Microcirculation and the effects of blood transfusion in liver transplantation

MD(Res)

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Declaration

I, Ben Clevenger, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Liver disease represents a growing public health issue and is a significant cause of death worldwide. Liver transplantation remains the definitive treatment for end stage liver disease. Liver transplantation is traditionally associated with high rates of haemorrhage and intraoperative blood transfusion. There is evidence that both blood loss, and, although often necessary and lifesaving, blood transfusions are associated with adverse outcomes after liver transplantation.

Cirrhosis represents a chronic inflammatory state that develops due to progressive liver damage. Cirrhosis results in circulatory alterations throughout the body, affecting different tissues and organs, and their microcirculation, in a heterogeneous manner. Non-invasive methods of microcirculatory monitoring can now be utilised to examine the effects of liver disease upon microcirculatory blood flow and vascular reactivity.

Experiments to examine effect of blood transfusion, bleeding and surgery upon the microcirculation and vascular reactivity during liver transplantation were undertaken. Despite improvements in haemoglobin concentration with blood transfusion there was no significant improvement in microcirculatory flow. Over the duration of the liver transplant, in spite of alterations in haemoglobin and macrovascular measures of circulatory adequacy, there was no correlation with indices of microvascular flow. Microcirculatory flow and tissue oxygenation was well maintained even at low haemoglobin concentrations without significant alteration by blood transfusion, suggesting that even lower transfusion thresholds might safely be adopted.

Further work should assess the efficacy of treating preoperative anaemia and safe transfusion thresholds in liver transplantation.

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Impact Statement

Liver disease is a growing public health problem. Major surgery is associated with numerous modifiable risks that can affect a patient's outcome and subsequent recovery. Bleeding and blood transfusion are both associated with adverse outcomes. Liver transplantation is associated with both of these risks.

For the first time, video microscopy during liver transplantation recorded microcirculatory blood flow to assess the impact of blood loss and blood transfusions. Microcirculatory flow was well maintained in spite of blood loss. Even at low haemoglobin concentrations, blood transfusion did not alter microcirculatory flow. Within clinical intraoperative practice these microcirculatory monitoring techniques may provide an additional monitor to guide haemodynamic status and directly monitor the effect of interventions, including the decision to transfuse blood. They can be utilised in the post-operative period to monitor tissue perfusion and help to detect early changes that may be associated with post-operative complications.

Further research into the role of the microcirculation in patients with liver disease and undergoing liver transplantation using non-invasive techniques may improve the understanding pathophysiology of cirrhosis and the development of complications after liver-transplantation.

A large body of research has demonstrated that anaemic patients have increased adverse outcomes after surgery, including those undergoing liver transplantation. Nonetheless, anaemia is strongly associated with blood transfusions, which independently are associated with adverse outcomes after surgery. This work may aid the implementation of treatment strategies to reduce pre-liver-transplant anaemia and reduce blood transfusion rates in liver transplantation further still.

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Abbreviations

2,3-DPG	2,3-diphosphoglycerate
ABG	Arterial blood gas
ACD	Anaemia of chronic disease
ACE	Angiotensin converting enzyme
ACS	American College of Surgeons
ALD	Alcoholic liver disease
AIH	Autoimmune hepatitis
AT	Antithrombin
ATP	Adenosine triphosphate
AUC	Area under curve
AVA	Automated Vascular Analysis
BP	Blood pressure
CaO_2	Arterial oxygen content
CCC	Caval cross clamp
CCD	Charge-coupled device
CCT	CytoCam Tools

CI	Confidence interval
CKD	Chronic kidney disease
CLI	Clot lysis index
CNA	Capillary Network Analysis
CO	Cardiac output
CO_2	Carbon dioxide
CSV	Comma-separated values
CVP	Central venous pressure
DBD	Donation after brain-stem death
DBS	De Backer score
DCD	Donation after cardiac death
DO_2	Oxygen delivery
ECG	Electrocardiogram
eNOS	Endothelial nitric oxide synthetase
EPO	Erythropoietin
EQA	External Quality Assessment
ERAS	Enhanced recovery after surgery
ESA	Erythropoiesis stimulating agents

EuSOS	European Surgical Outcomes Study
FBC	Full blood count
FCD	Functional capillary density
FFP	Fresh frozen plasma
FID	Functional iron deficiency
FiO_2	Fraction of inspired oxygen
GI	Gastrointestinal
HAT	Hepatic artery thrombosis
Hb	Haemoglobin
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HI	Heterogeneity index
HIV	Human immunodeficiency virus
HR	Heart rate
HVPG	hepatic vein pressure gradient
ICU	Intensive care unit
IDA	Iron deficiency anaemia

IDF	Incident dark field
IL	Interleukin
iNOS	inducible nitric oxide synthetase
INR	International normalised ratio
IQR	Interquartile range
IVC	Inferior vena cava
LFT	Liver function tests
MA	Maximum amplitude
MAP	Mean arterial pressure
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
MD	Mean difference
MELD	Model of End Stage Liver Disease
MFI	Microvascular flow index
NASH	Non-alcoholic steatohepatitis
NATA	Network for the Advancement of Transfusion Alternatives
NHANES	United States National Health and Nutrition Examination Survey

NHS	National Health Service
NHSBT	National Health Service Blood and Transfusion
NIRS	Near infrared spectroscopy
NO	Nitric oxide
NSQUIP	National Surgical Quality Improvement Program
O_2	Oxygen
OLT	Orthotopic liver transplantation
OPS	Orthogonal polarised spectrum
OR	Odds ratio
PAC	Pulmonary artery catheter
PAI-1	Plasminogen activator inhibitor
PaO_2	Arterial partial pressure of oxygen
PB	Piggy back
PBM	Patient blood management
PCC	Prothrombin complex concentrate
PMNL	Polymorphonuclear neutrophils
PO	*Per os*
POCT	Point of care test

PPV	Proportion of perfused vessels
PREVENTT	Pre-operative intravenous iron to treat anaemia in major surgery
PRBC	Packed red blood cell
PSC	Primary Sclerosing cholangitis
PT	Prothrombin time
PVD	Perfused vessel density
RAAS	Renin-angiotensin-aldosterone-system
RBC	Red blood cell
RDW	Red cell distribution width
rEPO	Recombinant erythropoietin
RR	Relative risk
SD	Standard deviation
SDF	Sidestream dark field
SHOT	Serious Hazards of Transfusion
SI	Speed indicator
SMD	Standardised mean difference
SpO_2	Peripheral oxygen saturation
STfR	Serum transferrin receptor

StO_2	Tissue oxygen saturation
SvO_2	Mixed-venous oxygen saturation
SVR	Systemic vascular resistance
TEG	Thromboelastograph
TIBC	Total iron binding capacity
TNF-α	Tumour necrosis factor alpha
TOE	Trans oesophageal echocardiography
tPA	Tissue plasminogen activator
TSAT	Transferrin saturation
TVD	Total vessel density
TXA	Tranexamic Acid
UK	United Kingdom
VEGF	Vascular endothelial growth factor
VOT	Vaso-occlusive test
vWF	Von Willebrand Factor
WBC	White blood cell
WHO	World Health Organisation
ZPP	Zinc protoporphyrin

Chapter 1 Introduction

1.1 Liver transplantation and cirrhosis

Liver disease is common. In the UK 600,000 patients have liver disease, and 60,000 have cirrhosis. Between 2005 and 2015 there was a 49% increase in the numbers of admissions with a primary diagnosis of cirrhosis in England (NICE, 2016). It is the 14th most common cause of death worldwide.

Cirrhosis develops progressively as result of liver damage, usually over several years. The most common risk factors for cirrhosis in the UK and Europe include: alcohol misuse, hepatitis B and C infections and obesity. Most chronic liver disease is asymptomatic until cirrhosis with clinical decompensation occurs. The normal smooth structure of the liver becomes distorted with nodules surrounded by fibrosis. This affects the liver's synthetic, metabolic and excretory functions.

Liver transplantation is now a well-established operation which provides the definitive treatment for end stage liver disease of multiple aetiologies in both adult and paediatric patients. 7399 liver transplants were carried out in the UK between 2005 and 2015 (Statistics and Clinical Studies, 2015).

The first liver transplant in humans was carried out by Thomas Starzl in 1963 (Starzl et al., 1963). That patient bled to death on the table, four hours after the revascularisation of the transplanted liver. Over the subsequent years since the first human case reports of liver transplantation the refinement of surgical technique, immunosuppressant regimens and anaesthetic and haemostatic management has transformed this operation into one with five year survival rates of 80% in adults in the UK (Statistics and Clinical Studies, 2015).

Until the introduction of the immunosuppressive agent ciclosporin in 1979, survival after liver transplantation was variable, with 1 year survival rates of less than 30%. Thereafter, alongside advances in donor and recipient selection and refinements in surgical technique, survival rates rose to 70% at 1 year (Zarrinpar and Busuttil, 2013). The advances in immunosuppressive regimens over the past few decades have

greatly improved post-transplant survival with fewer toxic-side effects and more selective regimens such as those using mono-clonal antibodies to specific immune system target (Zarrinpar and Busuttil, 2013). Refinements to surgical techniques have continued, such as the introduction of split liver transplantation, the caval piggy back (PB) in 1989 and the use of temporary porto-caval shunts in the 1990s (Llado and Figueras, 2004). Most adult operations are orthotopic liver transplants (OLT) where the native liver is replaced by the donor in the same anatomic position.

Organ donation after death is an area of intense medical and ethical debate. 611 patients were on the UK active transplant list on 31st March 2015, approximately double that from March 2008. Approximately 76% of patients on the waiting list receive a transplant within two years of being added to the waiting list (Statistics and Clinical Studies, 2015). The concept of brain death became widely accepted in the late 1960s, and the UK published its first criteria for brainstem death in 1976. This allowed the transplantation of organs donated after brain-stem death (DBD) as well as those donated after cardiac death (DCD) (or non-heart beating donor organs), increasing the pool of donor organs, as well as improving the quality of the donor graft since DBD organs sustain much less warm ischaemic damage prior to reperfusion. Live liver transplants are also possible in which a living donor's liver is split by hepatectomy and a proportion transplanted to the recipient.

1.2 Cirrhosis

Cirrhosis represents a usually irreversible state of liver damage; however, it can be viewed as a dynamic process. It can be compensated when the liver still functions effectively or decompensated whereby the liver no longer functions adequately, leading to overt clinical complications including ascites, jaundice, variceal haemorrhage and hepatic encephalopathy. The leading cause of death in patients with cirrhosis is hepatocellular carcinoma. 1 year mortality ranges from 1% to 57% depending upon the occurrence of decompensating events (D'Amico et al., 2006).

Cirrhosis results from different mechanisms of liver injury that lead to necroinflammation and fibrogenesis. It is characterised histologically by diffuse nodular regeneration surrounded by dense fibrotic septa with subsequent

parenchymal destruction and collapse of liver structures, leading to distortion of the hepatic vascular architecture (Tsochatzis et al., 2014). Chronic liver disease proceeds to cirrhosis due to inflammation, activation of hepatic stellate cells, subsequent fibrogenesis, angiogenesis and parenchymal extinction lesions caused by vascular occlusion. This process leads to hepatic microvascular changes, characterised by sinusoidal remodelling (extracellular matrix deposition from proliferating activated stellate cells, leading to capillarisation of hepatic sinusoids), formation of intrahepatic shunts (due to angiogenesis and loss of parenchymal cells), and hepatic endothelial dysfunction (Tsochatzis et al., 2014). Capillarisation of sinusoids and intrahepatic shunts interfere with effective hepatocyte perfusion, predisposing to liver failure.

Endothelial dysfunction is characterised by insufficient release of vasodilators, particularly nitric oxide (NO). Nitric oxide release is inhibited by low activity by endothelial nitric oxide synthetase (eNOS). eNOS activity is constrained by insufficient protein-kinase-B-dependent phosphorylation, lack of cofactors, increased scavenging due to oxidative stress, and high concentrations of endogenous inhibitors of nitric oxide. Meanwhile vasoconstrictor production is increased – due to adrenergic stimulation and thromboxane A2, and activation of the renin-angiotensin-aldosterone-system, antidiuretic hormone and endothelin).

The distortion of the vasculature increases the resistance to portal blood flow, resulting in portal hypertension and hepatic synthetic dysfunction. Portal hypertension is a result of structural disturbances, which produce approximately 70% of total hepatic vascular resistance, endothelial dysfunction and increased hepatic vascular tone. Portal hypertension is present when hepatic vein pressure gradient (HVPG) is more than 5 mmHg. Clinically significant portal hypertension, and the threshold of developing oesophageal varices is HVPG greater than 10mmHg (Bosch et al., 2006). The formation of oesophageal varices is the first clinically important consequence of portal hypertension.

Portal hypertension is the most common cause of complications related to cirrhosis. Splanchnic vasodilatation is an adaptive response to the changes in intrahepatic haemodynamics in cirrhosis. The changes associated with portal hypertension are

driven by low grade inflammation, promoted by inflammation within the splanchnic system, which can switch to high grade inflammation with the development of life threatening severe complications (Aller et al., 2007).

Ascites represents fluid accumulation within the peritoneal cavity as a result of splanchnic vasodilatation and portal hypertension, with sodium and water retention, and is the commonest cause of hospitalisation related to cirrhosis. Cirrhosis with ascites predisposes patients to further complications including spontaneous bacterial peritonitis, hyponatraemia and the hepatorenal syndrome due to the development of renal vasoconstriction.

Hepatic encephalopathy is a dysfunction of the brain as a result of liver insufficiency and, or, portosystemic shunting. It is caused by the effect of nitrogenous waste products such as ammonia and glutamine on the brain. This leads to symptoms including cognitive and behavioural changes, personality changes, sleep disturbance, motor problems and altered level of consciousness. Severe cases lead to acute confusion, agitation and coma. Approximately half of patients with cirrhosis experience hepatic encephalopathy following diagnosis, affecting around 1 in 5 people with cirrhosis each year. It is often trigged by a predisposing event including constipation, dehydration, infection, gastrointestinal bleeding and the effect of some drugs including opioids, benzodiazepines and diuretics).

Oesophageal varices develop as a result of dilation of the veins in the lower oesophagus and stomach due to increased portal pressure. Angiogenesis is increased by increased vascular endothelial growth factor (VEGF) (Abraldes et al., 2006). The rupture of varices and associated haemorrhage has a high mortality rate. Risk factors for rupture include the size of varices, severity of liver disease, sepsis and hepatocellular carcinoma.

Patients with cirrhosis are at increased risk of bacterial infection and sepsis because of immune dysfunction. The most commonly occurring infections are spontaneous bacterial peritonitis; where the body's innate bacteria cause infection of ascitic fluid, urinary tract infections, pneumonia, cellulitis, and bacteraemia. Bacterial infections have a significant role in the progression of liver failure, complications and mortality

in cirrhosis. Because of the haemodynamic dysfunction of cirrhosis, the response to sepsis and infection increases the risk of shock and acute renal failure. Risk factors for infection in cirrhosis include reduced liver function, low protein ascites and variceal bleeding.

Infection in cirrhosis increases mortality four times and has a poor prognosis, with 30% of patients dying within a month of infection, and a further 30% within 1 year (Tsochatzis et al., 2014). Decreased bowel motility, bacterial overgrowth and increased intestinal permeability all increase the risk of translocation of intestinal microbes to the mesenteric lymph nodes, thus pre-disposing the patient to infection. This includes spontaneous bacterial peritonitis, but also is a source of endotoxin and other bacterial products that influence systemic haemodynamics. Bacterial DNA in non-infected cirrhotic patients is associated with aggravation of peripheral vasodilation and worsening of aggravation of intrahepatic endothelial dysfunction (Bellot et al., 2010).

As well as hepatic encephalopathy, the shunting of portal blood to the systemic circulation through the portosystemic collaterals leads to the decreased first pass metabolism of orally administered drugs, and decreased reticulo-endothelial system function.

1.3 Blood and the circulation

Oxygenated blood from the pulmonary circulation and heart is delivered to the tissues along a partial pressure gradient from the systemic circulation. Pressure from the contraction of the left ventricle drives flow around the systemic circulation. Gap junctions between vascular smooth muscle and endothelial cells transmit signals between adjacent cells to coordinate the constriction and relaxation of the vasculature. Blood flow is determined by: the pressure gradient applied, vessel length and diameter and blood viscosity.

Erythrocytes or RBCs are biconcave discs of 6-8 µm diameter, 2µm thick. RBC membranes shear and bend easily, but resist area changes. This makes RBCs highly deformable, allowing them to pass through capillaries with a diameter less than that

of an unstressed RBC (Pries et al., 1996). The RBC membrane is composed of proteins, lipids and carbohydrates and its elasticity depends on structural interactions between the membrane and the underlying protein skeleton (Piagnerelli et al., 2003).

The blood itself directly influences flow within vessels.

Fluid viscosity determines how 'slippery' the fluid is; how easily fluid layers slip over and interact with one another. Higher viscosity means greater interaction between adjacent layers. Newton's definition of fluid viscosity is a ratio of 'shear' stress' (force applied to a plate moving on the surface of a liquid in a container, divided by plate area – dyne/cm^2) to 'shear rate' (velocity of movement of the liquid below the plate divided by the depth of the liquid in the container - (cm/s)/cm). Low viscosity fluids have high velocity of movement for a given applied force with a low ratio of shear rate: shear stress. Higher viscosity fluids flow less easily. Poise is the unit of measurement of viscosity where 1 poise = 1 dyn.s/cm2.

Blood flow is highly dependent upon its viscosity, with flow inversely proportional to viscosity (to which RBCs are a primary factor) according to Poiseuille's equation:

Equation 1: Poiseuille's equation

$$Flow = \frac{\pi (P1-P0) r^4}{8 \times \eta \times L}$$

Where: (P1-P0) = hydrostatic pressure gradient; r = radius of tube; η = viscosity; L = tube length

The resistance to flow provided by blood vessels of the cardiovascular system is the ratio of the pressure drop: flow, proportional to the hydrostatic pressure gradient acting along the vessel and to the fourth power of the vessel radius; and inversely proportional to the fluid viscosity and vessel length.

Equation 2: Resistance

$$Resistance = \frac{8\eta L}{\pi r^4}$$

Where: η = viscosity; L = vessel length; r = radius of vessel

Therefore, resistance is directly related to viscosity (η) and vessel length (L). Given that resistance is dependent upon constant vessel length, a relatively constant blood viscosity, vessel diameter is the main variable of peripheral resistance, largely through altering arteriolar diameter. In laminar flow, the resistance arises between the viscous forces between the moving layers of fluid – determined by the liquid's viscosity. Increased viscosity thus increases resistance to flow. Temperature inversely changes fluid viscosity, decreasing viscosity with increased temperature. At 37 ^{o}c, plasma has a viscosity of 1.2 centipoise. The relative viscosity of blood to water is doubled when the haematocrit (the volume percentage of RBCs in the blood) rises from 0.45 to 0.6.

However, Poiseuille's equation is derived from measurements of steady laminar flow though rigid glass tubes, with constant driving pressures, where flow is proportional to the driving pressure. The cardiovascular system is very different, with a pulsatile driving pressure from the ventricle within the arterial system, distensible vessels that can alter their diameter, and blood viscosity related to vessel size and flow rate; and the potential for turbulent flow, altering the relationship between pressure and flow. Investigations have taken place for the effect of pulsatile and non-pulsatile flow upon the microcirculation (De Backer et al., 2009).

The blood is an aqueous solution containing elements and molecules, including RBCs, WBCs and platelets. Blood is a non-Newtonian fluid whereby viscosity is dependent upon shear forces and the concentration of RBCs suspended within it. Shear rate refers to the rate of change of velocity at which one fluid layer passes over an adjacent rate. The viscosity of blood decreases as the shear rate increases. This is known as 'shear thinning'. At high shear rates, the RBCs lie in the axial stream with their long axis in the direction of the flow, where the flow rate is faster. At low flow rates, the erythrocytes tend to aggregate, increasing the resistance to flow (Soutani et

al., 1995). At low shear rates blood becomes a high viscosity suspension. The electrostatic repulsion of the RBC is overcome by the presence of macromolecules which aggregate the cells (Piagnerelli et al., 2003).

In small vessels, RBCs occupy the central, axial, fast moving stream, whilst plasma flows slowly through the marginal streams. The Fahraeus effect refers to the decrease of average concentration of RBCs in blood as the diameter of the glass tube in which it is flowing decreases. Thus, the ratio of red cell volume to blood volume (i.e. the haematocrit) is effectively reduced when blood is in small vessels of <300 μm compared to larger vessels, due to reduced frictional forces, the Fahraeus effect, heterogeneous flow distributions and interactions between the luminal glycocalyx and molecules within the plasma.

Within the capillary, single-file flow further reduces the viscosity so that in tubes of 6μm, the viscosity of blood is as low as plasma (Levick, 2010). Additionally, most microcirculatory vessels are lined with an endothelial surface layer, which alters the flow resistance and leads to an apparent increase in blood viscosity within the microcirculation (Gompper and Fedosov, 2016). The main determinants of whole blood viscosity are thus shear rate, plasma viscosity, haematocrit, RBC deformability and RBC aggregation.

1.3.1 Oxygen Delivery

Oxygen is transported to the tissues along the oxygen cascade from atmosphere, through the passages of the airways, through diffusion into the RBCs in the blood at the alveoli then through the arterial, then capillary vasculature to the respiring cell and its mitochondria. The oxygen flux represents the amount of oxygen delivered to the peripheral tissues per minute.

The oxygen content of the blood is predominantly transported by red blood cells, bound to haemoglobin within. This accounts for 98.5% of the oxygen content of the blood, the remainder dissolved within the plasma calculated as follows:

Equation 3: Oxygen content

$$CaO_2 = (\{Hb\} \times SaO_2 \times H \times 0.01) + (PaO_2 \times S)$$

Where: CaO_2 = arterial oxygen content (ml/L); {Hb} = haemoglobin concentration; SaO2 = arterial oxygen saturation of haemoglobin; H = Huffner's constant – the value of capacity of Hb to carry oxygen on direct measurement (1.34 ml O_2 / g Hb) (in spite of the theoretical maximum oxygen carrying capacity of 1.39 ml O2/g Hb); PaO_2 = arterial partial pressure of oxygen; S = solubility coefficient for oxygen in blood (0.225 ml of O_2 dissolved per 1000ml/blood)

Thus, for a normal adult male with a Hb of 150 g/L and arterial PaO_2 of 13.3 kPa and oxygen saturations of 100%, CaO_2 is 204 ml per litre of blood (3ml of which is dissolved oxygen). If that same patient were anaemic, with a Hb of 100 g/L, the CaO_2 is reduced to 137 ml per litre.

Oxygen delivery is the amount of oxygen delivered to the peripheral tissues, calculated by:

Equation 4: Oxygen delivery

$$DO_2 = CaO_2 \times CO$$

Where DO_2 = oxygen delivery (ml/minute); CaO_2 = arterial oxygen content; CO = cardiac output

Oxygen delivery to the tissues can therefore be influenced by cardio-respiratory factors including respiratory adequacy, concentration of inspired oxygen and cardiac output. Critical oxygen delivery is the lowest DO_2 that can support aerobic metabolism. Haemoglobin concentration is central to the oxygen carrying capacity of the blood. At the peripheral tissues, oxygen delivery is mediated by several factors including the regulation of the microcirculation and the extraction of oxygen by the respiring tissues. This occurs down concentration gradients from the capillary to the mitochondria.

1.4 Microcirculation

The microcirculation refers to those blood vessels with a diameter of less than 100 micrometres (μm), comprising arterioles, venules and capillaries. These can be classified as small (diameter less than 20μm), medium (20-50μm diameter) and great (50-100 μm). The smallest vessels are 4-8 μm in internal diameter. The microcirculation should also be considered to include the blood circulating within it. Thus, the key cells of the microcirculation include erythrocytes, leukocytes and the plasma components of blood; as well as the endothelial cells of blood vessels and smooth muscle cells (predominantly within arterioles).

The structure and function of the microcirculation is highly heterogeneous within different organs. The vessels of the microcirculation are generally embedded within an organ, providing a large surface area of contact between the blood and organ tissue for the transport of substrates and metabolites to the tissue – including the delivery of oxygen as its central function. The main determinants of capillary flow are driving pressure, arteriolar tone, hemorheology and capillary patency.

Within organ tissues, the microcirculation regulates the distribution of blood flow to facilitate the demand of the tissue. This is largely controlled by arteriolar resistance, mediated by smooth muscle which contracts and relaxes in response to vasodilatory or vasoconstrictive stimuli facilitated by the autonomic nervous system. Beyond the arterioles red blood cells flow in single file through the capillaries, maximising the surface area for the release of oxygen from Hb within the RBC.

The regulation of the microcirculation is provided by multiple systems controlling perfusion. These are myogenic; sensing strain and stress, metabolic (based upon O2, CO2, lactate and H+), and neurohumeral. A combination of autocrine and paracrine interactions regulates the blood flow through the microcirculation to ensure adequate tissue oxygenation. Mediating substances include vasodilators: carbon dioxide, lactate, adenosine, potassium ions), nitric oxide, prostacyclin, endothelin (ET-1)) and local paracrine secretions: histamine, serotonin, thromboxane.

Endothelial cells lining the blood vessels are autoregulation to this by sensing flow, metabolic and other regulating substances to regulate arteriolar smooth muscle tone and capillary recruitment (Ince, 2005) Endothelial cell-to-cell signalling transmits information about haemodynamics downstream. Meanwhile, the endothelium has effects upon coagulation and immune function, which directly impacts microcirculatory function (Ince, 2005).

Much of the investigation of abnormalities of microcirculatory flow in critically ill patients has taken place in the context of sepsis. Decompensated cirrhosis, acute-on-chronic liver failure and sepsis commonly result in multi-organ dysfunction, and there is increasing evidence that the underlying pathophysiological mechanisms may be similar.

1.4.1 Microcirculation in Sepsis

In sepsis, activation of inflammation is central to the pathological microvascular changes seen. In decompensated cirrhosis, plasma concentrations of inflammatory cytokines TNF-α, IL-1, Il1- and regulatory cytokines (G-CSF and CM-CSF) promoting monocyte and neutrophil activation are present (De Backer et al., 2014b, Claria et al., 2016, Lin et al., 2007). Decompensated cirrhosis, despite an absence of infection, has been shown to be a state of endotoxaemia without overt infection (Lin et al., 2007).

Septic shock is characterised by profound haemodynamic alterations associated with organ dysfunction. Systemic alterations associated with septic shock include a degree of hypovolaemia, decreased vascular tone and myocardial depression. The microcirculation has been demonstrated to play a key role in sepsis. The pathological processes of sepsis affect almost every cellular component of the microcirculation including the endothelial and smooth muscle of the vessels, tissue cells and the erythrocytes and leukocytes circulating within. Even when systemic haemodynamic variables have been corrected, signs of impaired tissue perfusion may persist.

The autoregulation of the microcirculation is severely disrupted by sepsis. A defining feature is the heterogeneity of flow within the microcirculation – with some vessels

under perfused whilst others have normal or high blood flow (De Backer et al., 2002a).

When the autoregulation of the microcirculation is disrupted, systemic monitoring and oxygen derived variables may be unable to determine the microcirculatory insufficiency or distress, due to the flow heterogeneity and shunting, and an oxygen extraction deficit where the partial pressure of oxygen within the arterial end of the microcirculation drops below that of the venous blood (Ince, 2005).

Central to the autoregulation of the microcirculation is the nitric oxide system (NO). Sepsis disturbs the NO system by a heterogeneous expression of inducible nitric oxide synthase (iNOS) in different areas of organ microvasculature, heterogeneously altering the vasodilatation and vasoconstriction in different areas, leading to the shunting of flow (Ince, 2005). iNOS is not expressed homogenously within organ systems so areas with lower iNOS will have less NO-induced vasodilatation, leading to under perfusion.

Smooth muscle cells that line the arterioles and regulate perfusion lose their adrenergic sensitivity and tone in sepsis. Eventually uncorrected microcirculatory dysfunction leads to parenchymal cell distress and organ failure. The degree of microcirculatory dysfunction in sepsis has been shown to be correlated with the development of multi-organ failure (Sakr et al., 2004, De Backer et al., 2002b). However, it has been shown that correction of systemic haemodynamics and oxygenation may not correct microcirculatory failure. Shunting may disguise the microcirculatory distress (Ince and Sinaasappel, 1999).

1.4.1.1 The red blood cell in sepsis

Red blood cells play an important role in the regulation of microcirculatory blood flow by their ability to release NO in the presence of hypoxia and to cause vasodilatation. This can be disturbed by sepsis. Red blood cell rheology is influenced my multiple factors including alterations in intracellular calcium and adenosine triphosphate (ATP) concentrations, the effects of NO, levels of RBC membrane components including sialic acid and 2,3 diphosphglycerate. Other factors include interactions with leukocytes and reactive oxygen species. Integrations with WBCs

cause release of oxygen free radicals, which stimulate RBC intracellular proteolysis, membrane lipid peroxidation and NO production.

Red blood cells additionally become less deformable and aggregate more. Acute phase proteins (including fibrinogen and serum proteins such as a2 macroglobulin) increase RBC aggregation. RBCs aggregate to form rouleaux – stacks of RBCs – caused by plasma macromolecules, with weak attractive forces, which can be reduced by applying sufficient shear forces (Wagner, 2013). Rouleaux of cells can bind together, side-by-side, and continue to uptake individual cells, creating larger aggregates (Piagnerelli et al., 2003). Increased aggregation can lead to RBC entrapment in the microcirculation including within the spleen, lung, liver and femur, reducing regional blood flow in proportion to the number of trapped RBCs (Simchon et al., 1987).

Red blood cell apoptosis is also affected in the presence of sepsis. Alongside the coagulopathy that develops in sepsis, the blood has a significant impact upon microcirculatory function and perfusion in sepsis. Leukocytes are activated by septic inflammation, generating reactive oxygen species which can disrupt microcirculatory structures, cellular interactions and coagulation functions. Inflammatory mediators have direct effects upon the cell-to-cell junctions and endothelial glycocalyx, altering the barrier function of the microcirculation, leading to tissue oedema and further impacting the oxygen extraction ratio.

1.4.2 The microcirculation in cirrhosis

Systemic haemodynamics in cirrhosis produce a hyperkinetic system, with increased cardiac index, hypotension and reduced systemic vascular resistance. There are regional alterations within the splanchnic and hepatic circulations in cirrhosis, with variations in other regional tissue vasculature, in spite of a hyperdynamic circulation, representing a ‘splanchnic steal’ (Davies et al., 2017). The splanchnic circulation vasodilates, with reduced responsiveness to vasoconstrictors, angiogenesis and development of porto-systemic collaterals. Meanwhile, there is usually vasoconstriction within the kidney and brain, with vasodilatation in the lungs and skin (Gonzalez Ballerga et al., 2018). These heterogeneities are thought to be related

to regional differences in nitric oxide production (Wiest and Groszmann, 2002). NO activates soluble guanylate cyclase, which stimulates the formation of cGMP. In vascular smooth muscle, cGMP activates cGMP dependent protein kinase, which phosphorylates several proteins regulating calcium homeostasis. Phosphorylation leads to a decrease in intracellular Ca2+-concentration and ultimately smooth muscle relaxation and vasodilation (Wiest and Groszmann, 2002).

High levels of circulating inflammatory mediators including TNF-α, IL-1, IL-6 and soluble adhesion molecules have been demonstrated in decompensated cirrhosis (Sheikh et al., 2009). Thus, decompensation in cirrhosis involves severe systemic inflammation and oxidative stress, in a similar manner to sepsis, resulting in multi-organ dysfunction and haemodynamic disturbances.

Microvascular monitoring has been made increasingly achievable with non-invasive monitoring techniques including hand-held videomicroscopy devices. These have helped to elucidate the microvascular alterations in sepsis (Ince, 2005, Trzeciak et al., 2007). A dissociation between microvascular perfusion and global haemodynamic variables has been demonstrated in sepsis.

These have also been utilised in disease states including cirrhosis, diabetes and chronic kidney disease (Sheikh et al., 2009, Reynolds et al., 2013). Reynolds et al used sidestream dark field (SDF) videomicroscopy in stable patients with cirrhosis, demonstrating a trend towards increased microcirculatory flow and perfused vessel density compared to controls (Reynolds et al., 2013). Sheikh et al utilised SDF in cirrhotic patients with and without decompensation, and in non-cirrhotic patients with sepsis. They found that microvascular perfusion was significantly impaired in decompensated compared with compensated cirrhosis secondary to vasoconstriction. The alterations in sublingual microcirculatory flow in decompensated cirrhosis mimics the changes seen in sepsis, with less intensity (Sheikh et al., 2009).

1.5 Effect of surgery on the microcirculation

The effect of surgery upon the microcirculation has been investigated in a variety of pathologies and surgical specialities. Surgery is associated with a stress response,

with numerous hormonal and metabolic changes following tissue injury and trauma. Systemic responses include sympathetic nervous system activation, endocrine effects including pituitary hormone secretion and insulin resistance, immunological and haematological changes from cytokine production, acute phase reactants, neutrophil leucocytosis and lymphocyte proliferation (Desborough, 2000). This leads to increased secretion of catabolic hormones, as an evolutionary mechanism to allow survival following injury by using stored body fuels and retaining salt and water. After major surgery, the cytokines are released to mediate and maintain the inflammatory response to tissue injury. Il-1 and TNF-α are released from activated macrophages and monocytes in the damaged tissues. This stimulates the production of more cytokines, including IL-6 which induces the acute phase response (Desborough, 2000).

Therefore, surgery is associated with an inflammatory reaction with inflammatory mediators in a similar manner to the inflammatory response to sepsis and decompensated cirrhosis. Several studies have investigated microvascular flow in major surgery, although few studies have visualised the microcirculation in liver transplantation.

Surgical trauma produces microcirculatory alterations as a result of early systemic inflammatory responses, tissue hyperperfusion and direct tissue injury (Ni Choileain and Redmond, 2006). Prolonged hypoperfusion secondary to significant intraoperative blood loss can lead to microcirculatory disturbances. The sympathetic nervous system mediates the initial response to severe blood, producing vasoconstriction of arterioles and venules. This results in reduced capillary blood flow with an increase in hydrostatic pressure.

