	77 (11)	86 (12)	91 (12)	93 (13)	92 (12)
Mean arterial pressure (mmHg)	**CVP**	80 (22)	79 (20)	79 (15)	79 (15)	77 (14)
	SV	76 (15)	81 (15)	83 (14)	79 (14)	77 (14)
	† SV & DPX	80 (18)	83 (17)	84 (13)	77 (13)	74 (12)
Central venous pressure (mmHg)	**‡ CVP**	6 (5)	7 (5)	7 (5)	8 (5)	8 (5)
	*** SV**	4 (5)	6(4)	6 (5)	7 (4)	7 (5)
	† SV & DPX	5 (4)	7 (4)	7 (4)	8 (6)	8 (5)
Cardiac index ($l/min/m^2$)	**CVP**	3.5 (1.1)	3.5 (0.9)	3.5 (0.9)	3.5 (0.9)	3.4 (0.9)
	‡ SV	3.2 (0.9)	3.5 (0.9)	3.7 (1.0)	3.7 (1.0)	3.6 (1.0)
	§ SV & DPX	3.3 (0.8)	4.0 (0.9)	4.3 (1.0)	4.3 (0.9)	4.4 (1.1)
Oxygen delivery index ($ml/min/m^2$)	**CVP**	477 (146)	490 (144)	480 (152)	468 (168)	467 (159)
	† SV	449 (145)	492 (160)	495 (147)	499 (165)	484 (150)
	§ SV & DPX	498 (157)	594 (167)	635 (198)	631 (174)	614 (209)
Stroke volume (ml)	**CVP**	80 (23)	86 (25)	84 (24)	82 (21)	81 (21)
	‡ SV	78 (23)	85 (22)	84 (22)	85 (22)	83 (22)
	‡ SV & DPX	80 (23)	88 (24)	90 (24)	89 (23)	88 (26)
Serum lactate (mmol/l)	**† CVP**	1.4 (1.0-2.1)	1.1 (0.9-1.6)	1.1 (0.9-1.8)	1.2 (0.9-1.8)	1.2 (0.9-1.8)
	*** SV**	1.4 (0.9-2.7)	1.3 (0.9-2.2)	1.3 (0.8-2.4)	1.2 (0.8-1.9)	1.2 (0.8-1.8)
	SV & DPX	1.9 (1.3-2.8)	1.7 (1.0-2.4)	1.9 (1.0-2.9)	1.9 (1.0-3.1)	1.7 (1.1-2.4)
Base deficit (mmol/l)	**CVP**	-1.9 (2.6)	-2.2 (2.7)	-1.7 (2.8)	-1.7 (2.9)	-1.6 (2.6)
	*** SV**	-2.2 (2.4)	-2.1 (2.8)	-1.6 (3.1)	-1.0 (2.2)	-1.0 (2.3)
	‡ SV & DPX	-2.2 (2.1)	-2.3 (2.4)	-2.2 (2.4)	-1.9 (2.3)	-1.4 (2.4)

Table 5.3. Cardiovascular physiology for the three treatment groups during eight hour study period. **Data presented as mean (SD) or median (IQR). Significant changes over time signified by † (p<0.05), ‡ (p<0.01), * (p<0.001) and § (p<0.0001).**

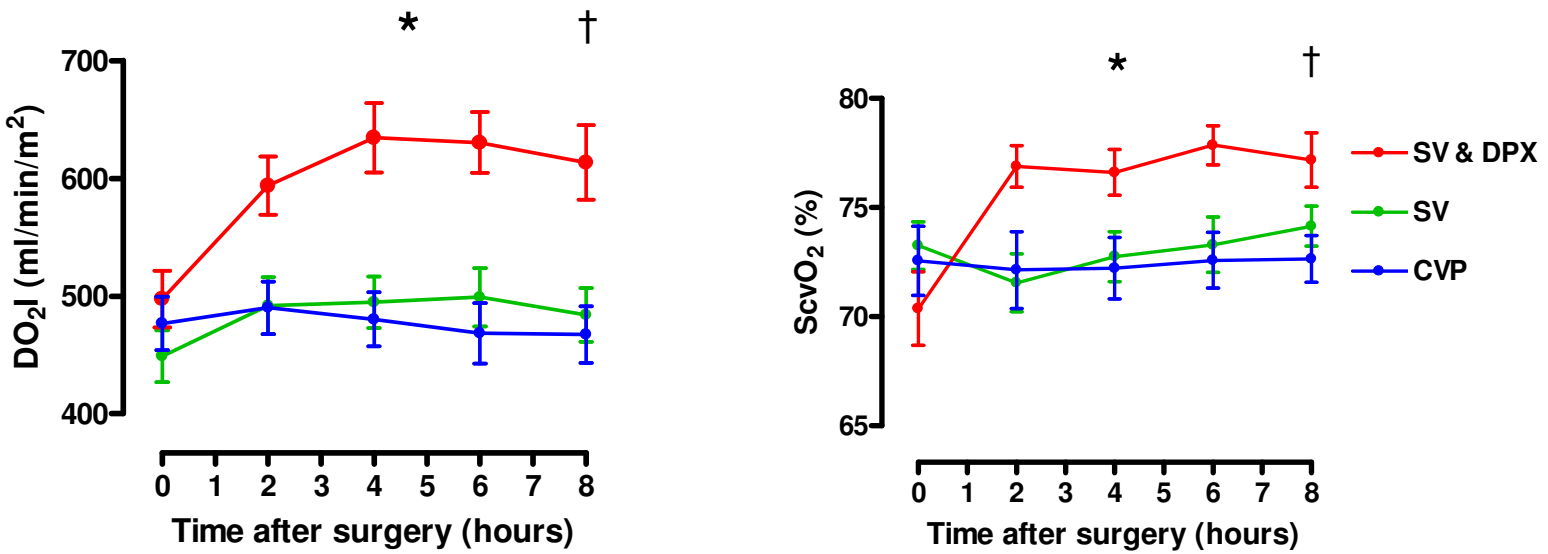


Figure 5.2. Changes in oxygen delivery index (DO_2I) (A) and central venous oxygen saturation ($ScvO_2$) (B) following surgery in the three treatment groups.

***Significant difference between groups over time for DO_2I and $ScvO_2$ ($p<0.0001$) (two way repeated measures ANOVA). Significant increase in DO_2I over time: SV group $p=0.003$; SV & DPX group $p<0.0001$. Significant increase in $ScvO_2$ over time: SV & DPX group $p<0.0001$; no change in the SV group ($p=0.22$) or CVP group ($p=0.98$). †At hour 8, there was a significant difference in DO_2I between the CVP and SV & DPX groups ($p<0.001$) but no difference between the SV and CVP groups ($p>0.05$). At hour 8, there was a significant difference in $ScvO_2$ between the CVP and SV & DPX groups ($p<0.05$) but no difference between the SV and CVP groups ($p>0.05$).**

		Hour 0	Hour 2	Hour 4	Hour 6	Hour 8
Microvascular Flow Index	**CVP**	2.5 (0.3)	2.5 (0.7)	2.6 (0.4)	2.6 (0.4)	2.5 (0.5)
	SV	2.5 (0.4)	2.5 (0.5)	2.6 (0.4)	2.7 (0.3)	2.6 (0.4)
	† SV & DPX	2.5 (0.4)	2.4 (0.5)	2.5 (0.4)	2.7 (0.3)	2.5 (0.4)
Perfused vessel density (mm^{-1})	**‡ CVP**	6.1 (2.4)	6.1 (1.7)	5.8 (2.0)	5.8 (1.9)	5.3 (1.8)
	SV	5.8 (2.5)	5.7 (2.6)	5.7 (1.9)	5.7 (1.9)	6.2 (3.0)
	† SV & DPX	5.8 (2.4)	5.5 (2.4)	5.9 (2.8)	6.2 (1.8)	6.3 (3.0)
Proportion of perfused vessels	**CVP**	0.83 (0.14)	0.83 (0.12)	0.81 (0.14)	0.82 (0.18)	0.81 (0.18)
	SV	0.80 (0.15)	0.80 (0.21)	0.82 (0.17)	0.84 (0.13)	0.80 (0.19)
	SV & DPX	0.81 (0.16)	0.77 (0.14)	0.81 (0.15)	0.85 (0.12)	0.87 (0.17)
Heterogeneity index	**CVP**	0.39 (0.23-0.51)	0.23 (0.12-0.41)	0.25 (0.17-0.48)	0.28 (0.16-0.38)	0.25 (0.10-0.54)
	SV	0.23 (0.10-0.43)	0.20 (0.07-0.31)	0.22 (0.04-0.41)	0.19 (0.06-0.31)	0.22 (0.08-0.46)
	† SV & DPX	0.27 (0.18-0.40)	0.20 (0.14-0.44)	0.18 (0-0.27)	0.13 (0.08-0.27)	0.17 (0-0.38)

Table 5.4. Sublingual microvascular flow for small vessels (<20μm) during eight hour study period. **Data presented as mean (SD) or median (IQR). Significant changes over time signified by † ($p<0.05$), ‡ ($p<0.01$).**

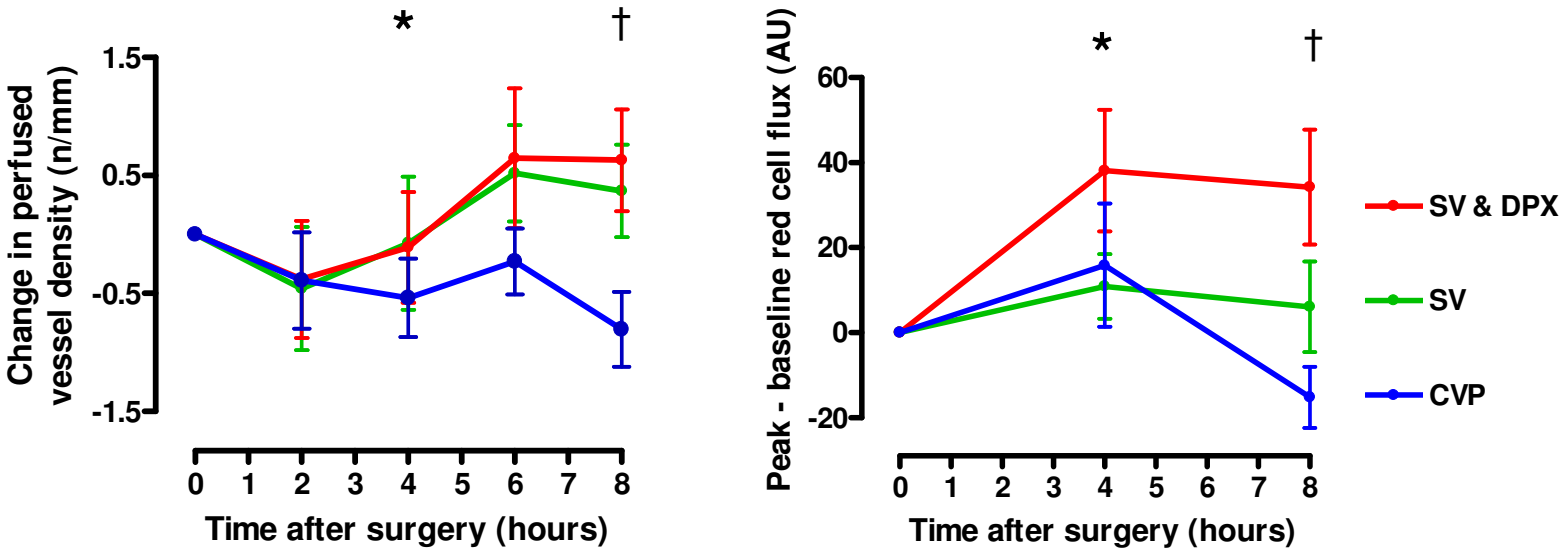


Figure 5.3. Changes in sublingual perfused vessel density (A) and peak-baseline cutaneous red cell flux following three minutes of vascular occlusion (B) from hour 0 following surgery in the three treatment groups. * **Significant difference between groups over time for sublingual vessel density ($p<0.05$) and cutaneous hyperaemic response ($p<0.01$) (two way repeated measures ANOVA). Significant increase in perfused sublingual vessel density over time in the SV & DPX group ($p=0.046$), no change in the SV group ($p=0.58$) and a decrease in the CVP group ($p=0.005$). Significant increase in cutaneous hyperaemic response over time in the SV & DPX group ($p=0.003$), no change in the SV group ($p=0.58$) and a decrease in the CVP group ($p=0.03$).**

† At hour 8, there was a significant difference in perfused sublingual vessel density between the SV & DPX and CVP groups ($p<0.05$) but not between the SV and CVP groups ($p>0.05$). At hour 8, there was a significant difference in cutaneous hyperaemic response between the SV & DPX and CVP groups ($p<0.001$) but not between the SV and CVP groups ($p>0.05$).

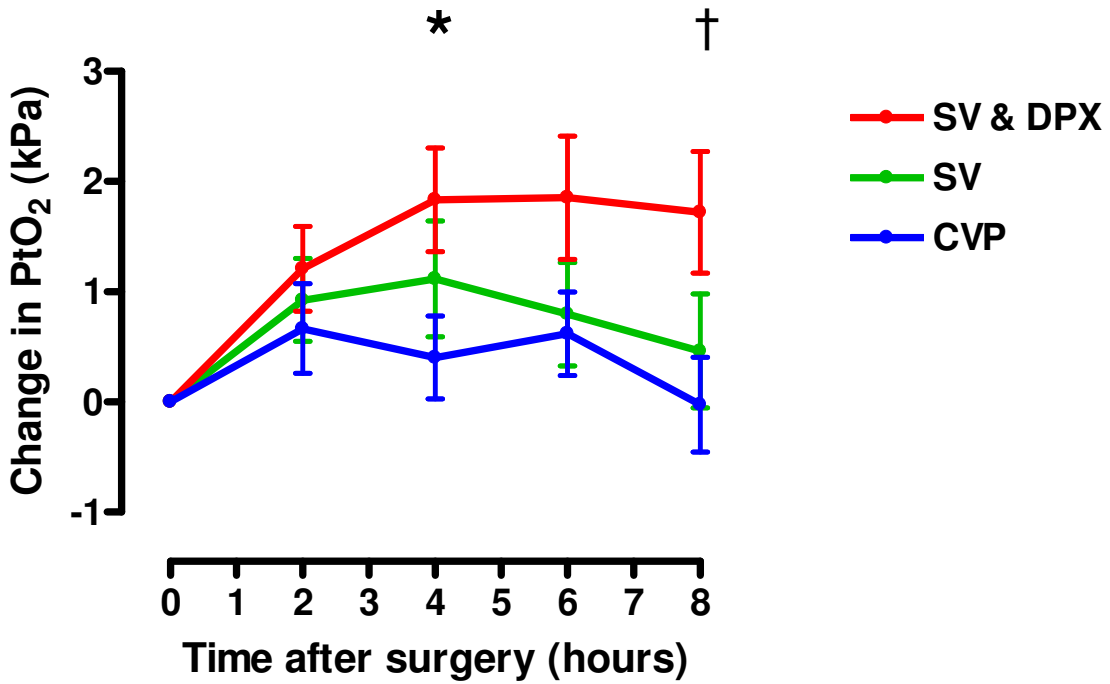


Figure 5.4. Changes in tissue oxygenation (PtO_2) following surgery in the three treatment groups. * Significant difference between groups over time (p=0.0005) (two way repeated measures ANOVA). Significant increase in PtO_2 over time in the SV & DPX group (p=0.0003), no change in the SV (p=0.14) or CVP groups (p=0.20).

† At hour 8, there was a significant difference in PtO_2 between the SV & DPX and CVP groups (p<0.005) but not between the SV and CVP groups (p>0.05).