The local microcirculatory inflammatory response is characterised by leucocyte accumulation and adherence to the endothelial lining of blood vessels (Ni Choileain and Redmond, 2006). This is associated with an increase in microvascular permeability, reflecting disruptions of endothelial integrity. Surgical trauma induced microvascular inflammation include endogenous TNF-α release and adhesion molecules. A combination of capillary leakage secondary to pro-inflammatory cytokine release, increased NO production and the interactions of adhesion

molecules leads to polymorphonuclear neutrophils (PMNLs) sticking together, leading to obstruction of the microcirculation and failure of transcapillary exchange (Ni Choileain and Redmond, 2006).

Non-invasive videomicroscopy techniques including orthogonal polarisation spectral (OPS) imaging and sidestream-dark field (SDF) imaging have been utilised to visualise the microcirculation by detecting erythrocytes moving through vessels (De Backer et al., 2012). Jhanji et al investigated the effect of surgery upon the microcirculation in major elective abdominal surgery using SDF. It was demonstrated that impaired sublingual microcirculatory flow was associated with more frequent postoperative complications and increased length of hospital stay (Jhanji et al., 2009). Bansch et al used SDF during major abdominal surgery - including 20 patients undergoing liver surgery. They found that flow and perfusion was higher during anaesthesia than before and afterwards, but that perioperative changes were minor throughout the surgery. They showed no correlation between microcirculatory parameters and perioperative lactate and $ScvO_2$ values (Bansch et al., 2014).

1.5.1 Effect of anaesthesia on the microcirculation

There is also evidence that anaesthetic agents themselves alter the microcirculation. The haemodynamic effect of anaesthetics includes central inhibition of the sympathetic nervous system, reduced cardiac output, and peripheral vasodilatation. As well as the compromised macrohaemodynamics, alterations in immune responses, and NO pathways by intravenous anaesthetics affect organ perfusion and the microcirculation (Turek et al., 2009).

Propofol and thiopentone act centrally to inhibit the sympathetic nervous function whilst ketamine has sympathomimetic actions. Thiopental, propofol and ketamine have vasodilatory effects mediated by L-type voltage gated Ca2+ channels (Holzmann et al., 1995, Longnecker et al., 1974, Wada et al., 1996, Hirota and Lambert, 1996). Propofol has been demonstrated experimentally to have a dose dependent effect on regional organ blood flow in the kidney, small intestine and large intestine (Piriou et al., 1999). In healthy humans anaesthetised with propofol,

OPS imaging demonstrated reduced total microvascular density and a reduced proportion of perfused small vessels (Koch et al., 2008b). Propofol inhibits iNOS and increases constitutive NOS stimulation by inflammatory mediators, altering nitric oxide production, as a mechanism for its effect upon the microcirculation (Vasileiou et al., 2009).

Volatile anaesthetic agents are halogenated ethers. Sevoflurane, desflurane and isoflurane are the commonly used agents in clinical practice and all are associated with dose dependent decreases in systemic vascular resistance and mean arterial blood pressure. Microcirculatory perfusion under volatile anaesthesia has been studied with conflicting results, and it has been shown that isoflurane and sevoflurane cause mild decrease in portal blood flow, but no change in arterial hepatic blood flow (Bernard et al., 1992). However, current evidence suggests that hepatic and intestinal perfusion is well maintained in relation to oxygen demand under volatile anaesthesia (Turek et al., 2009).

The microcirculation is further discussed in chapter 4 with focus upon the effects of liver disease, chronic inflammatory states and surgery.

1.6 Tissue oxygenation and microcirculatory monitoring

There are several invasive and non-invasive mechanisms utilised to monitor tissue oxygenation and the microcirculation. Assessment of the microcirculation can add a further measure of oxygen delivery to the tissues whilst providing a mechanism to measure the effect of haemodynamic manipulation. It has been shown that maintaining oxygen delivery and related parameters are associated with reduced adverse outcomes following major surgery(Hamilton et al., 2011, Arulkumaran et al., 2014).

Lactate measurement has been a traditional measure to assess tissue perfusion and tissue oxygenation in critically ill patients. However, lactate production is dependent upon many factors including decreased oxygen delivery, impairment of tissue oxygen extraction or mitochondrial dysfunction (Kraut and Madias, 2014, Singer, 2008).

Techniques to measure tissue oxygenation include central venous oxygenation ($ScvO_2$) or pulmonary artery oxygenation (SvO_2)- as a surrogate for whole body uptake –delivery of oxygen (vO2 –DO2), measuring the adequacy of perfusion and flow. O_2 electrodes to measure tissue PO_2. Gastric tonometry and sublingual capnometry both measure tissue CO_2 to reflect perfusion adequacy and anaerobic metabolism (De Backer et al., 2010).

In pathological inflammatory conditions including sepsis and critical illness, systemic oxygen indices may not represent tissue oxygenation due to shunting, vascular deregulation and mitochondrial dysfunction (Sakr, 2010). Near infrared spectroscopy (NIRS) can measure tissue O_2 saturation non-invasively. Tissue oxygen saturation can be calculated by the absorption of light by oxy- and de-oxyhaemoglobin, giving a measure of tissue oxygenation. However, it can also be used dynamically by measuring the effects of a period of occlusion upon the microvasculature's response. This assesses reactive hyperaemia, the tissues ability to adjust oxygen extraction capacity relative to oxygen delivery, which is integral to microcirculatory function and to calculate muscle oxygen consumption (Creteur, 2008).

Intravital fluorescence microscopy is the gold standard in experimental conditions to measure an individual blood vessel's flow. However, this can only be used on organs that can be trans illuminated from the opposite side, and uses specific dyes which are unlicensed for human use.

Historical measurement of the microcirculation included invasive measures such as implantable Doppler or microdialysis (measuring lactate, pyruvate and glycerol within the interstitial fluid of tissues or organs), and non-invasive techniques. Microvascular perfusion can be measured non-invasively using laser Doppler which can measure relative flow, haemoglobin content and microvascular reactivity. Laser Doppler flowmetry (LDF) is a non-invasive technique to measure blood flow within the microcirculation. Low energy Laser light is reflected back from moving red blood cells with a change in wavelength which is detected, providing a measure of flow in relative units (mV) within a specific tissue. LDF can be applied to ski, mucosal and internal organ surfaces.

Video microscopy techniques can measure vascular density, heterogeneity, perfusion and flow. These include nailfold video microscopy and mucosal spectroscopy techniques based upon orthogonal polarisation spectroscopy (OPS). OPS and its successors, sidestream dark field (SDF) and incident-dark field (IDF) microscopy are based on a technique of transmitted light through tissue, which is variably absorbed or reflected back to a detector, forming an image. These allow non-invasive visualisation of erythrocyte movement through capillaries of mucosal surfaces including organ surfaces and other accessible mucosae including sublingual, rectal, vaginal, Ileostomy and colostomy.

Further discussion of microcirculatory monitoring is undertaken in chapter 5.

1.7 Anaemia

Tissue oxygen delivery is regulated by RBC oxygen content and the RBC flow regulation within the microcirculation. RBCs have been shown to be both a sensor and effect for the local regulation of oxygen delivery (Crawford et al., 2006). This allows a constant negative feedback mechanism during oxygen offloading to ensure local vascular tone and tissue perfusion is adequate for local oxygen demands, mediated by vasodilators released by the RBCs (Raat and Ince, 2007). As oxygen is offloaded from the RBC the cell can signal to increase arteriolar blood flow through vasodilation by modulating nitric oxide (NO) release. As well as the RBC effect, peripheral tissues can increase oxygen extraction by altering microvascular blood flow, which leads to a reduction in venous oxygen content but maintained tissue oxygenation (Wang and Klein, 2010).

An increasing severity of anaemia can eventually lead to a critical oxygen delivery level which is inadequate for tissue requirements. Compensation can be made by increasing CO. In an adult male with a Hb of 150 g/L, and CaO_2 of 200 ml/l, and CO of 5 L/minute, the DO_2 is approximately 1000ml/minute. If the same patient were anaemic with Hb of 100 g/L, then DO_2 is reduced to 685 ml/minute. In order to maintain the same DO_2, that patient's CO would have to increase to 7.3 l/minute.

In animal studies, maximal oxygen consumption (VO2max) and endurance is decreased by anaemia, and more significantly so in untrained animals. Trained animals with anaemia had lower VO2 max, but had greater muscle oxidative capacity and greater endurance than untrained controls (Gregg et al., 1989). Meanwhile in humans, transfusion of allogenic packed red cells to anaemic adults (raising Hb from mean 83 (12) g/L to 112 (14) g/L), increased peak oxygen consumption (VO2peak) from 13.9 (3.6) to 15.4 (3.4 ml/kg/min) (p=0.016) on CPET testing a median of 4 days (range 2-6 days) post-transfusion. Anaerobic threshold increased by 0.4 ml/kg/min per g/dL increase in Hb (Wright et al., 2014). Such measures are associated with the stratification of risk and the risk of complications and death after major surgery.

Meanwhile severe haemodilution causing an isovolaemic anaemia can impair microvascular function as a consequence of reducing plasma viscosity. The reduction in plasma viscosity reduces the functional capillary density (FCD) – calculated by the number of capillaries with RBC's passing through them. The point at which the reduced blood viscosity leads to a reduction in FCD is the same as that at which oxygen consumption becomes supply limited (Tsai et al., 2010). This leaves insufficient RBCs to 'hold open' all of the vessels of the microcirculation, reducing perfusion to the tissue.

The most common cause of anaemia worldwide is iron deficiency, which affects a significant part of the population in nearly every country in the world (WHO, 2001), and this is the most commonly diagnosed cause of anaemia in the perioperative period. Discoveries in the pathways of iron metabolism have found that chronic disease, and particularly inflammation, can cause a state of functional iron deficiency leading to anaemia. Patients with cirrhosis are frequently anaemic due to direct blood loss from the gastrointestinal tract and from the consequences of chronic inflammation upon iron homeostasis and bone marrow suppression. Anaemia is caused directly by viruses and by medications including antiviral drugs, the direct effects of alcohol and due to malnutrition.

1.8 Blood transfusion

Whilst alterations in CO and oxygen extraction can compensate for anaemia in the presence of isovolaemia, treating anaemia with transfused RBCs to increase the concentration of Hb aims to increase DO_2. 40-80% of RBC transfusions in critical care are for low Hb or for alterations in tissue perfusion and is an independent predictor of worse clinical outcome (Corwin et al., 2004). However, there is considerable scientific debate as to which thresholds are optimal and when to transfuse, depending upon individual patient comorbidities, and equipoise about the evidence upon when transfusion decisions are based.

There is an increasing body of evidence demonstrating restrictive transfusion thresholds to be non-inferior to liberal transfusion thresholds. The landmark TRICC study (Transfusion Requirements in Critical Care) by Hebert et al, published in 1999, showed that a restrictive strategy (maintaining a Hb concentration in the range 70 to 90 g/L, transfusing RBCs if the Hb fell below 70 g/L) was at least as effective than a liberal strategy (maintaining Hb concentration 100 to 120 g/L, transfusing RBCs if the Hb fell below 100 g/L) in critically ill patients, (patients with acute myocardial infarction and unstable angina were excluded from this study) (Hébert et al., 1999).

In the two decades since that work, further studies have supported these findings and clinical practice has moved inexorably towards a progressively more restrictive transfusion strategy. In post-operative patients after hip fracture surgery Carson et al investigated a restrictive threshold of <80 g/L compared to a liberal threshold of 100 g/L in patients with a history or risk factors for cardiovascular disease. The liberal transfusion strategy did not reduce mortality rates or functional capacity in these patients (Carson et al., 2011). In a long-term follow up of this study cohort, mortality did not differ significantly between the restrictive and liberal transfusion groups (Carson et al., 2015).

A Cochrane review of over 6000 patients were reviewed, concluded that restrictive transfusion thresholds (with an average postoperative haemoglobin concentration 14.8 g/L (95% CI −1.92 to −1.03) lower than in the liberal transfusion groups) should be supported for most patients, including those with pre-existing cardiovascular

disease. Indeed, restrictive transfusion strategies were associated with a statistically significant reduction in hospital mortality (RR 0.77, 95% CI 0.62 to 0.95); although not 30-day mortality (RR 0.85, 95% CI 0.70 to 1.03). Similar findings were made in upper gastrointestinal bleeding where a restrictive transfusion strategy increased six-week survival probability (95% vs. 91%; hazard ratio for death with restrictive transfusion 0.55 (95% CI 0.33-0.92, p=0.02) (Villanueva et al., 2013). In cardiac surgery however, the TITRe-2 trial demonstrated uncertainty of restrictive strategies in the unstable cardiac patient, reporting a beneficial impact of liberal transfusion to an Hb > 90g/L on mortality following cardiac surgery. (4.2% vs. 2.6% in the restrictive vs. liberal group; hazard ratio, 1.64; 95% CI, 1.00 to 2.67; P=0.045) (Murphy et al., 2015). Interestingly a similar study in critically ill patients with septic shock, a restrictive transfusion threshold of 7 g/dL compared to 9 g/dL had similar numbers of ischaemic events, severe adverse events and required life support. The relative risk of death in the lower threshold group was 0.94; 95% CI 0.78-1.09 (p=0.44) (Holst et al., 2014). Subsequently another trial of liberal versus restrictive transfusion in cardiac surgery showed that a restrictive strategy was non-inferior to a liberal strategy for all-cause mortality and major adverse cardiac events (OR of death 0.85 (95% CI 0.62 – 1.16)) (Mazer et al., 2017).

Many studies have reported global oxygenation indices ($ScvO_2$, SvO_2, $DO2\text{-}VO_2$) to assess tissue response to RBC transfusion. In a systematic review of blood transfusion in critically ill patients by Nielsen et al, seven of the nine studies included did not improve global oxygenation indices(Nielsen et al., 2017). Adamczyk et al showed only an improvement in $ScvO_2$ in patients with a low pre-transfusion $ScvO_2$ of <70% (Adamczyk et al., 2009). Walsh et al failed to show no improvement in gastric tonometry indices (pH 7.19 to 7.12 (p=NS)) with blood transfusion irrespective of age of RBCs when comparing new vs older stored RBCs in mechanically ventilated patients with organ failure(Walsh et al., 2004) .

In sepsis, Sakr et al investigated the effect of transfusion in patients with severe sepsis using OPS imaging. They demonstrated improved oxygen delivery but no increase in oxygen utilisation (DO_2 increased from 349 mL/min/m2 to 391 (P<0.01), but showed considerable inter-individual microcirculatory response to transfusion;

The improvements were best in those with altered capillary perfusion at baseline (Sakr et al., 2007)

Sadaka et al evaluated the effect transfusion of 1-2 units RBCs in patients with severe sepsis using NIRS and SDF imaging. Sublingual microcirculation was globally unaltered, as was muscle oxygenation and consumption and microvascular reactivity overall. Again, there was considerable variation in individual response and muscle oxygen consumption improved in patients with a low baseline and deteriorated in those with preserved baseline (Sadaka et al., 2011).

Donati et al randomised 20 patients with sepsis to receive non-leucodepleted or leucodepleted RBCs and analysed the response using SDF sublingual imaging and NIRS pre-and post-transfusion. The proportion of perfused vessels was higher in the leucodepleted group and the MFI was significantly different after transfusion with StO_2 upslope increased in both groups and StO_2 downslope increased in the non-leucodepleted group only. They concluded that they could not show a clear benefit of leucodepleted vs non-leucodepleted blood, but there was a suggestion of benefit in leucodepleted blood (Donati et al., 2014).

NIRS has been widespread to assess tissue oxygenation in response to blood transfusion in critically ill patients. Kiraly et al monitored the effect of RBCs upon thenar StO_2 using NIRS in adult trauma patients and demonstrated no improvement in NIRS indices (Kiraly et al., 2009).

Weinberg et al prospectively evaluated the microvascular response to transfusion of 1unit RBC in stable, adult trauma ICU patients using SDF. They demonstrated a variable response to RBCs by measuring % change in proportion of perfused capillaries (PPC), and found that it this change correlated inversely with pre-transfusion PPC, with those with altered pre-transfusion PPC showing the greatest improvement after transfusion (Weinberg et al., 2012).

Cretuer et al investigated the impact of transfusion of 1 unit RBC on global oxygenation indices and NIRS. They too showed considerable inter-individual

variation, where transfusion improved some indices in those with low baseline indices (Creteur et al., 2009).

PRBCs provide a colloid solution that can increases the CO and thus DO_2 indirectly. Conversely, PRBCs can increase the viscosity of the blood, decreasing the cardiac output (Roberson and Bennett-Guerrero, 2012). However it has been shown that RBC transfusions or plasma expanders can maintain FCD and improve DO_2 by increasing the viscosity of the plasma by physical effects not reliant on the oxygen carrying ability (Tsai et al., 2010).

In addition to the influence of native RBCs upon the microcirculation, transfused RBCs require additional comment due to the effect of storage.

1.8.1.1 Effect of storage upon red blood cells

RBC transfusions are associated risks and hazards including mortality, as well as functional defects of the RBCs themselves. Stored, packed RBCs develop a 'storage lesion'. This 'storage lesion' includes altered rheology, reduced deformability, haemolysis, reduced in-vivo recovery, energy and membrane loss, altered oxygen release, reduced ATP (adenosine triphosphate) and NO secretion and the shedding of toxic products (Hess, 2014). The disc shape often changes and micro-vesicular blebs occur, along with the formation on echinocytic spines (bleb cells) (Hess, 2014).

Damage to stored RBCs can occur due to the accumulation of waste products, enzymatic and oxidative injury, and by metabolically programmed apoptosis (or eryptosis). Cells consume glucose and accumulate lactic acid, lose potassium, gain calcium and lose Hb bound NO, whilst concentrations of ATP and 2,3-diphosphoglycerate (2,3-DPG) decrease. These changes to ATP and 2,3-DPG are reversible once stored RBCs are transfused (Raat et al., 2005). RBC ATP levels drop to 60% after 5 weeks of storage but are restored within hours post-transfusion. Until ATP levels are below 50% of normal, RBC survival appears unaffected – requiring storage beyond the current maximum of 42 days.

This has led to interest in the age of stored RBCs, with studies associating older stored RBCs with increased mortality when compared to newer stored RBCs. The

RECESS trial randomised patients undergoing complex cardiac surgery to leucocyte reduce red cells stored for 10 days or less or to those stored for 21 days or more for perioperative transfusions. There was no significant change in multiple organ dysfunction score (MODS) between the two groups. Whilst 7-day mortality was 2.8% in the shorter-term storage group vs 2.0% in the longer-term group, this difference was not significant (P=0.43). 28-day mortality was 4.4% vs 5.3% for shorter-term storage vs longer-term storage RBCs (p=0.57) (Steiner et al., 2015). Fergusson randomised neonatal intensive care patients to RBCs stored 7 days or less (mean age 5.1 days (SD 2.0) or to standard issue RBCs (mean age 14.6 days (SD 8.3). A primary outcome of major neonatal morbidities and death was not significantly different (52.7% primary event rate vs 52.9% (RR 1.00 (95% CI 0.82 -1.121))) (Fergusson et al., 2012).

A systematic review of 409,966 patients found that older blood was associated with a significantly increased risk of death (odds ratio 1.16 (05% CI 1.07 – 1.24) (Wang et al., 2012). However, a recent randomised controlled trial of fresh (less than 8 days storage (mean 6.1 +/1 4.9 days) versus standard issue red cells (mean (22+/- 8.4 days)) in critically ill patients showed no significant difference in the hazard ratio for death between the groups (hazard ratio 1.1 (96% CI 0.9 – 1.2 (p=0.38))) (Lacroix et al., 2015).

Koch et al found a significant difference in espies or septicaemia rates in patients who had undergone cardiac surgery randomised to newer (mean 11 days) or older blood (mean 20 days) was significantly higher in older blood (4.0% vs 2.8% (p=0.01)) (Koch et al., 2008a). Meanwhile, da Cunha et al showed no difference in sepsis rates in premature infants transfused new or older blood (Fernandes da Cunha et al., 2005).

1.9 Bleeding and transfusion management during liver transplantation

A major challenge of the transplant operation has been the significant levels of bleeding during the operation due to portal hypertension, surgical complexity with multiple vascular anastomoses and due to existing coagulopathy and post-reperfusion coagulopathy. In the 1980s when the OLT had become an established operation with

improving survival rates, mean packed red blood cell (PRBC) transfusion rates were between 20 – 40 units per operation (Lewis et al., 1987). Transfusion rates have decreased alongside improvements in both surgical and anaesthetic management of patients undergoing OLT, with a median transfusion rate of 5 units RBCs in one multicentre study (Ozier et al., 2003). Increasing numbers of transplants are performed without any intraoperative blood transfusions (Massicotte et al., 2012).

It is well known that coagulopathy and catastrophic bleeding can accompany liver transplantation. There is wide variation in the approach to the management of coagulation and blood transfusion for liver transplantation between centres (Lopez-Plaza, 2007). Strategies to manage the coagulopathy and bleeding have been designed to reduce the rates of blood transfusion during these operations (Boin et al., 2008). These include pharmacological and non-pharmacological methods, as well as coagulation monitoring and treatment protocols.

1.9.1 Blood transfusion during liver transplantation

It is increasingly recognised that there is an association between increased mortality and blood transfusions during OLT surgery (Rana et al., 2013, Xia et al., 2006). There is a cumulative risk demonstrated between amount of blood transfused and increased mortality (Schroeder et al., 1999). In a cohort of 433 patients undergoing transplantation it was shown that any transfused blood was associated with negative outcomes after liver transplantation (de Boer et al., 2008). In another cohort study of 232 patients undergoing liver transplantation, survival was significantly lower in those patients who received more than six units of red blood cells compared to those who received less than six. 12-month survival (≥6 RBC vs. <6 RBCs) was 53.9% vs. 76.3% respectively. This survival advantage extended to five years with survival of 34.5% vs. 49.2% (Boin et al., 2008).

Experience from transplantation in Jehovah's Witness patients provided evidence for the practicalities of transfusion free transplantation, whilst providing strategies for minimising the risk of bleeding and transfusion (Jabbour et al., 2005, Darwish, 2011) In a study of 500 liver transplantations at a single centre, 79.6% of patients did not receive any blood transfusion during their operation (Massicotte et al., 2012). In that

series, bleeding did not correlate with the severity of patients' disease or with baseline coagulation status. The strongest correlation between transplantation without blood transfusion was with the starting haemoglobin (Hb) concentration (Massicotte et al., 2012).

Various factors that predict transfusion have been identified. The severity of the patient's liver disease has frequently been associated with increased risk of blood transfusion (Rana et al., 2013, Mangus et al., 2007). Blood urea and platelet count have been shown to be independently associated with transfusion (Deakin et al., 1993). In many studies, haemoglobin concentration has been shown to predict transfusion requirements (Sabate et al., 2012, McCluskey et al., 2006, Araujo et al., 2010, Wang et al., 2010, Massicotte et al., 2009). This has not been confirmed by all studies (Roullet et al., 2011).

Models such as the McCluskey Risk Index for the Prediction of Massive Blood Transfusion include: age >40 years, haemoglobin concentration <100 g/L, International normalised ratio (INR) >2.0, platelet count <70 $x10^9$, creatinine (>100 μmol/L for females, >120 μmol/L for males) and albumin <24 g/L; as well as repeat transplantation (McCluskey et al., 2006)

1.10 Summary of Introduction and Thesis Plan

Liver disease and cirrhosis is a common cause of disability and mortality, with high prevalence worldwide, and is a growing public health problem within Western society. Liver transplantation remains the definitive treatment for end stage liver disease and the liver transplant waiting list continues to grow.

Cirrhosis is associated with a chronic inflammatory state that leads to profound alteration of both the hepatic vasculature and the extra-hepatic circulation, with multi-organ effects. I will further discuss the circulatory and microcirculatory changes associated with cirrhosis throughout the body. Methods of monitoring the microcirculatory changes, including direct videomicroscopy now exist to non-invasively quantify the microcirculatory changes in cirrhosis. These techniques have also been applied in other high risk surgical populations to examine the impact of

surgery, blood transfusion and other factors such as cardio-pulmonary bypass. This thesis aims to investigate the microcirculation in liver transplantation, and the effects of blood transfusion during liver transplantation upon measures of microcirculatory flow and vascular reactivity.

Liver transplantation remains an operation with high rates of major bleeding and blood transfusion due to technically challenging surgery involving major blood vessels, and the complex patients suffering with the effects of portal hypertension and splanchnic vasodilatation, coagulopathy and fibrinolysis during the surgery. Patients with cirrhosis are frequently anaemic for multifactorial reasons. I aim to investigate the prevalence of anaemia in patients undergoing liver transplantation, and the associations with blood transfusion and outcome.

The haemodynamic goals during surgery are to maintain adequate perfusion and oxygen delivery to the respiring tissues. These are determined by the physiology of the vasculature, the circulating elements of the blood contained within, and the interaction between these components. Traditionally systemic measures of the circulation and surrogate measures of perfusion such as mixed venous oxygenation and lactate levels have been used to guide intraoperative haemodynamic management. There is little data however, regarding the effect of liver transplantation upon the microcirculation. Microcirculatory monitoring can also be used to assess the reactivity of the vasculature during liver transplantation and in response to blood transfusion, using dynamic tests of tissue oxygenation. The effect of blood transfusion upon the microcirculation has been studied in a variety of settings including critically ill patients and during surgery. In critically ill patients, significant inter-individual response to transfusion has been demonstrated. Meanwhile, there is a growing body of evidence for the harms of blood transfusion in patients undergoing surgery, including liver transplantation.

I aim to investigate the effect of intraoperative blood transfusion during liver transplantation upon microcirculatory flow using incident dark field videomicroscopy and on vascular reactivity measured using near-infrared spectroscopy. I aim to describe the changes in microcirculatory flow during the operation in the two main surgical modes – caval-cross clamp or piggy-back

hepatectomy and transplant - in patients with end stage liver disease and correlate these to routine haemodynamic parameters. I hypothesise that microvascular flow would reduce along with blood loss intraoperatively due to the de-recruitment of capillary networks, reducing the microcirculatory blood vessel density and that blood transfusion would increase the total vessel density of the sublingual microcirculation. I hypothesise that increasing anaemia with surgical blood loss would lead to a reduction in tissue oxygenation and an increase in tissue oxygen consumption, and that improved measures of microcirculatory perfusion would be reflected by improved tissue re-oxygenation.

Chapter 2 Liver disease and liver transplantation

2.1 Introduction

In this chapter I shall explore the literature relating to the pathophysiological processes of cirrhosis, focusing upon the effects upon the vasculature and microcirculation. I will discuss the changes to the hepatic circulation and microcirculation that drive the systemic and splanchnic circulatory changes as well. I will examine the literature related to microcirculatory monitoring of cirrhotic patients. I will discuss the haematological consequences of liver disease including anaemia and coagulopathy of liver disease before discussing the haemodynamic consequences of liver transplantation and the methods currently utilised to monitor the circulation intraoperatively.

2.2 Circulatory complications of cirrhosis

Cirrhosis is typically associated with a hyperdynamic circulation secondary to vasodilatation. characterised by a bounding pulse, low systemic blood pressure and palmar erythema (Moller and Henriksen, 2008). This hyperdynamic circulation comprises increased heart rate, increased cardiac output and plasma volume, with reduced systemic vascular resistance and arterial blood pressure.

Cardiac output is increased by a combination of increased venous return, increased heart rate and myocardial contractility under the control of the autonomic nervous system. As cirrhosis progresses, vasodilatation (with low systemic vascular resistance), the presence of arteriovenous communication, expanded blood volume and increased sympathetic nervous stem activity can increase the cardiac output further.

The central blood volume – comprising the heart, lungs and central arterial tree – is usually decreased, whilst the non-central blood volume, particularly the splanchnic blood volume, is increased. Total vascular compliance and arterial compliance (whereby an increased intravascular volume relative to the increased in transmural blood pressure) are increased in cirrhosis relative to the degree of decompensation.

The pathophysiological components of the hyperdynamic circulation and cardiovascular dysfunction in cirrhosis are widespread. They include peripheral and splanchnic arterial vasodilatation with a baroreceptor induced increase in heart rate; autonomic dysfunction: increased sympathetic nervous activity and vagal impairment; alterations in cardiac preload: increased portosystemic shunting, an increased blood volume and decreased blood viscosity. Alterations in oxygen exchange occur as a consequence of anaemia, hypoxaemia, the hepatopulmonary syndrome and portopulmonary hypertension (Moller and Henriksen, 2008).

Arterial compliance depends on the properties of the elastic and smooth muscle of the arterial wall and represents an important coupling between the heart and arterial system for the alterations in intravascular volume. Arterial compliance expresses the stoke volume relative to the pulse pressure and is directly related to the severity of cirrhosis. The changes in arterial mechanics are largely reversible. Arterial compliance is increased in advanced cirrhosis. It is related to age, body size, sex, arterial blood pressure, severity of cirrhosis and abnormal volume distribution.

Pulmonary vascular resistance is often decreased in cirrhosis – except in those patients with portopulmonary hypertension. Portopulmonary hypertension is characterised by pulmonary vasoconstriction, most likely due to endothelial dysfunction in the pulmonary circulation, particularly mediated by endothelin-1 (ET-1), a potent vasoconstrictor (Benjaminov et al., 2003).

The hepatopulmonary syndrome is the result of impaired ventilatory lung function and diffusion, combined with vascular abnormalities, reduced transfer factor and low arterial oxygen saturation. In cirrhosis, the reduced transfer factor correlated with low pulmonary blood volume – suggesting that central under filling plays a role in the impairment of pulmonary function.

The hyperdynamic circulation is mainly caused by circulatory alterations in the splanchnic area. Therefore, arteriolar vasodilatation would be a more localised event, whereas the elevation in arterial compliance may be more systemic.

Arteriolar vasodilation in cirrhosis in cirrhosis and portal hypertension can be caused by: overproduction of circulating vasodilators, vasodilators of intestinal or systemic origin, vasodilators that fail to be degraded by the failing liver or bypass the liver through porto-systemic collaterals, reduced resistance to vasoconstrictors or increased sensitivity to vasodilators.

Splanchnic arteriolar vasodilation leads to reduction of the systemic vascular resistance, central arterial under filling with relative hypovolaemia, activation of the vasoconstrictor systems including sympathetic nervous system and renin-angiotensin-aldosterone system (RAAS), and increased production vasoconstrictors vasopressin, endothelins and neuropeptide Y – resulting in a hyperdynamic circulatory state (Moller and Henriksen, 2008).

The splanchnic vasodilatation precedes the increase in cardiac output and heart rate. It has been shown that mild increases in portal pressure upregulate nitric oxide synthase (eNOS) (Abraldes et al., 2006).

As the disease progresses, splanchnic vasodilatation becomes more pronounced and the hyperdynamic circulation may not be sufficient to correct the effective hypovolaemia. The arterial blood pressure is mainly maintained by vasoconstriction in the renal, cerebral and hepatic vascular beds where there is diminished release of nitric oxide (NO) from endothelial cells (Wiest and Groszmann, 2002). Systemic NO production is increased and precedes the development of hyperdynamic circulation in cirrhosis, playing a major role in arteriolar and splanchnic vasodilatation and vascular hyporeactivity.

Vascular endothelial growth factor (VEGF) appears to stimulate angiogenesis and the development of portosystemic collaterals, and the blockage of VEGF receptor-2 has been shown to inhibit the processes and revert portal hypertension and the hyperdynamic circulation experimentally (Abraldes et al., 2006). Circulating vasodilators, including VEGF and adrenomedullin, are increased, whilst there is an inadequate haemodynamic response to vasoconstrictors, leading to vasodilation and vascular hyporeactivity, combined with a hyperdynamic circulation in cirrhosis.

As previously discussed, there is an imbalance between vasodilatory and vasoconstrictive forces in cirrhosis, which leads to an abnormal distribution of volume, abnormal vascular resistance and flow. The increased cardiac output represents substantial vasodilatation, but there is heterogeneous perfusion of different vascular beds. In the kidney, vasoconstriction is predominant, particularly due to RAAS and sympathetic nervous system activation.

The increased plasma volume is secondary to neurohumeral mechanism, low arterial blood pressure and reduced central and aerial blood volume.

Central hypovolaemia and arterial hypotension can be treated by intravenous fluids to expand the plasma volume. However, during volume expansion, most cirrhotic patients respond with a further reduction in systemic vascular resistance. Using hyperosmotic fluids or albumin, results in an initial shift of fluid from the interstitial space in to the plasma, increasing the plasma volume. This increases the stroke volume and cardiac output. It has been shown that in late cirrhosis, expansion is mainly confined to the non-central part, with a proportionally smaller increase in cardiac output, due to abnormal vascular compliance and cardiac dysfunction.

2.3 Hepatic circulation in cirrhosis

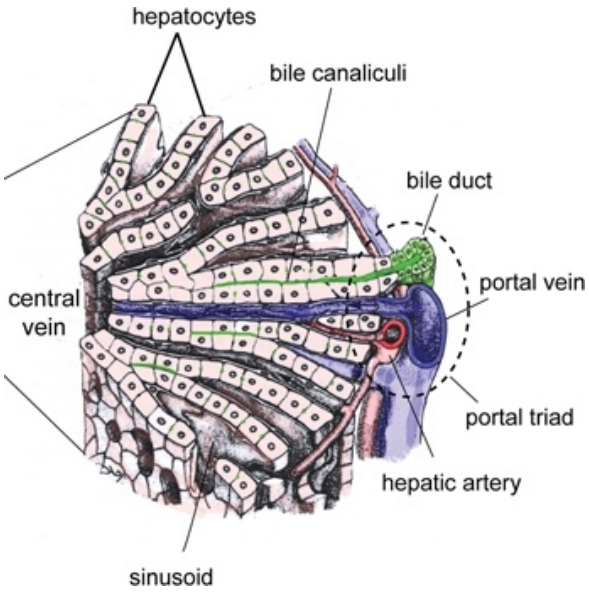
Hepatic vascular resistance and portal inflow dictate the portal pressure. Hepatic vascular resistance is dependent upon both structural and dynamic components. Structural components are cirrhotic steatosis, fibrosis and regeneration nodules. The dynamic structures are those cells with contractile properties including hepatic stellate cells, myofibroblasts and smooth muscle cells. Portal venous inflow is determined by splanchnic vasodilatation. In health, hepatic blood flow equals the splanchnic flow. In portal hypertension, the development of portosystemic collateral vessels, have an increased mesenteric in flow. Thus, a large part of the increased cardiac output is returned through portosystemic collaterals.