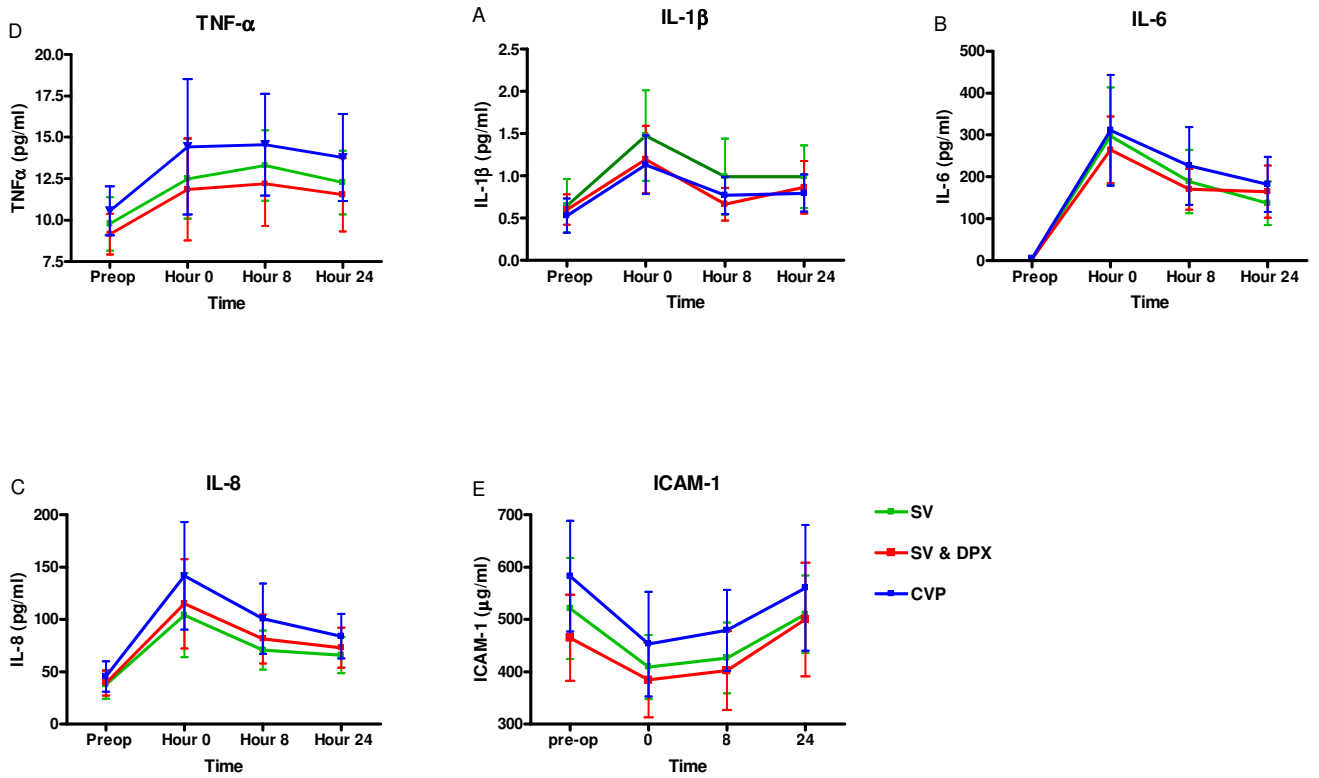


Figure 5.5. Changes in serum interleukin-1β (IL-1β) (panel A), interleukin 6 (IL-6) (panel B), interleukin 8 (IL-8) (panel C), tumour necrosis factor alpha (TNFα) (panel D) and soluble inter-cellular adhesion molecule 1 (ICAM-1) (panel E) between the three treatment groups. Data presented as mean (SE). There were no significant differences between the groups.

	CVP group n=45	SV group n=45	SV & DPX group n=45	p
Complications (number of patients, %)	30 (67%)	26 (58%)	31 (69%)	0.51
Cardiac complications (number of patients, %)	4 (9%)	3 (7%)	3 (7%)	0.90
Infectious complications (number of patients, %)	29 (64%)	24 (53%)	28 (62%)	0.52
Other complications (number of patients, %)	10 (22%)	14 (31%)	12 (27%)	0.63
Critical care free days within 28 days of surgery	24 (21-26)	24 (21-26)	26 (21-27)	0.45
Duration of hospital stay (days)	15 (10-26)	14 (11-26)	16 (11-28)	0.73
Hospital mortality (%)	6 (13%)	5 (11%)	4 (9%)	0.45

Table 5.5. Clinical outcomes in the three intervention groups. Data presented as median (IQR) or absolute values (%). Note: A number of patients developed more than one complication. Acute kidney injury at seven days not included in 28 day complication outcome.

5.4 Discussion

This is the first study to substantiate the theory that cardiac output guided haemodynamic therapy can improve tissue perfusion and oxygenation. Our principal finding is that a treatment algorithm incorporating stroke volume guided fluid therapy and a fixed low dose dopexamine infusion increased global oxygen delivery and $ScvO_2$ in association with significant improvements in sublingual and cutaneous microvascular flow and cutaneous tissue oxygenation. Stroke volume guided fluid therapy alone was associated with more modest improvements in global haemodynamics and microvascular flow. There were, however, no differences in circulating markers of the inflammatory response to surgery between treatment groups.

This randomised controlled trial used physiological end-points and was not designed to identify differences in clinical outcomes although a post hoc analysis did identify a possible improvement in renal outcomes (incidence of acute kidney injury – see chapter 6) associated with stroke volume guided therapy. This finding is consistent with a recent meta-analysis suggesting that haemodynamic optimisation protects renal function in surgical patients.[300] There was no reduction in overall complication rates in the intervention groups and the small difference in hospital mortality, although consistent with improved outcome was not significant. To achieve 80% power to detect a 25% reduction in the relative risk of complications would require a minimum of 150 patients in each of the three treatment groups.

In common with all trials of complex interventions, it was not possible to fully blind clinical staff to study group allocation. We did, however, conceal study group allocation from all investigators apart from the member of the research team delivering the intervention. This

included concealment of cardiac output data and the use of dummy infusions. All complications were assessed according to prospectively defined criteria and verified by the principal investigator who was unaware of study group allocation. Lastly our stratified randomisation procedure ensured that the three groups were comparable.

The importance of using cardiac output derived data to guide a carefully prescribed and consistently applied clinical intervention is illustrated by the findings of a previous multi-centre randomised trial in which perioperative pulmonary artery catheterisation, in the absence of improved haemodynamics, failed to influence outcome.[198] In the study reported here, three clinically relevant treatment algorithms were strictly implemented by members of the research team throughout the eight hour intervention period. Perhaps as a consequence, unlike most previous studies, the total volumes of intra-venous fluid administered were similar between the groups.[39,40,48,295] This suggests a high standard of care for all patients that may have limited the apparent treatment effect of stroke volume guided fluid therapy. Interestingly, the findings of one previous trial suggest that, even where median fluid administration is similar between groups, cardiac output guided fluid therapy may be associated with improved clinical outcomes.[41]

The relationship between derangements in cardiac output related variables and complications following major surgery is well described.[32,33,287] The findings of some, but not all clinical trials and a number of meta-analyses suggest that cardiac output guided haemodynamic therapy can improve post-operative outcomes.[39-41,298] It has long been assumed that the potential benefits of 'flow guided' peri-operative haemodynamic therapy relate to improved tissue perfusion and oxygenation. A number of studies have highlighted the significance of impaired tissue microvascular flow in the pathogenesis of post-operative complications.[96,100,162] In this context, it is interesting to note that the use of high

concentrations of inspired oxygen did not affect the incidence of post-operative wound infection or pneumonia in a recent large clinical trial.[173] In the current study, the use of a fixed low dose inotrope infusion coupled with stroke volume guided fluid therapy resulted in increases in heart rate and, to a lesser extent, stroke volume which in turn increased DO_2 and $ScvO_2$ to values previously associated with improved clinical outcomes.[33,287] We show for the first time that such increases in global haemodynamics are associated with improvements in tissue microvascular flow and oxygenation, thus validating the study hypothesis. Although stroke volume guided intra-venous colloid therapy alone led to much smaller increases in cardiac index and DO_2, with no change in heart rate or $ScvO_2$, microvascular flow was better maintained than in the CVP guided therapy group. The incremental effects of low dose dopexamine on both microvascular flow and tissue oxygenation are likely to relate to the β_2-adrenoceptor mediated inotropic and vasodilator actions of this agent. It is therefore possible that changes in microvascular flow relate to direct effects on the microcirculation as well as global cardiac output.

Interestingly, in a recent randomised trial, low dose nitroglycerin had no effect on sublingual microvascular flow in resuscitated patients with severe sepsis.[157] These contrasting findings may reflect differences in the nature and timing of the intervention as well the patient population and smaller sample size. In contrast, the use of vasopressor and inotropic agents has been shown to improve both tissue microvascular flow and oxygenation in patients with severe sepsis,[15,301] although these effects were not demonstrated in all such investigations.[194,302] Whilst, these studies do suggest potential effects of vasoactive drugs on microvascular flow, the current study is the first to investigate the effects of the use of cardiac output based end-points on tissue microvascular flow and oxygenation.

The simultaneous use of three different modalities to assess different aspects of tissue microvascular function was an important strength of this investigation. SDF imaging is a non-invasive technique which provides a real time video image of the intact microcirculation. However, this technique is limited by semi-quantitative analysis and the fact that it can only be used to image the microcirculation under mucosal surfaces. Laser Doppler flowmetry is a technique based on the Doppler shift of reflected laser light from moving red blood cells. This method cannot distinguish the size or type of microvessel, direction of flow or heterogeneity of flow, all of which may be important in critically ill or high-risk surgical patients. These limitations can be addressed through the measurement of post-occlusion reactive hyperaemia which provides a reproducible assessment of endothelium dependant microvascular response.[261] The cutaneous Clarke electrode measures the local partial pressure of oxygen by a polarographic method. If tissue perfusion decreases whilst PaO_2 remains constant, cutaneous PtO_2 will decrease thus linking peripheral perfusion and tissue oxygenation.[303] The consistent patterns of change identified with each of the three modalities is therefore of particular importance. However, these methods have been used to assess quite different aspects of microvascular function and cannot be directly compared. We have presented the changes in microvascular flow in terms of change from the baseline values. Whilst differences are less apparent on analysis of absolute values, the consistency of the changes we observed between three distinct measures of tissue perfusion strongly suggests that these findings are robust.

5.5 Conclusions

A treatment algorithm incorporating stroke volume guided fluid therapy plus low dose dopexamine infusion was associated with significant improvements in microvascular flow and tissue oxygenation but no change in the inflammatory response to surgery. These physiological changes may explain the beneficial effects of cardiac output guided haemodynamic therapy demonstrated in previous clinical trials. Our findings strongly support the need for large multi-centre trials to evaluate the clinical effectiveness of cardiac output guided haemodynamic therapy. Several such trials are now under way in patients with severe sepsis, those undergoing major surgery and in potential organ donors.

Chapter 6
Renal effects of post-operative haemodynamic optimisation

6.1 Introduction

Post-operative renal dysfunction is common and accounts for between 18 and 47% of all cases of hospital acquired renal failure.[304,305] Acute renal dysfunction is associated with increased mortality rates following both cardiac and non-cardiac surgery. [306 307] A number of factors play a role in its occurence including the administration of anti-inflammatory and nephrotoxic agents, pre-existing renal dysfunction as well as patient comorbidities and the type of surgery.[308] It is likely that hypoperfusion plays an important role in most cases of postoperative renal dysfunction.[305] Previous work has shown that 80% of patients who develop postoperative renal dysfunction had a documented period of haemodynamic instability perioperatively. There is no evidence that any specific pharmacological treatments help prevent postoperative renal dysfunction. Therefore, maintaining perfusion remains the mainstay in preventing deterioration in renal function. GDHT through optimising cardiac output and thus organ blood flow might be expected to ameliorate postoperative renal dysfunction. A recent meta-analysis examined 20 randomised controlled trials (4220 patients) of GDHT and the incidence of postoperative renal dysfunction.[49] These authors found that postoperative acute renal injury was significantly reduced by perioperative haemodynamic optimisation. This was true whether the intervention was commenced preoperatively, intraoperatively or postoperatively.

Similar to the systemic inflammatory response syndrome (SIRS) and sepsis, surgical stress causes a disseminated inflammatory response associated with changes in endothelial function influenced by the action of pro-inflammatory cytokines.[309,310] An early feature of the acute inflammatory process is capillary endothelial cell activation causing a rapid increase in capillary permeability to plasma proteins including albumin. The transcapillary escape of radiolabeled albumin from the circulation increases dramatically following cardiac surgery, as well as in patients with sepsis and malignancy.[311] In an experimental model of the early stages of inflammation following surgery and peritonitis, increases in systemic vascular permeability were accompanied by increases in renal permeability to macromolecules. Thus the finding of microalbuminuria following surgery is a reflection of systemic vascular endothelial permeability as part of the inflammatory process.

The degree of microalbuminuria and its time course post-operatively appears to be related to the extent of the surgical stress.[312,313] There is also some evidence in surgical patients that persistent microalbuminuria following surgery may be associated with a poor outcome. De Gaudio and colleagues found an increase in microalbuminuria post-operatively in those patients who became septic.[309] A threshold value of 3 mg/mmol (albumin / creatinine ratio) has been found to be a very sensitive predictor of poor outcomes, but at the expense of specificity and a low positive predictive value.[314] Szakmany and colleagues found a significant difference between survivors and non-survivors in microalbuminuria immediately following major abdominal surgery.[315] Whether there is any correlation between glomerular permeability and lung permeability remains unclear.[309,315]

Microalbuminuria has been used as a marker of inflammation following surgery in two interventional trials to date.[316,317] The oxygen free radical scavenger, N-acetylcysteine had no effect on microalbuminuria, whereas low dose steroids appeared to reduce microalbuminuria.[316,317]

There is some evidence to suggest that the use of inotropic therapy and intra-venous fluids results in a reduction in the inflammatory process and the degree of renal dysfunction.[41,49,318,319] The aim of this analysis was to determine the effects of three different haemodynamic strategies following major surgery on microalbuminuria and indices of renal function.

6.2 Methods

A detailed description of patient recruitment and clinical management is provided in Chapter 5. Urine samples were obtained from a urinary catheter which was inserted after induction of anaesthesia, but before surgery. It has been shown previously that uncomplicated catheterization does not alter urinary albumin concentrations.[320] Urine samples were taken at four timepoints. T0 was after induction of anaesthesia but before surgery commenced. T1 was following surgery on admission to the critical care unit before the intervention commenced. T2 was at the end of the eight hour intervention period. T3 was 24 hours following the end of surgery. Samples were placed into polypropylene tubes and placed in a -80 freezer within 15 minutes. The measurement of microalbuminuria to creatinine ratios is described in chapter 2. Serum creatinine was obtained from samples ordered and taken by the attending clinical team for up to seven days following surgery. Serum creatinine was measured using the kinetic Jaffe method (Roche modular P unit). Microalbuminuria was assessed using an immunoturbimetric method (see chapter 2). Estimated glomerular filtration rate (eGFR) was calculated preoperatively upto day 7 after surgery from serum creatinine, age, race and gender using the Modification of Diet in Renal Disease equation.[321] Patients were prospectively followed for the development of acute kidney injury within seven days of surgery.[322]

6.3 Results

135 patients were recruited. Baseline demographics and outcomes are detailed in Chapter 5. Urine samples were obtained in all patients except one. Microalbuminuria to creatinine ratios (MACR) increased immediately following surgery and fell in the subsequent 24 hours. Changes in MACR in the three different treatment groups are shown in figure 6.1. There was no difference in MACR in those patients who went on to develop complications versus those who did not (figure 6.2). There was no significant correlation between MACR and P/F ratio or hospital stay.

There was a significant fall in serum creatinine from preoperative levels to 7 days postoperatively in all three treatment groups. However, there was no significant difference between treatment groups in serum creatinine over the same time period ($p=0.47$, two way repeated measures ANOVA) (figure 6.3). There was, however, a significant difference between treatment groups in eGFR ($p=0.02$, two way repeated measures ANOVA) (figure 6.4). During the first seven days after surgery, eGFR increased significantly in the SV & DPX group but not in the SV or the CVP group (SV & DPX group 21 [20] ml/min, $p=0.001$; SV group 10 [33] ml/min, $p=0.09$; CVP group 2 [35] ml/min; $p=0.73$). Consequently, a post hoc analysis of the predefined renal outcome was performed. Ten (22%) patients in the CVP group developed acute kidney injury within 7 days of surgery. There was a trend towards fewer patients developing acute kidney injury in the SV (3[7%]) and SV & DPX groups (4[9%]) within seven days of surgery ($p=0.055$ One way ANOVA). There was a significant reduction in the incidence of acute kidney injury in the pooled SV and SV & DPX groups ($p=0.03$ t-test).