2.3.1 Hepatic microcirculation

As already described, the microcirculatory alterations in cirrhosis are widespread, and different organ microcirculatory vessels have distinct alterations in flow. The

hepatic microcirculation is unique due to the dual blood supply of the liver. The hepatic sinusoids correspond to the capillary bed of the liver and are the location of nutrient transport and waste removal. (Figure 1)

Figure 1: Architecture of the liver lobule



https://commons.wikimedia.org/wiki/File:Cellular_architecture_of_the_liver..jpg

The hepatic arterioles are wound around the portal venules and it has been demonstrated that communication occurs between the vessels. If portal venous blood flow is reduced, there is shunting via hepatic arterio-portal anastomoses to maintain microvascular perfusion and oxygen delivery (Vollmar and Menger, 2009). The sinusoids run between the liver cells. They are lined with specialised endothelial cells characterised by flattened processes perforated with small fenestrae and have no basement membrane. Hepatic stellate cells are located externally to the endothelium in the Space of Disse – a space between the hepatocytes and endothelium. They act as a contractile machinery, regulating vessel calibre and therefore, blood flow. Blood flows from the sinusoid into the central interlobular veins, which combine and organise to become sublobular veins which unite to form hepatic veins, which drain to the inferior vena cava (Mitra, 2012).

Sinusoidal pressure is raised in cirrhosis due to mechanical factors related to structural changes within the microcirculation and but dynamic factors including

endothelial dysfunction, reduced NO production, increased release of vasoconstrictors and contraction of hepatic stellate cells (Davies et al., 2017).

Vascular structural changes include angiogenesis and pathological sinusoidal remodelling. The sinusoidal endothelial cells lose their characteristic fenestrations and an organised basement membrane is formed, termed sinusoidal capillarisation. This impairs oxygen diffusion from the sinusoidal vessels to the liver parenchyma. Hepatic stellate cells migrate and promote coverage of sinusoidal vessels, leading to vasoconstriction, which contributes to the high vascular resistance. Collagen is deposited into the space of Disse, restricting microvascular flow.

As a result of these changes, the liver tissue becomes hypoxic. This leads to several consequences, including angiogenesis, migration of stellate cells and inflammation. Angiogenesis promotes new vessel formation and shunting between pre- and post sinusoidal vessels.

Hepatic endothelial dysfunction becomes apparent in cirrhosis. Sinusoidal endothelial cells normally release vasoactive substances to maintain sufficient blood flow. Endothelial dysfunction impairs endothelium-dependent relaxation in the liver microcirculation, increasing hepatic vascular resistance. Endothelial dysfunction leading to increased vascular resistance is driven by impaired production of nitric oxide (NO) by the endothelial cells and reduced vasodilatory response to NO. However, in the splanchnic circulation, there is an increase in vasodilator molecules, particularly NO, contributing to increased endothelium-dependent relaxation. These changes lead to portal hypertension and systemic vasodilatation in arterial and splanchnic circulations in cirrhosis (Iwakiri and Groszmann, 2007). The decrease in endothelial NO synthase (eNOS) activity is the key driver for the reduction in bioavailability of NO. This is the result of oxidative stress, regulatory defects and increased levels of eNOS inactivators.

2.4 Splanchnic microcirculation

As intrahepatic vascular resistance rises, portal pressure is raised and hepatic perfusion decreases. To maintain hepatic blood flow, splanchnic vasodilatation

increases the blood inflow, further increasing the portal pressure. The combination of splanchnic vasodilatation and portal hypertension leads to the development of a collateral circulation and porto-systemic shunting. NO is central to splanchnic vasodilatation. NO bioavailability is increased in the splanchnic microcirculation because of increased activity of eNOS in cirrhosis and portal hypertension. The upregulation of eNOS is proposed to be caused by an increase of vascular stretch within the splanchnic microcirculation due to portal hypertension (Abraldes et al., 2006). Portal hypertension also increases expression of (vascular endothelial growth factor) VEGF which promotes angiogenesis, promoting the development of porto-systemic collaterals, as well as acting as an activator of eNOS. Pro-inflammatory cytokines including tumour necrosis factor alpha (TNF-α) upregulate eNOS, whilst vasoactive mediators including prostaglandin-I2 (PGI2), carbon monoxide and endothelium-derived hyperpolarising factors (EHF) contribute to splanchnic vasodilatation (Davies et al., 2017).

Arterial blood pressure – dependent upon cardiac output and systemic vascular resistance – is low to normal in cirrhotic patients to compromise between the vasodilation and vasoconstrictive drivers affecting the vascular resistance and arterial compliance.

2.5 Microcirculatory monitoring in cirrhosis

It has been demonstrated that patients with cirrhosis who recover from bacterial infections have a worse prognosis when compared to patients with a similar disease severity without a history of infection. This may be similar to the persistence of microcirculatory alterations in sepsis after the restoration of systemic haemodynamics (Sakr et al., 2004, Dionigi et al., 2017).

Meanwhile, treatment of complications related to cirrhosis involve the use of vasoconstrictive medications, including terlipressin and noradrenaline for hepatorenal syndrome. Terlipressin is the most widely utilised vasopressor, acting to reduce portal inflow and reduce portal pressure, and to reduce splanchnic vasodilatation, with the aim of raising systemic arterial blood pressure and thus improving renal perfusion pressure (Wong, 2012). Meta-analysis shows terlipressin

to reverse hepatorenal syndrome in 42% of patients vs 26.2% of controls, without superiority compared to noradrenaline with adverse events – most frequently abdominal cramps, arrhythmias and cyanosis of the toes - in 25.4% (vs 10.6% in noradrenaline groups) (Wang et al., 2018). It is therefore likely that a direct effect upon the microcirculation is involved which would benefit from techniques of microcirculatory monitoring that have been utilised in sepsis and other critically ill patients.

Investigations into the vascular alterations in cirrhosis within different organs have been undertaken. Cerebral blood flow decreases in patients with liver cirrhosis(Kudo, 2001). These include the use of transcranial Doppler to monitor cerebral haemodynamics, which demonstrated significantly higher cerebral pulsatility and resistive indices in cirrhosis, which were related to the severity of disease (Kawakami et al., 2001). The cerebral vascular bed is thus affected by tissue hypoperfusion and organ dysfunction, contributing to the development of hepatic encephalopathy. This may be related to microvascular dysregulation and structural deterioration due to high levels of inflammatory cytokines and neutrophil dysfunction, alongside the activation of the RAAS and sympathetic nervous system (Macias-Rodriguez et al., 2015).

Sorensen et al retrospectively measured cerebral NIRS during liver transplantation along with end tidal CO_2 tension and minute ventilation. Cerebral oxygenation decreased during the anhepatic phase and increased during reperfusion, which were directly related to end-tidal CO_2 (Sorensen et al., 2014).

The microcirculation of the liver itself has been studied using a variety of techniques including NIRS applied directly to the organ (El-Desoky et al., 2000, Kitai et al., 1993, Fan et al., 1999). Whilst the critical care and sepsis research arena has moved beyond simple measures of haemodynamics to tissue oxygen delivery and perfusion, this has not yet happened in liver transplantation.

SDF imaging of the hepatic microcirculation has been utilised during liver transplant. Pulitano et al assessed the microcirculation of the liver using SDF probes upon the posterior surface of the liver to correlate these findings with hepatic blood flow,

haemodynamic parameters, and soluble mediators. Deterioration of the hepatic microcirculation after ischemia/reperfusion was associated with an increased risk of early graft dysfunction (Pulitano et al., 2017).They found that post reperfusion microvascular parameters of the liver did not correlated with central venous pressure or mean arterial pressure, donor age, recipient stay in intensive care or duration of anhepatic period (Pulitano et al., 2017).

As of yet, the systemic microcirculation during the liver transplant has not been assessed using videomicroscopy technology. Sublingual mucosa is an ideal area since it is well vascularised with capillaries and shares a common embryological origin to the splanchnic blood flow, making it an acceptable surrogate.

Ballerga et al investigated the sublingual microcirculation using SDF imaging in stable cirrhotic patients without active bleeding or infection. They demonstrated lower PVD and TVD, PPV and MFI for small vessels in cirrhotic patients compared to normal controls. An increase in heterogeneity was also demonstrated (Gonzalez Ballerga et al., 2018). They demonstrated that vascular remodelling also involved the sublingual mucosa and declared that sublingual mucosa might be a suitable non-invasive window for monitoring systemic microcirculatory alterations in cirrhotic patients. In Sheikh et al's study when comparing the MFI of compensated cirrhosis MFI was significantly reduced between compensated (MFI 2.40 +/- 0.46 (95% CI 2.07 – 2.72) vs decompensated without infection/sepsis (MFI 1.40 +/- 0.52 (95% CI 1.03 – 1.77) ($P<0.001$) and with infection/sepsis (MFI 0.80 +/- 0.48 (95% CI 0.45 – 1.15) (($p<0.001$) (Sheikh et al., 2009).

2.6 Cardiac dysfunction in cirrhosis

The cardiac output is persistently increased in cirrhosis due to blood volume expansion, eventually overloading the heart. Cardiac failure is often mitigated by the decreased afterload secondary to the reduced systemic vascular resistance and increased arterial compliance. However, this latent left ventricular failure can become apparent under strain or due to vasoconstriction. This has been termed cirrhotic cardiomyopathy. The diagnostic criteria are chronic cardiac dysfunction in cirrhotic patients characterised by impaired contractile responsiveness to stress

and/or altered diastolic relaxation with electrophysiological in the absence of other known cardiac disease. Diagnostic criteria for systolic dysfunction are: blunted increased in cardiac output on exercise, volume challenge or pharmacological stimuli; with a resting ejection fraction <55%. Supportive criteria for the diagnosis include: electrophysiological abnormalities, abnormal chronotropic response, electromechanical uncoupling/dys-synchrony, prolonged Q-Tc interval, enlarged left atrium, increased myocardial mass, increased BNP and pro=NBNP and increased troponin I.

After exercise, LVEF increases less in cirrhotic patients that in control. This may be due to blunted heart rate response to exercise, reduced myocardial reserve and skeletal muscle wasting with impaired oxygen extraction.

Diastolic dysfunction is common in cirrhosis, affecting left ventricular filling. The left ventricle becomes stiffer and less compliant due to cardiac hypertrophy, patchy fibrosis and subendothelial oedema. Diastolic dysfunction is diagnosed by: E/A ration <1.0 (age corrected), prolonged deceleration time (>200 ms) and prolonged isovolumetric relaxation time (>80 ms).

No specific treatment is recommended for cirrhotic cardiomyopathy, and cardiac failure should be treated as it would be in non-cirrhotic patients with sodium restriction and diuretics. Care should be taken with ACE inhibitors and angiotensin antagonists since severe hypotension may be provoked. B-blockers have been shown to reduced acutely the prolonged QT interval and may have cardio protective effects, although effects on morbidity and mortality remain to be demonstrated. Liver transplantation has been shown to reverse cardiac changes included diastolic dysfunction.

2.7 Haematological consequences of liver disease

2.7.1 Anaemia in chronic liver disease

Anaemia is common in chronic liver disease, occurring in approximately 75% of patients (Gonzalez-Casas et al., 2009). It is multifactorial in its aetiology, including: nutritional deficiencies, hypersplenism, haemodilution, haemolysis, bone marrow

suppression due to viruses or ethanol, renal insufficiency and variceal bleeding (Thachil, 2011). Malnutrition commonly occurs due to the reduced intake, absorption, processing and storage of nutrients (Saunders et al., 2010). As well as protein-calorie malnutrition, iron, vitamin B12 and folate deficiency are common, all of which contribute to the development of anaemia.

The key iron regulatory protein hepcidin, is produced in the liver and regulated by iron stores and erythropoietic activity. Hepcidin regulates intestinal iron absorption, plasma iron concentrations and tissue iron distribution by inducing the degradation of its receptor, ferroportin, the cellular iron exporter (Ganz and Nemeth, 2012). Ferroportin exports dietary iron from the duodenum through enterocytes and from iron stores within macrophages and hepatocytes, into the plasma. Hepcidin is also regulated by the iron requirements of erythroid precursors for haemoglobin synthesis. However, hepcidin is also upregulated by inflammation caused by infection, malignancy or autoimmune disease. This inhibits iron absorption from the duodenum and sequesters iron within macrophages.

Inflammatory mediators also upregulate the expression of transferrin receptors, increasing the uptake of transferrin bound iron into the macrophage. Transferrin, the protein that binds and transports iron, can be functionally blocked from carrying out its role due to increased levels of hepcidin. Individual macrophages are stimulated by inflammatory mediators to take up ferrous iron (Fe^{2+}). Activated macrophages phagocytose ageing RBCs at the end of their lifespan (approximately 120 days) permitting the recycling of iron. Thus, a state of functional iron deficiency is created as a consequence of the sequestration of iron within the duodenum and macrophages in response to the effects of inflammatory mediators (Wessling-Resnick, 2010, Weiss and Goodnough, 2005). Tumour necrosis factor α (TNF-α) induces macrophages and directly damages red blood cell membranes, leading to early phagocytosis. Inflammatory mediators additionally reduce EPO production by the kidney, reducing erythropoiesis. The combination of these effects leads to anaemia.

2.8 Coagulopathy of liver disease

The coagulopathy of chronic liver disease is present pre-operatively and further disturbance of coagulation can occur intraoperatively, resulting in bleeding complications, but also thrombotic events.

In chronic liver disease, all procoagulant factors are decreased, except factor VIII and Von Willebrand factor (vWF), which increase. Levels of the endogenous anticoagulant factors, antithrombin (AT) and protein C fall. Levels of tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI-1) re-equilibrate (Sabate et al., 2012). This rebalancing of procoagulant and anticoagulant factors requires coagulation tests which demonstrate the net result of these haemostatic changes to accurately monitor the coagulation system in liver disease. Prothrombin time (PT) tests do not utilise thrombomodulin, a transmembrane protein on vascular endothelial cells that downregulates thrombin generation. This is the main physiological activator of protein C. By failing to measure the anticoagulant factors' effect on thrombin generation, and solely the effect of procoagulant factors, this balance of coagulation is inaccurately measured by PT, misrepresenting the risk of haemorrhage (Tripodi and Mannucci, 2011).

Thrombocytopaenia is common in liver disease and platelet function is affected in liver disease. This is multifactorial in aetiology, including hypersplenism secondary to portal hypertension, decreased thrombopoetin synthesis, immune complex associated platelet clearance and reticuloendothelial destruction (Giannini and Savarino, 2008).

Thus, primary haemostasis, coagulation and fibrinolysis are all altered by liver disease. Previously it was thought that the balance lay towards a bleeding tendency when assessed by conventional tests of coagulation. It is now understood that the low levels of pro-coagulant factors are to some extent "re-balanced" by the reduced levels of anticoagulant factors, so that thrombin generation remains normal or even enhanced, which can lead to a prothrombotic state in some patients (Gatt et al., 2010).

2.9 Liver transplantation

Liver transplantation is the therapeutic option for patients who develop decompensation or hepatocellular carcinoma with cirrhosis. Indications are cirrhosis with decompensation: with ascites as assessed with disease severity scores; (intractable pruritus, recurrent cholangitis and Hepatopulmonary syndrome are potential exceptions to listing based on prognostic scores). Hepatocellular carcinoma with background cirrhosis is usually scored using the Milan criteria – 1 lesion </= 5 cm or no more than 3 lesion s</= 3cm each with no macrovascular invasion and no extrahepatic disease. Refractory or difficult-to-control ascites can be an indication for assessment for liver transplantation.

2.9.1 Intraoperative phases

Liver transplant surgery is traditionally divided into 3 main phases: a dissection, an anhepatic and a reperfusion phase.

The initial phase of the operation is the dissection phase. Given that patients undergoing transplantation who have cirrhosis are vasodilated with low SVR, the effects of anaesthesia magnify this effect. After the initial abdominal incision, the liver is mobilised and the porta hepatis identified. The bile duct and hepatic artery are ligated, to allow for reconstruction and the portal vein divided. Ascites may be drained after the abdominal incision and patients may show signs of hypovolaemia at this point. If caval resection is not being undertaken the donor liver is mobilised entirely from the IVC. Caval cross clamping of the recipient infra- and supra-hepatic IVC is required. If a ‘piggy-back’ technique is utilised, partial caval clamping can be utilised, with an anastomosis made between the recipient and donor vena-cavae. The presence of abdominal adhesions following previous abdominal surgery increases the complexity of the dissection and the risk of bleeding is significantly increased (Steib et al., 2001, Feltracco et al., 2013).During the dissection phase of the transplant, excessive bleeding is significantly related to the degree of difficulty experienced during the surgical dissection and the presence of portal hypertension, which results in large, dilated collateral vessels prone to bleeding. Surgical bleeding thereby results

from coagulopathy, portal hypertension and oesophageal-gastric venous distension caused by compression and vascular clamping (Sabate et al., 2012).

The anhepatic phase begins with the occlusion of the vascular inflow to the liver. Cross-clamping of suprahepatic and infrahepatic IVC can decrease venous return by as much as 50% (Steadman, 2004). This can lead to a reduction in preload, reduced renal perfusion pressure, increased splanchnic congestion and metabolic acidosis. Traditionally veno-veno bypass was used routinely, however it is now seldom used. Alternatively, the piggy-back technique is employed.

The hepatectomy is then performed, followed by haemostasis and anastomoses created for supra- and infra-hepatic IVC and portal vein. During the anhepatic phase of the transplant there is reduced coagulation factor synthesis and clearance. Enhanced fibrinolytic activity occurs due mainly to lack of tPA clearance whilst levels of PAI-1 remain relatively unchanged, increasing the likelihood of fibrinolysis developing.

The portal vein anastomosis is then performed to allow reperfusion of the liver. Once this has been completed the hepatic artery anastomosis is performed followed by bile duct reconstruction. The donor liver will have been preserved with an intra-hepatic preservation solution which must be flushed to reduce the risk of hyperkalaemia and a reperfusion syndrome. Reperfusion is associated with rapid increases in potassium and hydrogen ion concentration, an increase in preload, and a decrease in systemic vascular resistance and blood pressure. Life threatening hyperkalaemia requires immediate treatment with calcium chloride, sodium bicarbonate and insulin. Vasopressors including noradrenaline, dopamine and adrenaline are frequently employed at reperfusion.

Post-reperfusion syndrome (PRS) is signified by systemic hypotension and pulmonary hypertension within the first 5 minutes of reperfusion (Steadman, 2004). Definitions of PRS vary, although a decrease in MAP of >30% for at least 1 minute relative to the end of the anhepatic phase, within the first 5 minutes of reperfusion is widely accepted, with an incidence of between 12-50% (Jeong, 2015). The underlying cause is not fully understood, and is multifactorial. Metabolic acidosis,

hyperkalaemia, hypocalcaemia, hypothermia, air embolism and the release of vasoactive substances are all involved. After unclamping of the portal vein, proinflammatory cytokines including TNF-α, IL-1, IL-2,and IL-8 are released into the systemic circulation from the liver graft (Jeong, 2015). In response to reperfusion of the graft, further proinflammatory cytokines are produced by the recipient, including kallikrein, bradykinin, chemokines and activated complement (Fiegel et al., 2012). The role of ischemia-reperfusion injury due to interruption to the oxygen supply during the ischaemic period, followed by reperfusion and subsequent responses remains controversial. The reperfusion injury produces proinflammatory cytokines and oxygen free radicals, and activates the complement system, leading to an inflammatory response leading to endothelial injury, vasoconstriction, and leukocyte sedimentation (Jeong, 2015). The severity of ischaemic-reperfusion injury, which creates a local inflammatory response that can progress to a systemic inflammatory response has not been correlated to the immediate haemodynamic responses of the PRS (Ramsay, 2008).

During reperfusion, profound coagulation abnormalities are common, due to the "heparin like effect" (Agarwal et al., 2008), platelet entrapment in the sinusoids of the donor liver, a global reduction of all coagulation factors, decreased PAI-1 and antifibrinolytic factors, with simultaneous generation of tPA. Fibrinolysis is normally carefully balanced by pro- and anti-fibrinolytic factors yet these are also disrupted in liver disease. During OLT some patients have accelerated release of t-PA from the donor graft endothelium once it is reperfused, leading to hyperfibrinolysis (Porte et al., 1989). Usually this hyperfibrinolysis resolves within an hour post reperfusion, but may persist if the graft is of marginal quality or functions poorly. The use of anti-fibrinolytic drugs can improve this state.

Post operatively, thrombocytopaenia due to consumption of platelets in the new liver is counteracted by their activation, leading to an increased risk of hypercoagulability and thrombosis (Agarwal et al., 2013). The most immediate and immediately life-threatening complication of liver transplantation is perioperative haemorrhage. Donor organ quality has a major impact on the immediate postoperative outcome. Post-operative coma following the cessation of sedation, a rising prothrombin time, acidosis, high insulin requirements, thrombocytopaenia or hyperkalaemia may be

symptoms of primary graft non-function, non-thrombotic graft infarction, or vascular thrombosis. This immediately requires imaging of the hepatic artery and portal vein. Sometimes a super-urgent re-transplantation is required in these situations.

2.9.2 Haemodynamic monitoring during liver transplant

Standard monitoring of the patient during liver transplant include MAP, CVP, and with the use of a pulmonary artery catheter, PAP and mixed venous oxygen saturation. SvO2 can allow the anaesthetist to monitor for signs of mismatch between oxygen demand and delivery. Transoesophageal echocardiography is increasingly employed to monitor for signs of haemodynamic compromise, including signs of right heart failure. Most cardiac output monitoring systems including waveform analysis (e.g. Flowtrac (Edwards Lifesciences, Irving, California), and bolus calibration systems such as PiCCO (Pulsion Medical System, Munich, Germany) and LiDCO (Lidco Cardiac Sensor System, London, UK) have not been universally validated for liver transplant surgery because of considerable variability during haemodynamic instability in liver transplantation (Feltracco et al., 2012).

Pulse pressure variation (PPV) analysis and stroke volume variation (SVV) has been investigated for use during liver transplantation. Pulse pressure changes proportionally to left ventricular stoke volume (Michard et al., 2000). During positive pressure ventilation, blood return to the right heart is decreased, thus a lower volume of blood passes through the pulmonary circulation, and the left ventricular end diastolic volume is reduced. This results in a lower stroke volume and a smaller pulse pressure after positive pressure ventilation; the magnitude of this difference is proportional to preload (Rudnick et al., 2015). It has been shown to predict fluid responsiveness more effectively than CVP in liver transplantation (Su et al., 2012), and to discriminate fluid responders from non-responders with a93% sensitivity and 94% specificity (Biais et al., 2009).

Chapter 3 Retrospective analysis of anaemia and transfusion in liver transplant

3.1 Introduction

A major challenge of the transplant operation has been the significant levels of bleeding during the operation due to portal hypertension, surgical complexity with multiple vascular anastomoses and due to existing coagulopathy and post-reperfusion coagulopathy. In the 1980s when the OLT was an established operation with improving survival rates, mean packed red blood cell (PRBC) transfusion rates were between 20 – 40 units per operation (Lewis et al., 1987). Transfusion rates have decreased alongside improvements in both surgical and anaesthetic management of patients undergoing OLT and increasing numbers of transplants are performed without any intraoperative blood transfusions (Massicotte et al., 2012).

Patients with liver disease are frequently anaemic due to acute blood loss, nutritional deficiencies including iron and folate, hypersplenism and haemolysis and bone marrow suppression by ethanol or viruses (Thachil, 2011). Anemia is implicated in worsening the bleeding tendency by increasing the hyper dynamic circulation and portal hypertension. In addition red blood cells stimulate thrombin generation and have an effect upon platelet function (Thachil, 2011, Horne et al., 2006) Therefore anaemia can have both a direct, and an indirect effect upon the blood transfusion requirements at orthotopic liver transplantation (OLT), by reducing the reserve before transfusion thresholds are reached and increasing bleeding tendency.

The first study of this thesis aimed to identify the prevalence of anaemia in patients undergoing liver transplantation, and its effect upon blood transfusion rates and outcome.

3.2 Methods

A retrospective study was performed at the Royal Free Hospital, a UK liver transplant centre. It was formally confirmed as a service evaluation to inform the delivery of best care by the Joint Research Office of UCL in June 2013. Data was

retrieved from a database of perioperative data including intraoperative fluid administration, blood transfusion and point of care results maintained by the anaesthetic department with reference to individual patient medical records and laboratory blood results. Data was routinely collected clinical data and not prospectively collected for the purpose of this project. Data elements collected included patient demographics (age, gender, weight), severity of liver disease (MELD score, concurrent hepatocellular carcinoma), pre-operative blood tests (full blood count, coagulation tests, haematinics, creatinine), intra operative fluid and blood product administration and post-operative outcomes (post-operative ventilator days and days in ICU and hospital, return to theatre, renal replacement therapy and survival (at 30 days and 1 year). Data was collected by myself and a fellow anaesthetic registrar.

Data were extracted for 200 patients undergoing liver transplantation between October 2008 and February 2012 to provide survival data to one year. The outcome of interest was intraoperative transfusion rates.

Within patients with liver disease, anaemia is highly prevalent – in up to 75% of patients with advanced disease when compared to the WHO definition (Hb <120 g/L for females and <130 g/L for males) (Gkamprela et al., 2017). Within cohorts of patients of liver disease, alternate definitions of anaemia have been used, using 100 g/L as a clinically significant threshold for anaemia (McHutchison et al., 2006). For this analysis patients were stratified into anaemic and non-anaemic groups using a haemoglobin concentration of <110 g/L and >=110 g/L for both male and female patients. This cut-off was chosen to effectively divide the sample population into two similarly sized groups.

Iron deficiency was defined by the following variables: either ferritin <100 ug/L, or ferritin 100-299 ug/L with iron saturation <20%) as per standard definitions of absolute and functional iron deficiency in the context of chronic disease (Anker et al., 2009).

Massive transfusion was defined as 6 or more units of RBCs in many studies of bleeding associated with liver transplantation (Esmat Gamil et al., 2012, McCluskey et al., 2006, Ramos et al., 2003). In many cases the massive transfusion is a consequence of surgical bleeding or profound coagulopathy, often unrelated to the haemoglobin concentration of the patient preoperatively. Ramos et al demonstrated that at a cut off value of six packed red cell units pre-operative anaemia ceased to be significant(Ramos et al., 2003). Separate analyses were conducted between anaemic and non-anaemic patients who received less than 6 units to better quantify the effect of anaemia without massive blood loss.

3.2.1 Statistical Analysis

Statistical analysis was performed using Microsoft Excel 2013 (Microsoft Corp, USA) and SPSS Statistics v22 (IBM Corp, USA).

Data was screened for impossible values by inspecting frequencies, maximum and minimum values of each variable, and recoding these as missing. Not all data elements were available for each patient. An assumption of missing at random was made, therefore only complete cases were included in all regression analysis, assuming that the probability of having no missing values did not depend on the outcome variable. Under this assumption the simple descriptive analysis may result in biased estimates. Pairwise exclusion of missing data was used.

Normal distribution was confirmed by a Shapiro-Wilk test ($p>0.05$) and visual inspection of data histograms and Q-Q plots.

Summary statistics were calculated using number (percentage) for categorical variables and median (interquartile range) for non-parametric or mean (standard deviation) for parametric data as appropriate. Comparisons between groups were explored using two-sample T tests or Mann Whitney U tests for parametric or non-parametric continuous variables respectively as appropriate and Pearson's Chi-squared tests for categorical variables. Correlations between variables and outcomes were made using Pearson's Correlation. The association of anaemia and transfusion, ICU and hospital length of stay, 30-day and 1 year mortality were then analysed

using multivariate analysis. Odds ratios and 95% confidence intervals were estimated using multivariable logistic regression.

A value of $p<0.05$ was considered statistically significant in all cases. All statistical analyses were performed by myself with statistical support provided by Timothy Collier from the London School of Hygiene and Tropical Medicine.

3.2.2 Surgical and anaesthetic protocols

Anaesthetic protocols, haemodynamic monitoring, antibiotic prophylaxis and immunosuppressive protocols were similar for all patients. All patients were pre-medicated with Temazepam 10mg on the ward prior to transfer to theatre. On arrival in theatre, patients were pre-oxygenated in 100% oxygen using a face mask. Induction of anaesthesia is intravenous using propofol and fentanyl, followed by atracurium as muscle relaxant. Following endotracheal intubation arterial and central venous access is placed. Routine access at our institution includes: radial arterial line, femoral arterial and femoral central venous line, and internal jugular 4-lumen CVP line, rapid infuser sheath and Swan sheath. A pulmonary artery catheter is placed transduced via a Vigilance II monitor (Edwards Lifesciences, Irving, California, USA). Anaesthesia is maintained with volatile anaesthetic agents (isoflurane or desflurane) in oxygen and air, with continuous infusions of fentanyl and atracurium perioperatively to maintain muscle relaxation, with noradrenaline infusions as the standard vasopressor.

Four experienced surgeons performed the operations, with a mixture of piggyback and caval replacement techniques based upon surgical preference. 9 experienced consultant anaesthetists were involved throughout the study period. Veno-veno bypass was not used in any case analysed. All the livers were harvested from cadaveric donors and were ABO-Rh compatible.

All patients have a full panel of laboratory bloods measured on admission for transplant including: Full blood count (FBC), Coagulation screen, fibrinogen, urea and electrolytes (including calcium and magnesium), Liver function tests (LFTs) and full cross match. Patients are routinely cross-matched 10units RBC, 10u FFP and 4

pools platelets. 4g of Fibrinogen concentrate and 3000 IU of Prothrombin Complex Concentrates (PCCs) are available in the OLT theatre.

Haematinics are routinely measured when being pre-assessed for transplant and whilst on the transplant waiting list. All haematinic data is the most recent for each patient.

After induction of anaesthesia and placement of invasive monitoring, routine baseline point of care (POCT) bloods are drawn for thromboelastography (TEG) (Haemonetics Corp., Braintree, MA, USA), INR (ITC Haemochron Signature Elite, Edison, NJ, USA), FBC (Sysmex POCH-100i, Milton Keynes, UK), and arterial blood gas (ABG) (Siemens Rapidlab, Munich, Germany) analysis. These are repeated at defined points throughout the transplant and as clinically indicated: baseline; dissection phase – every 60 minutes; anhepatic phase: at the beginning and every 30 minutes; reperfusion – 5 minutes and 30 minutes post reperfusion; 10 minutes after therapy with coagulation factors or platelets.

Cell salvage was used routinely for all patients unless specifically contraindicated. Local departmental protocols for transfusion are used: Hb <80 g/L and in the case of acute massive blood loss, aiming Hb >90 g/L. Platelets are to be administered if platelet count <40 x 10^9 and bleeding; Cryoprecipitate & fibrinogen concentrate if TEG Maximum amplitude (MA) <40mm with platelet count >40 $x10^9$ (with administration of 2g fibrinogen or 2 u cryoprecipitate); PCC if bleeding with evidence of clotting factor deficiency (TEG heparinase r time >30 minutes); Tranexamic acid 2g if TEG evidence of lysis with clot lysis index (CLI) >15%, or prophylactically prior to reperfusion in DCD (donation after cardiac death) grafts.

3.3 Results

Two hundred consecutive OLT operations (132 males and 68 females) between 2008 and 2012 were retrospectively analysed. 4 cases during the period were not analysed because the intraoperative transfusion data was not available for analysis.