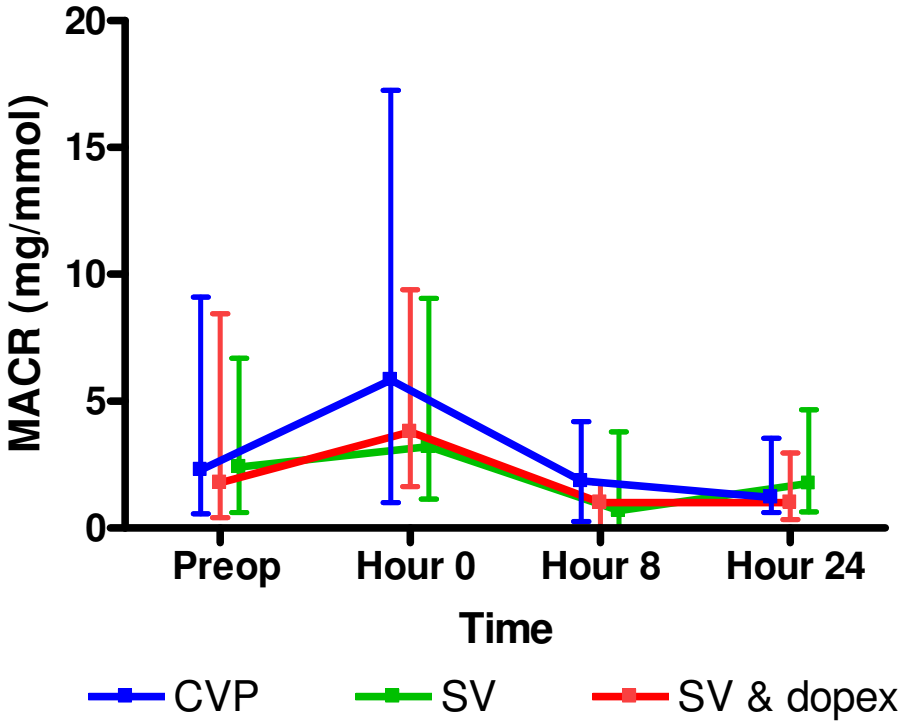


Figure 6.1 Changes in microalbumin to creatinine ratio (MACR) following major abdominal surgery with three different haemodynamic therapies. Data presented as median (IQR). No significant difference between groups.

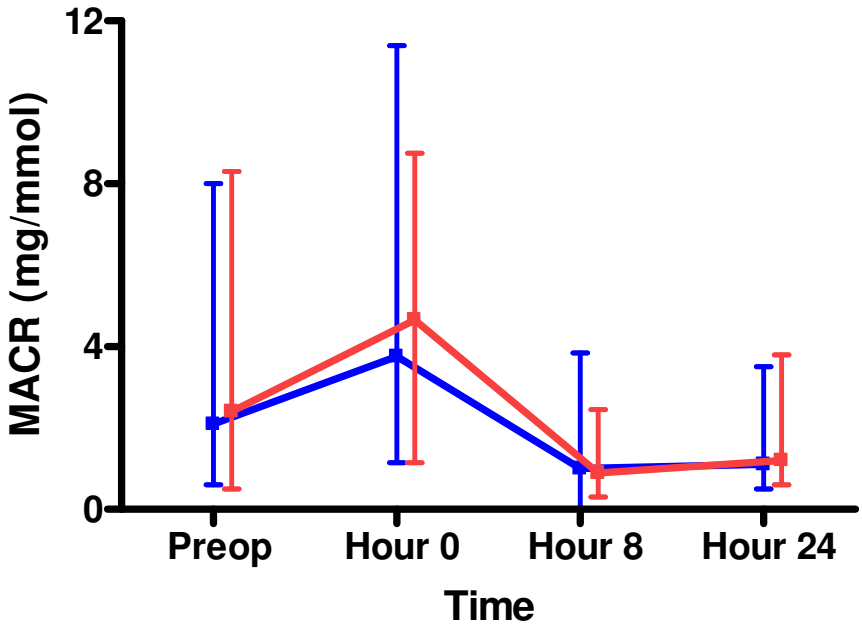


Figure 6.2 Microalbumin to creatinine ratio (MACR) in those patients who did and did not develop complications. Data presented as median (IQR). No significant difference between groups.

Blue : complications, **Red** : no complications.

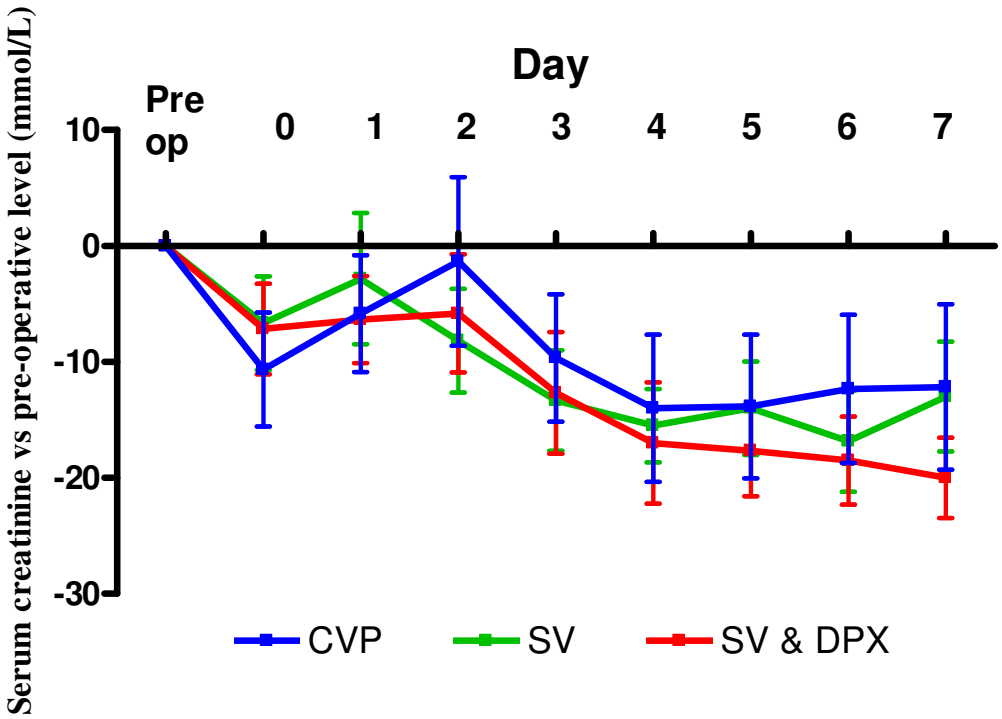


Figure 6.3 Change in serum creatinine for first seven days following surgery in the three treatment groups. **Data presented as median (IQR). No difference between groups (p=0.47 Two way repeated measures ANOVA)**

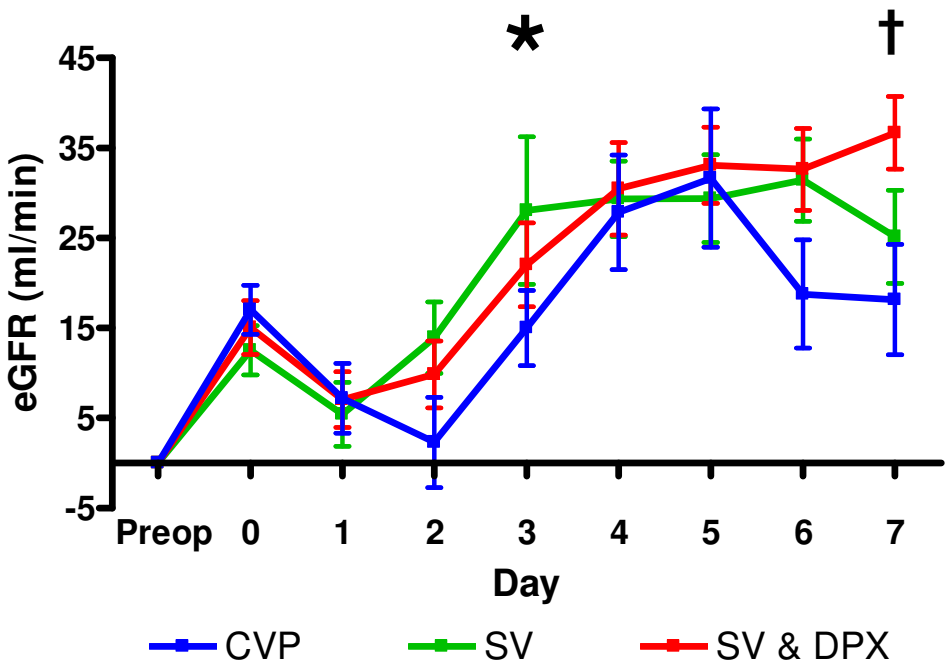


Figure 6.4 Changes in estimated glomerular filtration rate (eGFR) following surgery in three treatment groups. * Significant difference between groups over time (p=0.02) (two way repeated measures ANOVA).

† At day 7, there was a significant difference in eGFR between the SV & DPX and CVP groups (p<0.05) but not between the SV and CVP groups (p>0.05).

6.4 Discusssion

We found a significant decrease in serum creatinine and a corresponding increase in the estimated glomerular filtration rate in all groups over the first seven days postoperatively. There was a significant difference between groups in the change in eGFR post-operatively with greater improvements in the group receiving stroke volume guided fluid therapy plus dopexamine. This was associated with a reduced incidence of acute kidney injury in the pooled stroke volume optimised groups compared to the CVP group. There was no significant difference in the volume of fluid given either during the operation or the intervention period between groups. As expected MACR increased immediately following surgery and then fell over the subsequent 24 hours. We did not find any difference in MACR between treatment groups. In contrast to previous work, we did not find any association between changes in MACR and outcome or any correlation between MACR and PaO_2 / FiO_2 ratio.

No studies of perioperative optimisation to date have analysed postoperative renal dysfunction as a specific outcome measure. A recent meta-analysis found that the incidence of postoperative AKI was significantly reduced by perioperative haemodynamic optimisation.[49] One of the major difficulties in this area is the lack of a uniform definition of renal failure. The main outcome measure in this meta-analysis was worsening of renal function, using whichever definition had been used by the authors of the individual studies. Sub-group analyses were performed examining the different definitions of AKI (eg RIFLE, AKIN, need for RRT) and the timing of optimisation (pre-, intra- or postoperative). All of these analyses produced similar findings to the main outcome measure. The exact biological mechanism for this protective effect remains unclear, although it is possible that

an inadequate cardiac output perioperatively may directly lead to renal hypoperfusion, as well as activating neurohumoral responses that promote renal vasoconstriction. Maintainance of cardiac output may thus ensure adequate renal blood flow and limit renal vasoconstriction.

We found a significant improvement in eGFR associated with GDHT, as well as a trend towards a reduction in the incidence of acute kidney injury (AKI). Although this analysis was post-hoc, it was performed on an a priori defined post-operative complication using the Acute Kidney Network definition of AKI. Similar to our findings, previous work has found an increase in MACR associated with surgery.[315] In contrast to previous work, however, we did not find any association between MACR and clinical outcomes.[314,315,323] We also did not find any difference in MACR between treatment groups. De Gaudio and colleagues found that post-operative patients that developed septic complications showed an increase in MACR compared to those who had an uncomplicated post-operative course. These increases, however, occurred at the time of the septic complication and since we only took samples up to 24 hours post-opertaively, we would not have captured this data. Both Gosling and Szakmany found significant increases in MACR immediately following surgery in non-survivors.[315,323] The mortality rate in both of these studies was higher than in the present study, so it is possible that our patient group was underpowered to find an association between MACR and mortality. Interestingly, in both of these studies further samples were taken within six hours of admission to ICU. In both studies, the MACR fell significantly over this time period. This may partly explain why we found no difference between treatment groups in MACR given that we sampled urine only at eight and 24 hours following commencement of treatment.

In conclusion, we found a significant improvement in estimated glomerular filtration rate associated with GDHT, together with a trend towards a reduction in the incidence of AKI. Although MACR increased with surgery, there was no association with outcome or any difference between treatment groups. Larger prospective studies are required to investigate whether GDHT reduces the incidence of AKI following major abdominal surgery.

Chapter 7
Haemodynamic changes according to outcome following post-operative haemodynamic optimisation

7.1 Introduction

Links between surgical outcomes and perioperative haemodynamics were first demonstrated by Clowes and colleagues in 1960.[32] William Shoemaker followed up this work with a series of observational studies demonstrating that a number of haemodynamic and oxygen transport variables were correlated with survival following major surgery.[33-35] Since then a number of investigators have evaluated changes in perioperative physiology and their association with outcome.[324,325] This has led to the use of a number of these variables as endpoints for perioperative haemodynamic therapy. Although the validity of comparing clinical outcomes from within a randomised controlled trial of three different interventions may be questionable such an analysis might generate some interesting hypotheses and could help to improve understanding of perioperative physiology. The aim of this analysis, therefore, was to compare changes in post-operative physiology in those patients who did or did not develop complications following surgery using the cohort recruited to the randomised controlled trial described in Chapter 5.

7.2 Methods

Data has been analysed from the randomised controlled trial of post-operative haemodynamic optimisation described in chapter 5. Recruitment, monitoring and the cardiovascular management of patients during the first eight hours after surgery are described in detail in the methods section of chapter 5. All patients received usual care following this eight hour period for the rest of their hospital stay.

Routine demographic data were captured and P-POSSUM scores and ASA scores calculated. Complications and deaths up to 28 days following surgery were included in this analysis. Complications were prospectively defined (see Appendix). Clinical outcome data were collected by a blinded member of the research team and verified by the principal investigator. Physiological, microvascular and tissue oxygenation data were collected as described in Chapters 2 and 5.

7.2.1 Statistical analysis

Comparisons between groups were performed by t-test or a Mann-Whitney test depending on whether the data was normally distributed. Two way repeated measures ANOVA were used to compare differences between groups over time. Categorical variables were tested with the Chi squared or Fisher's exact tests. Receiver operating characteristic (ROC) curves were constructed to identify optimal cut-off values for association with complications. Statistical analysis was performed using GraphPad Prism version 4.0 (Graphpad software, USA). Significance was set at $p<0.05$. Data are presented as mean (SD) where normally distributed or median (IQR) if not normally distributed. Categorical

variables are described as a percentage of the group from which they are derived. Normality was ascertained using the D'Agostino-Pearson test.

7.3 Results

One hundred and thirty five patients were recruited between December 2007 and February 2009 and randomised into one of three treatment groups (see Chapter 5). 87 (64%) of these patients developed pre-defined complications following surgery (see Appendix for definitions of complications). There was no difference in age, p-POSSUM score or the use of GDHT between those patients who went on to develop complications and those who did not (Table 7.1). Unsurprisingly, critical care stay and hospital stay were longer in those patients who developed complications (Table 7.1). There was no difference between groups in baseline or minimum heart rate, mean arterial pressure or cardiac index. The lowest recorded central venous oxygen saturations were significantly lower in those patients who went on to develop complications (Table 7.2). The optimal value of minimum $ScvO_2$ for discriminating those patients who did and did not develop complications was 65.0% (sensitivity 49% and specificity 78%) (Figure 7.1). Both baseline and the lowest recorded transcutaneous tissue oxygenation tension were also lower in those patients who went on to develop complications (Table 7.2). The optimal value of PtO_2 for discriminating those patients who did and did not develop complications was 5.9kPa (sensitivity 45% and specificity 83%) (Figure 7.2). There was no difference in global haemodynamics between those patients who developed complications compared to those who did not except in central venous oxygen saturations (Figure 7.3). Base deficit was greater in those patients who went on to develop complications though there was no difference in serum lactate (Figure 7.4). There was no difference in microvascular flow

found over the study period between groups (Figure 7.5). Interestingly, however, transcutaneous tissue oxygenation was significantly lower throughout the whole study period in those patients who went on to develop complications (Figure 7.5). There were no significant differences in inflammatory markers between those patients who developed complications and those who did not (Figure 7.6).

	No complications (n=48)	Complications (n=87)	p
Age	66.2 (11.2)	66.8 (11.8)	0.76
P-POSSUM	36 (6)	37 (6)	0.26
GDHT	33/48 (69%)	57/87 (65%)	0.85
Alive and 28 day Crit care free days	26 (24-27)	23 (17-26)	<0.0001
Hospital stay (days)	10 (8-13)	20 (15-31)	<0.0001

Table 7.1 Patient demographics and hospital stay in those patients who developed complications and those who did not

	No complications (n=48)	Complications (n=87)	p
Baseline heart rate (bpm)	75 (14)	77 (15)	0.53
Maximum heart rate (bpm)	85 (16)	91 (16)	0.053
Baseline mean arterial pressure (mm Hg)	80 (17)	78 (20)	0.24
Minimum mean arterial pressure (mm Hg)	71 (12)	68 (13)	0.28
Baseline global oxygen delivery index (ml/min/m^2)	441 (126)	491 (158)	0.08
Minimum global oxygen delivery index (ml/min/m^2)	385 (322-488)	411 (338-521)	0.13
Baseline cardiac index (L/min/m^2)	3.3 (1.0)	3.4 (0.9)	0.52
Minimum cardiac index (L/min/m^2)	3.0 (0.7)	3.2 (0.8)	0.32
Baseline central venous oxygen saturations (%)	75 (9)	71 (10)	0.06
Minimum central venous oxygen saturations (%)	70 (7.2)	65 (10)	0.0076
Baseline cutaneous tissue oxygenation (kPa)	9.6 (3.8)	8.0 (3.3)	0.009
Minimum cutaneous tissue oxygenation (kPa)	8.4 (3.0)	6.5 (2.8)	0.0004
Baseline perfused vessel density (n/mm)	5.8 (4.7-7.5)	5.1 (4.1-6.4)	0.07
Minimum perfused vessel density	4.5 (1.5)	4.4 (2.0)	0.99

Table 7.2 Haemodynamic, microvascular and tissue oxygenation variables in those patients who developed complications and those who did not. Data presented as mean (SD) or median (IQR) as appropriate. Baseline refers to immediately following surgery.

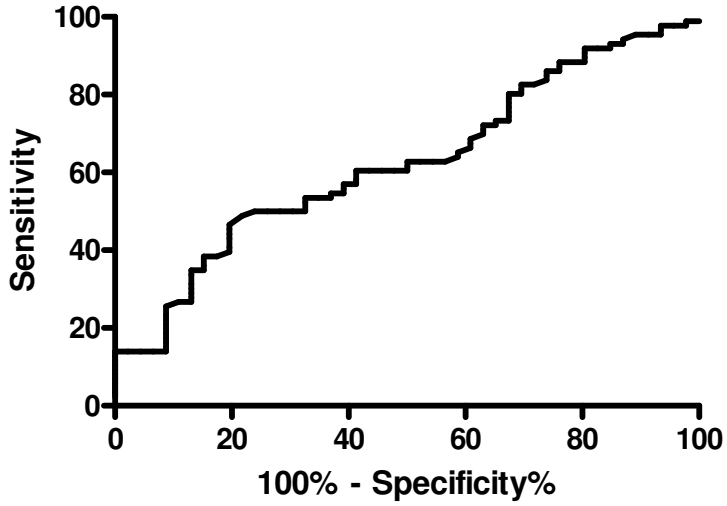


Figure 7.1 Receiver Operating Characteristic (ROC) curve for predicting complications with minimum $ScvO_2$. Area under curve 0.63 (95%CI 0.53-0.72)

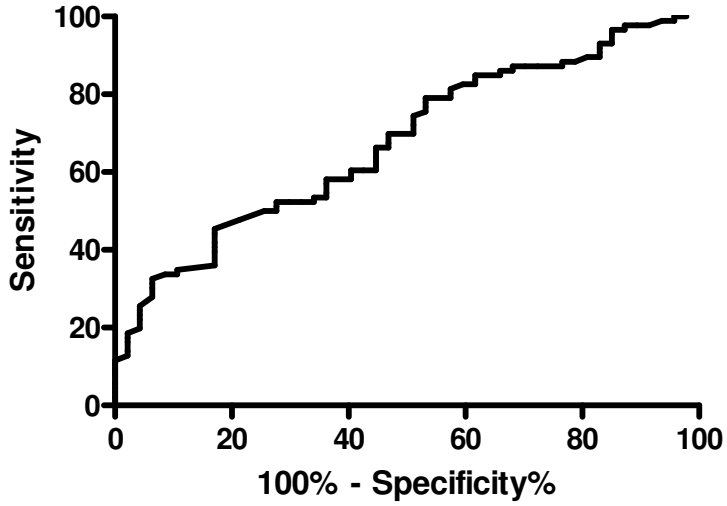


Figure 7.2 Receiver Operating Characteristic (ROC) curve for predicting complications with minimum PtO_2. Area under curve 0.68 (95%CI 0.58-0.77).

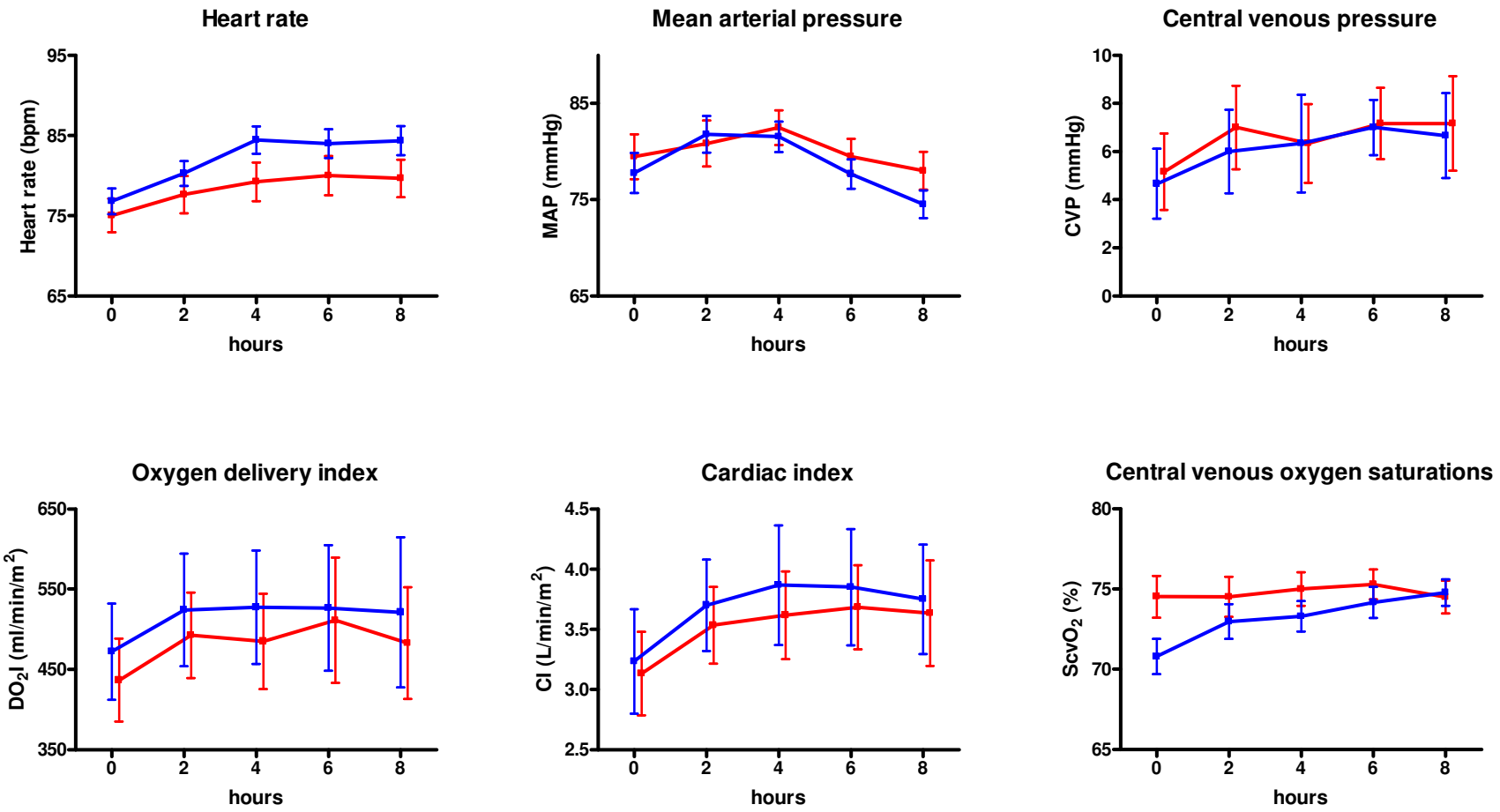


Figure 7.3. Global haemodynamics during eight hour study period. Data presented as mean (SEM). No significant difference between groups except $ScvO_2$ $p<0.05$ (two way repeated measures ANOVA).
Blue : complications, **Red** : no complications.

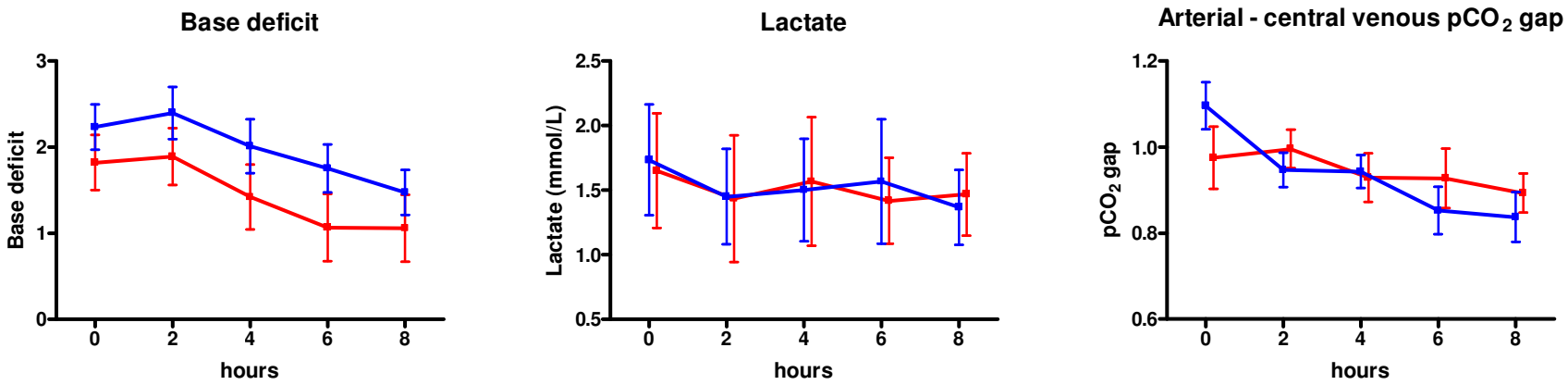


Figure 7.4 Changes in markers of tissue perfusion during study period. Data presented as mean (SEM). No significant difference between groups except base deficit ($p<0.05$) (two way repeated measures ANOVA).
Blue : complications, **Red** : no complications.

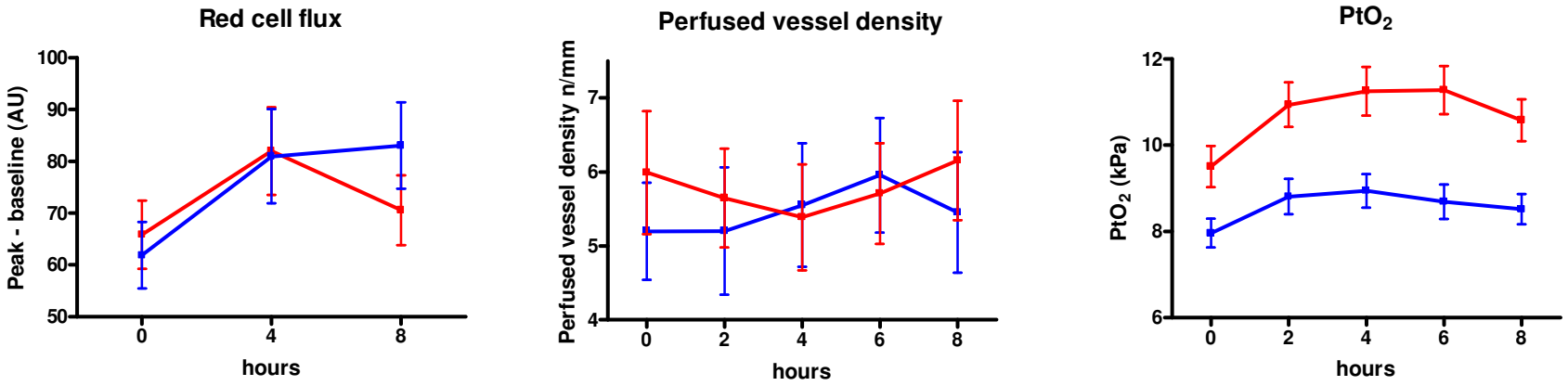


Figure 7.5 Changes in microvascular flow and tissue oxygenation during study period. Data presented as mean (SEM). No significant difference between groups in microvascular flow. Significant difference in PtO_2 between groups ($p<0.0001$) (two way repeated measures ANOVA).
Blue : complications, Red : no complications.

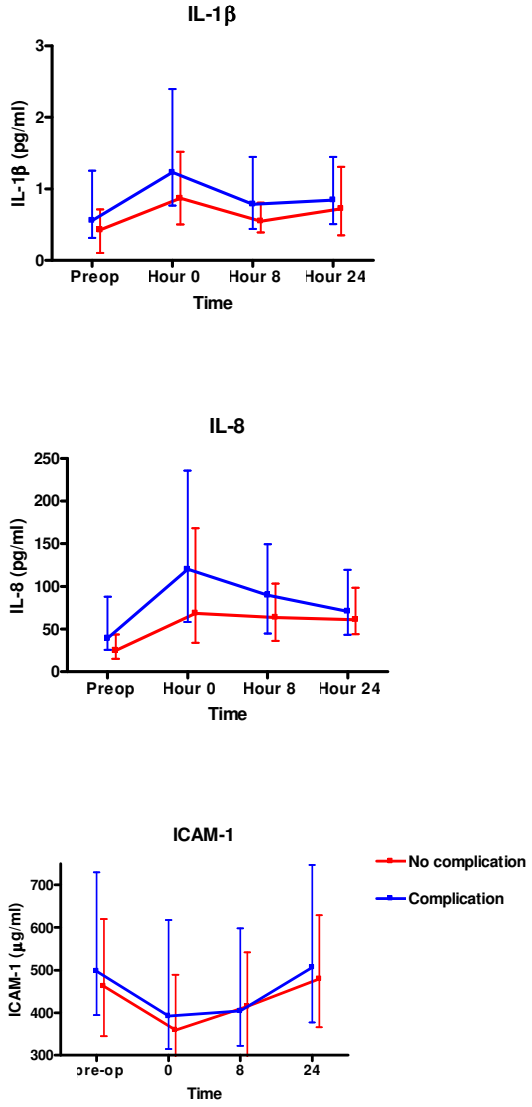


Figure 7.6 Inflammatory markers in those patients who did and did not develop complications. Data presented as median (IQR).

Blue : complications, Red : no complications.

7.4 Discussion

The main finding from this analysis was that there were differences in central venous oxygen saturations, base deficit and transcutaneous tissue oxygenation between those patients who did and did not develop complications post-operatively. Surprisingly, there were no other differences in global haemodynamics or markers of tissue perfusion between groups. Interestingly, despite the difference in tissue oxygenation we only found a trend towards lower baseline sublingual microvascular flow in those patients who went on to develop complications and there was no difference in cutaneous microvascular flow.

Clowes and colleagues provided the first data linking global haemodynamics with perioperative outcomes.[32] They found that survivors of thoracic and emergency abdominal surgery tended to achieve higher cardiac outputs than those who died. William Shoemaker built on this early work publishing a list of haemodyanmic variables that correlate with survival after major surgery.[33] More recent work has investigated changes in central venous oxygen saturations associated with surgery.[324,325] Surprisingly, we found no difference in global oxygen delivery or cardiac index between those patients who went on to develop complications and those who did not. There were differences in central venous oxygen saturation and cutaneous tissue oxygenation, however. Although there was a trend to a difference in baseline sublingual microvascular flow immediately following surgery in those who went on to develop complications, this trend did not remain over the subsequent treatment period. Why we found differences in some variables but not others is unclear. The total volumes of intra-venous fluid administered were similar between the groups despite the differing endpoints. This suggests a high standard of care for all patients that may have minimised any differences. As mentioned previously, the validity of

comparing clinical outcomes from within a randomised controlled trial of three different interventions may have compromised this secondary analysis. A lowest recorded central venous saturation value of 65% was associated with complications in this analysis. This value is very similar to a previous analysis also performed within a randomised controlled trial of GDHT.[287] A European multicentre study of perioperative patients undergoing major abdominal surgery receiving usual care, found a higher central venous saturation cut-off of 73% for prediciting complications.[325] Tissue oxygenation was also found to be lower in those patients who went on to develop complications. Previous investigators have reported similar findings, with Hopf and colleagues finding that low subcutaneous tissue oxygen tension was associated with an increase in the incidence of wound infections.[178] Baseline oxygen tensions in those patients who developed complications in the study performed by Hopf were similar to the cut-off found in the current analysis. Although we found marked differences between groups in transcutaneous tissue oxygenation, we found no difference in microvascular flow. The reason for this is unclear. We found no statistical difference in the pro-inflammatory response to surgery between those patients who developed complications and those who did not.