A range of indications for transplant was present within the group, and 26% of cases had concurrent HCC (hepatocellular carcinoma). Median Model of End Stage Liver Disease (MELD) score (not weighted for HCC) was 15 (10-19) (MELD score was not presented for six cases where the patient was listed for super-urgent transplantation due to acute liver failure). (Table 1)

Table 1: Baseline demographic data

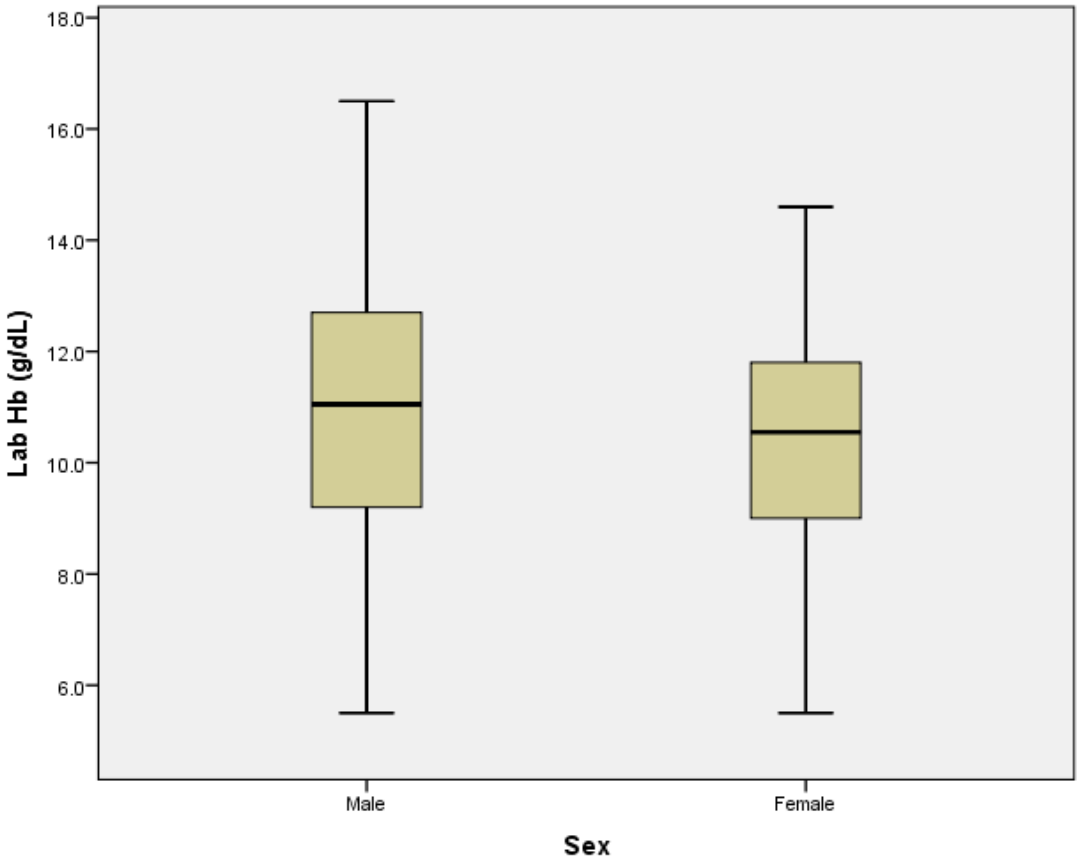
Preoperative Characteristics	n	Total	Anaemic (Hb <110 g/L) N=104 (52%)	Non-Anaemic (Hb ≥110 g/L) N=96 (48%)	P-value
Sex (male) %		132 (66 %)	65 (62.5%)	67 (70%)	0.277
Age (years)	200	54 (47.3-60)	53.5 (44.3-60)	54.5 (50-60.8)	0.183
Weight (Kg)	198	74.9 (64-87.6)	73.8 (60.0-88.5)	76.5 (66.3-86.3)	0.105
MELD score	194	15 (10-19)	17 (11.3-22)	13 (9.0-16.0)	<0.001
HCC n (%)		52(26 %)	20(19%)	32(33%)	0.023
Hb (g/L)	199	109 (22.5)	91.4 (12.4)	128 (13.7)	<0.001
Platelets ($x10^9$)	198	95 (61-148.5)	92 (60-144)	97 (63-153)	0.827
INR	194	1.5 (1.3-1.8)	1.6 (1.4-1.9)	1.4 (1.2-1.7)	<0.001
Fibrinogen (g/L)	149	2.1 (1.6-3)	1.9 (1.4-2.8)	2.3 (1.8-3.4)	0.002
Iron (μmol/L)	132	18.7 (11.5-27.7)	18.0 (7.9-26.1)	19.6 (12.5-29.1)	0.159
TIBC (μmol/L)	131	42.3 (35.1-52.6)	40.3 (32.0-50.4)	46.7 (37.7-54.7)	0.034
Iron Saturation (%)	131	45.4(27-76.8)	46.3 (24.0-77.7)	44.8 (27.1-70.8)	0.890
Ferritin (mcg/L)	130	228 (53.3-469.3)	232.5 (45.8-585.5)	227.5 (85.3-449.3)	0.989
Vitamin B12 (pg/ml)	100	978 (672.3-1353.3)	942 (745-1358)	1010 (594-1364.5)	0.858
Folate (ng/ml)	100	9.7 (7.3-12.5)	9.4 (7.4-12.0)	9.9 (7.3-14.1)	0.546
Creatinine (mmol/L)	164	76 (61.3-93.8)	81 (62-99)	74 (60-88)	0.164
NOTE: Data is presented as number (%) or median (Interquartile range) and the Two-sample t-test or Mann-Whitney U test or Pearson chi-square test used as appropriate.					

Utilising the Shapiro-Wilk test, only Hb was demonstrated to be normally distributed within the pre-operative data.

Baseline mean Hb (SD) was 109 (22.5) g/L. Male mean Hb was 110.9 (23.7) g/L and for females mean Hb was 105.3 (19.7) g/L (p=0.93) (Figure 2). 104 patients (52%) had a baseline Hb <110 g/L, thus were anaemic by the criteria assigned in this study.

There were significant differences between the anaemic and non-anaemic groups for MELD score – anaemic patients had a higher MELD (17 (11.3-22) vs 13 (9-16.0) respectively (P<0.001). However, there was a lower frequency of concurrent HCC for anaemic patients (19%) vs 33% of non-anaemic patients (p=0.023).

Figure 2: Box plot of preoperative haemoglobin concentration



Box represents 25 - 50 - 75th quartiles, whiskers represent 5% and 95% limits.

Median (IQR) transfusion was 3 (0-6) units of RBC. Patients with a baseline Hb <110 g/L received 4 (2-6) units RBCs, whilst those ≥110 g/L received 2 (0-4) units (P<0.001). Cell salvaged RBCs were returned to the patient in 79 cases. Comparing anaemic and non-anaemic patients there were significant differences in the units of FFP required (4 (2-6) vs 2 (0-5.75) p=0.031) and cryoprecipitate transfused (0 {range 0-2} vs 0 {range 0-6}) p=0.041). (Table 2)

Table 2: Intraoperative fluid and transfusion

	All patients	**Anaemic (Hb <110 g/L)**	**Non-Anaemic (Hb ≥110g/L**	**P-value**
RBC (units)	3 (0-6)	4 (2-6)	2 (0-4)	<0.001
		N (%)	N (%)	
0 u (n=52)	0	15 (14%)	37 (39%)	<0.001
FFP (units)	3 (0-6)	4 (0-7.75)	2 (0-5.75)	0.031
Platelets (pools)	1 (0-2)	1 (0-2)	0 (0-2)	0.13
Cryoprecipitate (units)	0 {0-6}	0 {0-6}	0 {0-2}	0.041
Prothrombin Complex Concentrate (u)	0 {0-3500}	0 {0-3500}	0 {0-2500}	0.496
Fibrinogen concentrate (g)	0 {0-10}	0 {0-6}	0 (0-10)	0.111
Cell Salvaged RBCs (ml)	663 (333-1656)	828 (335-1900)	553 (306-1617)	0.397
Colloids (l)	2 (1.5-3)	2 (1.5-3)	2.4 (1.5-3)	0.468
Crystalloids (l)	4.5 (3-6)	4.5 (3-6)	4.5 (3-5.6)	0.993

NOTE: Data presented as medians (interquartile range) ({total range} is used where IQR=0), or mean (Standard Deviation) when appropriate. Chi-squared or Mann Whitney U test was used to determine p-values.

52 (26%) OLTs took place without any intraoperative transfusion, 48% received 1-5 units, and 26% received 6 or more units (considered a massive transfusion). Of those patients who received no blood, 71.2% had an Hb ≥110 g/L at baseline. Only 14.4% of those with a starting Hb<110 g/L received no RBCs intraoperatively. Of those

patients who received a massive transfusion (≥6 units RBCs), 65.4% had a baseline Hb <110 g/L.

Of those patients with a baseline Hb <110 g/L, haematinic data was available for 70 patients. Of these, iron deficiency was present in 28 patients (40%): 26 had ferritin <100 μg/L, with 2 having ferritin 100-299 μg /L with Iron saturation <20%.

Between anaemic and non-anaemic patients, there was no significant difference in: age (median) 54 (45-60) vs. 55 (50-61) (p=0.165), platelet count (92 (60-144) vs. 97 (63-153) (p=0.827) ($x10^9$), creatinine (81 (63-98) vs. 74 (60-88) (p=0.164) (mmol/L) respectively. However, there was a significant difference between MELD score (p=<0.001), INR (p=<0.001), total RBC transfused (p=<0.001).

Post operatively between anaemic and non-anaemic patients there was a statistically significant difference in the number of days ventilated post operatively (2 (1-5) vs 1 (1-2)) days respectively (p=0.029). Length of stay in ICU (days) (anaemic 3 days (1-6) vs non-anaemic 2 days (2-5) (p=0.187) and total hospital length of stay between the 2 groups (22 (15-38) vs 19 (15-27) (p=0.098) was not significantly different, despite longer median values in anaemic patients.

3.3.1 Patients Receiving Less than Six Units RBCs

52 patients received a massive transfusion – defined as 6 or more units of RBCs (range 6-30 units, median 8.5 (7-12)). In many cases the massive transfusion is a consequence of surgical bleeding or profound coagulopathy, often unrelated to the haemoglobin concentration of the patient preoperatively.

137 patients received 4 or less units of RBCs. There was significant difference between anaemic and non-anaemic patients whether they were transfused or not (p=0.003), with non-anaemic patients receiving 1 (0 – 2) units and anaemic patients receiving 2 (0.75 – 3) units amongst this group.

148 patients received less than 6 units of RBC. There was no significant difference in age, weight, concurrent HCC, platelet count, creatinine and fibrinogen. There was

significantly higher MELD score (p=0.004) and INR (p=0.018) in anaemic patients compared to non-anaemic. (Table 3)

Table 3: Baseline data of those patients who did not receive a massive transfusion

Preoperative Characteristics	n	Anaemic	Non-Anaemic	P-value
Sex (male) %	91 (61.5%)	51(66%)	40(57%)	p=0.218
Age (years)	148	54.0 (49.5-60)	52.0 (43.5-58.3)	0.173
Weight (Kg)	148	75.1 (65.5-85.3)	71.5 (59.8-90.3)	0.244
MELD score	144	13 (9-16)	15.5 (11-19.8)	0.004
HCC %		25 (32%)	14 (20%)	0.080
Hb (g/L)	148	126 (118-135)	93 (85-103)	<0.001
Platelets ($x10^9$)	147	91 (61-153.5)	112.5 (61.8-164.5)	0.299
INR	143	1.5 (1.2-1.7)	1.6 (1.2-1.4)	0.023
Fibrinogen (g/L)	108	2.3 (1.8-3.4)	2 (1.5-3)	0.046
Creatinine (mmol/L)	148	70 (60-83)	79.5 (59.5-91.3)	0.281

35.1% of patients, when excluding massive transfusion, received no intraoperative RBCs. (Table 4) 71.2% of the patients who received no intraoperative RBCs had a starting Hb ≥110 g/L. Performing Pearson's χ-square test, there was a significant difference in transfusion between anaemic and non-anaemic patients (p=0.008) (Table 5).

Table 4: Units of RBCs Transfused (excluding massive transfusion)

RBC units transfused		Anaemic (Hb <110 g/L) n=71		Non-Anaemic (Hb ≥110 g/L) N=77	
	N (%)	N (%)	Hb (g/L) mean(SD)	N (%)	Hb (g/L) mean(SD)
0	52 (35%)	15 (21%)	89 (11.7)	37 (48%)	129 (10.6)
1	15 (10%)	8 (11%)	91 (18.1)	7 (9%)	126 (15.0)
2	30 (20%)	14 (20%)	99 (9.1)	16 (21%)	129 (13.8)
3	20 (14%)	12 (17%)	91 (12.1)	8 (10%)	125 (10.3)
4	20 (14%)	14 (20%)	91 (9.9)	6 (8%)	121 (8.4)
5	11 (7%)	8 (11%)	99 (11.4)	3 (4%)	125 (21.4)

Table 5: Intraoperative fluid and transfusion requirements, anaemic vs non-anaemic patients

Intraoperative	Anaemic	Non-anaemic	P value
RBC units	2 (1-4)	1 (0-2)	<0.001
FFP units	2 (0-4)	2 (0-4)	0.401
Platelets (pools)	0 (0-1.3)	0 (0-1)	0.832
Cryoprecipitate (units)	0 (0-0)	0 (0-0)	0.411
Cell Salvage (ml)	563.5 (300-1067.25)	515 (225-1059.3)	0.773
PCC (g)	0 (0-0)	0 (0-0)	0.620
Tranexamic Acid (g)	0 (0-0)	0 (0-0)	0.405
Fibrinogen Concentrate (g)	0 (0-0)	0 (0-0)	0.386
Colloid (ml)	1500 (1000-2025)	2000 (1000-2500)	0.256
Crystalloid (ml)	4400 (3000-6000)	4750 (3000-6000)	0.87

NOTE: Data is presented as number (%) or median (Interquartile range) and the Mann-Whitney U test used.

There was significant difference between anaemic and non-anaemic patients in number of ventilator days (p=0.047) and total length of stay (p=0.036). (Table 6)

Comparing patients who received no blood (n=52) and those who received 1-5u (n=96) there was a significant difference between length of ICU stay (2 (1-3) vs. 2.5 (2-6)) (p=0.002) and ventilator days (1 (1-2) vs. 1 (1-4)) (p=0.006).

Table 6: Postoperative outcome data of patients receiving 0-5 units RBCs

Postoperative	Anaemic	Non-anaemic	P-value
Ventilator days	1 (1-4.3)	1 (1-1)	0.039
ICU days	2 (1-6)	2 (1-3)	0.364
Days admitted	23 (16-38)	19 (15-27)	0.041
Return to theatre (yes/no)	51/9	58/7	0.581
Renal Replacement Therapy (yes/no)	47/13	57/8	0.164
Survival (30 days) (Survival / non-survival)	68/2	77/0	0.139
Survival (1 year) (Survival / non-survival)	66/4	75/2	0.351

NOTE: Data is presented as median (Interquartile range); p-value from the Mann-Whitney U test.

3.3.2 Correlations

Analysing the entire cohort of 200 patients, MELD score had the strongest correlation to transfusion (r=0.258 (p=<0.001)), followed by Hb which was negatively correlated ((r=-.237 (P=0.001)), platelet count (r=-0.208 (p=0.003) and INR (p=0.170 (p=0.018).

Partial correlation was used to explore the relationship between Hb and units of RBCs transfused, whilst controlling for MELD. When comparing all 200 cases, the correlation between Hb and number of RBCs was not significant (r=-

0.019)(p=0.790). When comparing those who received <6 units RBCs (n=148), the correlation became significant (r=-200)(p=0.016).

Ferritin concentration was negatively correlated to both 30-day survival (r=-0.235 (p=0.007)) and 1year survival (r=-0.281 (p=0.001)), as well as days ventilated (r=0.352 (p<0.001)) and on ITU (r=0.446 (p<0.001)).

When analysing only those who received less than six units of blood, MELD (r=0.264) (p=0.001), Hb (r=-.241) (p=0.003), female sex (r=0.233) (p=0.004), INR (r=0.189) (p=0.024) and platelet count (r=-0.187) (p=0.023) were significantly correlated to blood transfusion. MELD score was the only preoperative variable associated with survival at l year (r=0.183 (p=0.028)). There were no pre-operative variables that strongly correlated to 30day survival. MELD and anaemia were correlated to ventilator days (r=0.204 (p=0.015) and r=0.193 (p=0.019) respectively) and length of stay in hospital (r=0.224 (p=0.008) and r=0.177 (p=0.036) respectively.

Multiple regression for units of red cells was performed including all pre-operative variables to assess for confounding effects. These were removed in a stepwise manner if they were not significant in the model according to the beta of the coefficients.

The multivariable analysis for units of RBC transfused for all cases showed that only MELD score made a statistically significant contribution to the number of RBCs transfused (beta=0.324 (p=0.013)), controlling for Hb, platelets, INR, age, weight, creatinine and ferritin. However, this model was not statistically significant for predicting units of red cells (r2=0.132, p=0.060). After adjusting for age, MELD< Hb and platelet count, the model was predictive (r2=0.115; p<0.001).

However, when considering only those patients who received less than 6 units, the effect of MELD, Hb and platelet count became significant in the constructed model for units of RBC transfused (r2=0.165, p<0.001). The standardised coefficients for MELD (beta=0.228 (p=0.013)), Hb (beta= -.240 (p=0.004)) and platelet count (beta= -.229 (p=0.005)) were all significant within the model, emphasising the stronger influence of preoperative factors upon smaller transfusion amounts intraoperatively.

In those patients who received less than six units of blood, after adjustment for age, sex, platelet count and MELD, the odds of transfusion were significantly higher among anaemic patients compared to non-anaemic patients with an estimated odds ratio 3.69, 95% CI (1.59 - 8.55) (p=0.002). Treating haemoglobin concentration as a continuous variable, the odds of transfusion decreased significantly with increasing haemoglobin (odds ratio 0.79 95% CI (0.65 - 0.96) per 10 g/L increase (p=0.021) after controlling for age, sex, MELD and platelet count. (Table 7).

Table 7: Logistic regression analysis of transfusion

	Number (%) transfused	Unadjusted Odds Ratio (95% CI)	p-value	Odds Ratio Adjusted for age and sex (95% CI)	p-value	Adjusted Odds Ratio (95% CI)*	p-value
Anaemia (Hb <110 g/L)							
No	37 (48%)	Reference					
Yes	15 (21%)	3.45 (1.67,7.12)	0.001	3.28 (1.55, 6.91)	0.002	3.69 (1.59, 8.55)	0.002
Haemoglobin concentration (per g/dL)		0.78 (0.65-0.92)	0.004	0.80 (0.67, 0.96)	0.014	0.79 (0.65, 0.96)	0.021
NOTE = * adjusted for age, sex, MELD and platelet count; MELD scores not documented for 4 patients due to super-urgent listing for transplant							

3.4 Discussion

Even using a conservative measure of anaemia, over half of patients undergoing OLT in this cohort were anaemic. MELD score was significantly different between anaemic and non-anaemic patients, linking severity of liver disease with anaemia. Both increasing MELD score and decreased Hb were the most significant factors upon regression analysis for units of RBC transfused.

26% of patients had a transfusion free transplant, and 71.2% of those patients who received no intraoperative RBCs had a starting Hb ≥110 g/L. Patients who were anaemic were significantly more likely to require an intraoperative blood transfusion,

and the probability of achieving a transfusion free transplant was significantly reduced.

Regression analysis of patients who did not require a massive intraoperative transfusion to eliminate the influence of major surgical bleeding demonstrated that patients with a haemoglobin <110 g/L at the beginning of surgery had an odds ratio of 3.69 of receiving an intraoperative transfusion compared to those who were not anaemic. Per 10 g/L rise in haemoglobin concentration the adjusted odds ratio of transfusion was 0.79 (0.65 – 0.96). Thus, pre-operatively incrementing these patient's haemoglobin may have raised it high enough above the transfusion threshold in the case of those who received a small intraoperative transfusion which was demonstrated by the difference in the significance of Hb in the regression models. However, these regression models only predicted a small amount of the variation in transfusion rates, demonstrating the significant interplay of other factors in predicting transfusion.

Patients who were not transfused intraoperatively had a significantly shorter duration of postoperative ventilation and ICU stay. The reasons for this are multifactorial and include many factors not controlled for in this retrospective analysis. Major haemorrhage intraoperatively is associated with decreased survival (Boin et al., 2008). Meanwhile longer surgeries (Massicotte et al., 2004, Feltracco et al., 2013), using marginal grafts (for example DCD), delayed graft function, post reperfusion coagulopathy are all associated with bleeding and increased complication rates post operatively (Broomhead et al., 2012)

Intensive care ventilation time and total length of stay was significantly shorter in non-anaemic patients. The cause of this may be secondary to sicker patients having lower haemoglobin (Baron et al., 2014), but also due to the consequences of higher exposure to blood transfusion. Non-anaemic patients had a higher proportion of HCC and a lower MELD score, suggesting less severe cirrhosis prior to liver transplant.

The causes of anaemia are multifactorial in chronic liver disease. Iron deficiency was demonstrated to be prevalent in 40% of those patients who were anaemic in this study. The liver is a major contributor to iron homeostasis and liver disease can be

responsible for abnormalities in iron homeostasis. The liver is responsible for the majority of hepcidin production, the main iron regulatory hormone. Several studies have shown that hepcidin levels are decreased in chronic liver disease in proportion to the degree of inflammation (Tsochatzis et al., 2010, Lyberopoulou et al., 2015) Meanwhile, cirrhotic patients classically present with significantly lower serum transferrin and serum iron levels but higher levels than non-cirrhotic patients and healthy controls (Gkamprela et al., 2017).

Ferritin was negatively associated with survival in this cohort and positively correlated to length of stay in ITU and ventilator days. This has been shown in other studies where of liver disease, where high ferritin is associated with adverse outcomes in decompensated cirrhosis (Oikonomou et al., 2017), and acute liver failure (Anastasiou et al., 2017). Ferritin is an acute phase protein influenced both by iron stores, but also inflammation, malignancy and liver disease- making it difficult to interpret in the context of liver disease. Nonetheless, a ferritin level of <100 ug/L is associated with iron deficiency in both inflammation and chronic liver disease (Gkamprela et al., 2017, Weiss and Goodnough, 2005).

The first pillar of patient blood management is the recognition and treatment of pre-operative anaemia. Patients on the liver transplant waiting list provide an ideal population since they are regularly reviewed whist being assessed for suitability for listing on the transplant waiting list. However, concerns exist regarding the use of iron in patients with liver disease, due to its association with parenchymal liver damage in states of overload, including haemochromatosis, due to increased oxidative stress (Alla and Bonkovsky, 2005). Newer intravenous iron preparations, including ferric carboxymaltose and iron sucrose, have been shown to liberate less free iron, reducing oxidative stress and tissue damage (including hepatic) than traditionally utilised compounds such as iron dextrans (Toblli et al., 2010). Experience of OLT in Jehovah's Witness patients has provided evidence for the optimisation of patients with iron and erythropoietin to reduce the risk of perioperative anaemia, with encouraging results (Jabbour et al., 2005, Darwish, 2011). Jabbour et al reported on 27 consecutive transplants in blood refusing patients. Patients were treated with erythropoietin, iron sulphate and folic acid to aim for a

normal or hypernormal red cell mass preoperatively without adverse events (Jabbour et al., 2005).

There are several limitations to this study. The retrospective nature of the analysis has inherent weaknesses. As a historical cohort of patients, the findings of this study may not fully reflect the current approach to transfusion management intraoperatively. The transfusion guidelines for liver transplantation have not significantly changed from this cohort. The associations seen between anaemia, transfusion and outcome in this study cannot assume causation nor account for other confounding factors, such as preoperative management, surgical or donor factors. Additionally, missing data, particularly relating to haematinics can affect the validity of the findings.

3.5 Conclusion

This retrospective study demonstrated that patients undergoing liver transplantation were significantly more likely to receive an intraoperative blood transfusion if they were anaemic pre-operatively. Given the high prevalence of anaemia in patients with chronic liver disease and strong evidence correlating blood transfusion with adverse perioperative outcomes in liver transplant (Rana et al., 2013, de Boer et al., 2008, Boin et al., 2008), a focus should be made to diagnose the aetiology and institute treatment for anaemia in patients awaiting liver transplantation.

Patient blood management has the potential to improve outcomes after OLT. Large steps have been taken over the years since the introduction of liver transplantation in intraoperative bleeding and coagulation management to reduce transfusion rates in OLT. There have been no controlled trials of preoperative anaemia correction in OLT. Further research and clinical focus should now be applied to the pre-operative period, including the diagnosis and treatment of anaemia, particularly focusing upon iron and other nutritional deficiencies to improve functional status. In this manner, we can pre-optimise these patients in preparation for their surgery and recovery.

Chapter 4 Tissue oxygenation and vascular reactivity

4.1 Introduction

Tissue oxygenation is commonly assessed by global measures with surrogate markers of perfusion such as blood pressure, cardiac output, oxygen-derived variables and blood lactate levels. Near infrared spectroscopy (NIRS) permits the non-invasive monitoring of muscle oxygenation. This permits the total tissue oxygenation to be recorded, but also allows measurement of tissue oxygen consumption and microvascular reactivity using dynamic measures such as the vascular occlusion test (VOT)

4.1.1 Near Infrared Spectroscopy

Near Infrared Spectroscopy (NIRS) allows measurement of tissue oxygenation in end organ beds, including the brain and muscle. It was initially described in the 1970s and was first used to transilluminate the newborn head (Pellicer and Bravo Mdel, 2011). Near-infrared light is absorbed by chromophores within muscle; the most important of which is haemoglobin. Deoxy- and Oxy-haemoglobin have different absorption spectra, so that light of different wavelengths emitted through muscle tissue is absorbed to differing degrees depending upon the relative deoxy- and oxy-haemoglobin concentrations (Gerovasili et al., 2010). The Beer-Lambert law is based upon the absorbance of light being proportional to the concentration of the medium, and the path length. Applying the Beer-Lambert law allows the calculation of haemoglobin saturation in the microvasculature of the tissue of interest, calculating the tissue oxygen saturation (StO_2) as an average of all vessels (arterioles, capillaries and venules). In peripheral muscle, such as found at the thenar eminence, the infrared light can only obtain data from the small vessels (arterioles, capillaries and venules) less than 1mm in diameter. 75% of blood in skeletal muscle is venous, so the NIRS value is largely a venous haemoglobin saturation of the blood after oxygen delivery and extraction (De Backer et al., 2010).

In acute circulatory failure, such as haemorrhagic shock, blood flow is diverted to vital organs. Therefore, oxygenation monitoring in less important tissues such as

muscle can provide an early marker to tissue hypoperfusion. In severely injured trauma patients, McKinley compared changes in StO2 with resuscitation over 36 hours. Mean StO2 increased in parallel with oxygen delivery, suggesting that StO2 may have a potential as a non-invasive indicator of adequate resuscitation (McKinley et al., 2000).

4.1.1.1 The vascular occlusion technique

The absolute value of StO_2 provides an average of tissue oxygenation. However, a dynamic physiological challenge can provide information about the adequacy of microcirculation and oxygen utilisation. Using a technique whereby a brief episode of forearm ischaemia is precipitated, analysing the changes in StO_2 allow quantification of the microvascular system. Implementing such a vascular occlusive test (VOT) provides reliable data reflecting underlying tissue oxygenation (Martin et al., 2013). This technique has been validated (Kragelj et al., 2000), and utilised in multiple settings including perioperatively, in sepsis, shock and trauma (Lipcsey et al., 2012).

The procedure typically involves placing a blood pressure cuff around a limb, rapidly inflating it above systolic blood pressure for a period of three to five minutes and then releasing the cuff. During the procedure, StO_2 is measured continuously by NIRS, distal to the cuff, and the measurements plotted to generate a StO_2-time curve. (Figure 3) Temporary arterial, and venous, occlusion throughout cuff inflation results in muscle ischaemia, and a steady decline in StO_2, measured as the decrease in saturation of Hb (decrease rate % / minute). This is extrapolated to represent the skeletal muscle oxygen consumption rate, reflecting the basic metabolic rate of the muscle, as well as the competence of the microcirculation to provide the oxygen required by the tissue (De Blasi et al., 1993).

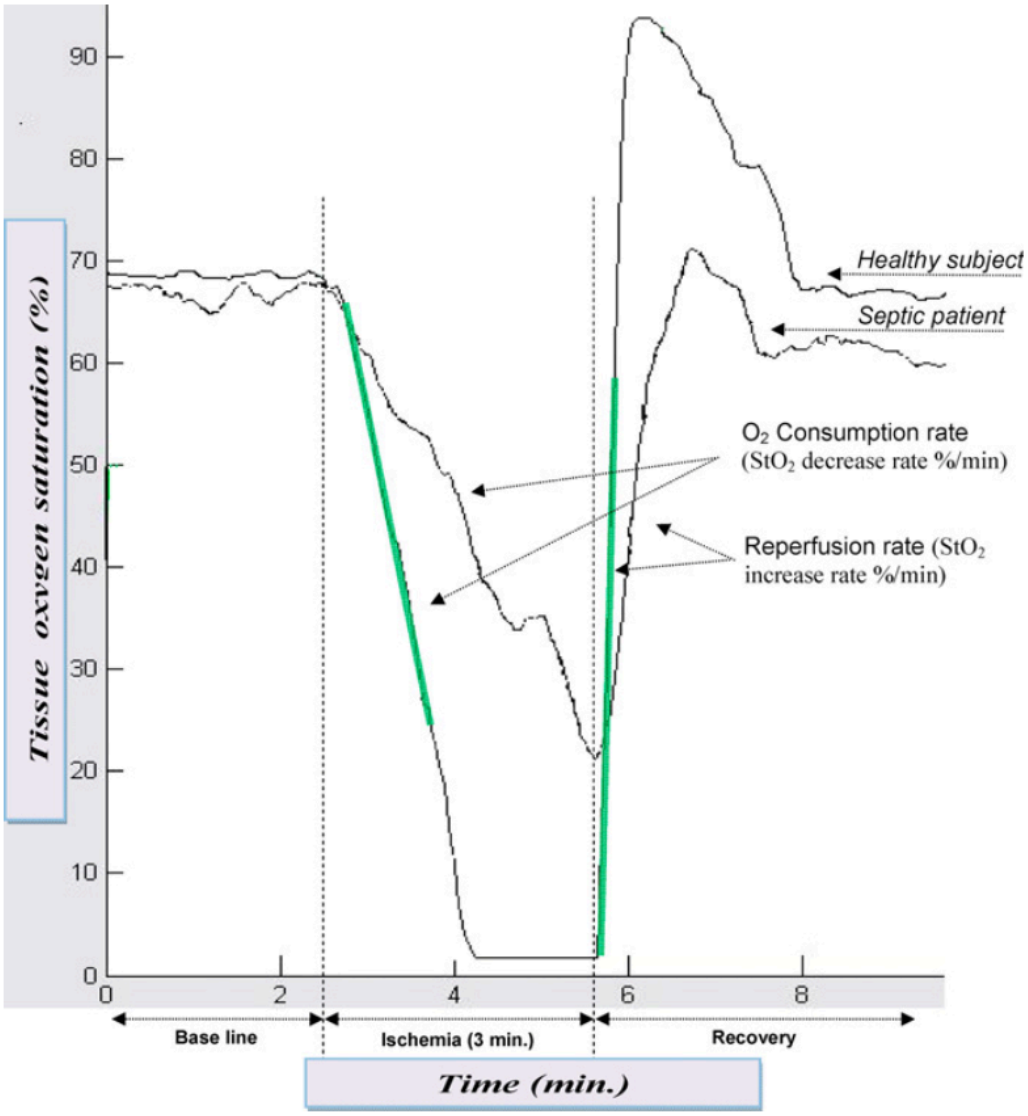
The StO_2 reaches an ischaemic nadir prior to cuff release, at which point rapid reperfusion is seen as the re-oxygenation of haemoglobin occurs, which depends on the integrity and function of the vascular endothelium. The release of the cuff restores oxygenated blood to the tissue and washout of the deoxygenated blood (increase rate % / minute). The initial rapid re-saturation of haemoglobin is primarily dependent upon endothelial unction, with the rate reflecting endothelium dependent

vasodilation (Gerovasili et al., 2010).

Following reperfusion of the tissue, there is a period of reactive hyperaemia (marked by a period of over oxygenation, with the StO_2 raised above baseline), representing by regional post-ischaemic vasodilatation. The degree of hyperaemia is dependent upon the ability of the microcirculation to respond to ischaemia adequately. The area under the curve above baseline following reperfusion has been used to estimate vascular reserve. There is then a slow return back to baseline StO_2.

Reactive hyperaemia is considered an integral test of microcirculatory reactivity by evaluating the tissue's ability to adjust oxygen extraction capability to oxygen delivery after the hypoxia created by a temporary interruption to blood flow (Creteur, 2008). It is mediated by myogenic and endothelial-derived factors, increasing flow within patent capillaries and recruiting additional capillaries.

Figure 3: NIRS tissue oxygenation during the vascular occlusive test (VOT)



NIRS StO2 traces from a 3-minute vascular occlusion test (VOT) comparing the response of a healthy participant vs a patient with established sepsis. StO2: tissue saturation. Reprinted from International Journal of Industrial Ergonomics, Gerovasili, V., et al, Utilising the vascular occlusion technique with NIRS technology, 40, 218-222, Copyright 2010, with permission from Elsevier. (Gerovasili et al, 2010)

4.2 Vascular reactivity in sepsis

Much of the research utilising the vascular occlusion testing to evaluate dynamic changes in tissues oxygenation to assess microvascular function and blood flow has been in patients with sepsis. Studies have demonstrated that the oxygen consumption

rate is significantly lower in patients with septic shock than those with severe sepsis, and in healthy volunteers (Pareznik et al., 2006).

Creteur et al showed a lower rate of increase StO2 (RincStO2) after reperfusion and lower delta-StO2 (difference between maximum reperfusion StO2 and baseline) in septic patients than ITU controls and healthy volunteers. RincStO2 was lower in patients with shock than those without; and higher in survivors than non-survivors (Creteur et al., 2007). This is thought to represent the inability of the thenar microvasculature to respond appropriately to ischaemia by augmenting blood flow (Creteur, 2008).

In septic patients, the oxygen consumption closely correlated with the degree of organ dysfunction. De Blasi et al demonstrated prolonged haemoglobin re-saturation rate in septic shock, reflecting the absence of normal, rapid increase in arterial blood delivery (De Blasi et al., 2005). Meanwhile Donati et al demonstrated that alterations in desaturation slope, slower reperfusion and less-pronounced reactive hyperaemia were associated with mortality in critically ill patients (Donati et al., 2016).

4.3 Vascular reactivity in liver disease

In 1973, Lunzer et al measured skeletal blood flow in patients with chronic liver disease, measuring the clearance of clearance of Xenon, and then changes in skeletal blood flow after head-up tilting. They demonstrated normal skeletal muscle blood flow in patients with liver disease. The patients with liver disuse showed significantly less vasoconstriction than control subjects, whilst patients with liver disease showed significantly less tachycardia in response to tilting (Lunzer et al., 1973). There is abundant evidence for endothelial dysfunction in cirrhosis. In cirrhosis, endothelial dysfunction contributes to increased intrahepatic vascular resistance whilst increased production of vasodilator molecules contribute to increased endothelium-dependent relaxation in the arteries of the systemic and splanchnic circulation (Iwakiri and Groszmann, 2007).

Investigations into peripheral blood flow in cirrhosis using various techniques have had contrasting results. Carella et al showed no change using a isotope clearance

technique (Carrella et al., 1992), whilst Seino demonstrated increased forearm muscular flow using laser Doppler spectroscopy (Seino et al., 1993). However, in keeping with most models of peripheral vascular alterations in cirrhosis, increased blood flow and peripheral dilatation have been demonstrated experimentally using venous occlusion plethysmography (Helmy et al., 2003) and flow mediated dilation using Doppler (Cazzaniga et al., 2008). Utilising a VOC, this study showed higher rates of flow-mediated dilation in the brachial artery in decompensated cirrhosis than in compensated cirrhosis, who had higher rates than healthy controls (Cazzaniga et al., 2008).

NIRS VOT has been utilised to elucidate the microvascular function in cirrhotic patients by Thompson et al. They hypothesised that an exaggerated hyperaemic response would be seen due to the vasodilated state of cirrhosis. Stable cirrhotic patients were recruited. They had lower resting baseline muscle StO2 – which may represent increased peripheral oxygen extraction or decreased supply of oxygenated haemoglobin. Cirrhotic patients demonstrated significantly larger post-occlusive hyperaemic variables compared to volunteers (17 vs 15% overshoot ($p=0.009$), and the magnitude of change increased with disease severity (Thomson et al., 2010). They also performed serial VOT measurements and found that there was ischaemic adaptability in healthy subjects where rates of deoxygenation decreased and reactive parameters increased. These changes were not present in the cirrhotic patients. This may represent the already vasodilated state of cirrhosis has no further capacity to increase the hyperaemic response.