In conclusion, we found an association between the development of postoperative complications and low central venous oxygen saturations and impaired tissue oxygenation. We found no differences in other measures of global haemodynamics or in microvascular flow between those patients who developed complications and those who did not.

Chapter 8
Early microvascular changes in sepsis and severe sepsis

8.1 Introduction

Sepsis is a major cause of death and disability worldwide. It is estimated that 750,000 people develop sepsis each year in North America alone.[9] In the UK, approximately 35,000 patients are admitted to intensive care with sepsis each year, of whom 45% do not survive to leave hospital.[10] Approximately one third of these patients are admitted directly from the emergency department.[326,327] Sepsis related deaths result from multi-organ failure which generally develops in the early stages of the disease.[11] There is increasing recognition that early therapeutic intervention is key to improving survival following sepsis. This has led to a particular emphasis on the cardiovascular management of the septic patient within the first few hours of hospital admission.[328]

Sepsis related cardiovascular changes are complex and include vasodilatation, hypovolaemia and myocardial depression.[66,329,330] Sepsis also results in important derangements of the microcirculation which are associated with organ failure and death.[94] Such effects include increased endothelial permeability,[331,332] endothelial-leucocyte adhesion,[333] and a characteristic heterogeneity of blood flow which is associated with tissue hypoxia.[84,297] Several investigations have identified abnormalities of microvascular flow in patients with established severe sepsis. However, understanding of the natural history of microvascular dysfunction remains incomplete. Recent investigations by

Trzeciak and colleagues have identified abnormalities in sublingual microvascular flow in patients soon after the commencement of early goal directed haemodynamic therapy.[95,161] These data illustrate the significance of early impairments in microvascular flow and suggest potentially beneficial effects of haemodynamic resuscitation on such abnormalities. However, these data only describe microvascular changes in severely ill septic patients receiving a specific form of haemodynamic resuscitation. Because of the considerable interest in interventions which may improve microvascular function,[15,155,157] there is a need to further describe patterns of microvascular flow in a wider group of septic patients at the earliest stages after presentation. The aim of this study was to evaluate the sublingual microcirculation of patients with sepsis and severe sepsis within six hours of hospital admission and compare these data to that of healthy volunteers.

8.2 Methods

This single centre, observational study was approved by the Local Research Ethics Committee. Adult patients within six hours of admission to the Emergency Department at The Royal London Hospital with confirmed or strongly suspected sepsis were eligible for recruitment. [334] In addition, adult hospital in-patients within six hours of identification of the onset of sepsis were also eligible for inclusion. Written informed consent was sought from patients with capacity. Where a patient did not have capacity to give consent, a relative or healthcare practitioner was approached to provide assent. Retrospective consent for data use was sought once capacity was regained. There were no exclusion criteria except refusal of patient consent or relatives assent. Treatment in the emergency department was guided by local policies. Patients admitted to the intensive care unit received treatment in

accordance with the Surviving Sepsis guidelines current at that time. [335] All treatment decisions were made by the attending physician in charge of the intensive care unit.

8.2.1 Measurements

In addition to routine haemodynamic data, sublingual microvascular flow and cardiac output were measured using non-invasive technology. Sublingual microvascular flow was evaluated using sidestream darkfield (SDF) imaging with a x5 objective lens (Microscan, Microvision Medical, Amsterdam, Netherlands).[59] Image acquisition and subsequent analysis was performed according to published consensus criteria as described in chapter two.[277] Analysis of the videos was performed by two blinded observers (AS and AVS- see acknowledgements). Cardiac output was measured using the supra-sternal Doppler technique (USCOM Ltd., Australia).[336] Patients were followed up for 28 day in-hospital mortality.

8.2.2 Statistical analysis

There was no formal sample size calculation. Instead we planned to recruit 20 patients with sepsis, severe sepsis and septic shock as well as 20 healthy volunteers.

Normally distributed data were tested with unpaired t-tests or one-way analysis of variance (ANOVA) with post-hoc Bonferroni multiple comparison tests as appropriate. The Mann-Whitney U test or Kruskal-Wallis test with post-hoc Dunn's tests were used when data were not normally distributed. Data are presented as mean (SD) where normally distributed or median (IQR) where not normally distributed. Normality was tested with the

D’Agostino-Pearson test. Significance was set at $p<0.05$. Analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, USA).

8.3 Results

A total of 48 patients and 16 healthy volunteers were recruited between September 2007 and April 2008. The healthy volunteers had a median age of 39 (36-43) years and eight (50 %) were male. Septic patients had a median age of 50 (30-70) years and 24 (50 %) were male. Baseline patient data are presented in table 8.1. Four hospital in-patients who had developed severe sepsis were recruited. The remaining 44 patients were recruited in the Emergency Department. The median time from arrival at the emergency department to recruitment was 159 (104-218) minutes. Nineteen patients were classified as having severe sepsis on the following basis: fluid resuscitation required to treat hypotension (n=9), persistent hypotension despite fluid resuscitation (n=7), serum lactate >4mmol/L (n=3). The remaining 29 patients were classified as having uncomplicated sepsis. Eight patients were admitted to critical care within 24 hours of enrolment. At the time of admission to critical care four of these patients required invasive ventilation and five required vasopressor or inotropic therapy. None of these patients required renal replacement therapy. Sublingual microvascular flow and haemodynamic data were collected in all patients. Two patients were receiving vasopressor therapy at the time measurements were taken. There were no significant differences in microvascular flow between these patients and the rest of the cohort. 30 patients had received intravenous fluid prior to measurements (volume infused 1000 mls [500-1750]). The intravenous fluids used were 0.9% sodium chloride, compound sodium lactate solution, or a gelatin colloid solution (Gelofusine B Braun). For large vessels (>20μm) MFI was 3.0 (2.9-3.0) and the proportion of perfused vessels was 100% (100-100) in all patients. The kappa coefficient with linear weighting for inter- observer variability for calculation of MFI was 0.76 (0.68-0.84). The inter-observer coefficient of variability for vessel density was 5.9%.

8.3.1 Comparison to healthy volunteers

Heart rate was significantly greater in all patients and mean arterial pressure was reduced in the severe sepsis group. There was no difference in cardiac index between groups (Figure 8.1).For small vessels (<20μm), MFI, heterogeneity index and the proportion of perfused vessels were significantly reduced in both groups compared to healthy volunteers (table 8.2 & figure 8.3). Significant reductions in perfused vessel density were identified in the severe sepsis group but not the sepsis group when compared to healthy volunteers.

8.3.2 Comparison between patients with sepsis and severe sepsis

Abnormalities of MFI and perfused vessel density for small vessels (<20μm) were more marked in the severe sepsis group compared to the sepsis group (table 8.2 and figure 8.3). There was no difference in cardiac index between the sepsis and severe sepsis groups (figure 8.1). Mean arterial pressure was lower and serum lactate was greater in the severe sepsis group.

8.3.3 Comparison between survivors and non-survivors

In non-survivors, mean arterial pressure was lower and heart rate was greater but there was no difference in cardiac index (Table 8.3 and Figure 8.2). There was also a trend towards higher serum lactate concentration (p=0.05) in non-survivors. The proportion of perfused vessels was significantly reduced in those patients who did not survive to leave hospital (figure 8.4).

	Sepsis n=29	Severe sepsis n=19	p
Age (years)	33 (25-58)	66 (45-82)	0.0007
Male	12 (41%)	12 (63%)	0.24
Apache II score	8 (5)	18 (7)	<0.0001
Critical care admission	2 (7%)	6 (32%)	0.04
Hospital stay (days)	2 (1-7)	12 (5-20)	0.001
Mortality	1 (3%)	7 (37%)	0.004
Source of sepsis			
Respiratory	11 (38%)	8 (42%)	>0.99
Abdominal	6 (21%)	5 (26%)	0.73
Urological	8 (28%)	2 (11%)	0.28
Soft tissue	2 (7%)	1 (5%)	>0.99
Neurological	1 (3%)	0 (0%)	>0.99
Other	1 (3%)	3 (16%)	0.29

Table 8.1. Patient demographics. Data presented as mean (SD), median (IQR) or absolute values (%) (Fisher's exact test or Mann- Whitney U test).

	Healthy Volunteers n=16	Sepsis n=29	Severe sepsis n=19	p
Heart rate	69 (7)	115 (15) ***	114 (25) ***	<0.0001
Mean arterial pressure (mm Hg)	84 (5)	88 (15)	70 (16) * ‡	0.0001
Cardiac index (l min^{-1} m^{-2})	2.8 (0.7)	3.6 (1.2)	3.5 (2.3)	0.29
Serum lactate (mmol l^{-1})	-	1.8 (0.9)	3.1 (2.1) †	0.02
Base excess (mmol l^{-1})	-	-0.5 (3.7)	-1.1 (5.5)	0.68
Microvascular flow Index	3.0 (2.9-3.0)	2.8 (2.5-2.9) *	2.7 (2.2-2.8) *** †	0.0005
Vessel Density (n mm^{-1})	8.6 (1.6)	9.2 (1.4)	8.8 (1.6)	0.38
Perfused Vessel Density (n mm^{-1})	8.5 (1.5)	8.0 (1.5)	6.8 (2.0) * †	0.0085
Heterogeneity index	0.0 (0.0-0.09)	0.18 (0.09-0.39) *	0.21 (0.10-0.38) **	0.0025

Table 8.2. Haemodynamic data and microcirculatory indices of small vessels (<20µm). Data presented as mean (SD) or median (IQR).

***p<0.05 vs healthy volunteers, **p<0.01 vs healthy volunteers, ***p<0.001 vs healthy volunteers.;**

† p<0.05 vs sepsis group, ‡ p<0.001 vs sepsis group

(One-way analysis of variance or Kruskal-Wallis test between groups with post-hoc Bonferroni's multiple comparison test or Dunn's test).

	Survivors n=40	Non survivors n=8	p
Heart Rate	113 (19)	124 (21)	0.17
Mean arterial pressure (mm Hg)	85 (16)	64 (16)	0.001
Cardiac Index (l min^{-1} m^{-2})	3.7 (1.6)	2.9 (2.0)	0.25
Serum lactate (mmol l^{-1})	1.8 (1.2-2.4)	2.7 (1.4-5.4)	0.05
Base Excess (mmol l^{-1})	-0.5 (3.8)	-1.9 (4.8)	0.40
Microvascular flow index	2.8 (2.5-2.9)	2.4 (1.8-2.8)	0.07
Vessel Density (n mm^{-1})	9.0 (1.4)	9.1 (2.0)	0.88
Perfused vessel density (n mm^{-1})	7.7 (1.4)	6.5 (3.0)	0.06
Heterogeneity index	0.17 (0.09-0.33)	0.31 (0.20-0.65)	0.11

Table 8.3. Haemodynamics and microvascular flow indices of small vessels (<20 µm) for survivors and non survivors. Data presented as mean (SD) or median (IQR) (unpaired t-test or Mann-Whitney U test)

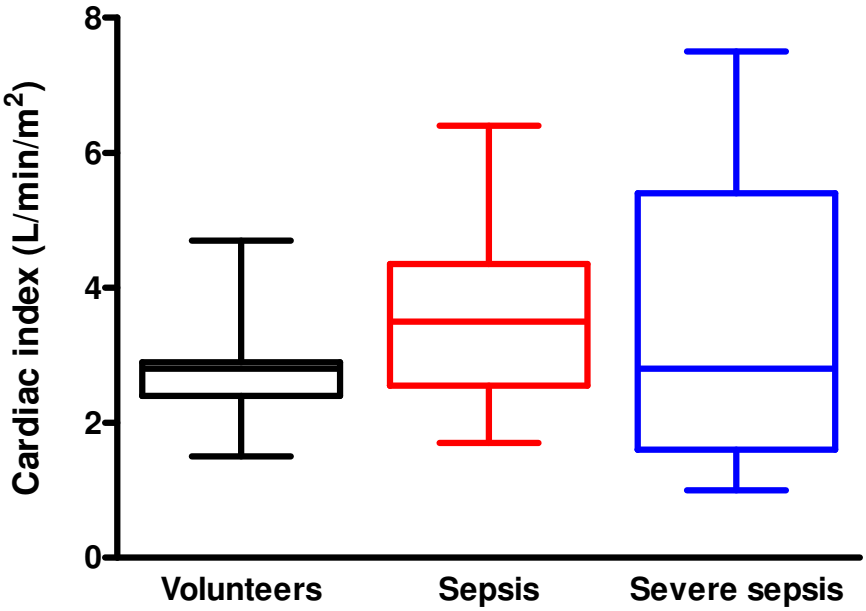


Figure 8.1 Cardiac index in healthy volunteers, patients with sepsis and patients with severe sepsis. No difference between groups (p=0.29, one way ANOVA)

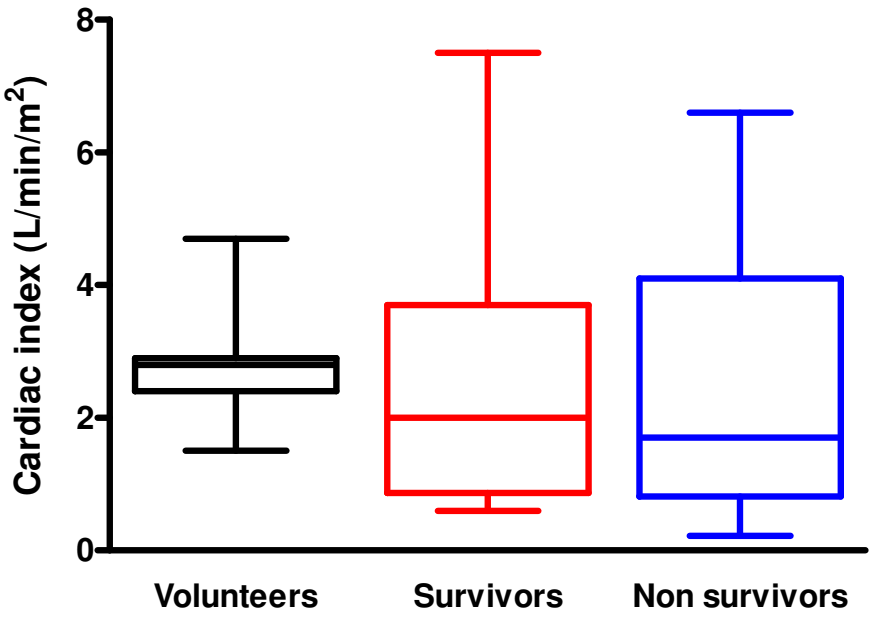


Figure 8.2 Cardiac index in healthy volunteers and survivors and non-survivors of sepsis.

No difference between groups (p=0.36, Kruskal-Wallis test)

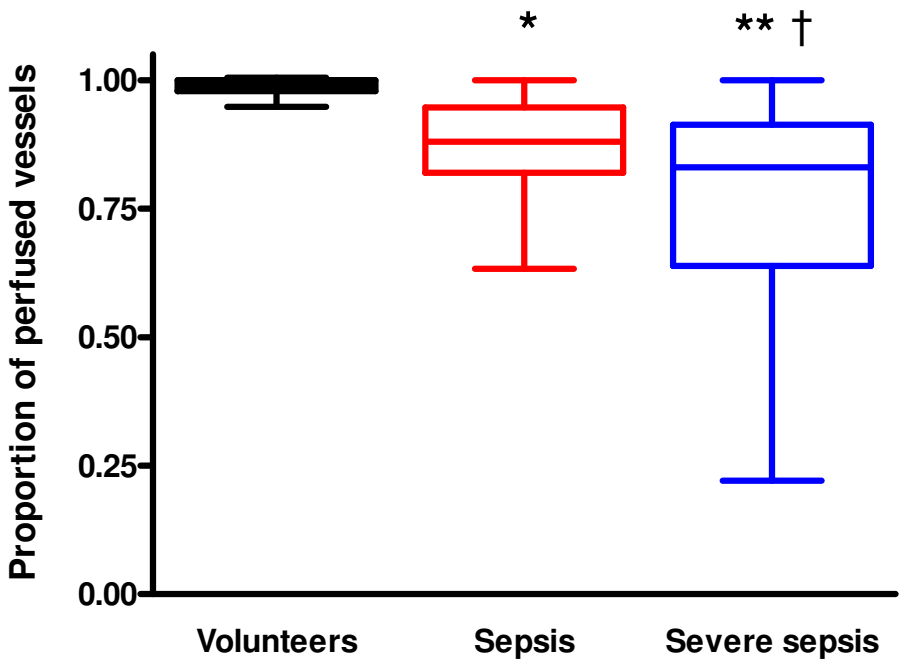


Figure 8.3. Proportion of perfused small vessels (<20 µm) in healthy volunteers, patients with sepsis and patients with severe sepsis. Data presented as median (IQR). *p<0.05 and **p<0.001 vs healthy volunteers, † p<0.05 vs sepsis group.