As yet, the NIRS VOT has not been used to study vascular reactivity in patients with cirrhosis who are hospitalised with decompensation, or during liver transplantation.

4.4 Vascular reactivity in surgery

In surgery NIRS has been utilised to measure the response to fluid and pharmacological treatments. Futier et al investigated the changes in response to hypovolaemia and volume expansion in patients undergoing major abdominal surgery. They demonstrated that hypovolaemia was associated with significant alterations in NIRS variables measured at the thenar eminence and that restoring

intravascular volume significantly improved the StO2 upslope (Futier et al., 2011). They found a 50% increase in STO2 upslope with fluid loading suggesting that restoration of intravascular volume in preload dependent-patients improves muscle tissue oxygenation. There was no significant change in haemoglobin levels before and after the fluid administration. (Futier et al., 2011).

NIRS VOT demonstrated no consistent effect of RBC transfusion on muscle tissue oxygenation or microvascular reactivity in haemodynamically stable critically ill patients (Creteur et al., 2009). In keeping with patients with sepsis, patients with altered baseline microvascular reactivity showed a greater improvement; whilst those with preserved baseline microvascular reactivity showed deterioration.

4.5 Conclusion

Vascular reactivity is part of the regulatory mechanism that ensures that tissue oxygen perfusion and delivery matches demand. Inflammatory disease states including sepsis, critical illness, and cirrhosis can interfere with this balance. Red blood cells themselves may have an influence upon vascular reactivity by acting as local vasodilators by the release of NO.

NIRS provides a basic measure of tissue oxygenation, predominantly reflecting venous haemoglobin oxygen saturation, which cannot determine the function of the microcirculation. Using dynamic tests such as the NIRS VOT, the alterations in oxygen consumption and restoration of flow can provide measures of microcirculatory competence.

This technique has been used in patients with liver disease, which has shown alterations in keeping with the inflammatory vascular dysfunction of cirrhosis. Whilst NIRS has been used to assess the oxygenation of donor liver grafts, it has not been utilised to monitor microvascular reactivity of the recipient during liver transplantation. It has also been used to assess the response to blood transfusions in a variety of situations, but these experiments have not been conducted during liver transplantation.

Chapter 5 Monitoring the microcirculation

5.1 Introduction

Numerous methods have been used to monitor the microcirculation during the perioperative period, and to assess the influence of blood transfusion. Improvements in technology and refinements to earlier techniques have allowed the production of devices that can monitor microcirculatory flow, perfusion and tissue oxygenation at the patient.

In this chapter I shall discuss in further detail the techniques which I have chosen to utilise in my research. As discussed in the previous chapter, utilising NIRS and a vaso-occlusive test can provide an objective measure of tissue oxygen delivery and utilisation. By combining these data with direct observations of the microcirculation at the capillary level can provide information about changes in both vessel density and perfusion. Hand-held videomicroscopy and NIRS both offer non-invasive monitors of the microcirculation that can be used throughout surgery without interfering with the operation. IDF imaging with the Cytocam is the latest generation of videomicroscope with significant imaging improvements over its predecessors and was therefore selected for use in my studies.

5.2 Video microscopy

5.2.1 Orthogonal polarisation spectroscopy

Microscopy of the microcirculation has advanced in sophistication over recent decades. Orthogonal Polarisation Spectral (OPS) was the first generation of videomicroscope that could observe red blood cells flowing within vessels of mucosal beds (Slaaf et al., 1987, Groner et al., 1999). OPS requires the illumination of tissue containing the microcirculation with linearly polarized (and thus in a single plane of direction) green light from around a probe placed over the tissue. The emitted light has a wavelength of 548nm to ensure sufficient optical absorption by deoxyhaemoglobin containing RBCs (Goedhart et al., 2007). An orthogonally (perpendicularly) placed polariser blocks the light reflected from tissues with

unchanged polarisation. Light that has been deflected and scattered in the deeper layers of the tissue changes its polarisation direction and is allowed to pass through the second polariser. This serves as a virtual light source within the depth of the tissue and eliminating the reflected light, imaging on a charge-coupled device (CCD) camera creates an image of the subsurface structures – including the microcirculation (Lindert et al., 2002). The light absorbing RBCs appear as black moving particles within surrounding white tissue, which has reflected away the emitted light. Such studies found that observing sublingual microcirculatory perfusion correlated better with patient outcome from sepsis and shock than standard vital observation measurements (Goedhart et al., 2007).

However, the technique had many limitations to its use including suboptimal images caused by motion artefact and blurring, which made it difficult to observe the individual cells within the vessel and thus measure the blood flow velocities within.

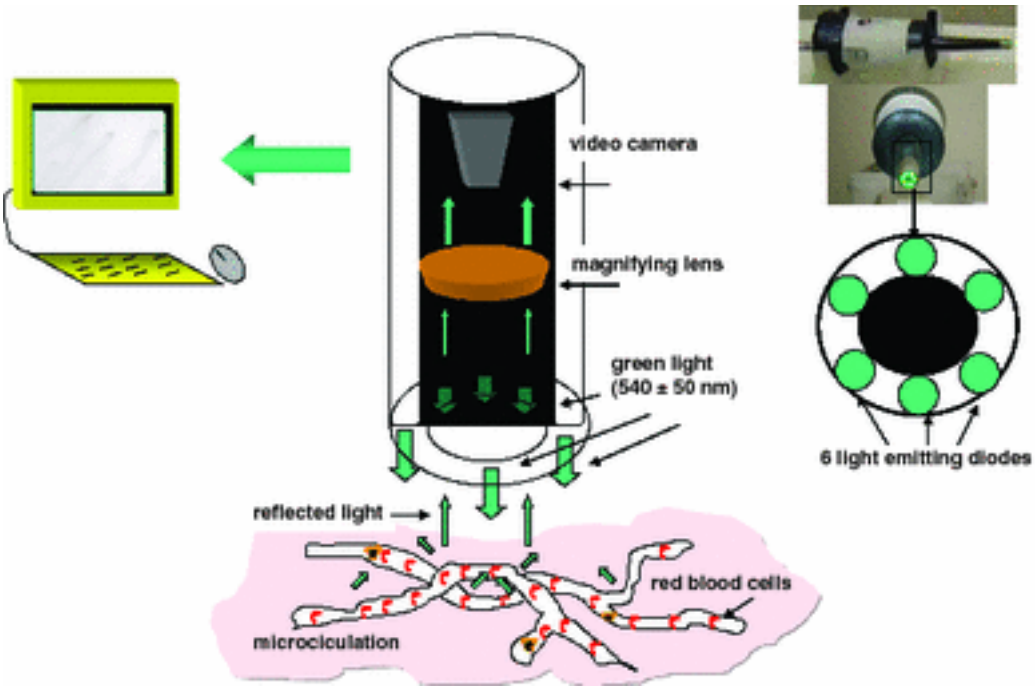
5.2.2 Sidestream dark field imaging

Sidestream Dark Field (SDF) and Incident Dark Field (IDF) microscopy are the successors of this technique with improved image quality in increasingly portable forms. In SDF imaging, green light is emitted from a ring of light emitting diodes (LEDs) surrounding a central light guide at the tip of a microscope probe. The lens is isolated from the illuminating outer ring, preventing image contamination by tissue surface reflections, only allowing light reflected by deeper tissues – to a depth of 3mm – to reach the lens.

The light is emitted at a central wavelength of 530 nm, an isobestic point of oxy and deoxyhaemoglobin to ensure optimal optical absorption by the haemoglobin within the RBC, regardless of the state of oxygenation. The emitted light is absorbed by haemoglobin, and reflected by surrounding tissue, so RBCs are visualised as dark moving images against a grey-white background, moving along the axis of flow. The light from the LEDs is emitted in pulses in synchrony with the CCD frame rate, known as intravital stroboscopy, to improve the image quality by reducing blurring by using short illumination intervals, enabling the imaging of individual RBCs (Goedhart et al., 2007). After passing through the lens, the reflected light is captured

on CCD camera. The image is magnified by a 5x objective lens for human sublingual measurements (De Backer et al., 2007), which is further magnified on-screen up to 380x. (Figure 4)

Figure 4: Sidestream dark field (SDF) imaging



Reprinted by permission from Springer Nature: Springer Nature, Archives of Dermatological Research, Sidestream dark field imaging: the evolution of real-time visualization of cutaneous microcirculation and its potential application in dermatology, Treu, C. et al, 303;2: 69-78, Copyright 2010 (Treu et al., 2010)

5.2.3 Incident Dark Field Spectroscopy

A further refinement to microcirculatory video microscopy has been the development of a device called Cytocam-IDF (incident dark field) (Cytocam, Braedius Medical, Huizen, The Netherlands). This is a handheld video microscope with a high-density pixel-based imaging chip and short pulsed illuminating ring of LEDs around a central light guide with a stepping motor for quantitative focussing. The image acquisition and sensor are under computer control and electronically synchronised to the illumination pulses, projecting the images onto a computer controlled image sensor. The camera has a 3.5-megapixel frame size, an optical magnification factor of 4, producing a field view of 1.55 x 1.16 mm – approximately three times larger

than the fields of the previous generation devices. The quantitative focusing mechanism has an integrated distance measuring system allowing the sensor to be positioned within 2 μm, creating a repeatable focal depth that need not be readjusted on each measurement of the same patient. (Aykut et al., 2015).

The Cytocam camera has the following specifications:(Braedius-Medical, 2015a)

- 4xoptical magnification
- field of view 1.1 x 1.5 mm
- 14-megapixel colour sensor
- 25 fps
- 2ms flash pulse duration
- 2 μm focusing accuracy
- recording 4-5 second images
- Optical resolution 4416 x 3312 pixels

The camera is connected to a dedicated computer that allows image storage and analysis, including using proprietary software (Cytocam tools, Braedius Medical, Huizen, The Netherlands). Recorded files can be analysed automatically or stored and exported.

The IDF video microscope has been demonstrated to produce significantly better images of the sublingual microcirculation than SDF (MicroVision Medical, Amsterdam, The Netherlands) when comparing for image quality and 30% more microvessels than SDF (Aykut et al., 2015). It records 25 frames per second (FPS) and standardly records a 6 second clip, of therefore 150 frames. In a comparative study, all films acquired using the IDF device were judged acceptable for data analysis (Gilbert-Kawai et al., 2016).

5.3 Reporting the microcirculation

SDF and IDF video microscopes produce high quality video images which require analysis for quantitative measurement of the variables used to report on the status of

the microcirculation. Importantly when considering the use of microcirculatory measurement is the time required to analyse the data obtained.

To standardise reporting, a round table conference was organised to discuss the acquisition and reporting of these images, producing a consensus statement using Delphi methodology (De Backer et al., 2007). The recommendations of this statement have standardised the reporting of microcirculation analysis in most studies in this area over the past decade, with over 500 citations. At that point, two scoring systems were used clinically – the De Backer Score (De Backer et al., 2002a) and the Microvascular Flow Index (MFI) (Spronk et al., 2002).

5.3.1 Vessel Density

Microcirculatory density is assessed as the total vessel density (TVD). TVD is calculated by dividing the total length of the vessels divided by the area of vessels within the area of analysis (mm/mm^2).

5.3.1.1 Perfused Vessel Density

The perfused vessel density (PVD) is defined as the total perfused vessel length divided by the total surface area of vessels within the area analysed (mm/mm^2). This provides an estimate of functional capillary density (FCD). FCD is considered the main determinant of microcirculatory blood supply.

5.3.2 The De Backer Score

The De Backer score (DBS) measures three equidistant horizontal and three equidistant vertical lines on the screen, with vessel density calculated by the number of vessels crossing the lines divided by the total length of the lines. Perfusion was then subjectively determined by the proportion of time during which there was flow in the vessels. The proportion of perfused vessels (PPV (%) could then be calculated by 100 x (total number of vessels – {no flow + intermittent flow})/total number of vessels.

Categories of flow are determined by eye as: continuous for at least 20 seconds, intermittent (at least 50% of the time with no flow for 20 seconds) and absent (no flow for 20 seconds).

Perfused vessel density (PVD) could then be calculated by multiplying vessel density by the PPV – giving an estimate of functional capillary density (FCD)(De Backer et al., 2007). 20 μm was the cut-off between small vessels and large vessels. The DBS has minimal intra-observer variability for vessel density and vessel perfusion. If flow is continuous, it takes no account of red blood cell velocity. However, the score is sensitive to isotropy due to manipulation by software, changing the final image magnification from that of the original. If the magnification of the image is altered, as can happen during image stabilisation, the length of the line could be altered.

5.3.3 Microcirculatory Flow Index

The MFI is calculated by dividing the image into four quadrants and assigning a score for flow using an ordinal scale: 0 = absent, 1 = intermittent, 2 = sluggish, 3 = normal. The value of each quadrant is then used to give an average. This has good inter-observer agreement (85% (Kappa score 0.78)) and intra-observer agreement (90% (Kappa score 0.85))(Boerma et al., 2005). However, it does not provide information about FCD in which the number of perfused vessels may decrease but the flow in those remaining appear improved following an intervention, thus ignoring an actual decrease in TVD.

5.3.4 Flow heterogeneity index

The flow heterogeneity index (HI) provides information relating to alternations in distribution of flow and shunting. It is calculated as the highest site flow velocity (as per the MFI) minus the lowest site flow velocity, divided by the mean flow velocity of all sublingual sites at that time point.

5.3.5 Image quality

Utilising the IDF video microscope can be challenging in order to retrieve meaningful data. The operator needs to be well trained in the use of the tool and

techniques to ensure the quality of the images acquired, with five key points for image acquisition (De Backer et al., 2007):

- Five sites per organ

Since the microcirculation is intrinsically variable, several sites of the organ of interest should be averaged - at least three sites that can be reliably evaluated per patients and if possible five sites.

- Avoidance of pressure artefacts

Since capillaries and venules are collapsible they are very sensitive to pressure applied to the organ. Since the microcirculation being analysed sits just below the microscope when applied to the tissue excessive pressure could compress and collapse the microcirculation in the area under investigation, leading to spurious results. This can be identified by decreased flow in large venules (>30 um), with sluggish, absent, alternate or backwards flow recorded.

A technique of applying pressure then withdrawing the microscope being pulled back slowly until contact is lost, then re-advanced until contact is regained is advocated (De Backer et al., 2007).

- Elimination of secretions
- Adequate focus and contrast adjustment
- High quality recording

Secretions can interfere with image acquisition by obscuring the vessels from the microscope and altering the depth of focus required to achieve clear images. This is particularly challenging in intubated and paralysed patients in whom saliva pools within the oropharynx.

Focus should be adjusted so that small vessels in the field of view are optimally focused to permit accurate analysis of vessel diameter, flowrate and capillary density. Out of focus vessels lose contrast and are less visible.

The Cytocam-IDF offers significant improvements to the focussing and auto-contrast adjustment required to obtain good quality images for recording. These are automatically recorded in digital format within the Cytocam tools software where they can be either analysed or exported for analysis within other software in a variety of file formats.

5.3.6 Image analysis

Other challenges of the technique are the analysis of the images acquired. As the quality of images acquired from the microcirculation has improved with the development of IDF imaging (Gilbert-Kawai et al., 2016, Aykut et al., 2015), the optimal method for analysing these images remains undetermined.

The Automated Vascular Analysis (AVA 3.2, Microvision Medical BV, Amsterdam, Netherlands) software was designed for use with the Microscan SDF camera (Microvision Medical BV, Amsterdam, Netherlands). It performs semi-automated pre-processing of images, video stabilisation, vessel tracing, flow scoring and calculates the parameters of microvascular flow discussed previously. This is the gold standard method for analysing microcirculatory video microscopy.

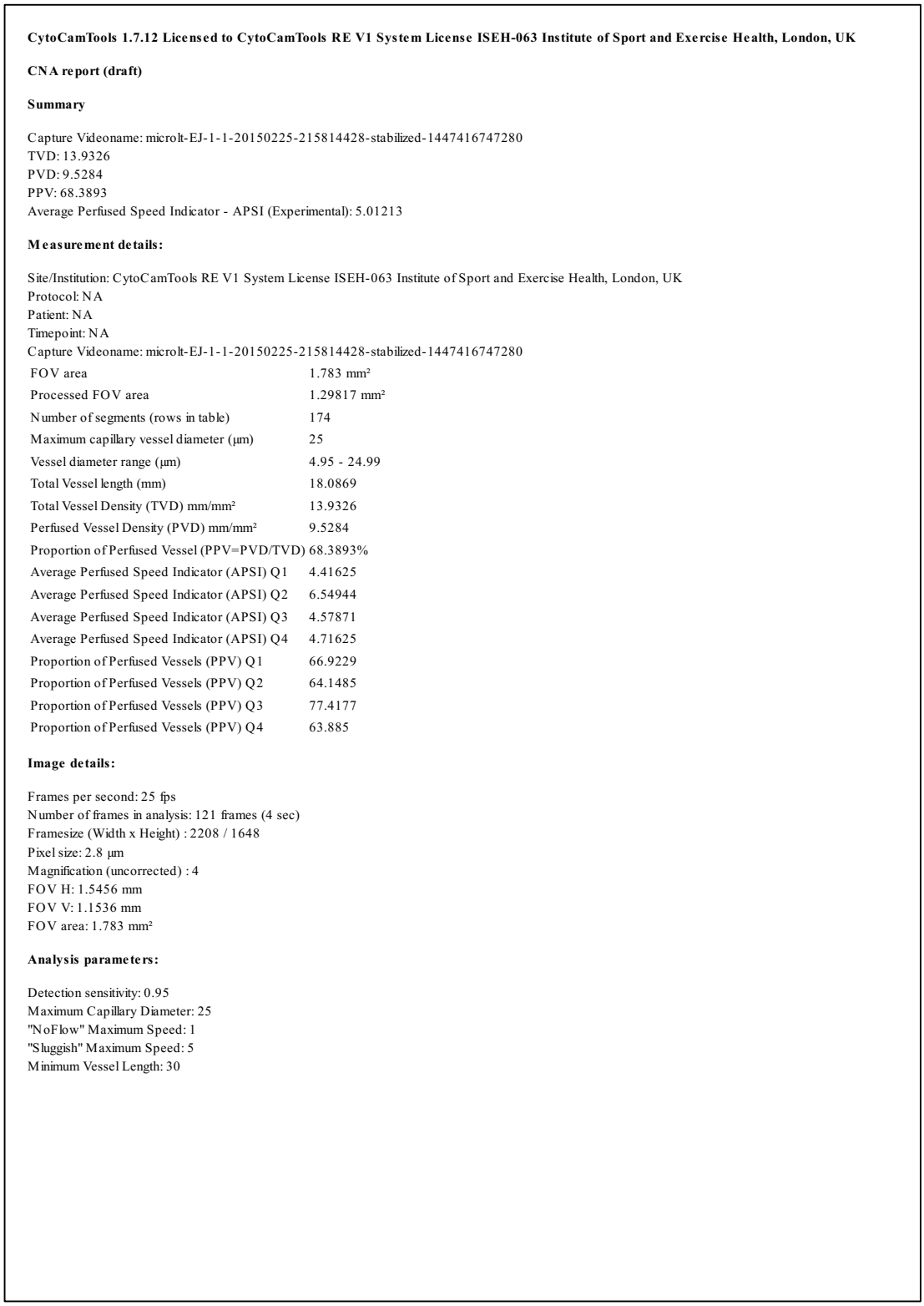
However, the automatic vessel detection algorithms are not accurate enough for reliable image analysis (Massey and Shapiro, 2016). Investigators are required to manually 'draw' vessels for analysis. There is a significant degree of subjectivity in the manual analysis and this is extremely time consuming.

These challenges have contributed to these devices not entering routine clinical practice and remaining a tool of research. The manufacturers have attempted to improve the automation of image analysis with the proprietary software in the CytoCam computer (Cytocam Tools 1.7.12, Braedius Medical, Huizen, Netherlands).

The manufacturers of the Cytocam state that "the CytoCam is equipped with an application for direct microcirculation assessment where the images are recorded digitally and analysed automatically." (Braedius-Medical) CytoCam tools is a fully automated analysis programme that allows no manual input in the way that AVA 3.2 allows. A report is generated containing the variables of microcirculatory flow

(Figure 5). At present there is limited data to validate such automated systems (Carsetti et al., 2017).

Figure 5: Cytocam Tools analysis report



CytoCamTools 1.7.12 Licensed to CytoCamTools RE V1 System License ISEH-063 Institute of Sport and Exercise Health, London, UK

CNA report (draft)

Summary

Capture Videoname: microlt-EJ-1-1-20150225-215814428-stabilized-1447416747280
TVD: 13.9326
PVD: 9.5284
PPV: 68.3893
Average Perfused Speed Indicator - APSI (Experimental): 5.01213

Measurement details:

Site/Institution: CytoCamTools RE V1 System License ISEH-063 Institute of Sport and Exercise Health, London, UK
Protocol: NA
Patient: NA
Timepoint: NA
Capture Videoname: microlt-EJ-1-1-20150225-215814428-stabilized-1447416747280

FOV area	1.783 mm²
Processed FOV area	1.29817 mm²
Number of segments (rows in table)	174
Maximum capillary vessel diameter (µm)	25
Vessel diameter range (µm)	4.95 - 24.99
Total Vessel length (mm)	18.0869
Total Vessel Density (TVD) mm/mm²	13.9326
Perfused Vessel Density (PVD) mm/mm²	9.5284
Proportion of Perfused Vessel (PPV=PVD/TVD)	68.3893%
Average Perfused Speed Indicator (APSI) Q1	4.41625
Average Perfused Speed Indicator (APSI) Q2	6.54944
Average Perfused Speed Indicator (APSI) Q3	4.57871
Average Perfused Speed Indicator (APSI) Q4	4.71625
Proportion of Perfused Vessels (PPV) Q1	66.9229
Proportion of Perfused Vessels (PPV) Q2	64.1485
Proportion of Perfused Vessels (PPV) Q3	77.4177
Proportion of Perfused Vessels (PPV) Q4	63.885

Image details:

Frames per second: 25 fps
Number of frames in analysis: 121 frames (4 sec)
Framesize (Width x Height) : 2208 / 1648
Pixel size: 2.8 µm
Magnification (uncorrected) : 4
FOV H: 1.5456 mm
FOV V: 1.1536 mm
FOV area: 1.783 mm²

Analysis parameters:

Detection sensitivity: 0.95
Maximum Capillary Diameter: 25
"NoFlow" Maximum Speed: 1
"Sluggish" Maximum Speed: 5
Minimum Vessel Length: 30

Chapter 6 Validation of Incident Dark Field (IDF) image analysis with CytoCam software and Automated Vascular Analysis software

6.1 Introduction

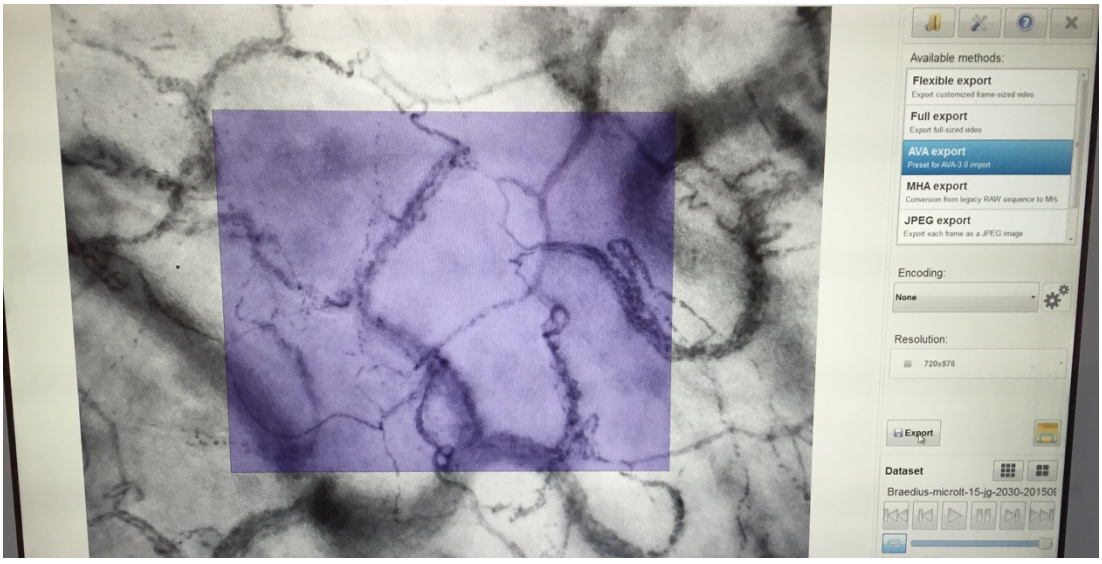
Prior to commencing my analysis of data recorded with the CytoCam-IDF I performed a comparison of the results from the CytoCam Tools 1.7.12 automated analysis with manually analysed films using AVA 3.2 as the usual gold standard method.

6.2 Methods

For the first 5 patients' data recorded with the IDF video microscope from the tests in the assessment of the microcirculation in liver transplantation (see Chapter 7 and 8) all films were compared, yielding 19 comparisons.

Each file was exported from the CytoCam tools into an AVA compatible file. (Figure 6)

Figure 6: Export from CytoCam Tools to AVA



Note that the lilac box representing the portion of the complete field captured by the CytoCam that is exported to AVA results in a much smaller image for analysis

I then analysed each of the 19 films using the CytoCam Tools software CNA approach.

Using the analysis dialogue within CytoCam Tools, each video was individually selected, then stabilised, then analysed. The software denotes vessels less than 25 um as small vessels. The analysis report is then exported as a pdf (as above) or in a CSV file for spreadsheet input. This process was repeated for each video file. Results from these analyses were not compared until all evaluations had been completed.

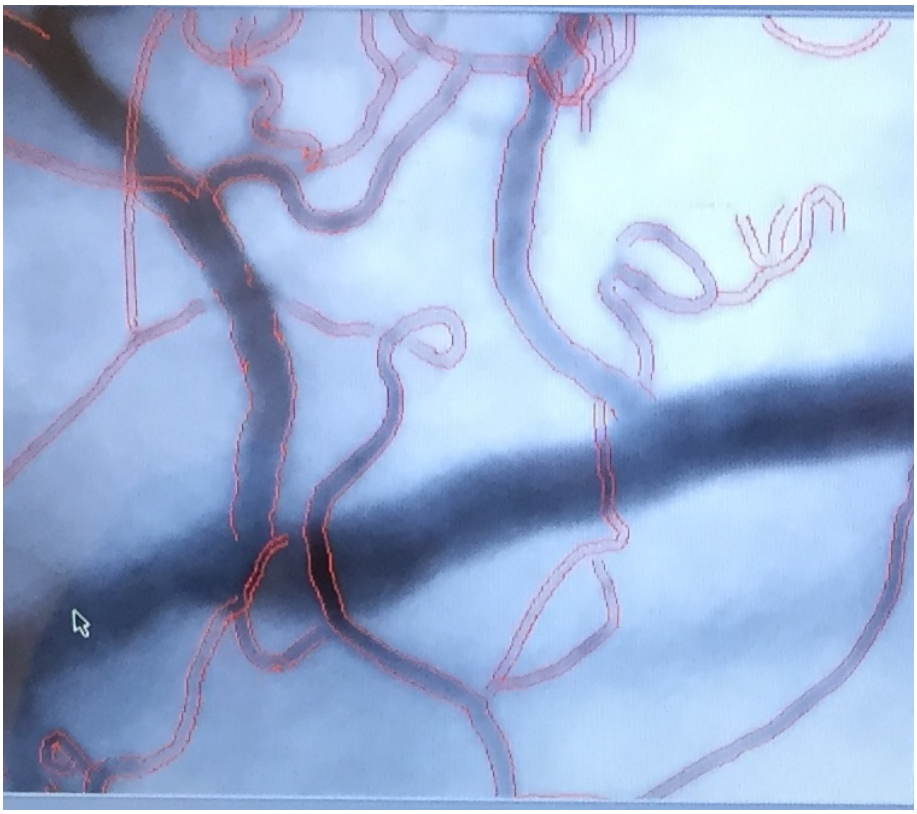
6.2.1 AVA analysis

Using the AVA 3.2 software package, the video fragment was selected and the contrast was enhanced manually to increase definition of haemoglobin from the background tissue. The absorption of emitted light by haemoglobin carrying RBCs provides the outline of each vessel by virtue of containing blood, meaning that the vessel itself is not shown, but a 'silhouette' created by the RBCs within.

The software uses the gradient of change in colour to determine the edge of vessels for automated analysis. A poorly focused image will create a blurred image which causes the vessel to appear wider than it actually is.

Stabilisation of the image is then performed, whereby the software produces a composite image of each frame of the video file, compensating for movement artefact and time-averaging of each frame to fill interruptions in the pseudo-vessel image caused by plasma gaps or WBCs, where the IDF light is not absorbed, that would appear using single frames (Dobbe, 2007).

Figure 7: AVA screenshot with vessel segments outlined

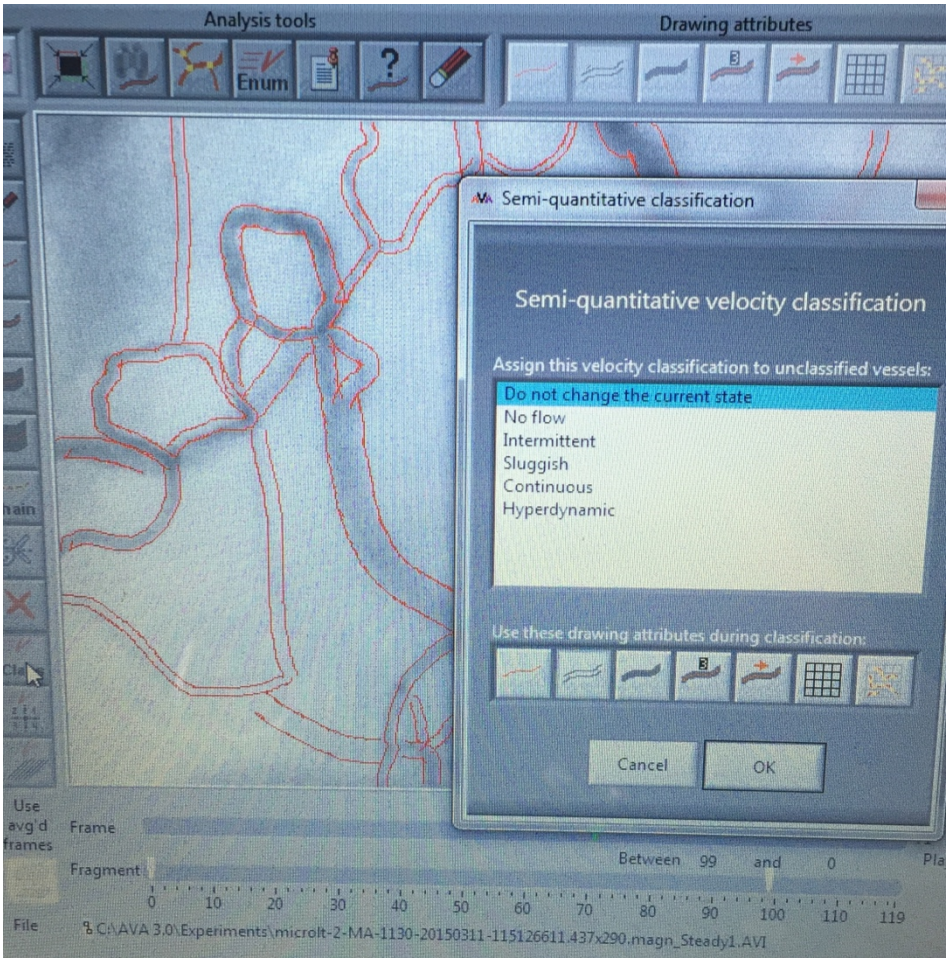


The averaged image can then be automatically analysed by the software to detect blood vessels. Vessels segments are automatically detected and the focus threshold adjusted to exclude vessel segments that our out of focus or spurious due to image noise. However, the results are inaccurate, incorrectly identifying spurious vessels and failing to include other vessels. AVA allows manual analysis whereby incorrectly detected vessel segments can be deleted and undetected segments manually traced. Segments of vessels could then be chained together when incorrectly disconnected, or unchained where inaccurately connected. This was performed for every image. (Figure 7)

Velocity was subjectively assigned to each vessel in a vessel-by-vessel approach using the AVA software. Velocity, in a semi-quantitative manner, was graded: 0 = no flow, 1 = intermittent flow, 2 = sluggish flow, 3 = continuous flow and 4 = hyper dynamic flow. The software could then generate the % of each flow classification per vessel type: small 0 - <25 μm, medium 25- 50 μm and large 50-100 μm (as well as very large >100 μm). An overlaid grid then divided the image into quadrants so that

the flow is scored per quadrant based upon the predominant type of flow, per vessel size. This allowed calculation of the MFI and is used to calculate the HI. (Figure 8)

Figure 8: AVA Semi-quantitative velocity classification



The results were then exported for analysis.

6.2.2 CytoCam Tools analysis

As previously mentioned, the CytoCam Tools software is automated and does not allow the manual tracing of vessels in the same manner as AVA. Films are cropped to a suitable length, then image stabilised to remove movement artefact before vessel detection occurs. CytoCam Tools offers a Capillary Network Analysis (CNA) or De Backer Score (DBS) method of analysis. CNA detects each vessel individually. The DBS method divides the screen using 3 horizontal and 3 vertical lines and detects the number of vessels crossing these lines, producing a score in n/mm. Liaison with the company stated that I should not use the DBS analysis since their algorithm was

creating 'undetermined' results. By default, CytoCam Tools only detects vessels smaller than 25 μm.

The CytoCam report included TVD, PVD and PPV values. Thus, CytoCam CNA results for small vessels (<25 μm) were compared with AVA results for small vessels where I had manually detected all vessels and subjectively assigned the semi-quantitative flow velocities.

6.2.3 Statistical analysis

Results are presented as mean (standard deviation). Discrepancies between the two approaches were compared using intra-class correlation coefficient.