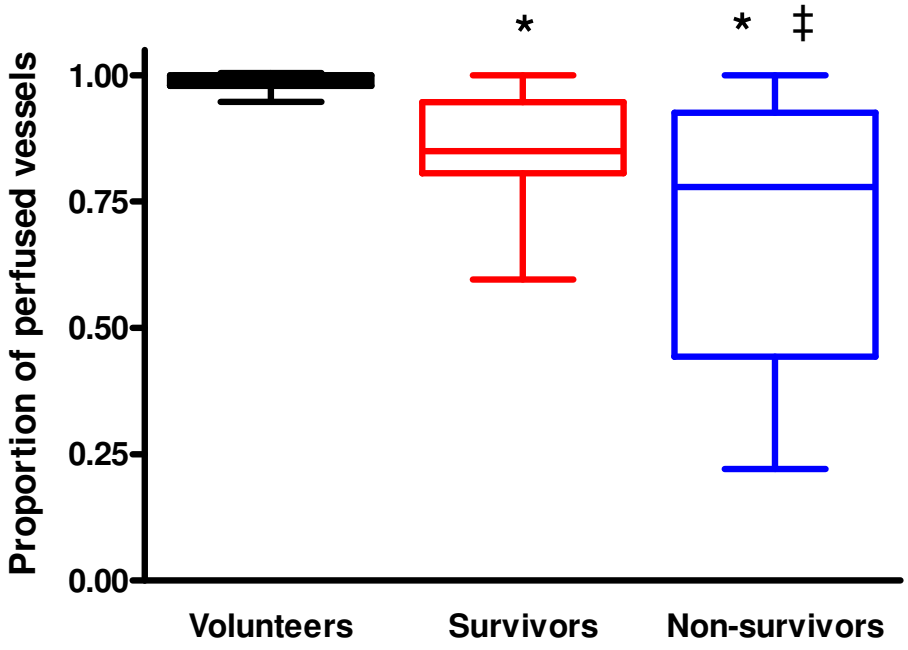


Figure 8.4. Proportion of perfused small vessels (<20 µm) in healthy volunteers and survivors and non-survivors of sepsis. Data presented as median (IQR). *p<0.01 vs healthy volunteers, ‡ p<0.01 vs survivors.

8.4 Discussion

The principle finding of this study was that there were significant derangements in sublingual microvascular flow in patients early after the diagnosis of sepsis which were more marked in the most severely ill patients. However, these abnormalities were less severe than those previously described in patients with established severe sepsis.[66,156] These findings confirm that the characteristic abnormalities of microvascular flow in established sepsis are already present in the earliest stages of the disease. Clinical interventions to improve microvascular flow should be considered at hospital presentation and might prevent progression to more severe microvascular abnormalities.

Previous investigations have characterised changes in sublingual microvascular flow in patients with established severe sepsis and demonstrated that persistent microvascular derangements are associated with poor outcome.[66,94] This work has led to interest in the effects of various therapeutic interventions on microvascular flow.[15,155,157] However, only one group has investigated microvascular derangements in patients with early sepsis.[95,161] In keeping with our findings, these investigators described derangements of sublingual microvascular flow which were more severe in non-survivors,[95] and improved following goal directed haemodynamic therapy guided by central venous oxygen saturation.[161] It is important to note that the majority of patients recruited to these studies were hospital in-patients (62 % and 48% respectively) representing a different population to those presenting with sepsis in the emergency department. In the current study, 92% of patients were recruited in the emergency department. This may partly explain the differences in the severity of abnormalities of microvascular flow. It remains uncertain to what extent sublingual microvascular flow correlates with that in other vascular beds.[99,274]

Sublingual SDF imaging is a valuable technique, which allows visualisation of the intact microcirculation in the clinical environment. Whilst several studies have demonstrated improvements in sublingual microvascular flow associated with specific clinical interventions,[15,155,156] at present there are no published data describing the use of this technique to guide clinical interventions. In a small number of individual cases, my supervisor has found it helpful to use sublingual SDF imaging as part of the clinical assessment of complex critically ill patients. However, at present this technique remains primarily a research tool and until further research establishes a clear role for routine clinical use, the authors would urge caution. Existing scoring systems for SDF image analysis remain semi-quantitative and data reliability may be affected by technical expertise and inter-observer bias.[277] We specifically evaluated these factors and our data indicate that they did not contribute to a significant degree of bias. All patients were recruited by AS (see Ackowledgements) who recorded detailed clinical notes as well as collecting haemodynamic and microvascular data. At the end of the study the notes were reviewed in a blinded fashion to confirm the diagnostic category. This review, which resulted in reclassification of 13 patients, may have introduced bias and reduced the number of significant findings. Although we identified a pattern of global haemodynamic and microvascular changes which was consistent with our expectations, some comparisons between groups did not achieve statistical significance because of the small number of patients recruited. A much larger investigation would be required to confirm the frequency and severity of early sepsis related abnormalities of microvascular flow. Patients in the severe sepsis group were significantly older than those in the sepsis group. Age may be associated with depletion of microvessels and may therefore represent a confounding factor.[337]

In conclusion, our findings confirm that the abnormalities of sublingual microvascular flow characteristic of established sepsis are already present in the earliest stages of the disease. These abnormalities are greatest in the more severely ill patients. Further research is needed to confirm these findings in a larger population and to investigate the efficacy of therapeutic strategies which maintain and improve microvascular flow.

Chapter 9
The effect of increasing doses of norepinephrine on tissue oxygenation and microvascular flow in patients with septic shock

9.1 Introduction

Sepsis is characterised by a complex combination of cardiovascular derangements including vasodilatation, hypovolaemia, myocardial depression and altered microvascular flow.[66,329,330,338] In severe cases, arterial hypotension may persist despite aggressive intravenous fluid resuscitation, a condition termed septic shock.[339] In health, constant organ blood flow is maintained by autoregulation over a range of mean arterial pressures (MAP) between 60 and 100 mmHg. When MAP falls below this range, organ blood flow also decreases in a linear fashion.[340] Consequently, in septic shock, vasopressor therapy is recommended in order to maintain tissue perfusion and oxygenation,[341] although sepsis related changes in vascular reactivity may alter the normal autoregulatory range [342] and the optimal arterial pressure end-point for vasopressor therapy remains uncertain.[343] Indeed the current recommendation to maintain MAP at greater than 65 mmHg is supported by only limited evidence,[194,344] and some have advocated routine use of higher arterial pressure targets, or alternatively the restoration of MAP to pre-morbid values. Because the consequences of both inadequate and excessive vasopressor therapy can be serious,[46] it is important to clarify the optimal end-points to which these potent agents should be titrated, as well as to investigate the effects of alternative vasopressor agents.[345]

Sepsis results in a variety of deleterious microvascular changes that are associated with organ failure and death.[94] These derangements include increased endothelial permeability, endothelial-leucocyte adhesion and a characteristic heterogeneity of blood flow that is associated with tissue hypoxia.[66,84,297] The causes of heterogeneous microvascular flow are not fully understood but reduced vascular tone due to impaired endothelial signal transduction may be one explanation.[61] It is possible, therefore that vasopressors might correct the abnormalities of microvascular flow and hence tissue oxygenation associated with septic shock.

The dose related effects of vasopressor therapy on microvascular flow and tissue oxygenation in sepsis have not previously been fully investigated. The aim of this investigation was to evaluate in more detail the effects of increasing doses of norepinephrine, targeted to achieve successively greater MAP, on microvascular flow and tissue oxygenation in patients with septic shock.

9.2 Methods

This single centre, interventional study was approved by the local research ethics committee and the Medicines and Healthcare products Regulatory Agency. Adult patients admitted to intensive care with a diagnosis of septic shock who were deemed to require vasopressor therapy despite adequate fluid resuscitation were eligible for recruitment. Written informed consent was sought from the patient when possible. Where patients lacked capacity to give consent because they were unconscious, the consent of a relative was sought. Patients who survived were subsequently asked for written permission to use their data once capacity had been regained. Exclusion criteria were refusal of patient's or relative's consent, concurrent lithium therapy, acute myocardial ischaemia, acute arrhythmias, pregnancy, patients receiving palliative treatment only and weight less than 40 kg.

9.2.1 Clinical management and study interventions

The clinical management of each patient was determined by clinical staff in accordance with care bundles based on the Surviving Sepsis Guidelines that were current at the time of the study.[346] This included compliance with the guidelines for fluid management, low-dose steroid therapy, tight glycaemic control, administration of drotrecogin alfa activated, red cell transfusion and invasive ventilation. Adherence to these care bundles is regularly audited on our Intensive Care Unit. The norepinephrine infusion was initially titrated by the research team to achieve a mean arterial pressure of 60 mmHg. Following a period of 45 minutes observation to ensure that the blood pressure and haemodynamics had stabilized, initial measurements were taken. The norepinephrine infusion was then increased to achieve a mean arterial pressure of 70 mmHg, followed by another 45 minute stabilization

period prior to the next set of measurements. This process was repeated with the norepinephrine infusion rate being increased to achieve a mean arterial pressure of 80 mmHg and finally 90 mmHg. Patients were monitored throughout the four hour intervention period for adverse events, in particular, tachycardia, arrhythmias, myocardial ischaemia, hypertension or other signs of excessive vasoconstriction. The physician in charge was free to request dose adjustment or cessation of the study at any time.

9.2.2 Measurements

In addition to routine clinical measurements, cardiac output and oxygen delivery index were determined using transpulmonary lithium indicator dilution combined with continuous arterial waveform analysis software (LiDCOplus, LiDCO Ltd, Cambridge, UK). Arterial and central venous blood samples were taken from indwelling cannulae after stabilization at each time point for analysis of blood gas tensions, lactate and haemoglobin concentration (ABL 600 and OSM3, Radiometer, Copenhagen, Denmark). Cutaneous tissue P_tO_2 was measured at the same time point at two sites overlying the deltoid muscle using a Clark electrode (TCM400, Radiometer, Copenhagen, Denmark). Laser Doppler flowmetry (Moorlab, Moor Instruments, Axminster, UK) was utilized to measure red cell flux at two sites overlying the deltoid muscle. To account for changes in blood pressure, cutaneous vascular conductance was also calculated by dividing red cell flux by mean arterial pressure (PU/mmHg).[261] Sublingual microvascular flow was evaluated using sidestream darkfield (SDF) imaging with a x5 objective lens (Microscan, Microvision Medical, Amsterdam, Netherlands). Image acquisition and subsequent analysis was performed according to published consensus criteria.[277] Analysis of the videos was performed by two

observers blinded to each others observations and the clinical data. All of these measurement tools are described in more detail in Chapter two.

9.2.3 Statistical Analysis

The primary outcome measure was the difference in sublingual microvascular flow index at different mean arterial pressures. Assuming a type I error rate of 5% and a type II error rate of 20%, we calculated that 16 patients would be required to detect a difference of 0.5 perfused vessels mm^{-2} (SD of difference between measurements: ± 0.7 vessels mm^{-2}) between a MAP of 60mmHg and 90mmHg. This calculation was based on data from a previous study of heterogeneity of microvascular flow in patients with severe sepsis. [66] For continuous data, differences over time were tested using a repeated measure analysis of variance (ANOVA) with Dunnett's post-hoc test for comparison against baseline. Non-normally distributed data were tested with the Friedman test. Analysis was performed using GraphPad Prism version 4.0 (GraphPad Software, San Diego, USA). Significance was set at $p < 0.05$. Data are presented as mean (SD) where normally distributed and median (IQR) where not normally distributed.

9.3 Results

Sixteen patients were recruited a median of one day (0.5-3.5) after the onset of septic shock. Baseline characteristics are presented in table 9.1. Increasing doses of norepinephrine were required to increase mean arterial pressure from 60 mmHg to 90 mmHg ($p<0.0001$). There were no changes in sedation or crystalloid infusion during the study period (table 9.2). Three patients each received a single 250ml bolus of colloid solution, one whilst the MAP was 60 mmHg, one at 70 mmHg and one at 80 mmHg. One patient was receiving an infusion of dobutamine and one an infusion of vasopressin, the doses of which remained constant throughout the study period (2.63 µg kg^{-1} min^{-1} and 0.04 U min^{-1} respectively). A third patient received an infusion of drotrecogin alfa activated. All patients received low dose steroids (hydrocortisone 50mg every six hours). Four patients were receiving continuous veno-venous haemodiafiltration during the course of the study and passed no measurable volume of urine. In four patients the attending physician requested that we did not increase MAP to 90 mmHg due to theoretical concerns regarding the possibility of impaired splanchnic perfusion. None of the patients developed tachycardia, arrhythmias, myocardial ischaemia, signs of excessive vasoconstriction or any other adverse effects which could be attributed to the intervention. In one patient, it was not possible to collect reliable SDF images due to patient movement.

There was a significant increase in global oxygen delivery ($p<0.01$), cutaneous PtO_2 ($p<0.0001$) and P_tO_2:P_aO_2 ratio ($p<0.0001$) as MAP increased from 60 to 90 mmHg (table 9.3 and figure 9.1). Cardiac index and central venous oxygen saturation ($ScvO_2$) also increased whilst base deficit improved. There were no changes in serum lactate or urine output (table 9.3). There was an improvement in cutaneous red cell flux and a decrease in

cutaneous vascular conductance as assessed by laser Doppler flowmetry (table 9.4). There were no changes in small (<20µm) or large (>20µm) vessel MFI, vessel density, the proportion of perfused vessels, perfused vessel density or heterogeneity index as assessed by SDF imaging (table 9.4, table 9.5 and figure 9.2). The kappa coefficient with linear weighting for inter-observer reliability for calculation of MFI was 0.82 (95%CI 0.78-0.86).

Patient characteristics	
Age (years)	67 (55-72)
Gender	9 males (56%)
Body weight (kg)	75 (60-80)
APACHE II score	23 (17-30)
Duration of intensive care stay (days)	8 (5-14)
Mortality	10 deaths (62.5%)
Source of sepsis	
Respiratory infection (%)	7 (44%)
Abdominal infection (%)	6 (38%)
Urological infection (%)	2 (12%)
Soft tissue (%)	1 (6%)

Table 9.1 Baseline patient characteristics. **Data presented as median (IQR) or absolute values (%).**

	n	60 mmHg	70 mmHg	80 mmHg	90 mmHg	p
Norepinephrine ($\mu g\ kg^{-1}\ min^{-1}$)	16	0.18 (0.18)	0.25 (0.22)	0.35 (0.27)	0.41 (0.26)	<0.0001
Propofol ($mg\ kg^{-1}\ hour^{-1}$)	12	1.4 (1.3-2.3)	1.4 (1.3-2.3)	1.4 (1.3-2.3)	1.4 (1.3-2.3)	>0.99
Fentanyl ($\mu g\ kg^{-1}\ hour^{-1}$)	16	1.6 (1.4-2.0)	1.7 (1.4-2.1)	1.7 (1.4-2.1)	1.7 (1.4-2.1)	0.39
Midazolam ($mg\ kg^{-1}\ hour^{-1}$)	4	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.1 (0.1-0.2)	>0.99
Intravenous crystalloid (ml)	11	109 (89-160)	118 (74-142)	89 (67-134)	96 (48-143)	0.68

Table 9.2 Clinical management at or between measurement time points. **Data presented as infusion rate at each time point or, in the case of intravenous crystalloid, the volume administered during the stabilization period between time points. Data presented as mean (SD) or median (IQR). Significance testing with repeated measures ANOVA and Friedman test.**

	60 mmHg	70 mmHg	80 mmHg	90 mmHg
Heart rate (bpm)	87 (17)	84 (18)	86 (18)	87 (17)
Cardiac index (l min^{-1} m^{-2}) [b]	3.86 (1.22)	4.24 (1.26)	4.43 (1.43) †	4.79 (1.61) ‡
Oxygen delivery index (ml min^{-1} m^{-2}) [c]	487 (418-642)	536 (446-720)	550 (474-800)	662 (498-829)
Systemic vascular resistance index (dynes·sec $cm^{-5}m^{-2}$) [b]	1195 (448)	1260 (392)	1402 (467) ‡	1451 (571) ‡
$ScvO_2$ (%) [d]	71 (6.4)	72 (6.7)	73 (7.1)	74 (6.7) †
Serum lactate (mmol l^{-1})	2.2 (1.4)	2.3 (1.3)	2.1 (1.2)	2.2 (1.2)
Serum base excess (mmol l^{-1}) [d]	-2.3 (0.2, -4.2)	-1.6 (0.1, -4.7)	-1.5 (1.3, -3.7)	-1.9 (1.3, -3.9)
Urine output (ml $hour^{-1}$)	53 (38)	63 (41)	61 (39)	55 (38)
PaO_2 (kPa)	11.3 (2.3)	11.9 (2.3)	11.5 (2.4)	10.8 (2.3)
Cutaneous PtO_2 (kPa) [a]	5.9 (1.7)	6.7 (2.1) †	7.1 (2.0) ‡	7.3 (2.0) ‡
PtO_2 : PaO_2 ratio [a]	0.53 (0.19)	0.57 (0.21)	0.62 (0.17) ‡	0.67 (0.19) ‡