6.3 Results

19 videos from 5 patients were analysed.

The mean value of TVD was 17.68 (3.38) mm/mm^2 for CCT and 12.78 (2.02) mm/mm^2 for AVA. A paired samples T-test for TVD demonstrated a significant difference between the mean TVDs (p=<0.001). The mean value of PVD was 10.55 (1.81) mm/mm^2 for CCT and 12.50 mm/mm^2 for AVA. The mean value of PPV was 60.81 (10.47) % for CCT and 86.58 (11.51) % for AVA. (Table 8) (Figures 9-11)

Table 8: Comparison of CytoCam Tools software with Automated Vascular Analysis software

Measure	Intra-class correlation coefficient (95% CI)
Total vessel density (TVD)	.122 (-.096 – .427)
Perfused vessel density (PVD)	.205 (-.125 - .550)
Proportion of perfused vessels (PPV)	-0.039 (-.143 – 0.165)

Figure 9: Scatterplot of total vessel density (TVD) for CytoCam Tools vs Automated Vascular Analysis

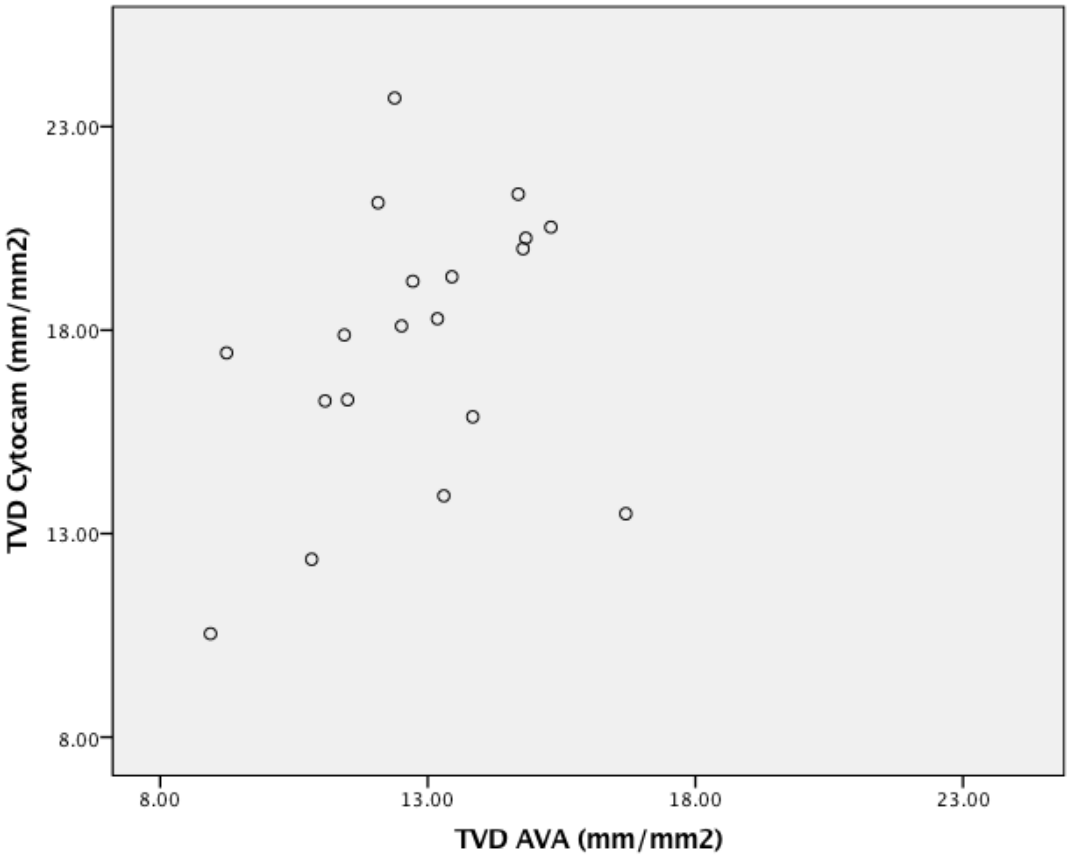


Figure 10: Scatterplot of perfused vessel density (PVD) for CytoCam Tools vs Automated Vascular Analysis

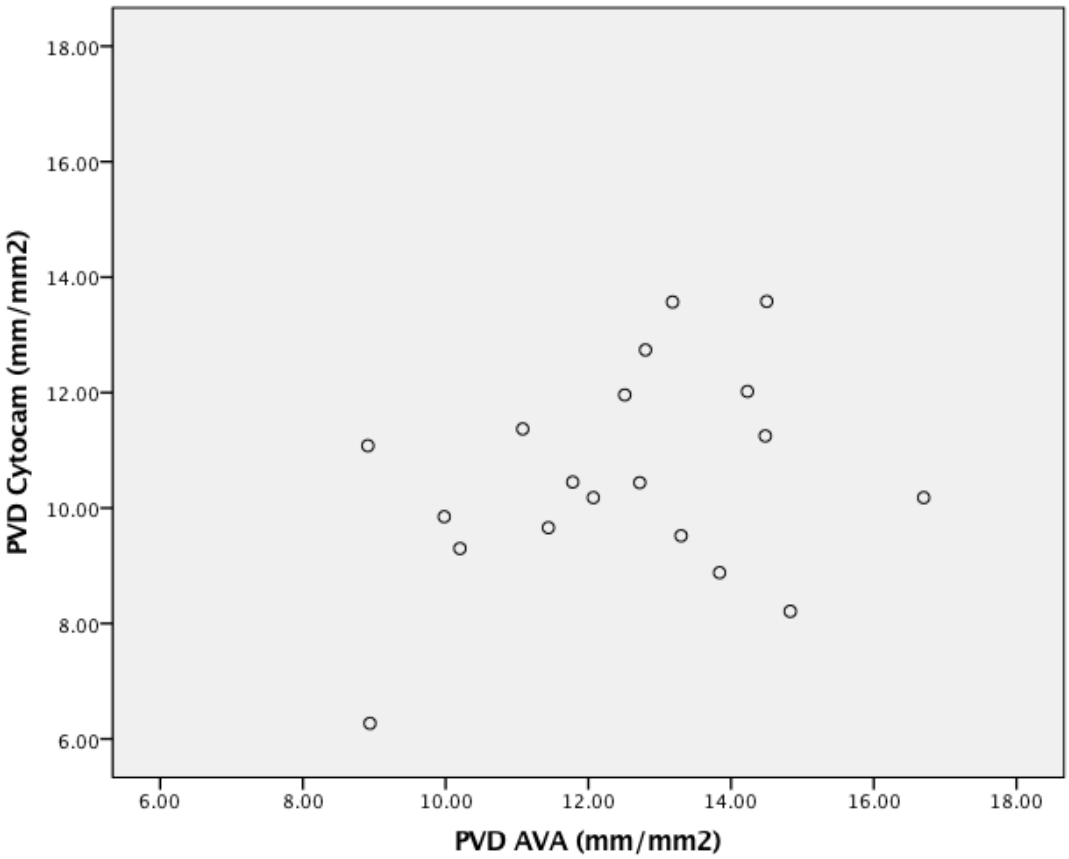
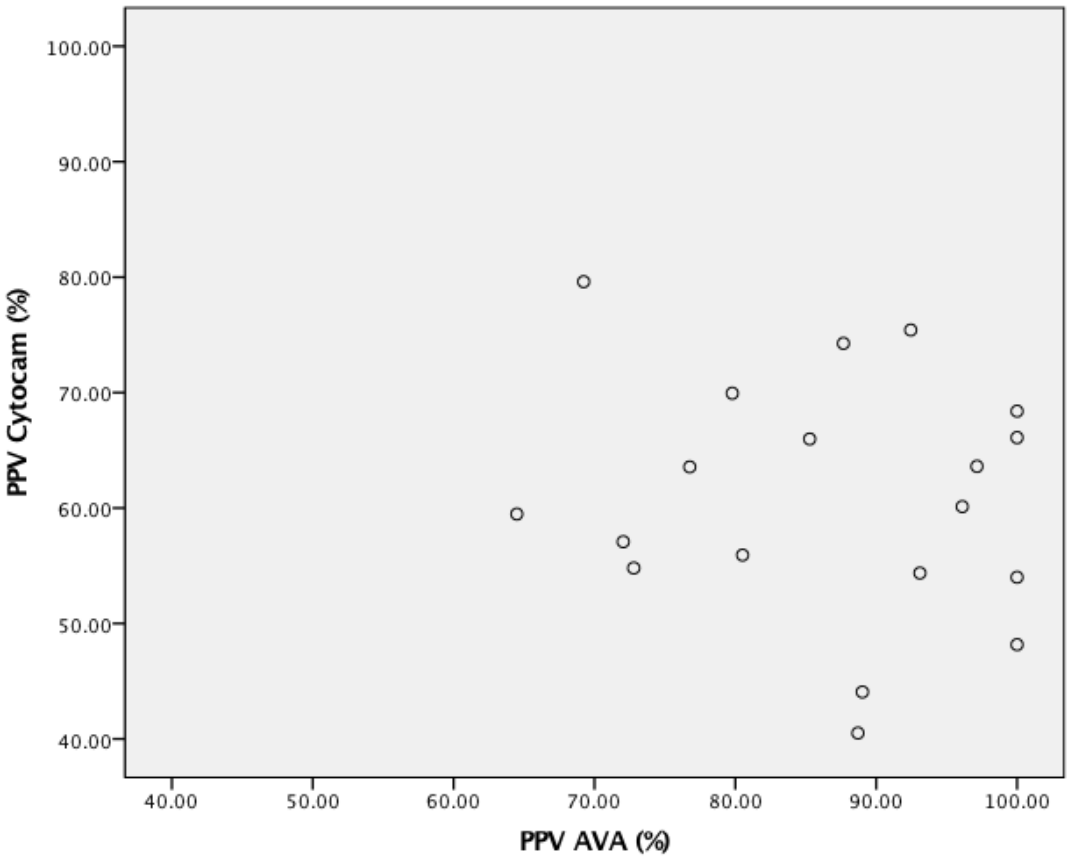


Figure 11: Scatterplot of proportion of perfused vessels (PPV) for CytoCam Tools vs Automated Vascular Analysis



6.4 Discussion

These results demonstrate very poor correlation between the two analysis tools for all of the measures. The variables account for the detection of vessels measured by TVD; and the flow within these vessels, measured by PVD and PPV.

This is unsurprising given the different analysis techniques are utilising two entirely different videos, even though obtained from the same source. It is notable that when exporting files recorded by the CytoCam to AVA, a significant loss of area of focus is demonstrated (see Figure 6). It is well recognised that the IDF camera has a much larger field of view due to its larger image sensor (Aykut et al., 2015), when compared to the Microscan SDF camera for which AVA was designed to be used.

The difference in TVD is significant. Thus, despite analysing the same film, the area being analysed by CCT is much larger, whilst AVA contains only the central portion of the film. This may be advantageous to the AVA user since image acquisition errors such as pressure artefact are most likely to be evident at the edge of the image rather than the centre.

However, despite this there was no linearity between the results, most likely due to the mass overestimation of vessels by automated systems compared to a manual tracing of vessels.

Flow within vessels is determined by completely different methodologies between the two programmes. AVA uses a manually assigned value to categorise whether a vessel is perfused (assigned category 2 (sluggish) or 3 (continuous) or not perfused (assigned category 0 (no flow) or 1 (intermittent), and calculates the area of these indices within each film. CytoCam Tools calculates the proportion of perfused vessels using a dimensionless relative number derived from the intensity variation along the centre line of a vessel, which describes the speed detected in the vessel; denoted SI (speed indicator) (Braedius-Medical, 2015c). The total length of all vessels with an SI higher than a threshold value are then divided by the processed area. This technique and its underlying algorithm has not been externally validated.

The PPV results are extremely dissimilar with extremely poor correlation. These results are similar to those found to those found by Carsetti et al in a comparison of AVA 3.2 with CCT (Carsetti et al., 2017).

This is disappointing since the timescales for analysis are considerably different between the two. Carsetti et al showed that the analysis time with CCT was 2:42 minutes: seconds (IQR 2:12, 3:31) versus 16:12 minutes: seconds (IQR 13:38, 17:57) with AVA ($p<0.001$). A truly automated system would certainly increase the acceptability of using these techniques at the bedside for routine monitoring, with rapidly turned around results upon which to base therapy and assess the response. At present, the need to manually analyse films taking more than a quarter hour per film, means that for a single measurement (i.e. taking three to five videos at different sites on an organ at a single time point) an hour would be needed to analyse the data,

reducing its applicability for active patient interactions and limiting it to simply record parameters at a historical time point in the patient's care.

6.5 Conclusion

Despite the CytoCam manufacturers' claims as to the improvements to microcirculatory analysis with CCT over existing software the correlation between the outputs from these methods are extremely poor. There remains minimal published data using the CCT software and the AVA software remains the gold standard for analysing the microcirculation. In light of these findings I decided that it was not acceptable to use the automated CCT for analysis of my data, but to continue to use the validated AVA method.

Chapter 7 The effect of blood transfusion on sublingual microcirculation in liver transplantation: an observational study

7.1 Introduction

A robust body of data has accumulated to associate anaemia and blood transfusion as independent variables for adverse outcomes after liver transplantation (Ramos et al., 2003, de Boer et al., 2008, Boin et al., 2008). Meanwhile, alterations in microcirculatory flow have been demonstrated to be associated with outcomes after major surgery and disease states such as sepsis (Sakr et al., 2004, De Backer et al., 2002b, De Backer et al., 2009). Data from the microcirculation of patients during liver transplantation may help to quantify the physiological changes intraoperatively, and record the effects of anaemia, haemorrhage and blood transfusion upon post-operative outcomes. The effect of anaemia and transfused RBCs upon the microcirculation may help to guide the pre-operative optimisation of haemoglobin mass and guide blood transfusion thresholds intraoperatively as the principles of patient blood management are applied to this surgical population.

This is the first time that such variables have been recorded during liver transplantation surgery.

7.2 Study aims and hypotheses

The primary research aim was to measure the effect of blood transfusion upon the microcirculation during liver transplantation. The primary outcome was the change in SDF variables before and after blood transfusion: total vessel density (TVD), perfused vessel density (PVD), proportion of perfused vessels (PPV), microcirculatory flow index (MFI) and heterogeneity index (HI).

Secondary outcomes were: changes in SDF variables from baseline to the first transfusion (representing surgical bleeding).

This may help to determine the optimum transfusion threshold for these patients, and allow optimisation of micro-circulatory parameters of oxygen delivery in addition to

those of the macro-circulation already measured in routine practice. I hypothesised that transfusion of packed red blood cells would increase the total vessel density. I hypothesised that the microcirculation blood vessel density would decrease as the haemoglobin decreases intraoperatively (from baseline to the need for transfusion) due to the de-recruitment of capillary networks.

7.3 Methods

7.3.1 Study design

This single centre observational study of the microcirculation in patients undergoing Orthotopic Liver Transplantation (OLT) took place at the Royal Free Hospital, London. Patients were managed according to standard intraoperative care at that institution by an anaesthetic team led by a liver transplant consultant anaesthetist. In addition to routine intraoperative data measurement and recording for OLT, sublingual microcirculatory data was collected and analysed at set time points during the operation beginning after the induction of anaesthesia until the end of surgery:

1. Baseline (after induction of anaesthesia)
2. Hourly thereafter during Dissection (phase 1)
3. Anhepatic (phase 2)
4. Pre-reperfusion
5. 5 minutes Post-reperfusion (phase 3)
    a. Hourly thereafter
6. End of surgery (closure of skin)
7. Any Transfusion Event
    a. Pre-transfusion (if not already measured)
    b. 30 minutes post transfusion

7.3.2 Ethical approval

The study design, documentation and protocols were in accordance with the Declaration of Helsinki, and were approved by the Research Ethics Committee of the East of England – Essex (Reference 14/EE/1275).

7.3.3 Participant selection and recruitment

Participants were patients presenting for liver transplantation surgery at the Royal Free Hospital, London. Participants were recruited upon attendance for their assessment for addition to the transplant waiting list. They were given a participant information sheet on admission. At this point, patients were routinely consented for their transplant operation, prior to being told if they had been deemed eligible for addition to the transplant waiting list.

Those patients already on the waiting list who had not been consented were sent a patient information sheet by post or email, which was followed up by a telephone call giving the patient opportunity to ask any questions and give an indication of their feelings towards further involvement in the study. If they expressed an interest they would be approached to give consent at the transplant outpatient clinic (attended every 1-3 months) or, in the event that it took place before their next appointment at this hospital, on attendance for their operation.

7.3.3.1 Inclusion criteria

Participants must have been willing and able to give informed consent for participation in the study, male or female aged 18 years or above. They were either referred for assessment for addition to the active liver transplant waiting list, or on the waiting list at the Royal Free Hospital, London.

7.3.3.2 Exclusion criteria

Those adults unable to give informed consent, having combined liver and other organ transplant, a transplant for symptomatic reasons only in cholestatic liver disease, pregnant patients and prisoners were excluded from the study.

7.3.4 Sample size

The primary outcome if interest is total vessel density after blood transfusion. A previous study by Yuruk et al investigating the effect of blood transfusion upon the microcirculation during cardiac surgery showed that RBC transfusion significantly increased functional capillary density (FCD) from 10.5 (1.2) to 12.9 (1.2) mm/mm2

($p<0.01$). Using these values to calculate a sample size using an 80% power and 5% significance level, 11 patients would be required to detect an increase in total vessel density of 1 mm/mm2. The sample size calculation was performed using an online sample size calculator (Institute, 2018). Adjusting for a non-parametric test, 12.7 patients would need to be recruited. A decision was made to recruit 20 patients to the study to allow for transplants without transfusion and dropout.

7.3.5 Investigations

The following measurements were taken at each time point during the study:

1. IDF microcirculation in at least 3 to 5 different areas sublingually.
2. NIRS VOT (see Chapter 9)

Alongside these measurements, the following routinely measured tests and observations were taken:

1. ABG (Rapidlab 1200, Siemens AG, Munich, Germany)
2. FBC (PocH-100i, Sysmex, Illinois, USA)
3. INR (Haemochron Signature, ITC, New Jersey, USA)
4. TEG {Native/Heparinase} (TEG 5000, Haemonetics, Massachusetts, USA)

Haemodynamic parameters:

1. FiO2, SpO2 (%)
2. MAP, SBP (mmHg)
3. Heart Rate
4. C.O (Pulmonary artery catheter (PAC))
5. CVP (cm H_20)
6. IVC (cm H_20)
7. Urine output (ml)
8. Vasopressor requirement

Using the Vigilance II Cardiac Output monitor (Edwards Lifesciences, Irving, California, USA) the patient's height and weight was entered, with entry of haemodynamic variables from the anaesthetic monitor at each time point would provide calculation of the patient's SVR, SVRI, CO and CI.

Fluids administered

1. RBC / Platelets / FFP
2. Colloid
3. Crystalloid
4. Cell Salvage RBCs

7.3.6 Conduct of anaesthesia

All patients were cared for by a consultant liver transplant anaesthetist with a liver transplant fellow and operating department practitioner assisting. An additional biomedical scientist was in attendance for all cases for the conduct of point of care monitoring and cardiac output monitoring. Conduct of anaesthesia was as routine practice for this operation at our institution.

All patients were pre-medicated with Temazepam 10mg on the ward prior to transfer to theatre. On arrival in theatre, patients were pre-oxygenated in 100% oxygen using a face mask and intravenous access was secured. Induction of anaesthesia was intravenous using propofol and fentanyl, followed by atracurium as muscle relaxant. Following endotracheal intubation arterial and central venous access was placed. Routine access at our institution includes: radial arterial line, femoral arterial and femoral central venous line, and internal jugular 4-lumen CVP line, rapid infuser sheath and Swan sheath. A pulmonary artery catheter was then floated via the Swann sheath and transduced via a Vigilance II monitor (Edwards Lifesciences, Irving, California, USA).

At this point baseline blood samples were taken, and simultaneous microcirculatory recordings were taken.

Anaesthesia was maintained with volatile anaesthetic agents (isoflurane or desflurane) in oxygen and air, and in all cases an infusion of fentanyl was continued throughout the operation alongside a continuous infusion of atracurium to maintain muscle relaxation.

7.3.7 IDF imaging protocol

Sublingual microcirculation was visualised using a IDF video microscope (Braedius Medical, Huizen, The Netherlands). Six second images were obtained in anaesthetised patients during at the time points previously noted during the operation (Chapter 8.3.1) by best practice for obtaining adequate images (Massey et al., 2013):

1. A single use disposable sterile cover was placed over the non-invasive IDF video microscope probe.
2. Pooled secretions were gently mopped using gauze swabs.
3. The probe was gently inserted into the patient's mouth in the region of the frenulum with the tip in contact with the sublingual mucosa
4. The probe was moved until the microcirculation was visible on the monitor, with care to avoid causing pressure between the endotracheal tube (ETT) and video microscope.
5. The probe was slowly advanced until flow was partially or completely hindered.
6. The probe was then gently retracted from the sublingual surface until the on-screen image was lost due and contact with the tissue was lost.
7. The probe was then gently re-advanced until contact was made to avoid pressure artefact; the optimal point being at which the probe is just about to lose contact with the mucosa when being withdrawn.
8. Image focus could be adjusted via the Cytocam computer to provide optimal focus upon those vessels most of interest – small vessels. Focussing aimed to make those vessels in which RBCs were passing single-file in best focus.
9. Six seconds of video were then captured onto the Cytocam computer.

This process was repeated until 5 good quality recordings had been acquired at different sites within the sublingual mucosa.

The figure below shows the intraoperative setup. At the head end of the operating table I was able to make all intraoperative IDF measurements with the Cytocam at the time points throughout the operation by wheeling the CytoCam to the patient's head and taking the sublingual videos as per the above protocol. I could select the optimum images at that point and export them using the Cytocam Tools software onto a portable hard drive for analysis on a separate laptop containing the AVA software.

Figure: Intraoperative Setup

Intraoperative setup: L-R of photo: patient (head end of bed and drapes), infusion pumps, anaesthetic machine monitor, anaesthetic machine with cardiac output monitor and NIRS monitor on top, wheeled trolley with Cytocam (IDF probe and sterile cap attached).

7.3.8 IDF data analysis

Data recorded on the Cytocam camera could be directly analysed by the inbuilt CytocamTools software, or exported for analysis using other software such as Automatic Vascular Analysis (AVA), discussed in Chapter 4. AVA remains the gold standard for analysis of microcirculatory images and after assessing the feasibility of utilising the inbuilt CCT software (Chapter 5) I utilised this for my analyses.

All films were exported for AVA analysis.

The video files were then randomly anonymised numerically to blind myself as the assessor as the time point within the operation at which they were recorded.

Films were then loaded in to the AVA 3.2 (Microvision Medical, Amsterdam, Netherlands) software for analysis. Once loaded, films were first optimised for contrast. Films were then set to the maximum number of frames and then stabilised to compensates for movement artefact by time-averaging each film to fill interruptions in the vessels.

Films which were of inadequate quality due to: poor illumination, unacceptable focus whereby no small vessels could be seen, artefacts or more than 30-50% of vessels being looped upon themselves, where movement was more than ½ of the field of view and motion blurring or signs of pressure impeding flow in large vessels; were excluded as per the Microcirculation Image Quality Score (MICS) (Massey et al., 2013). 5 videos (and a minimum of 3 videos) of different sites per time point were analysed per patient using AVA. Values for TVD, PVD, PPV, MFI and HI were calculated (see chapter 4).

7.3.9 Statistical analysis

Statistical analysis was performed using Microsoft Excel (Microsoft, USA) and SPSS Statistics 24 (IBM, USA). Normal distribution was confirmed by a Shapiro-Wilk test ($p>0.05$) and visual inspection of data histograms and Q-Q plots. A non-normal distribution was evident for the measures of the microcirculation, so non-parametric tests were utilised, with values summarised by median and interquartile ranges or mean and standard deviation as appropriate. Differences between time points were analysed using related samples Friedman's Two-way Analysis of Variance by ranks test and between related samples with Wilcoxon Signed Rank test with Bonferroni correction applied. Comparisons between groups at different time points were performed using unpaired T-tests or Mann-Whitney U tests as appropriate. Results are presented as box-whisker plots (boxes represent median and interquartile range, whilst whiskers represent 5% and 95% limits with outliers represented as dots). Correlation between microcirculation and other physiological

variables was assessed using Spearman's rank correlation. In all cases a P value <0.05 was considered to be statistically significant.

7.4 Results

7.4.1 Patients

20 patients (14 male, 2 female) were studied. Mean age was 55.40 (SD 10.81) years. Aetiology and comorbidities are listed in tables 9 and 10. All patients with hepatocellular carcinoma had cirrhosis.

Table 9: Aetiology of liver disease

Aetiology	N (%)
3 autoimmune hepatitis	3 (15)
9 alcoholic liver disease (ALD) - 1 with HCC	9 (45) - 1 (5)
1 Hepatitis B	1 (5)
1 Hepatitis B + D	1 (5)
3 Hepatitis C cirrhosis (with Hepatocellular Carcinoma (HCC))	3 (15)
1 Non-Alcoholic Steatohepatitis	1 (5)
2 Primary Sclerosing Cholangitis	2 (10)

Table 10: Coexisting conditions

Diagnosis	n
Type II diabetes	2
Insulin dependent diabetes	1
Hypertension	2
Chronic renal failure	1
Hepatopulmonary syndrome	2

12 patients underwent caval cross clamp (CCC) hepatectomy (two of which were a split graft), and 8 patients had a caval piggyback (PB) (one of which was a redo OLT) (Llado and Figueras, 2004).

Mean pre-operative blood pressure was 118 (13.07) / 73 (9.69) mmHg; with a mean HR of 76 (12.26) BPM. Mean height 167 cm (13) and weight 73.6 kg (18.02). Baseline data is presented in Table 11. There was a significant difference in the blood pressure readings in those patients who were transfused and those who were not (P=0.009).

Table 11: Baseline Characteristics

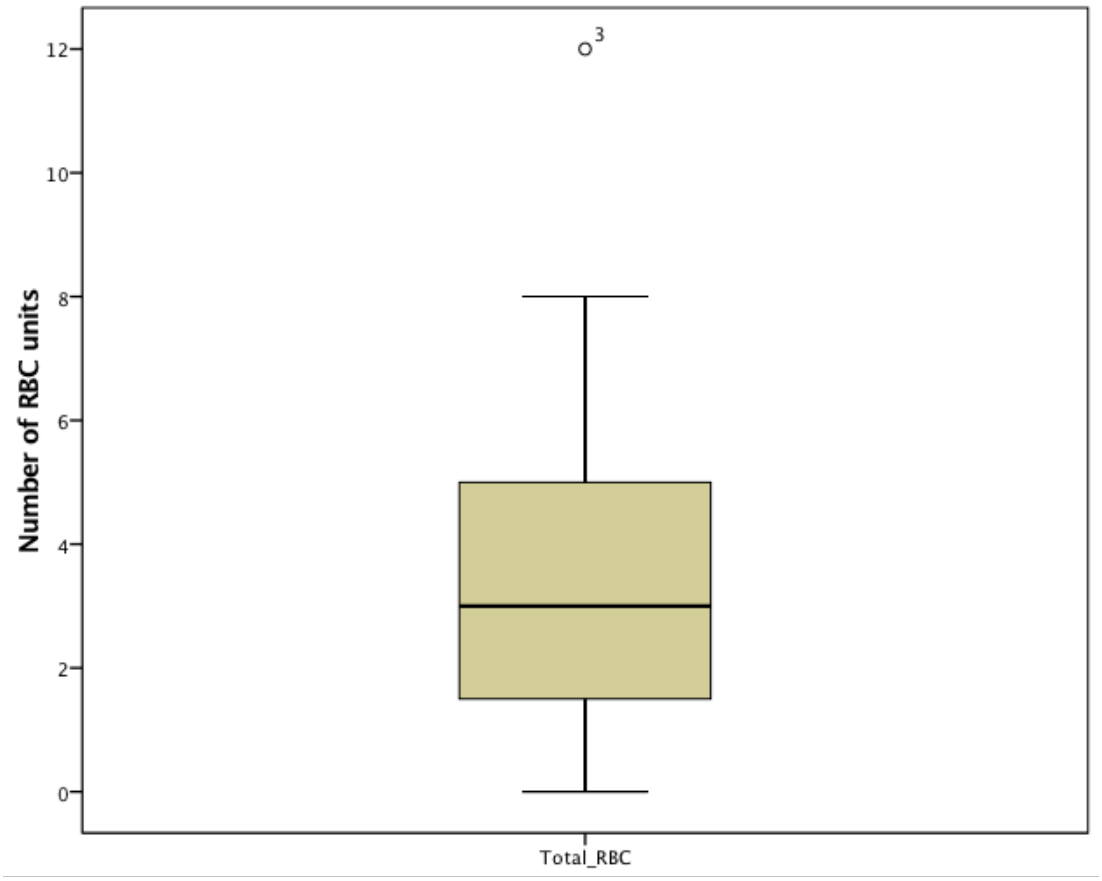
	All patients n=20	Not transfused n=3	Transfused n=17	P
SpO2	98.3 (1.7)			
Systolic / diastolic BP	102 (17.7) / 51.2 (11.2)	82 (4.6) / 41 (4.6)	107 (17.3) / 53.4 (11.7)	0.008* / 0.039*
MAP	70.5 (14.7)	54.3 (2.3)	74.2 (14.7)	0.009*
HR	72.8 (15.1)	68.7 (14.0)	73.5 (15.7)	.560
SVR	684.7 (294.2)	565.0 (155.2)	720.9 (326.8)	.459
SVRI	1247.65 (513.9)	993.67 (161.2)	1319 (574.4)	.368

SV	103.3 (26.8)	99.7 (24.8)	104 (27.9)	.859
CO	7.9 (1.6)	7.0 (1.5)	7.9 (1.6)	.223
CI	4.3 (0.9)	3.8 (1.0)	4.3 (1.0)	.368
Platelet count	86.4 (70.8)	81.3 (63.1)	92.2 (77.4)	.874
INR	1.6 (0.6)	1.4 (0.2)	1.7 (0.6)	.789
Hb	94.35 (19.8)	113.67 (19.4)	88.47 (17.7)	.09
HCT	0.288 (.06)	.346 (0.06)	.271 (0.05)	.101
Lactate	1.74 (.5)	1.28 (0.1)	1.80 (.5)	.050

7.4.2 Effect of transfusion

17 of the 20 patients were transfused RBCs (median 3 units, range 0-12 units) (IQR = 1.25 – 5 units) (Figure 12).

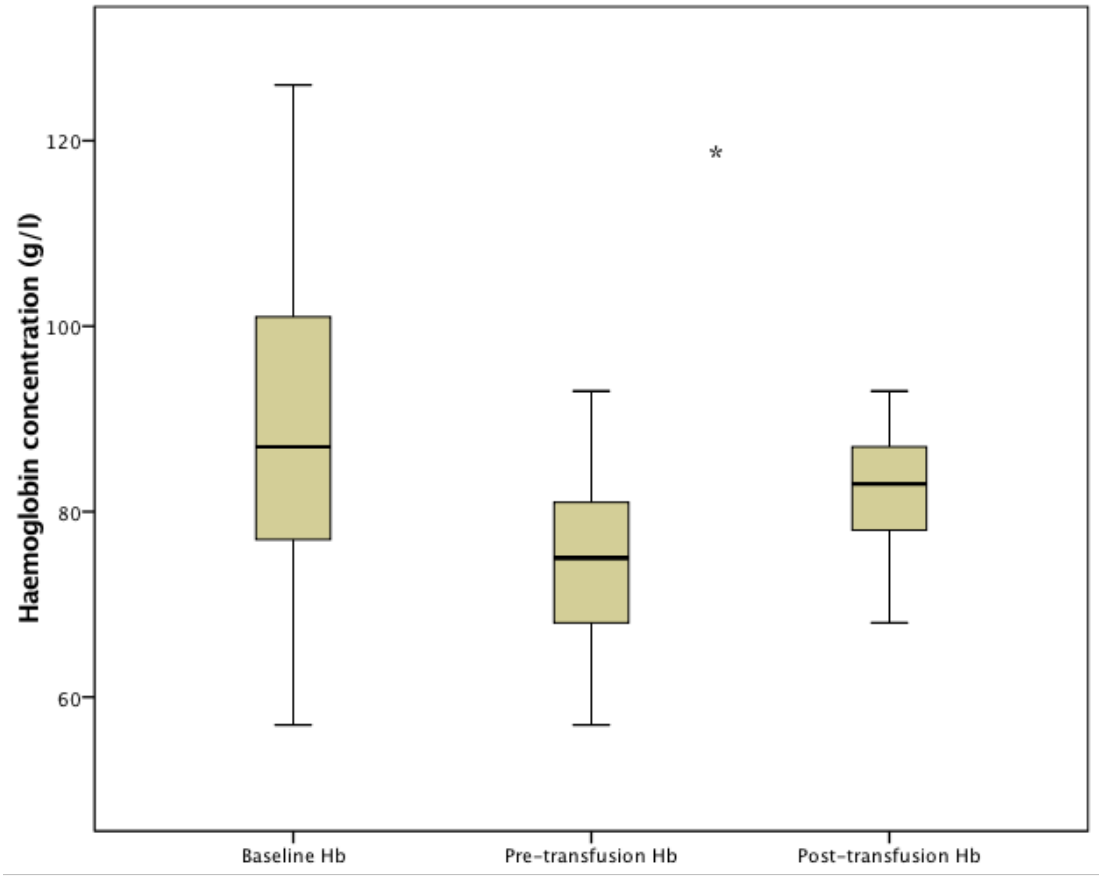
Figure 12: Overall number of RBCs transfused



Box-whisker: boxes represent median and interquartile range, whilst whiskers represent 5% and 95% limits with outliers represented as dots

For the first RBC transfusion, pre-and post-transfusion Hb readings were significantly different: Pre-transfusion 74.94 (10.0) (g/l) vs 82.63 (8.9) (g/l) post transfusion (p=0.048) (Figure 13).