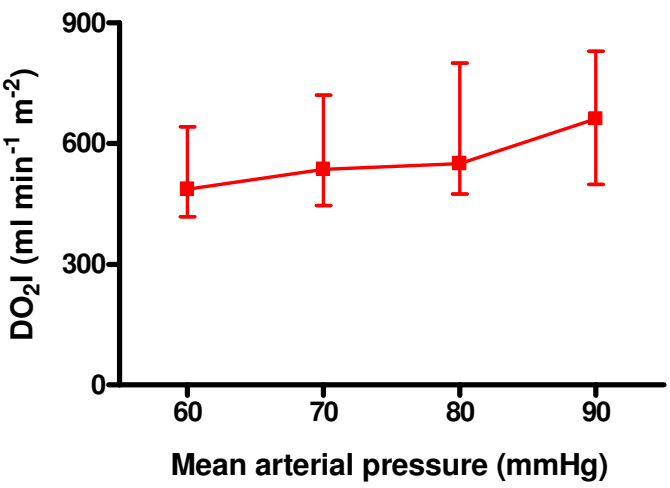
Table 9.3 Physiological data at different measurement points with increasing mean arterial pressure. **Data presented as mean (SD) or median (IQR). [a] p<0.0001 [b] p<0.001, [c] p<0.01 and [d] p<0.05 over time (repeated measures ANOVA and Friedman test). ‡p<0.01 and †p<0.05 compared to baseline of MAP 60mmHg (post-hoc Dunnett's test).**

	60 mmHg	70 mmHg	80 mmHg	90 mmHg	p
Microvascular flow index	2.3 (0.4)	2.5 (0.3)	2.4 (0.3)	2.3 (0.4)	0.45
Vessel density (mm^{-1})	6.9 (1.5)	7.1 (1.5)	7.1 (1.3)	6.9 (0.9)	0.96
Proportion of perfused vessels (%)	75 (66-87)	84 (74-90)	85 (71-93)	77 (72-84)	0.57
Perfused vessel density (mm^{-1})	5.3 (1.9)	5.9 (1.8)	5.8 (1.5)	5.3 (1.3)	0.75
Heterogeneity Index	0.41 (0.28)	0.37 (0.25)	0.32 (0.12)	0.33 (0.22)	0.84
Cutaneous red cell flux (PU)	26 (16-42)	27 (18-44)	27 (20-47)	33 (20-47)	0.04
Cutaneous vascular conductance (PU/mmHg)	0.44 (0.27-0.70)	0.39 (0.25-0.63)	0.34 (0.24-0.59)	0.37 (0.23-0.52)	0.003

Table 9.4 Indices of heterogeneity of sublingual microvascular flow for small vessels (<20μm), cutaneous red blood cell flux and cutaneous vascular conductance with increasing mean arterial pressure. Data presented as mean (SD) or median (IQR). Significance testing with repeated measures ANOVA and Friedman test.

	60 mmHg	70 mmHg	80 mmHg	90 mmHg	p
Microvascular flow index	2.9 (2.5-3.0)	2.9 (2.8-3.0)	2.9 (2.8-3.0)	3.0 (2.9-3.0)	0.92
Vessel density (mm^{-1})	2.0 (0.6)	1.7 (0.6)	2.1 (0.6)	2.0 (0.7)	0.20
Proportion of perfused vessels (%)	100 (100-100)	100 (100-100)	100 (100-100)	100 (100-100)	>0.99
Perfused vessel density (mm^{-1})	1.9 (0.6)	1.7 (0.6)	2.1 (0.6)	2.0 (0.7)	0.20
Heterogeneity Index	0.09 (0.0-0.09)	0.09 (0.0-0.14)	0.09 (0.0-0.09)	0.0 (0.0-0.09)	0.90

Table 9.5 Indices of heterogeneity of sublingual microvascular flow for large vessels (>20µm) with increasing mean arterial pressure. **Data presented as mean (SD) or median (IQR). Significance testing with repeated measures analysis of variance (ANOVA) for parametric data and Friedman test for non-parametric data.**



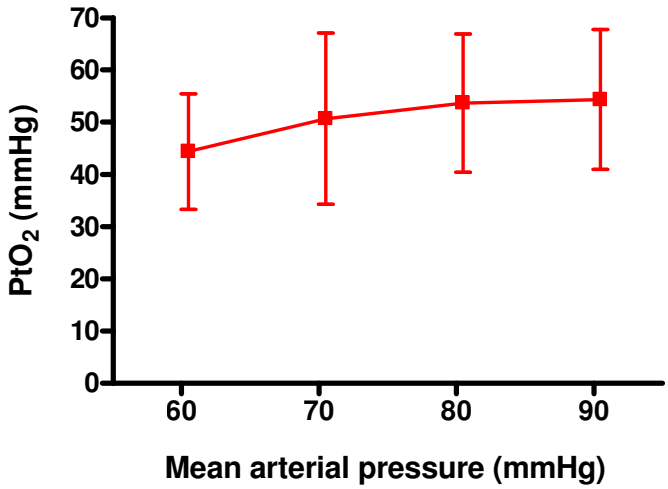


Figure 9.1 Increases in global oxygen delivery (DO_2I) ($p<0.01$, Friedman test) and cutaneous PtO_2 ($p<0.0001$, repeated measures ANOVA) associated with the increasing dose of norepinephrine required to achieve an increasing target mean arterial pressure.

PtO_2 data presented as mean (SD), DO_2I data presented as median (IQR).

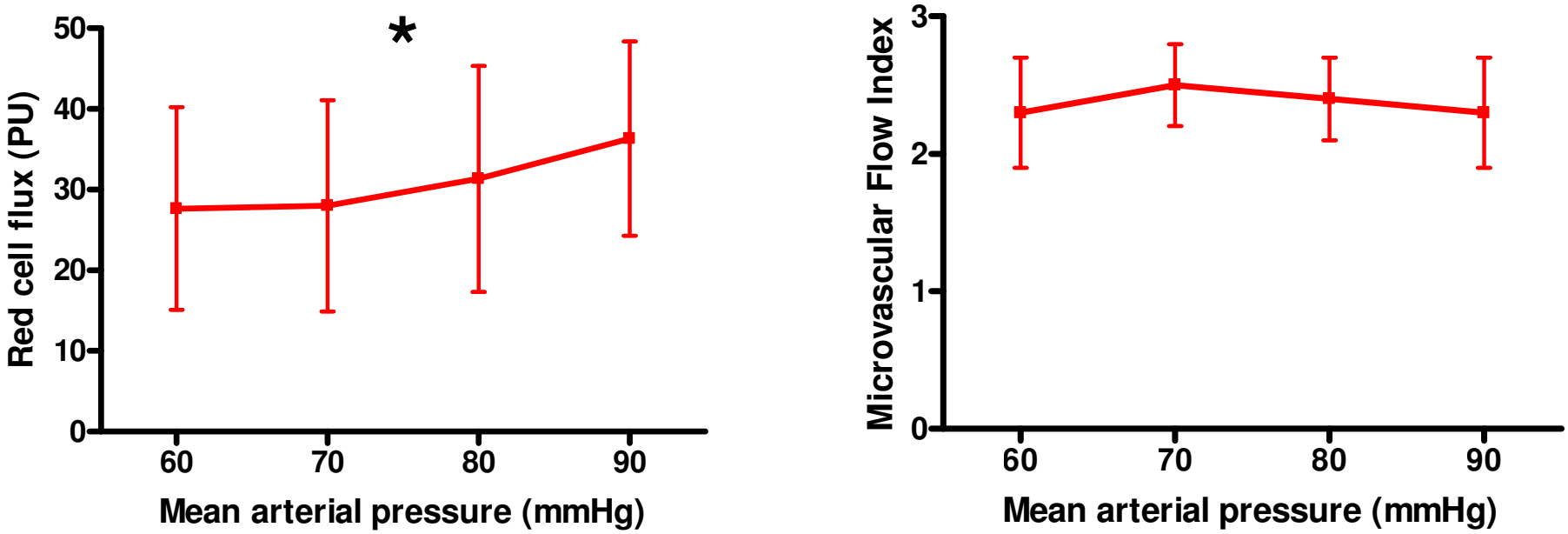


Figure 9.2 Significant changes in cutaneous red blood cell flux ($p<0.05$, Friedman test) but not sublingual microvascular flow index (MFI) ($p=0.45$, repeated measures ANOVA) associated with the increasing dose of norepinephrine required to achieve an increasing target mean arterial pressure.

MFI data presented as mean (SD), Red cell flux data presented as median (IQR).

9.4 Discussion

The principle finding of this study is that the use of incremental doses of norepinephrine to achieve increasing targets for mean arterial pressure was associated with increases in global oxygen delivery, cutaneous PtO_2 and cutaneous red cell flux. Deranged indices of sublingual microvascular flow remained unchanged. We also identified an increase in $ScvO_2$ and a decrease in base deficit as the dose of norepinephrine increased. However, in both cases these changes were small. There were no changes in serum lactate or urine output.

Although a number of pharmacological interventions have been shown to improve microvascular flow in patients with septic shock,[15,155,156] there is only limited data from clinical studies describing the microvascular effects of different doses of norepinephrine in such patients. LeDoux and colleagues reported that despite an increase in global oxygen delivery, gastric mucosal pCO_2 and cutaneous microvascular flow were unchanged when mean arterial pressure was increased from 65 mmHg to 85 mmHg using escalating doses of norepinephrine.[194] In a subsequent study, patients were randomised to MAP targets for vasopressor therapy of 65 mmHg or 85 mmHg.[344] Global oxygen delivery and mixed venous oxygen saturation were greater in the 85 mmHg group whilst there were no differences in renal function. [344] However, in a recent clinical investigation, urine output improved as MAP increased in response to noradrenaline,[347] contrasting with our findings and those of others.[344] None of these investigations included a visual assessment of the microcirculation, although improvements in sublingual microvascular flow

have been described with the use of both dobutamine and vasodilator therapy in septic shock.[15,156] A recent investigation, by Dubin and colleagues, also used SDF imaging to investigate the effects of increasing noradreanline to achieve MAP's of 65mmHg to 85mmHg.[302] These investigators found no improvement in sublingual microvascular flow with increasing MAP. They found considerable variations in the interindividual responses which appeared to depend upon the baseline condition of the microcirculation. Laboratory investigations of the effects of norepinephrine on global haemodynamics, tissue oxygenation and the microvasculature in sepsis have also proved inconsistent. In a rodent endotoxaemia model, treatment with norepinephrine improved tissue oxygenation,[348] whereas in a more detailed investigation in a rodent faecal peritonitis model, norepinephrine was not associated with any changes in microvascular flow or tissue PO_2.[349] In porcine models of sepsis, the increased dose of norepinephrine required to achieve higher MAP targets was associated with improved global haemodynamics.[350,351] In the first of these studies, this haemodynamic improvement was associated with an increase in regional and microvascular flow to the viscera,[350] whilst in the subsequent study, both regional and microvascular visceral flow were decreased.[351] In another study in non-septic pigs, no changes were identified in gut microvascular flow or oxygenation during norepinephrine infusion.[352]

It is unclear why cutaneous microvascular flow and tissue oxygenation improved as the noradrenaline dose increased whilst sublingual microvascular flow remained unchanged. Previous reports suggest that microvascular flow and tissue oxygenation may differ between organs in sepsis.[99,353] It is also

possible that local responses to norepinephrine differ between vascular beds either because of differences in metabolic demand between organs, heterogeneity in receptor distribution [354] or differential expression of inducible nitric oxide synthase (iNOS).[355] It should also be emphasised that while SDF imaging allows direct visualisation of the microcirculation, laser Doppler flowmetry measures overall microvascular flow in the underlying tissues. Moreover, in our patients the abnormalities in sublingual microvascular flow were less severe than those identified in previous reports, perhaps because of differences in the severity of illness or vasoactive drug therapy.[66,156] It is also possible that subtle changes in sublingual microvascular flow would have been identified had a much larger population of patients been studied. In health, autoregulation would be expected to maintain constant tissue blood flow within this range of arterial pressures. Consequently, one would expect a decrease in vascular conductance with increasing arterial pressure and hence constant tissue blood flow. Interestingly, despite a decrease in cutaneous vascular conductance there was still an increase in cutaneous red cell flux. This finding suggests disruption of autoregulatory mechanisms, either due to sepsis,[342] or the administration of exogenous norepinephrine.[356]

We used a variety of methods to obtain a more comprehensive assessment of alterations in microvascular flow. SDF imaging is a valuable technique which has been used successfully to evaluate the intact microcirculation in the clinical environment. However, this technique remains semi-quantitative and accuracy may be affected by inter-observer bias and pressure artifact from the SDF camera. We specifically evaluated these potential sources of error

and excluded any significant bias (see methods). Although a recent study has shown that sedative drugs can affect sublingual microvascular flow,[117] doses of sedation remained unchanged throughout the current study. SDF imaging was complemented by laser Doppler flowmetry, a technique that provides an objective measure of overall microvascular flow but does not allow discrimination between flow in larger and smaller vessels. In addition we evaluated cutaneous PtO_2 using a Clark electrode. With this method, the skin underneath the probe is warmed slightly to minimise artefact due to vasoconstriction; this active warming may therefore have counteracted the local vasoconstrictive effects of norepinephrine. However, the degree of warming remained constant throughout the experiment and cannot, therefore explain the progressive increase in PtO_2.

Although all patients were in established septic shock and had been adequately fluid resuscitated, variation in the delay between the diagnosis of septic shock and study enrolment is a potential limitation of this study. This and other differences between the patients such as the use of other vasoactive agents, haemofiltration and drotrecogin alfa activated, may have influenced microvascular function and hence the response to norepinephrine. Nevertheless, we were able to identify consistent and significant changes in microvascular flow and tissue oxygenation. Finally, it is important to emphasise that, in the current study, we explored only the short term effects of norepinephrine on global haemodynamics, tissue microvascular flow and oxygenation. Our understanding of the longer term effects of escalating vasopressor doses on these variables remains incomplete.

In summary, the use of norepinephrine to achieve incremental targets for mean arterial pressure, was associated with increases in global oxygen delivery, cutaneous microvascular flow and tissue oxygenation in patients with established septic shock. However, there were no associated changes in the pre-existing abnormalities of sublingual microvascular flow. These findings suggest that in patients with septic shock, significant improvements in global haemodynamics and tissue oxygen delivery can be achieved using escalating doses of norepinephrine to achieve higher values for MAP without exacerbating microcirculatory flow abnormalities. Further research is required to confirm our findings and to explore in more detail the long-term effects of higher arterial pressure targets for the administration of vasopressors.

Chapter 10
Conclusions and future work

10.1 Summary of findings

The care of the surgical patient is an important public health issue. Within the large surgical population is a smaller sub-population that is at high risk of morbidity and mortality. We found within our own institution that a high risk group of patients existed that accounted for less than 10% of surgical procedures but over 70% of surgical in-hospital deaths. Despite having a mortality rate of 12%, only one third of this high risk group were admitted to critical care at any stage following surgery. It seems possible that greater provision of critical care services may improve clinical outcomes for this population.

The microcirculation is essential for the delivery of oxygen and nutrients to the tissues. It would seem, therefore, that understanding microvascular flow is fundamental to the treatment of critically ill patients. To date, however, monitoring the microvasculature has not been routine in clinical practice, in part due to a lack of practical techniques. The advent of OPS and SDF imaging has brought clinical monitoring of the intact microcirculation to the bedside. Whilst microvascular abnormalities have been demonstrated in many conditions including established sepsis, it remains unclear whether these changes exist in other groups of critically ill patients. We found alterations in sublingual microvascular flow in a group of patients undergoing major

abdominal surgery that were significantly greater in those patients who went on to develop complications following surgery. Interestingly, this difference in microvascular flow was present even prior to surgery. There were no related differences in cutaneous microvascular flow, tissue oxygenation or global haemodyanmics. With increasing interest in the cardiovascular management of septic patients in the first few hours of hospital admission and in interventions that alter microvascular flow, we aimed to further describe the pattern of microvascular flow in a group of patients within six hours of hospital admission for sepsis. We found similar, but less severe, abnormalities of microvascular flow to those previously reported in established sepsis. These changes were more marked in the more severely ill patients and in those with poor outcomes.