Figure 13: Overall haemoglobin concentrations at measured time points

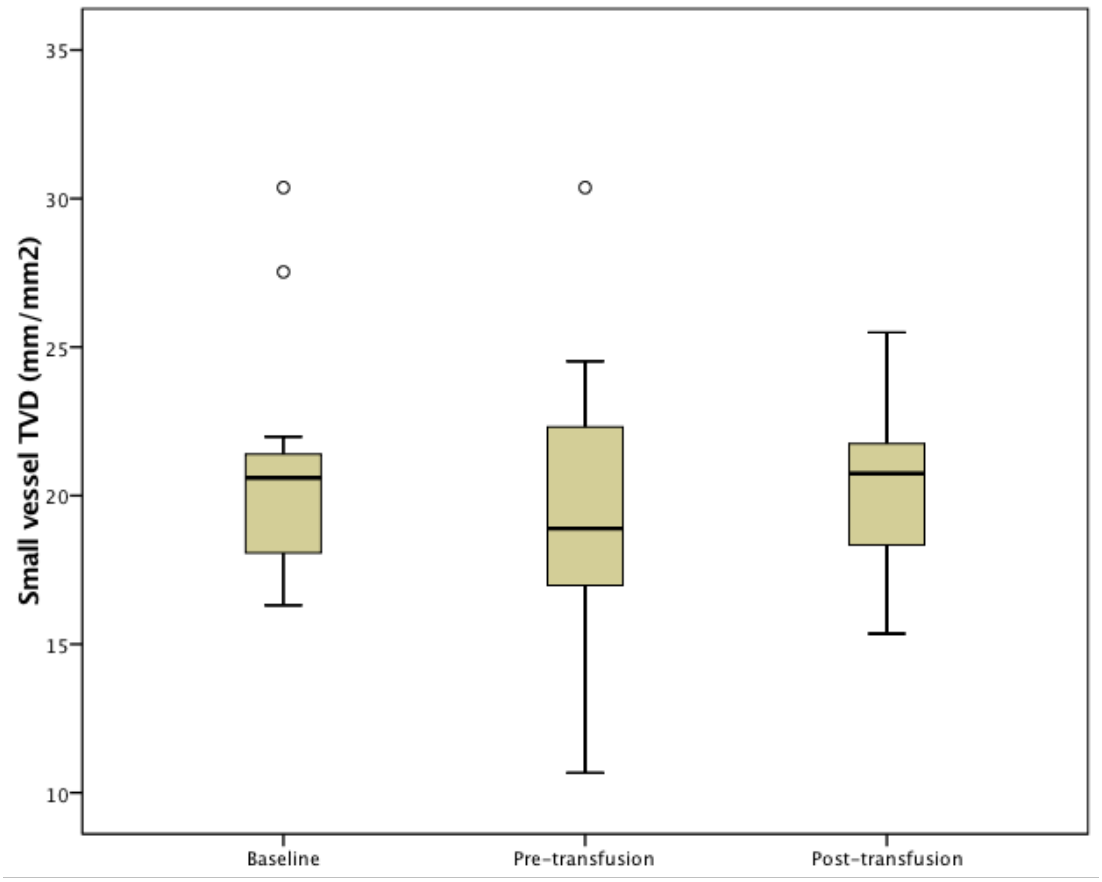


* = $p<0.05$

7.4.2.1 Total Vessel Density

There was no statistically significant difference between small vessel TVD from pre-transfusion to post transfusion. Baseline small vessel TVD was 20.6 (17.8 – 21.6) mm/mm2, and was 18.9 (15.71 – 22.47) mm/mm2 pre-transfusion, rising to 20.7 (18.1 – 22.3) mm/mm2 post transfusion (p=0.144) (Figure 14).

Figure 14: Total vessel density (small vessels)



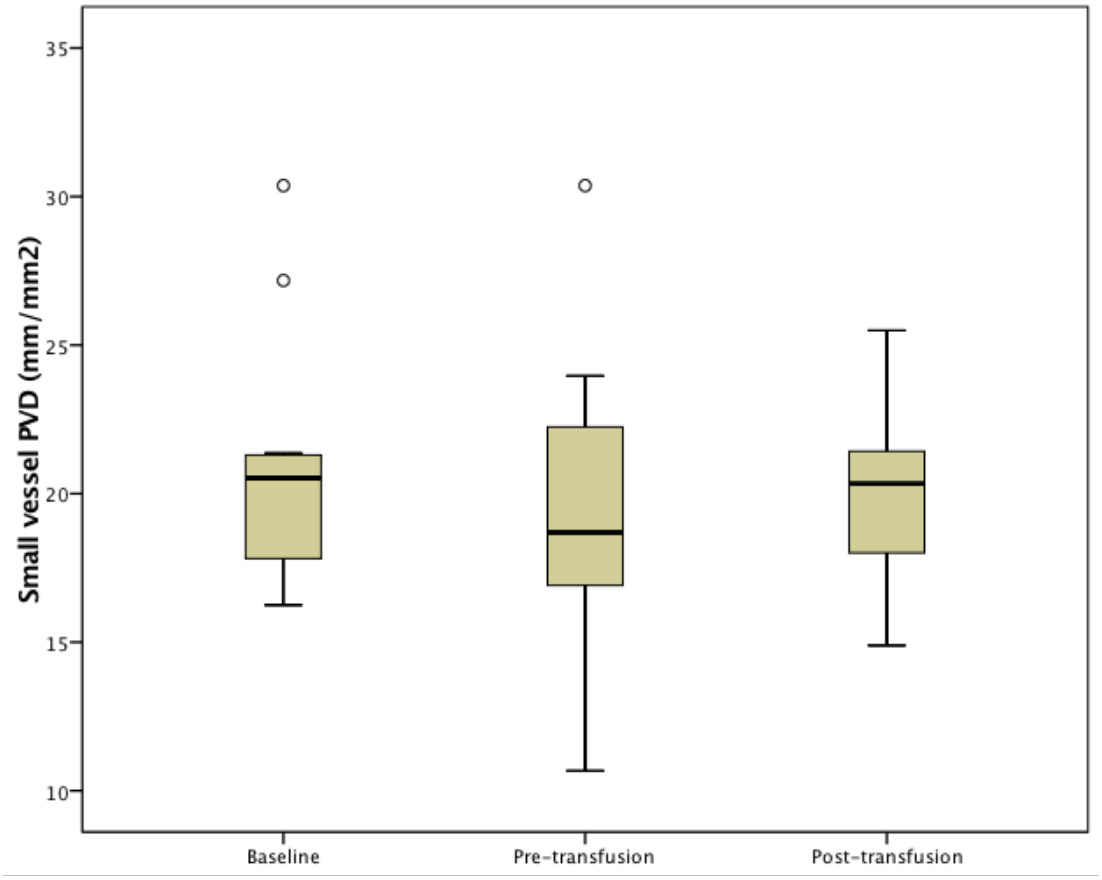
Other vessel (comprising vessels >25 μm diameter) TVD was not significantly different between baseline 1.51 mm/mm^2 (1.12 – 2.70), pre-transfusion 1.33 mm/mm^2 (0.64 – 2.34) and post-transfusion 1.17 mm/mm^2 (0.50 – 1.81) (p=0.570).

7.4.2.2 Perfused Vessel Density

There was no significant difference in small vessel PVD at the different time points: Baseline 20.52 mm/mm^2 (17.51 – 21.31), pre-transfusion 18.69 (15.68 – 22.45), post transfusion 20.34 mm/mm^2 (17.91 – 22.13) (p=0.144) (Figure 15). Other vessel PVD

was not significantly different: baseline 1.51 mm/mm^2 (1.12 – 2.70), pre-transfusion 1.33 mm/mm^2 (0.64 – 2.33) and post-transfusion 1.17 mm/mm^2 (0.50 – 1.81) (p=0.570).

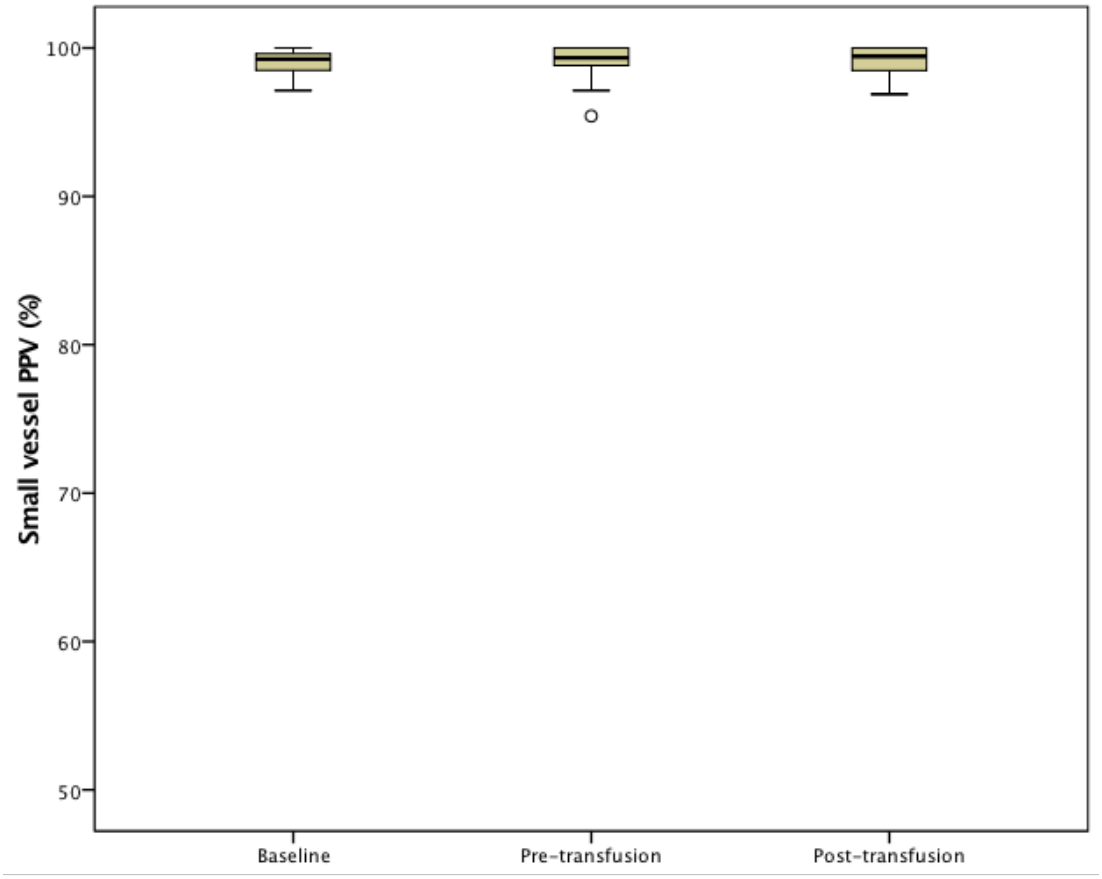
Figure 15: Perfused vessel density (small vessels)



7.4.2.3 Proportion of perfused vessels

There was no significant difference between small vessel PPV at the different time points: baseline 99.25 % (98.5 – 99.7), pre-transfusion (99.32 % (98.3 – 100) and post transfusion (99.43 % (98.2 – 100) (p=0.05) (Figure 16). Other vessel PPV: baseline 100 % (100 – 100), pre-transfusion 100 % (100 – 100) and post-transfusion 100 % (100 – 100) (p=0.607).

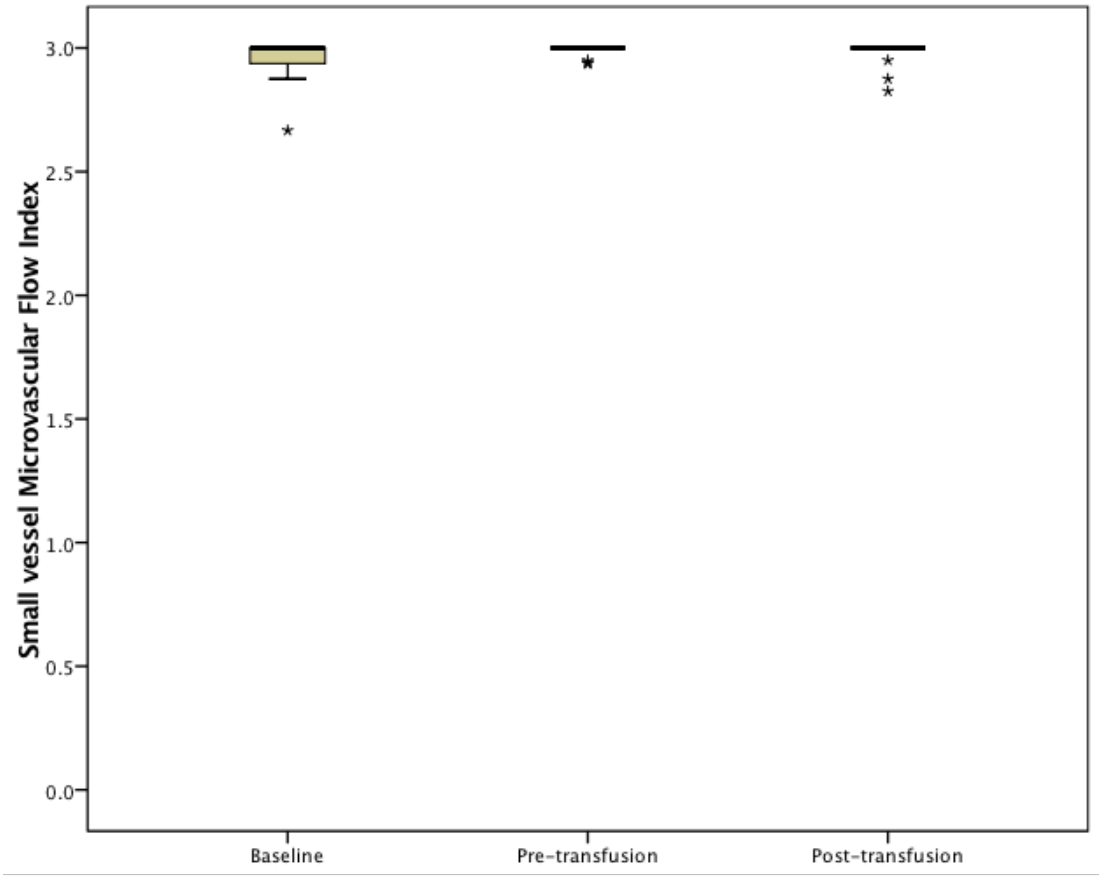
Figure 16: Proportion of perfused vessels (small vessels)



7.4.2.4 Microvascular flow index

There was no difference between small vessel MFI at any of the time points: baseline 3 (2.9 – 3), pre-transfusion 3 3.0 – 3.0 and post transfusion 3 (3 – 3) (p=0.1). Other vessel MFI: baseline 3 (2.9 – 3), pre-transfusion (3 (2.9 – 3) and post-transfusion 3.0 (2.9 -3) (p=.441) (Figure 17).

Figure 17: Microvascular flow index (small vessels)



7.4.2.5 Heterogeneity Index

There was no significant difference in the heterogeneity index (HI) in small vessels at the different time points: baseline HI 0 (0 – 0.09), pre-transfusion 0 (0 – 0.05) and post transfusion 0 (0 – 0) (p=0.587). In the other vessels, there was no significant difference between the time points: baseline HI 0 (0 – 0), pre-transfusion (0 (0 – 0.13) and post-transfusion 0 (0 -0.15) (p=0.092).

7.4.3 Correlations with other parameters

7.4.3.1 Correlations between small vessel TVD and physiological data

There were no significant correlations at any stage between small vessel TVD and physiological variables (Table 12).

Table 12: Relationship between total vessel density (small vessels) and physiological data at each time point

	Time point for correlation (r) with TVD		
Variable	**Baseline**	**Pre-transfusion**	**Post-transfusion**
SpO2	0.034	0.155	0.099
MAP	-0.148	0.18	0.377
HR	-0.155	-0.124	-0.134
SVRI	-0.216	-0.255	0.347
SVI	0.122	0.07	-0.254
CI	0.187	0.123	-0.145
Noradrenaline concentration	-0.233	-0.266	-0.314
Lactate	0.108	-0.146	-0.176
Hb	-0.044	0.057	-0.134
HCT	-0.092	0.226	0.193

7.4.3.2 Correlations between small vessel PVD and physiological data

There were no significant correlations between small vessel PVD and physiological variables at any time point (Table 13).

Table 13: Relationship between perfused vessel density (small vessels) and physiological data at each time point

	Time point for correlation (r) with PVD		
Variable	**Baseline**	**Pre-transfusion**	**Post-transfusion**
SpO2	0.053	0.186	0.068
MAP	-0.119	0.216	0.372
HR	-0.147	-0.093	-0.13
SVRI	-0.164	-0.208	0.341
SVI	0.045	0.054	-0.25
CI	0.146	0.134	-0.145
Noradrenaline concentration	-0.192	-0.245	-0.324
Lactate	0.118	-0.14	-0.196
Hb	-0.037	0.071	-0.161
HCT	-0.074	0.224	0.162

7.4.3.3 Correlations between small vessel MFI and physiological data

MFI had the strongest correlations with the physiological variables (Table 14), and was strongest for noradrenaline concentration post-transfusion. MFI had a moderate correlation with Hb concentration in the baseline and post transfusion measurements, but this association was inverted pre-transfusion, suggesting that this association may be spurious.

Table 14: Relationship between microvascular flow index (small vessels) and physiological data at each time point

	Time point for correlation (r) with MFI		
Variable	**Baseline**	**Pre-transfusion**	**Post-transfusion**
SpO2	0.168	0.078	0.018
MAP	0.041	0.041	0.455
HR	-0.128	0.11	0.158
SVRI	0.278	0.282	0.164
SVI	-0.013	-0.14	-0.204
CI	-0.272	-0.076	-0.131
Noradrenaline concentration	-0.069	0.022	-0.512 *
Lactate	0.036	0.015	-0.004
Hb	-0.414	0.401	-0.395
HCT	-0.367	-0.017	-0.305

7.5 Discussion

7.5.1 Statement of principal findings

This study demonstrated no statistically significant impact of anaemia upon parameters of microcirculatory flow and no correlation between Hb concentration and parameters of microcirculatory density or perfusion. There was a drop in both Hb and small vessel density and perfusion between baseline and blood transfusion, all of which improved post transfusion. Whilst blood transfusion significantly improved Hb concentration, the effect upon microcirculatory flow was not statistically significantly higher.

No other physiological parameters correlated well with any microcirculatory parameter.

7.5.2 Discussion

To my knowledge, no previous study has investigated whether RBC transfusions improve microcirculatory density and perfusion in patients undergoing liver transplantation.

There was no significant change in the parameters measuring the microcirculation (TVD, PVD, PPV, MFI or HI). Both TVD and PVD did increase after transfusion, though not significantly. At all points, the PPV was greater than 99% and the median MFI the maximum of 3, resulting in a median HI of 0 at all points.

In a study of 35 patients with sepsis who required blood transfusion (baseline median Hb 71 (g/L) (67 -76), the microcirculation was assessed using OPS (Sakr et al., 2007). Blood transfusion caused a rise to 81 g/L (75 – 86). However, no significant difference was demonstrated in total vascular density, perfused vessel density or the proportion of vessels perfused. They found significant variability between patients in the response of both macro circulatory and microcirculatory variables to transfusion. Those patients with altered perfusion at baseline showed an improvement, whilst those with normal baseline perfusion showed worsened microvascular perfusion. The authors postulated that transfusions may be deleterious in those with preserved RBC deformability, whilst providing improvements in those with already altered RBC deformability. Patients whose microcirculation was improved had similar baseline oxygen delivery, vasopressor dose and macro-haemodynamic parameters to those who did not improve (Sakr et al., 2007). Those findings are in keeping with the findings from this cohort of surgical patients.

In patients undergoing cardiac bypass surgery, it has been shown that blood transfusions significantly increased microcirculatory density (mm/mm^2) from 10.5 (1.2) to 12.9 (1.2) ($p<0.01$) measured with SDF imaging. Meanwhile MFI was unaffected (2.97 (0.03) pre-transfusion and 2.96 (0.03) post-transfusion ($p=0.95$) (Yuruk et al., 2011).

My data had much higher small vessel TVD and PVD than that study, which may have had an influence upon the magnitude of difference recorded in my study.

However, that study utilised SDF imaging which has a significantly lower vessel detection than IDF (Aykut et al., 2015).

During the course of the liver transplant, haemoglobin concentration, indices of coagulopathy and organ perfusion are constantly monitored and acted upon rapidly by the anaesthetic team caring for the patient. These responses are even more rapid than would be seen in the intensive care unit given the one-to-one nature of care given in the operating theatre. The pre-transfusion Hb sat in the region of most currently accepted transfusion thresholds (74. 9 g/L). It is possible that the changes in microvascular indices would have been at a more significant level had this Hb concentration been even lower. Importantly however, it showed that even at the lowest haemoglobin concentration recorded, microcirculatory flow was preserved, suggesting that this is an adequate transfusion trigger for these patients – before any interruption in flow has developed. It then allows us to ask the question of whether further assessment could be made of the tolerance of lower haemoglobin thresholds within the microcirculation, as part of the third pillar of blood transfusion.

These results demonstrate that microcirculatory perfusion was very good, with very few unperfused vessels (where flow was either absent or intermittent). Much data relating to these microcirculatory indices comes from septic patients in critical care. In those patients, a common finding is significant heterogeneity of flow with large numbers of vessels not perfused (De Backer et al., 2002a). In an observational study of patients with sepsis, the PPV of small vessels measured using SDF imaging was significantly reduced when comparing normal controls to those with sepsis, and significantly further reduced in those with severe sepsis (Spanos et al., 2010).

Numerous changes to the microcirculation have been reported as a result of sepsis and critical illness. These include changes to the red blood cell itself, including altered metabolism and decreased 2,3-DPG, reduced RBC deformability and redistribution of membrane phospholipids (Bateman et al., 2017). The dysregulation of endothelial cell signalling is central to the dysfunction of the microcirculation in sepsis, leading to a mismatch between oxygen requirements and flow (De Backer et al., 2014a). Septic shock results in severe vasodilatation and hypotension that is

refractory to fluid volume replacement. This can result in organ dysfunction in spite of the oxygen transport.

Such alterations may be expected from the cirrhotic patient. As the liver becomes cirrhotic, fibrosis develops. This increases the resistance to blood flow in within the hepatic vasculature. The increased resistance leads to portal venous dilatation and congestion, elevating the portal venous pressure – leading to portal hypertension. The increased hepatic resistance is also contributed by hepatic vasoconstriction largely contributed by the reduction in NO production (Kim et al., 2010). Increased hepatic blood pressure and flow lead to angiogenesis from the proliferation of endothelial cells and smooth muscle.

Because of the increased portal pressure, collateral vessels develop and a hyperdynamic splanchnic circulation results from porto-systemic shunting. The venous return to the heart is increased, raising the cardiac output, with a compensatory decreased systemic vascular resistance modulated by raised NO production released by upregulated endothelial NO synthase (eNOS) (Kim et al., 2010). Thus, cirrhotic patients have a hyperdynamic circulation with a high cardiac output, arterio-venous shunting, tachycardia, reduced SVR, hypotension and bounding pulses.

In this cohort of subjects with end stage liver disease, there were excellent microcirculatory flow indices with good homogeneity of flow, unlike patients with sepsis. These findings are similar to a study of microvascular flow using SDF imaging that included 18 patients with cirrhosis in an outpatient setting with an MFI of 3.0 (2.83 – 3) for small vessels (Reynolds et al., 2013). In that study, MFI was higher than in healthy volunteer controls. This suggests that unlike sepsis a homogenous blood flow is maintained, with better microvascular perfusion and oxygen delivery to the tissues, possibly as a result of adaptation of the circulation to this state, instead of the acute changes found in the septic patient with multiple organ dysfunction.

In other surgical groups studied using SDF imaging MFI was maintained. Bansch et al measured MFI in patients undergoing upper gastrointestinal surgery at time points

following induction of anaesthesia, during the last hour of surgery, and in recovery (as well as pre-operatively and the first postoperative day). MFI in those patients without complications and with complications was not significantly different. Median MFI was 2.8 (2-2-3.0), 2.8 (2.4-3.0) and 2.7 (1.9 -3.0) at the three time points in those without complications, with no mean estimated difference to those who developed complications (Bansch et al., 2014). However, in another study of patients undergoing major abdominal surgery, post-operative SDF imaging showed a significant difference in MFI between those who developed complications and those who did not (2.6 (2.5-2.9 vs 3.0 (3.0-3.0)) ($p<0.05$). (Jhanji et al., 2009).

Many studies have assessed the efficacy of interventions to improve haemodynamics and perfusion in sepsis and their effect upon the microcirculation. These include the administration of fluids, including blood and the use of vasoactive drugs.

I had predicted that increasing doses of noradrenaline may have a detrimental impact upon the microcirculation by with profound vasoconstriction of vessels and reducing the measured vessel density.

Noradrenaline has been studied in patients with septic shock. In a study of critically ill patients requiring noradrenaline to maintain adequate MAP, SDF imaging of the microcirculation was conducted at differing MAPs. Whilst CI rose in line with noradrenaline dose, TVD trended downwards with the increasing dose. There was no significant difference in MFI or PPV, with a trend to decreased PVD. The authors found significant variation in individuals' responses to noradrenaline dependent upon the baseline condition of the microcirculation (Dubin et al., 2009).

Jhanji et al studied post-operative microvascular flow and oxygenation in patients who had undergone major gastrointestinal surgery comparing fluid therapy strategies and a strategy combining low-dose dopexamine. The group with stroke volume guided fluid and low dose dopexamine had improved global oxygen delivery, tissue oxygenation and microvascular flow when compared to those receiving fluids alone. Microvascular flow improved over eight hours post op in the stroke volume and fluid group, whilst it was constant in the stroke volume group, and deteriorated in the control group. Additionally cutaneous tissue oxygen partial pressure (PtO_2) had a

sustained increase in the dopexamine group whereas it decreased to baseline in the fluid only groups (Jhanji et al., 2010).

Dobutamine has been demonstrated to increase microvascular perfusion in septic shock, independent to the global haemodynamic effects on blood pressure and cardiac index. De Backer et al demonstrated an increase in portion of perfused capillaries from 48 % (+/- 16) to 67% % (+/- 11) (p=0.001), yet capillary perfusion was not normalised (De Backer et al., 2006).

In my study, noradrenaline concentration was not correlated with microcirculatory values. The underlying adequacy of the microcirculation in these patients may have contributed to the lack of influence of noradrenaline or changes in SVR upon the results from my study. However, to confirm this finding a study powered to compare differences in patients with different vasopressors drugs or dosing strategies would need to be undertaken.

Conversely, vasodilatation may be able to improve microcirculatory flow by opening areas which are poorly perfused. Nitroglycerin infusions have been investigated using OPS imaging in sepsis, and demonstrated to significantly improve MFI in small vessels which had significant baseline impairment in flow (Spronk et al., 2002). As previously discussed, the naturally vasodilated state of cirrhosis with high NO generation, may explain the well perfused microcirculation that I have demonstrated.

7.5.2.1 Strengths and limitations

This study is the first to record microcirculatory indices of blood flow during liver transplantation. Nonetheless, it was a small study and a larger study population may be required to be powered to reveal significant results. Since the effect of individual data points have a greater effect in a smaller population, this may be particularly pertinent in the constantly varying situation of liver transplantation. Since there are so many different intentions and factors at play during the operation – from patient factors such as their co-morbidities and medications, anaesthetic factors and surgical factors including duration, mode of operation and donor organ quality – there is significant physiological variation throughput that could interfere with the intervention of interest.

The use of a control group of patients without cirrhosis undergoing surgery may have been of use to demonstrate the differences between the cohort undergoing liver transplantation and non-cirrhotic patients, and the effects of the liver transplantation surgery upon the microcirculation in comparison to other surgery, for example during hepatic resection surgery for colorectal metastases in non-cirrhotic patients.

The IDF device is one that has large degrees of inter-user variability and requires practice and experience in order to capture optimal images in keeping with the consensus guidelines. As a single operator conducting the experiments the possible confounding of inter-operator variability was removed. Nonetheless, during my time capturing this data I gained significant experience and was able to improve my technique over the course of the study. There is a learning curve for using the IDF device and for the first few patients (as demonstrated in chapter 7) the PPV was lower than demonstrated amongst the entire cohort, suggesting that there was pressure artefact within those films as compared to later subjects within the study as I improved my technique.

A significant limitation was access to the patient during the surgery, particularly during challenging parts of the surgery, particularly during the anhepatic phase and after reperfusion due to the position of the surgical team and the need for the anaesthetist to have access to the patient and their infusions. In addition, given the intraoperative setup in which the anaesthetist and their monitors are positioned at the head end of the patient, at times of clinical activity, it was not always possible to get adequate access to the patient. In addition, when the surgeons were actively working at the cranial end of their operative field, and using cranially placed Thompson's retractors, the made getting the probe easily into the mouth of the patient challenging to take sublingual recordings due to its vertical height.

Utilising sublingual IDF imaging in a paralysed and anaesthetised patient is advantageous by eliminating movement artefact. However, it adds additional challenges, particularly negotiating around the endotracheal tube to avoid causing pressure artefact if mucosa were compressed between the endotracheal tube and the probe. Significant amounts of secretion are produced whilst anaesthetised, in the absence of swallow, so these had to be carefully removed using gauze.

As mentioned in previous chapters, the manual analysis process is time-consuming, and can potentially lead to inaccuracies in data. Again, since all films were analysed by myself, inter-observer variation was eliminated. No intra-observer variability checks were performed. Nonetheless, the manual analysis could potentially lead to inaccuracies.

An area of improvement in the analysis will be better quantification of flow velocity through vessels. Although flow velocity may be many magnitudes different, the software is unable to account for this. Therein lies an inherent problem with the analysis of these films, since vessels with uninterrupted flow may have significantly different velocities but would be applied the same score of '3 = normal'. Additionally, the MFI score as per consensus guidelines only permits a highest flow score of 3 for normal flow – there is no scope in that for hyperdynamic or accelerated flow (De Backer et al., 2007). Nonetheless, the AVA software, does allow flow to be labelled as '4 = hyperdynamic'. I chose not to keep to the 0-3 scoring system in order to meet the consensus recommendations and to permit easier comparison between these results and those of other studies using this technique. Space-time diagrams can be created to describe red blood cell velocity profiles from pixels in the vessel centre line. These measurements can be biased, especially in larger vessels where velocity distribution is different with reduced velocity at the vessel wall and maximal velocity in the centre (Dobbe, 2007). Additionally, performing this analysis on all vessels in a single video is time prohibitive and unfeasible for multiple analyses (Massey and Shapiro, 2016). As the subject area evolves and the analysis software improves, these differences in flow velocity should become better distinguishable. This is of importance since oxygen offloading from RBCs is affected by the transit time through the capillary, thus altering DO2. Future consensus guidelines will need to take this into consideration.

A major limitation to this study was the lack of follow up data to see whether there was any correlation between the microcirculatory parameters recorded and outcome. In such a small cohort, such correlations would be difficult to find, given the huge variability in these patients – and in particular the quality of graft liver – which would not be distinguishable by these images.

7.6 Conclusions

This study examined the effect of blood transfusion in liver transplantation on the microcirculatory blood flow. It suggests that anaesthetic management based upon standard macrocirculatory parameters of haemodynamics maintained good microcirculatory flow. Whilst blood transfusion improved microcirculatory flow, these differences were not significant.

Future studies could be aimed at investigating the microcirculatory changes in different vascular beds during surgery, including correlating microcirculatory measurements of the liver itself during the dissection phase with sublingual findings, and examining the impact of blood transfusion in these different circulations.

Research is ongoing into the areas of transfusion threshold in many different patient populations. As more emphasis is placed upon reducing blood transfusion rates and utilising restrictive transfusion triggers the tipping point at which inadequate organ perfusion or oxygen delivery needs to be determined, for the risk of causing harm by under-transfusing patients. I have demonstrated that the current haemoglobin concentration at which transfusion was triggered was adequate, we do not yet know how low we can safely go in this population.

Chapter 8 Effect of technique of liver transplantation upon the microcirculation: observational study

8.1 Introduction

The second part of this study was to investigate the effect of surgical technique on microcirculatory flow. The effect of surgical technique comparing caval cross clamp and piggyback technique on IDF measurements were investigated.

8.1.1 Hypothesis

Primary outcome was the difference in perfused vessel density (PVD) after re-perfusion of the transplanted liver between caval cross clamp (CCC) and piggy-back (PB) groups.

Secondary outcomes were differences in: total vessel density (TVD), proportion of perfused vessels (PVD), microvascular flow index (MFI) and heterogeneity index (HI) after reperfusion; and, (including PVD) at each time point.

I hypothesised that caval cross clamp (CCC) technique would be associated with greater microvascular dysfunction post-reperfusion secondary to a greater disturbance to systemic haemodynamics and greater ischaemia-reperfusion effects.

8.2 Methods

The methods, participant selection and conduct of anaesthesia are as described in the methods section of chapter 7.

To assess the effect of mode of surgery (CCC vs PB technique), microcirculatory parameters between the two groups were compared at five set time points during the operation:

1. Baseline after induction of anaesthesia
2. Final reading during dissection phase (phase 1 of OLT)
3. During the anhepatic phase (phase 2 of OLT)

4. Post-reperfusion (phase 3 of OLT)
5. At end of surgery prior to cessation of anaesthesia

8.2.1 Ethical approval

The study design, documentation and protocols were in accordance with the Declaration of Helsinki, and were approved by the Research Ethics Committee of the East of England – Essex (Reference 14/EE/1275).

8.2.2 Statistical analysis

Statistical analysis was performed using Microsoft Excel (Microsoft, USA) and SPSS Statistics 24 (IBM, USA). Normal distribution was confirmed by a Shapiro-Wilk test ($p>0.05$) and visual inspection of data histograms and Q-Q plots. A non-normal distribution was evident for the measures of the microcirculation, so non-parametric tests were utilised, with values summarised by median and interquartile ranges or mean and standard deviation as appropriate. Differences between time points were analysed using related samples Friedman's Two-way Analysis of Variance by ranks test and between related samples with Wilcoxon Signed Rank test with Bonferroni correction applied. Comparisons between groups at different time points were performed using unpaired T-tests or Mann-Whitney U tests as appropriate. Results are presented as box-whisker plots (boxes represent median and interquartile range, whilst whiskers represent 5% and 95% limits with outliers represented as dots). Correlation between microcirculation and other physiological variables was assessed using Spearman's rank correlation. In all cases a P value <0.05 was considered to be statistically significant.