The use of protocolised fluid and vasoactive therapy aimed at improving global haemodynamics has been demonstrated to improve patient outcomes in early sepsis and in perioperative patients. It is believed that this approach may improve tissue perfusion and oxygenation but this hypothesis has yet to be proven. There is currently no data investigating the effects of GDHT on microvascular flow in perioperative patients. Work in patients with sepsis has shown that the use of GDHT in early sepsis using central venous oxygen saturations as a goal is associated with an improvement in sublingual microvascular flow. In established sepsis, however, there appears to be a discrepancy between improvements in the macro- and microcirculation associated with vasoactive therapy.

We investigated the effects of GDHT in a randomised controlled trial of patients undergoing major abdominal surgery. In particular we investigated the incremental benefit of stroke volume guided fluid resuscitation with dopexamine over stroke volume guided fluid therapy alone. The control group received fluid resuscitation guided by central venous pressure. We found significant improvements in global haemodyanamics including global oxygen delivery and central venous oxygen saturations in those patients receiving stroke volume guided fluid therapy plus dopexamine. Associated with these changes in global haemodynamics we found significant improvements in sublingual microvascular flow, cutaneous microvascular flow and tissue oxygenation. There were no changes in the inflammatory response to surgery. This study was not powered to identify differences in clinical outcomes but a post hoc analysis did suggest an improvement in renal outcomes. Stroke volume guided fluid therapy alone was associated with more modest changes. The incremental effects of low dose dopexamine are likely to relate to the β-adrenoreceptor mediated inotropic and vasodilator actions of this drug. It is thus possible that the improvements in microvascular flow may relate to direct effects of the drug on the microcirculation as well as improvements in cardiac output.

In septic shock, noradrenaline is recomeneded following fluid resuscitation to maintain adequate perfusion pressure. The recommendation of a mean arterial pressure of 65 mmHg as an endpoint for the administration of noradrenaline is based on limited data. We investigated the effects of incremental doses of noradrenaline titrated to achieve successively higher

mean arterial pressures on tissue microvascular flow and oxygenation in a group of patients with septic shock. To our surprise, we found increasing noradrenaline was associated with improvements in cutaneous microvascular flow and cutaneous tissue oxygenation with no detrimental effect on sublingual microvascular flow. Further work is needed to explore the ideal endpoint for the administration of vasopressors in septic shock.

Overall this thesis was successful in exploring the effects of haemodyanmic therapies on microvascular flow. In general, we found that haemodynamic therapies targeting global haemodyanmic goals in perioperative and septic patients were associated with improvements in microvascular flow and tissue oxygenation.

10.2 Strengths and weaknesses

The description of the high risk surgical population at our local institution found a high risk surgical population accounting for a small proportion of surgical procedures but over 70% of surgical deaths. Only approximately one third of these patients were admitted to critical care at anytime following surgery. The use of large healthcare databases in this way is associated with a number of limitations, particularly when accessing data that the database was not specifically designed to collect. Although we cross-referenced individual patient's information between databases and where possible with medical notes, it is possible that errors may have occurred. The study performed was retrospective in design. Performing a prospective study of surgical patients may allow identification of the reasons underlying decisions

regarding the allocation of critical care resources to individual patients. The use of biomarkers and / or cardiopulmonary exercise testing are currently being investigated as methods for identifying high risk surgical patients prior to surgery.

The simultaneous use of three different modalities to assess different aspects of tissue microvascular function was an important strength of the subsequent studies. SDF imaging is a major advance in microvascular monitoring, providing high quality, real time videos of the intact microcirculation. The technique is currently limited, however, by semiquantative analysis. It is likely that with software development, quantitative analysis of microvascular flow and density will become routine. Whether sublingual flow correlates with microvascular flow in other vascular beds remains unclear, although it is likely that there is heterogeneity of flow not only within individual microvascular units but also between microvascular units. Laser Doppler flowmetry is a versatile technique but it cannot distinguish the size and type of microvessel, the direction of flow or most importantly heterogeneity of flow, all of which may be relevant in critically ill patients. These limitations can be partially offset by examining the post-occlusive hyperaemic response which can provide a reproducible assessment of endothelium dependent microvascular reponses. This technique was used for the study described in Chapter 5. The cutaneous Clark electrode measures the local partial pressure of oxygen by a polarographic method. If tissue perfusion falls with stable arterial oxygenation, cutaneous oxygenation will also fall, thereby linking peripheral perfusion and tissue oxygenation. An ideal measure of tissue oxygenation would have

involved measurement of the splanchnic circulation, but this would require invasive techniques not suitable to these patient groups.

The randomised controlled trial of periopertive haemodynamic therapy involved high standards of care for all patients involved. Patients were stratified according to surgical procedure prior to randomisation to ensure that the three groups were comparable. In common with many trials of complex interventions, it was not possible to fully blind clinical staff to study group allocation. Study group allocation was concealed from all investigators apart from the member of the team delivering the intervention. This included concealment of cardiac output data and the use of dummy infusions. All complications were prospectively defined and verified by the blinded principal investigator.

Patients with established, fluid resuscitated, septic shock were recruited to investigate the effects of noradrenaline and increasing mean arterial pressure on microvascular flow. It is possible that variation in the delay between the diagnosis of septic shock and study enrolment is a potential limitation of this study. This and other differences between the patients such as the use of other vasoactive agents, haemofiltration and drotrecogin alfa activated, may have influenced microvascular function and hence the response to noradrenaline. Importantly, we explored only the short term effects of noradrenaline on global haemodynamics, tissue microvascular flow and oxygenation. Our understanding of the longer term effects of escalating noradrenaline doses on these variables remains incomplete.

10.3 Suggestions for further work

This work suggests a number of investigations that may contribute to improving the care of the perioperative and septic patient. Postoperative outcomes are a result of a complex interplay between the patient's health status, the surgical procedure performed and perioperative care. Outcomes may be influenced by the availability and use of critical care services. Appropriate triage of patients to intensive care postoperatively may have an impact on outcomes after non-cardiac surgery. Further work investigating critical care use for surgical patients may help delineate more clearly the factors which influence the decision making process of which patients are admitted to critical care following surgery. There is also ongoing work looking at pre-operative risk predictors of poor outcomes following surgery including the use of cardiopulmonary exercise testing and the use of biomarkers. This will hopefully allow clinicians to identify prior to surgery, which patients may benefit from critical care following surgery.

In this thesis, microvascular abnormalities following major abdominal surgery have been associated with poor outcomes. The use of dopexamine alongside stroke volume guided fluid therapy has been demonstrated to have an incremental benefit on tissue microvascular flow and oxygenation over stroke volume guided fluid therapy alone in patients following surgery. The question of whether this would translate into an improvement in clinical outcomes, however, remains somewhat unclear. A number of small single centre studies have demonstrated improvements in clinical outcomes with GDHT. A large multicentre effectiveness study of GDHT in surgical patients is now needed to

demonstrate whether a simple protocol that could be introduced easily into the current healthcare system improves outcomes.

Microvascular flow has been shown to improve with a number of therapies in critically ill patients. Currently rapid quantitative analysis of microvascular videos allowing changes in therapy is not possible. It is conceivable, however, that in the future the microcirculation may be utilised as an endpoint for resuscitiation at the bedside. Therapies that recruit the microcirculation could then be commenced in those patients who demonstrate impaired microvascular flow. It should be noted, however though that there is not, as yet, any evidence of improved clinical outcome associated with improvements in microvascular flow.

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Appendix – definition of complications

Infectious Complications

Sepsis, Severe sepsis and Septic shock

Two more of the following associated with documented infection (consensus definition):

i) core temperature <36 °C or >38 °C

ii) white cell count >12 x 10^9 l^{-1} or <4 x 10^9 l^{-1}

iii) Respiratory rate >20 breaths per minute or $PaCO_2$ < 4.5 kPa

iv) Pulse rate >90 bpm. Sepsis associated with organ dysfunction will be defined as severe sepsis. Sepsis associated with hypotension and need for vasopressors or inotropes despite fluid resuscitation will be defined as septic shock.

Urinary tract infection

A symptomatic urinary tract infection must meet at least one of the following criteria:

i) Patient has at least one of the following signs or symptoms with no other recognized cause: fever (>38 °C), urgency, frequency, dysuria, or suprapubic tenderness

And

patient has a positive urine culture, that is, >10^5 microorganisms per cm^3 of urine with no more than two species of microorganisms.

ii) Patient has at least two of the following signs or symptoms with no other recognized cause: fever (>38 °C), urgency, frequency, dysuria, or suprapubic tenderness

And at least one of the following:

a. Positive dipstick for leukocyte esterase and/or nitrate
b. Pyuria (urine specimen with >10 WBC mm^{-3})
c. Organisms seen on Gram stain of unspun urine
d. At least *two* urine cultures with repeated isolation of the same uropathogen with $>10^2$ colonies/ mL in non-voided specimens
e. $>10^5$ colonies/mL of a single uropathogen in a patient being treated with an effective antimicrobial agent for a urinary tract infection
f. Physician diagnosis of a urinary tract infection
g. Physician institutes appropriate therapy for a urinary tract infection

Other infections of the urinary tract (kidney, ureter, bladder, urethra, etc)
Other infections of the urinary tract must meet at least one of the following criteria:

i) Patient has organisms isolated from culture of fluid (other than urine) or tissue from affected site.

ii) Patient has an abscess or other evidence of infection seen on direct examination, during a surgical operation, or during a histopathologic examination.

iii) Patient has at least two of the following signs or symptoms with no other recognized cause: fever (>38 °C), localized pain, or localized tenderness at the involved site

And at least one of the following:

a. Purulent drainage from affected site
b. Organisms cultured from blood that are compatible with suspected site of infection
c. Radiographic evidence of infection, for example, abnormal ultrasound, computed tomography or magnetic resonance imaging
d. Physician diagnosis of infection of the kidney, ureter, bladder, urethra, or tissues surrounding the retroperitoneal or perinephric space
e. Physician institutes appropriate therapy for an infection of the kidney, ureter, bladder, urethra, or tissues surrounding the retroperitoneal or perinephric space

Surgical site infection (superficial incisional)

A superficial SSI must meet the following criteria:

i) Infection occurs within 30 days after the operative procedure And involves only skin and subcutaneous tissue of the incision And patient has at least one of the following:

a. Purulent drainage from the superficial incision
b. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision
c. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat, and superficial incision is deliberately opened by surgeon, unless incision is culture-negative
d. Diagnosis of superficial incisional SSI by the surgeon or attending physician

Surgical site infection (deep incisional)

A deep incisional SSI must meet the following criteria:

i) Infection occurs within 30 days after the operative procedure if no implant is left in place or within 1 year if implant is in place and the infection appears to be related to the operative procedure

And involves deep soft tissues (e.g., fascial and muscle layers) of the incision

And patient has at least one of the following:

a. Purulent drainage from the deep incision but not from the organ/space component of the surgical site
b. A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (>38C) or localized pain or tenderness, unless incision is culture-negative
c. An abscess or other evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathologic or radiologic examination
d. Diagnosis of a deep incisional SSI by a surgeon or attending physician

An infection that involves both superficial and deep incision sites should be classified as a deep incisional SSI.

Surgical site infection (organ/space)

An organ/space SSI involves any part of the body, excluding the skin incision, fascia, or muscle layers, that is opened or manipulated during the operative procedure. Specific sites are assigned to organ/space SSI to further identify the location of the infection. Listed later are the specific sites that must be used to differentiate organ/space SSI. An example is appendectomy with subsequent sub-diaphragmatic abscess, which would be reported as an organ/space SSI at the intra-abdominal specific site. An organ/space SSI must meet the following criteria:

i) Infection occurs within 30 days after the operative procedure if no implant is left in place or within 1 year if implant is in place and the infection appears to be related to the operative procedure

And infection involves any part of the body, excluding the skin incision, fascia, or muscle layers, that is opened or manipulated during the operative procedure

And patient has at least one of the following:

a. Purulent drainage from a drain that is placed through a stab wound into the organ/space
b. Organisms isolated from an aseptically obtained culture of fluid or tissue in the organ/ space
c. An abscess or other evidence of infection involving the organ/space that is found on direct examination, during reoperation, or by histopathologic or radiologic examination
d. Diagnosis of an organ/space SSI by a surgeon or attending physician

Laboratory confirmed bloodstream infection

Laboratory-confirmed bloodstream infection must meet at least one of the following criteria:

i) Patient has a recognized pathogen cultured from one or more blood cultures

And organism cultured from blood is not related to an infection at another site.

ii) Patient has at least one of the following signs or symptoms:

Fever (>38°C), chills, or hypotension

And at least one of the following:

a. Common skin contaminant is cultured from two or more blood cultures drawn on separate occasions
b. Common skin contaminant is cultured from at least one blood culture from a patient with an intravascular line, and the physician institutes appropriate antimicrobial therapy
c. Positive antigen test on blood

And signs and symptoms and positive laboratory results are not related to an infection at another site.

Nosocomial pneumonia

Ventilator-associated pneumonia (i.e. pneumonia in persons who had a device to assist or control respiration continuously through a tracheostomy or by endotracheal intubation within the 48-hour period before the onset of infection) will be classified separately. Care will be to distinguish between tracheal colonization, upper respiratory tract infections and early onset pneumonia. Nosocomial pneumonia will be characterized as early or late onset ie before or after first 4 days of hospitalization. Where repeated episodes of nosocomial pneumonia are suspected, a combination of new signs and symptoms and radiographic evidence or other diagnostic testing will be required to distinguish a new episode from a previous one.

Nosocomial pneumonia must meet the following criteria:

i) Two or more serial chest radiographs with at least one of the following:

a) New or progressive and persistent infiltrate
b) Consolidation
c) Cavitation

And at least one of the following:

a) Fever (>38°C) with no other recognized cause
b) Leucopaenia (<4,000 WBC mm^{-3}) or leucocytosis (>12,000 WBC mm^{-3})
c) For adults >70 years old, altered mental status with no other recognized cause

And at least two of the following:

a) New onset of purulent sputum or change in character of sputum, or increased respiratory secretions, or increased suctioning requirements
b) New onset or worsening cough, or dyspnoea, or tachypnoea
c) Rales or bronchial breath sounds
d) Worsening gas exchange

Cardiovascular complications

Myocardial ischaemia or infarction

Acute ECG changes with appropriate clinical history and biochemical findings (cardiac troponin rise)

Arrhythmia

ECG evidence of rhythm disturbance resulting in hypotension or considered by clinical staff to be severe enough to require treatment

Cardiac or respiratory arrest

Clinical criteria according to UK Resuscitation Council guidelines

Limb or digital ischaemia

Sustained loss of arterial pulse (as determined by palpation or Doppler) or obvious gangrene

Respiratory Complications

Pulmonary oedema

Appropriate clinical history and examination with consistent chest radiograph

Pulmonary embolism

Computed tomography (CT) pulmonary angiogram with appropriate clinical history

Acute respiratory distress syndrome

According to consensus criteria:

i) Suitable precipitating condition (many causes exist)

ii) Acute onset diffuse bilateral pulmonary infiltrates on chest radiograph

iii) No evidence of cardiac failure or fluid overload (PAOP < 18 mmHg)

iv) Either

a) PaO_2:FiO_2 < 40 kPa = **Acute Lung Injury**PaO_2:FiO_2 < 27 kPa = **Acute Respiratory Distress Syndrome**

Pleural effusions

Appropriate clinical history and examination with consistent radiograph, ultrasound or CT scan of the chest

Gastrointestinal Complications

Gastro-intestinal bleed

Unambiguous clinical evidence or endoscopy showing blood in gastro-intestinal tract

Bowel infarction

Demonstrated at laparotomy

Anastamotic breakdown

Demonstrated at laparotomy or by contrast enhanced radiograph or CT scan

Paralytic ileus

Persistent clinical evidence of intestinal ileus and failure to tolerate enteral fluid or feed associated with valid cause

Post-operative haemorrhage

Overt blood loss requiring transfusion of two or more units of blood in two hours.

Renal Complications

Acute kidney injury

As per AKIN criteria

Neurological Complications

Stroke

Clinical diagnosis with confirmation by CT scan

Transient ischaemic attack

Clinical diagnosis