8.3 Results

Baseline physiological data are presented in table 15.

Table 15: Baseline physiological data

Variable	All patients n=20	Caval Cross Clamp n=12	Piggyback n=8	P
Systolic / diastolic BP	102 (17.7) / 51.2 (11.2)	101.5 (18.0) / 50.3 (10.8)	103.1 (18.6) / 52.5 (12.3)	.938 / .817
MAP	70.5 (14.7)	69.4 (14.7)	72 (15.6)	.699
HR	72.8 (15.1)	73.5 (13.6)	71.8 (17.9)	.589
SVR	684.7 (294.2)	639.4 (210)	752.5 (396)	.512
SVRI	1247.65 (513.9)	1126.9 (315.9)	1428.8 (705.2)	.316
SV	103.3 (26.8)	101.0 (17.2)	106.1 (36.8)	.824
CO	7.9 (1.6)	7.8 (1.4)	8.0 (1.9)	.817
CI	4.3 (0.9)	4.3 (0.9)	4.2 (1.1)	.589
Platelet count	86.4 (70.8)	98.6 (87.4)	68 (30.9)	.589
INR	1.6 (0.6)	1.6 (0.5)	1.8 (0.6)	.483
Hb	94.35 (19.8)	99.75 (17.9)	86.25 (20.8)	.122
HCT	0.288 (.06)	.304 (.05)	.264 (.06)	.177
Lactate	1.74 (.5)	1.7 (.4)	1.8 (.5)	.877

There were no significant differences between the two groups at baseline when compared by haemodynamic and haematological parameters.

8.3.1.1 Haemoglobin concentration

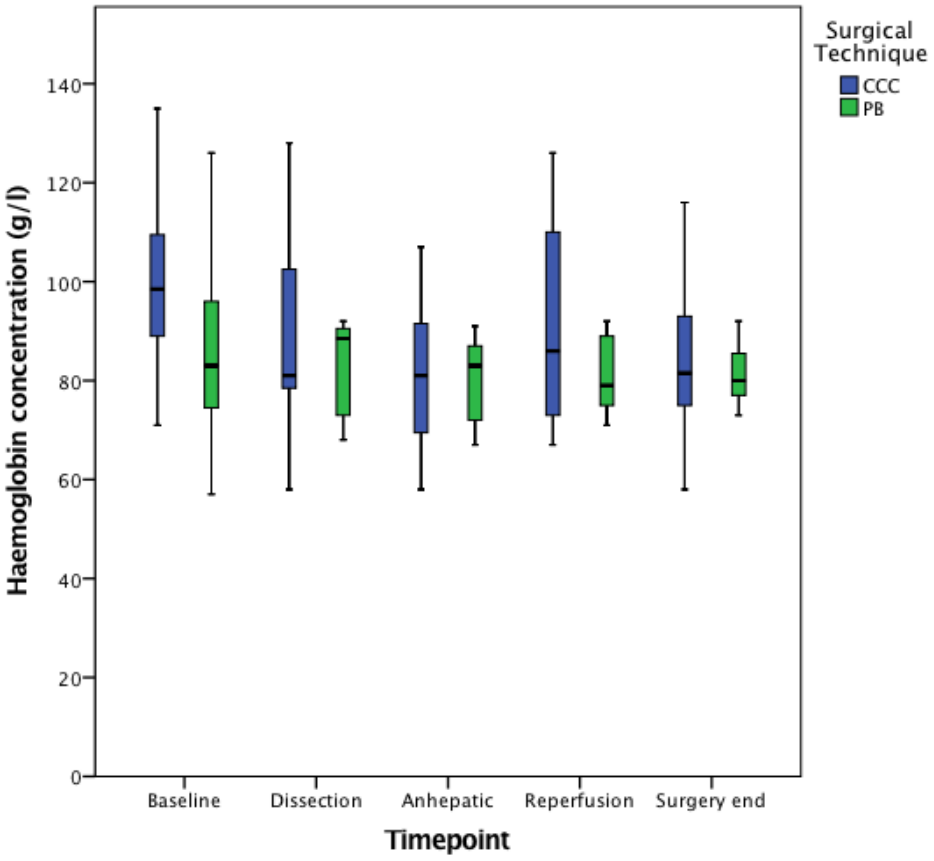
Haemoglobin concentration was higher in the CCC group at each stage of the operation, but this was not statistically significant different at any point between

CCC and PB groups' Hb (Table 16 and Figure 18), suggesting that blood loss was similar in each stage throughout the procedure, independent of the mode of surgery.

Table 16: Haemoglobin concentration throughout the operation

Time point	CCC Hb (g/L)	PB Hb (g/l)	P
1. Baseline	99.75 (17.9)	86.25 (20.8)	.122
2. Dissection	89.00 (22.3)	83.00 (10.1)	.969
3. Anhepatic	81.18 (16.3)	79.86 (9.7)	.964
4. Reperfusion	90.20 (19.7)	80.83 (8.1)	.515
5. Surgery end	83.83 (15.7)	81.43 (6.7)	.735

Figure 18: Haemoglobin concentration for both cohorts at each time point



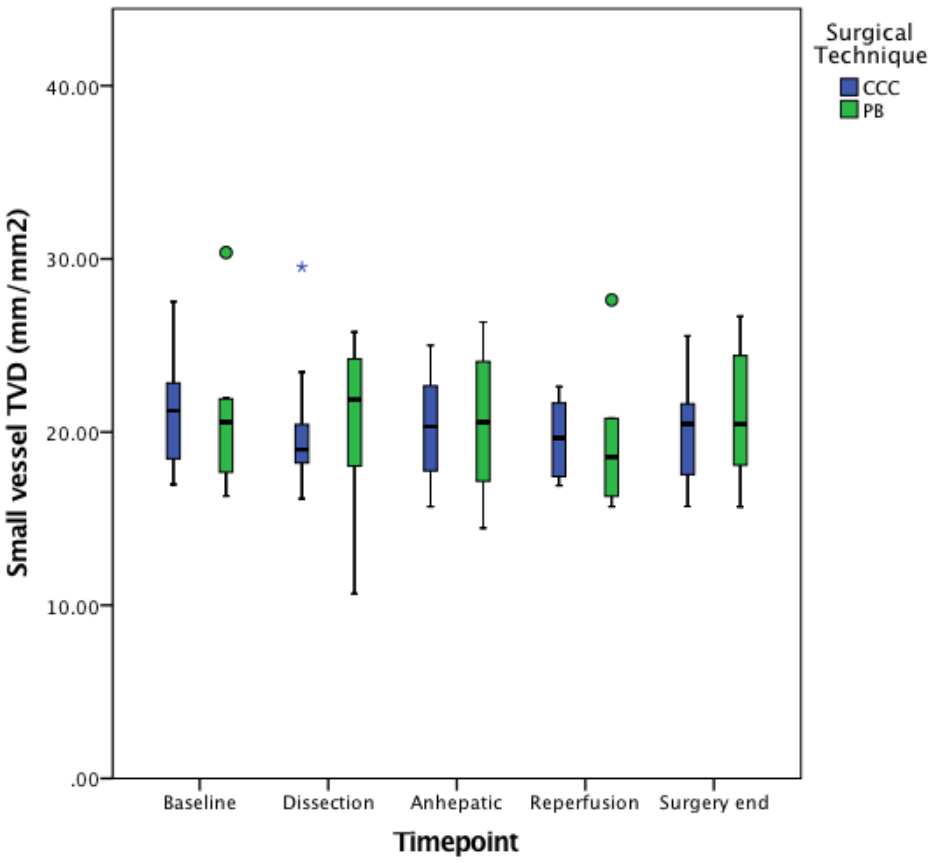
Key: Box-whisker: Box = median and 25-75th centile; whiskers = 5th and 95th centile

8.3.1.2 Total vessel density

The TVD for small vessels did not differ significantly between caval cross clamp (CCC) and piggyback (PB) technique groups at baseline (CCC: 21.23 mm/mm2 (18.27 – 23.19); PB: 20.58 mm/mm2 (17.09 – 21.95) (p=.537)), dissection (CCC: 19.00 mm/mm2 (18.16 – 20.54); PB: 21.88 mm/mm2 (17.41 – 24.38) (p=.385)), anhepatic (CCC: 20.32 mm/mm2 (17.72 – 23.13); PB: 20.57 mm/mm2 (15.35 – 25.50)(p=1)), reperfusion (CCC: 19.67 mm/mm2 (17.43 – 21.73); PB: 18:55 mm/mm2 (16.13 – 22.50) (p=.635)) or surgery end (CCC: 20.47 mm/mm2 (17.33 – 21.86); PB: 20.45 mm/mm2 (15.99 – 25.28)(p=.837)) (Figure 19).

Within each cohort there was no significant difference in TVD at any of the different time points (CCC: p=.615; PB: p=.900).

Figure 19: Total vessel density (small vessels) for both cohorts at each time point



Key: Box-whisker: Box = median and 25-75th centile; whiskers = 5th and 95th centile. Star (CCC) and Dot (PB) represent outliers.

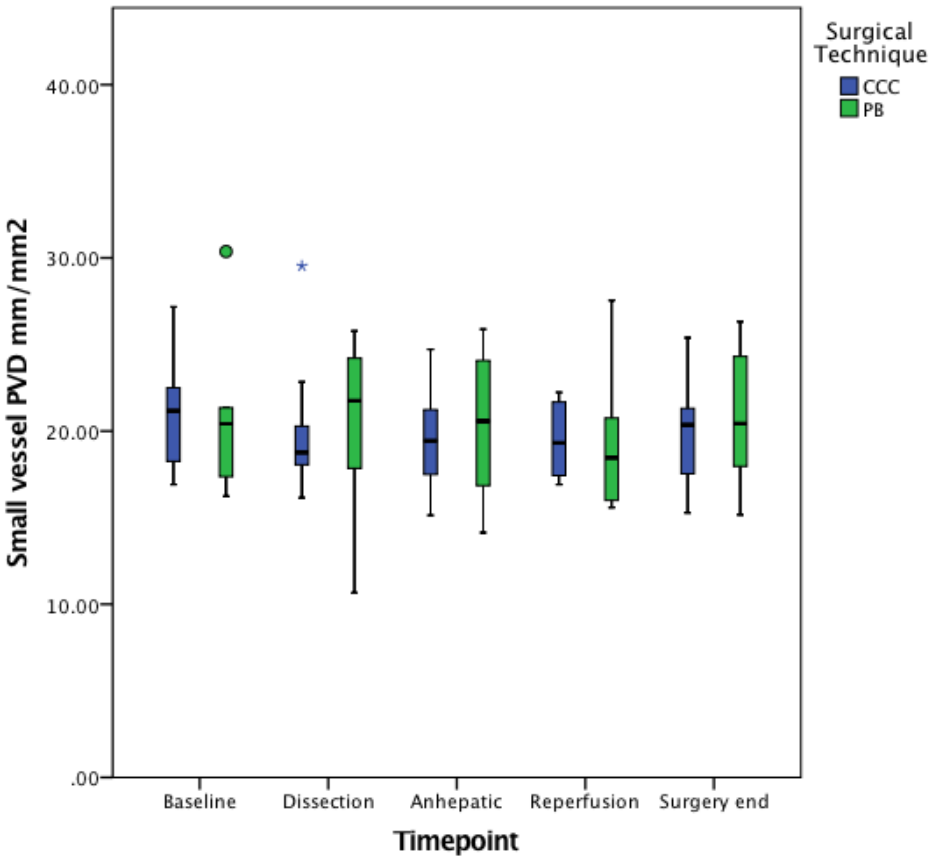
There were no significant differences in 'other vessel' (>25 μm) TVD between the CCC and PB groups at baseline (CCC: 1.48 mm/mm2 (1.11 – 2.83); PB: 1.59 mm/mm2 (.58 – 2.16) (p=.734)), dissection (CCC: 1.34 mm/mm2 (.80 – 1.93); PB: 1.13 mm/mm2 (.56 – 1.81) (p=.521)), anhepatic (CCC: 1.68 mm/mm2 (.79 – 2.51); PB: 0.65 mm/mm2 (.51 – 1.86) (p=.270), reperfusion (CCC: 1.35 mm/mm2 (.82 – 1.93); PB: 1.27 mm/mm2 (.82 – 2.40) (p=1)), and surgery end (CCC: 1.58 mm/mm2 (1.09 – 2.52); PB: 1.28 mm/mm2 (.67 – 1.77) (p=.227)).

8.3.1.3 Perfused vessel density

The PVD for small vessels did not differ significantly between CCC and PB groups at baseline (CCC: 21.17 mm/mm2 (18.03 – 22.95); PB: 20.41 mm/mm2 (16.86 – 21.36) (p=.487)), dissection (CCC: 18.76 mm/mm2 (18.03 – 20.32); PB: 21.76 mm/mm2 (17.21 – 24.36) (p=.316)), anhepatic (CCC: 19.43 mm/mm2 (16.91 – 21.91); PB: 20.57 mm/mm2 (14.89 – 25.50) (p=.770)), reperfusion (CCC: 18.32 mm/mm2 (17.43 – 21.73); PB: 18.46 mm/mm2 (15.90 – 22.45) (p=.588), or at surgery end (CCC: 20.35 mm/mm2 (17.30 – 21.59); PB: 20.43 (15.74 – 25.23) (p=.800)).

Within each cohort there was no significant difference in PVD at any of the time points (CCC: p=0.950, PB: p=.900). (Figure 20)

Figure 20: Perfused vessel density (small vessels) for both cohorts at each time point



Key: Box-whisker: Box = median and 25-75th centile; whiskers = 5th and 95th centile. Star (CCC) and Dot (PB) represent outliers.

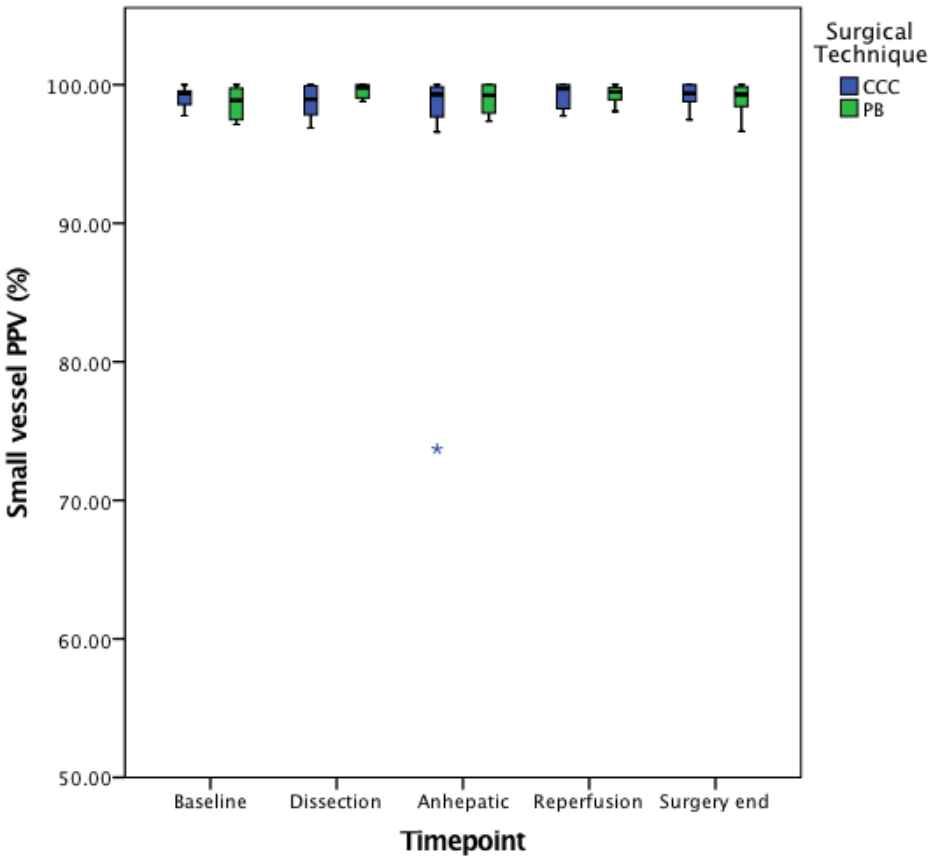
The PVD for 'other vessels' did not differ significantly between CCC and PB groups at baseline (CCC: 1.48 mm/mm2 (1.11 – 2.83); PB: 1.59 mm/mm2 (.58 – 2.16) (p=.734)), dissection (CCC: 1.34 mm/mm2 (.80 – 1.93); PB: 1.13 mm/mm2 (.56 – 1.81) (p=.521)), anhepatic (CCC: 1.49 mm/mm2 (.79 – 2.51); PB: .65 mm/mm2 (.51 – 1.86) (p=.270)), reperfusion (CCC: 1.52 mm/mm2 (1.03 – 1.93); PB: 1.27 mm/mm2 (.82 – 2.40) (p=.792)), and surgery end (CCC: 1.58 mm/mm2 (1.09 – 2.52); PB: 1.28 mm/mm2 (.67 – 1.77) (p=.227)).

8.3.1.4 Proportion of perfused vessels

The small vessel PPV did not differ significantly between CCC and PB groups at baseline (CCC: 99.37 % (98.54 – 99.59); PB: 98.87 % (97.34 – 99.81) (p=.512)), dissection (CCC: 98.95 % (97.76 – 99.96); PB: 99.81 % (98.96 – 100) (p=.148)), anhepatic (CCC: 99.30 % (97.41 – 99.88); PB: 99.24 % (97.58 – 100) (p=.693)), reperfusion (CCC: 99.71 % (98.24 – 100); PB: 99.47 % (98.74 – 99.83) (p=.616)) and surgery end (CCC: 99.38 % (98.74 – 100); PB: 99.3 % (98.22 – 99.91) (p=.495)).

Within each cohort there was no significant difference in PPV at any of the time points (CCC: p=.167, PB: p=0.982) (Figure 21).

Figure 21: Proportion of perfused vessels (small vessels) for both cohorts at each time point



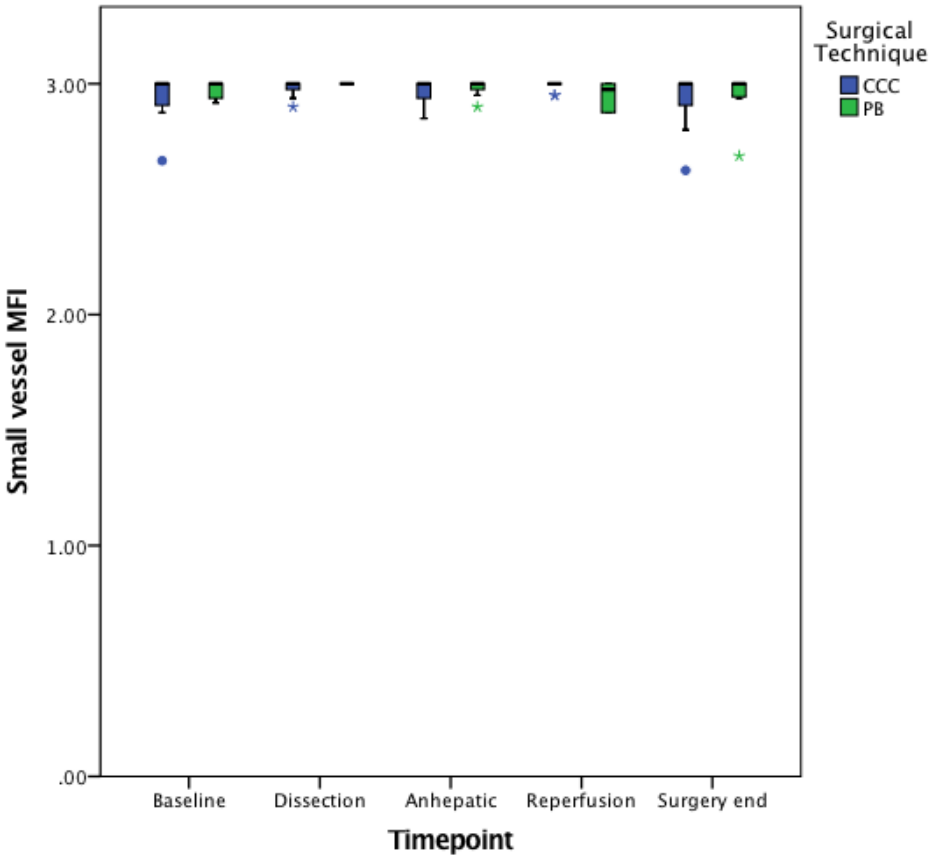
Key: Box-whisker: Box = median and 25-75th centile; whiskers = 5th and 95th centile. Star (CCC) and Dot (PB) represent outliers.

8.3.1.5 Microvascular flow index

The MFI of small vessels did not differ significantly between CCC and PB groups at baseline (CCC: 3 (2.89 – 3); PB: 3 (293 – 3) (p=.910)), dissection (CCC: 3 (3 – 3); PB: 3 (3 – 3) (p=.384)), anhepatic (CCC: 3 (2.94 – 3); PB: 3 (2.95 – 3) (p=.887)), reperfusion (CCC: 3 (2.99 – 3); PB: 2.98 (2.88 – 3) (p=.263)), and surgery end (CCC: 3 (2.89 – 3); PB: 3 (2.94 – 3) (p=.902)).

Within each cohort there was no significant difference in PPV at any of the time points (CCC: p=.929; PB: p=0.159) (Figure 22).

Figure 22: Microvascular flow index (small vessels) for both cohorts at each time point



Key: Box-whisker: Box = median and 25-75th centile; whiskers = 5th and 95th centile. Star (CCC) and Dot (PB) represent outliers.

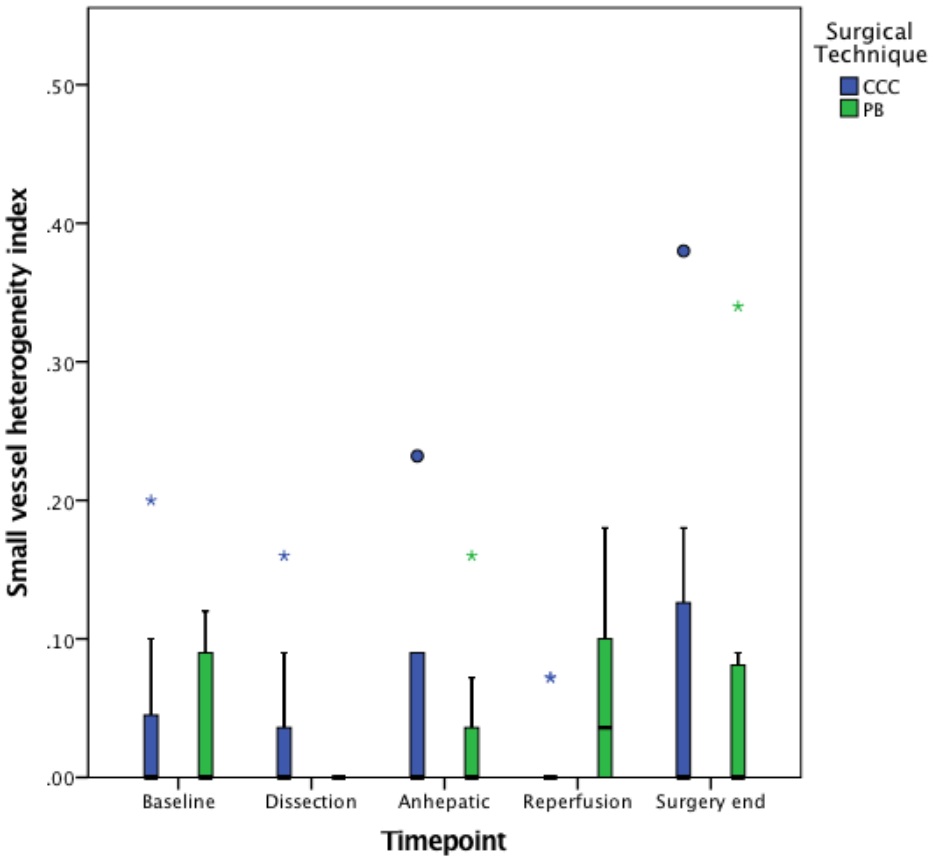
8.3.1.6 Heterogeneity index

The HI of small vessels did not differ significantly between CCC and PB groups at baseline (CCC: 0 (0 -.068); PB: 0 (0 - .090) (p=.734)), dissection (CCC: 0 (0 - .045); PB: 0 (0 - .045) (p=.384)), anhepatic (CCC: 0 (0 - .090); PB: 0 (0 - .072) (p=.887)), reperfusion (CCC: 0 (0 - .018); PB: .036 (0 - .120) ((p=.263)), and surgery end (CCC: 0 (0 - .144): PB: 0 (0 - .090) (p=.902)).

There was no significant difference in small vessel HI at any time point within each cohort (CCC: p=.965; PB: p=.159) (Figure 23).

CCC and PB groups 'other' vessel HI did not differ at baseline (CCC: 0 (0 - .163); PB: 0 (0 – 0) (p=.851)), dissection (CCC: 0 (0 - .148); PB: .034 (0 - .144) (p=.792)), anhepatic (CCC: .021 (0 - .077); PB: 0 (0 - .180) (p=.887)), reperfusion (CCC: .101 (0 - .189); PB: 0 (0 - .180) (p=.635)), and surgery end (CCC: .147 (0 - .266): PB: 0 (0 - .198) (p=.299).

Figure 23: Heterogeneity index (small vessels) for both cohorts at each time point



Key: Box-whisker: Box = median and 25-75th centile; whiskers = 5th and 95th centile. Star and Dot represent outliers.

8.4 Discussion

This study examined the effect of the main mode of surgical technique in liver transplantation on the microcirculatory blood flow. There was no difference in microcirculatory flow in the two cohorts of surgical technique.

Investigation of the microcirculation in high-risk surgery has shown that in patients who had postoperative complications, the density and proportion of perfused capillaries was lower than in those that did not, and these differences were present before surgery. Small vessel (<20 μm) density and proportions in those patients who developed complication was significantly lower before and after surgery compared to

those who did not develop complications. Tissue PO_2 and laser Doppler blood flow did not differ between the groups and there was no significant difference in global O_2 delivery between the groups (Jhanji et al., 2009). However, in comparison to the results gained in my experiments, the MFI was lower in both cohorts, alongside much reduced PPV in these post-operative patients (Jhanji et al., 2009).

During the liver transplant, significant haemodynamic differences can be noted between patients having caval cross clamp hepatectomy and those who have a piggy-back technique. The temporary cross-clamping of the inferior vena cava and portal vein in the CCC technique reduces venous return to the heart, creates congestion in the caval and splanchnic beds and reduces renal perfusion. The clamp is removed once the graft is re-vascularised, at which point venous return to the heart can drop significantly, reducing cardiac output and potentially reducing organ perfusion. The piggyback technique preserves the recipient vena cava during the hepatectomy where the venous outflow from the donor liver is 'piggy backed' onto the IVC in situ, thereby reducing the observed haemodynamic instability associated with a caval cross clamp. The PB technique has become the main technique of choice in much of the literature, although outcomes are largely similar, and often come down to surgical preference (Vieira de Melo et al., 2011).

In the present study, there were no significant differences between either group on any measure of microcirculatory flow, and indeed there was no significant difference between the macrocirculatory parameters of blood pressure, stroke volume or cardiac output after reperfusion in these patients; at which point a significant difference may have been expected given the physiological alterations made by the release of the caval cross clamp in the CCC group. This finding is similar to other studies comparing the two techniques where no significant difference has been shown in morbidity, re-transplantation rate and post-operative kidney and liver function (Miyamoto et al., 2004, Nikeghbalian et al., 2014).

The strengths and weaknesses of this study are discussed in Chapter 7. The study was powered to examine for differences between pre- and post-transfusion microcirculatory indices. Therefore, with the size of the groups, it may have been underpowered to determine significant differences between the two modes of surgery.

Nonetheless, this is the first time that microcirculatory parameters have been compared between the main modes of liver transplant surgery.

In summary, microcirculatory function was not significantly affected by mode of liver transplant. Larger trials of patients undergoing liver transplantation powered to identify differences between surgical technique may detect significant differences to investigate whether intra and post-operative microcirculatory parameters can be correlated with outcome. Post-operative microcirculatory tests in intensive care should now be undertaken to help to elucidate if intraoperative measures have an effect on post-transplant microcirculation and oxygen delivery.

Chapter 9 Tissue oxygenation and vascular reactivity in liver transplantation: Observational study

9.1 Introduction

It has been shown that vascular reactivity is disrupted in cirrhosis. The primary goal of intraoperative management is the adequate perfusion and oxygenation of the tissues whilst maintain depth of anaesthesia and providing analgesia to facilitate surgery. Adequate delivery of oxygen to the tissues is therefore paramount. Given that DO_2 is a directly proportional to the oxygen carrying capacity of the blood it is of interest whether changes in these variables have an effect upon tissue oxygenation. Peripheral tissue saturation (StO2) can be measured non-invasively using NIRS and the adequacy of the microcirculation calculated using variables derived from a vascular occlusion test (VOT). The effect of haemoglobin concentration and subsequent blood transfusion to correct blood loss upon tissue oxygenation can help to guide intraoperative management.

In addition, correlation of StO2 with the measures of microcirculatory flow using IDF discussed in the previous chapters may help to quantify the direct effect of microcirculatory competence upon tissue oxygenation.

NIRS is employed intraoperatively as a tool to monitor cerebral oxygenation, particularly in paediatric practice, cardiac surgery, or for surgery upon the carotid such as carotid endarterectomy (Murkin and Arango, 2009). There is limited literature relating to the use of peripheral NIRS with the VOT intraoperatively, and this is the first time that such values have been recorded during liver transplantation.

9.2 Study aims and hypothesis

The primary outcome was the change in vascular reactivity before and after transfusion in patients undergoing liver transplantation.

Secondary outcomes were change in: ischaemic phase (StO2 downslope), ischaemic area, minimum tissue oxygen saturation during VOT, reperfusion phase (StO2 upslope), post reperfusion hyperaemia area; before and after blood transfusion.

This may help to determine the optimum transfusion threshold for these patients, and allow optimisation of micro-circulatory parameters of oxygen delivery in addition to those of the macro-circulation already measured in routine practice.

I hypothesised that the that increasing anaemia will lead to a fall in tissue oxygenation. The effect of the transfusion of packed red cells will be measured, hypothesising that they shall have a smaller effect tissue oxygenation than native circulating red blood cells. Improved parameters of microcirculatory flow (total vessel density and perfused vessel density) will lead to an improvement in NIRS measures of reperfusion.

9.3 Methods

The NIRS-VOT data was collected simultaneously alongside the IDF data discussed in chapter 6. Thus, the methods, participant selection and conduct of anaesthesia are as described in chapter 6.

9.3.1 Ethical approval

The study design, documentation and protocols were in accordance with the Declaration of Helsinki, and were approved by the Research Ethics Committee of the East of England – Essex (Reference 14/EE/1275).

9.3.2 NIRS VOT protocol

Thenar eminence skeletal muscle has been selected for the site of NIRS-VOT. This approach has been used successfully in previous clinical studies and the thenar eminence is minimally susceptible to the confounding effects of excess adipose tissue and oedema (Gerovasili et al., 2010). StO_2 is measured using a tissue spectrometer (Inspectra Model 650, Hutchinson Technology, MN, USA or similar), fitted with a probe of 15mm optode spacing and is defined as the ratio of oxygenated

to total haemoglobin concentration. The device uses light at 680nm, 720 nm, 760 nm and 800 nm and measures StO_2 every three seconds. Data is recorded onto a laptop computer for later analysis (InSpectra Analysis Program v4.0, Hutchinson Technology, MN, USA).

A manual blood pressure cuff was placed around the upper arm, with the arms wrapped at the patient's side during surgery. A single use NIRS StO_2 sensor (Inspectra model 1615, Hutchison Technology, MN, USA) is attached to the thenar eminence of the passively supinated dominant hand and taped around its edges to reduce any effect that excessive ambient light might produce. Subjects will the anaesthetised and paralysed during all measurements so movement artefact will be eliminated. After a five-minute period of stabilisation, baseline StO_2 recordings are commenced. The blood pressure cuff is rapidly inflated to 250mmHg for three minutes then deflated. NIRS data collection is continuous following cuff release. The exact times of occlusion and deflation are electronically marked on the NIRS tracing.

This was repeated at each time point.

9.3.3 Data analysis

The electronic data is analysed using the automated InSpectra Analysis Program v4.0, (Hutchinson Technology, MN, USA. The following variables are derived: baseline, minimum and peak StO2 (%); StO_2 downslope (% per minute) and StO_2 upslope (% per second), ischaemic area under curve from cuff inflation to minimum, and hyperaemic area under curve above baseline post-reperfusion. Down slope and upslope are calculated by the software using the slope of the least squares error linear equation. The down slope measurement commences at the point where StO_2 is 0.98 times the baseline StO_2 and the endpoint a 1 minute later. The start point for up slope is determined when the recovery StO_2 first exceeds 1.2 times the minimum StO_2 reading and the endpoint is when the recovery StO_2 reaches 0.85 times the baseline StO_2.

9.3.4 Statistical analysis

Statistical analysis was performed using Microsoft Excel (Microsoft, USA) and SPSS Statistics 24 (IBM, USA). Normal distribution was confirmed by a Shapiro-Wilk test ($p>0.05$) and visual inspection of data histograms and Q-Q plots. Values are summarised by median and interquartile ranges or mean and standard deviation as appropriate. Differences between time points were analysed using related samples Friedman' Two-way Analysis of Variance by ranks test and between related samples with Wilcoxon Signed Rank test with Bonferroni correction applied. Correlation between NIRS variables and other physiological variables was assessed using Spearman's rank correlation. In all cases a P value <0.05 was considered to be statistically significant.

9.4 Results

NIRS measurements were taken from all patients at baseline and at points intraoperatively outlined in Chapter 8. 17 patients received blood transfusions. Due to technical challenges pre-transfusion readings were not taken in 2 patients, and thus pre- and post-transfusion NIRS readings were recorded in 15 patients.

Baseline characteristics are described in Chapter 8. Additional markers of oxygenation are detailed in table 17.

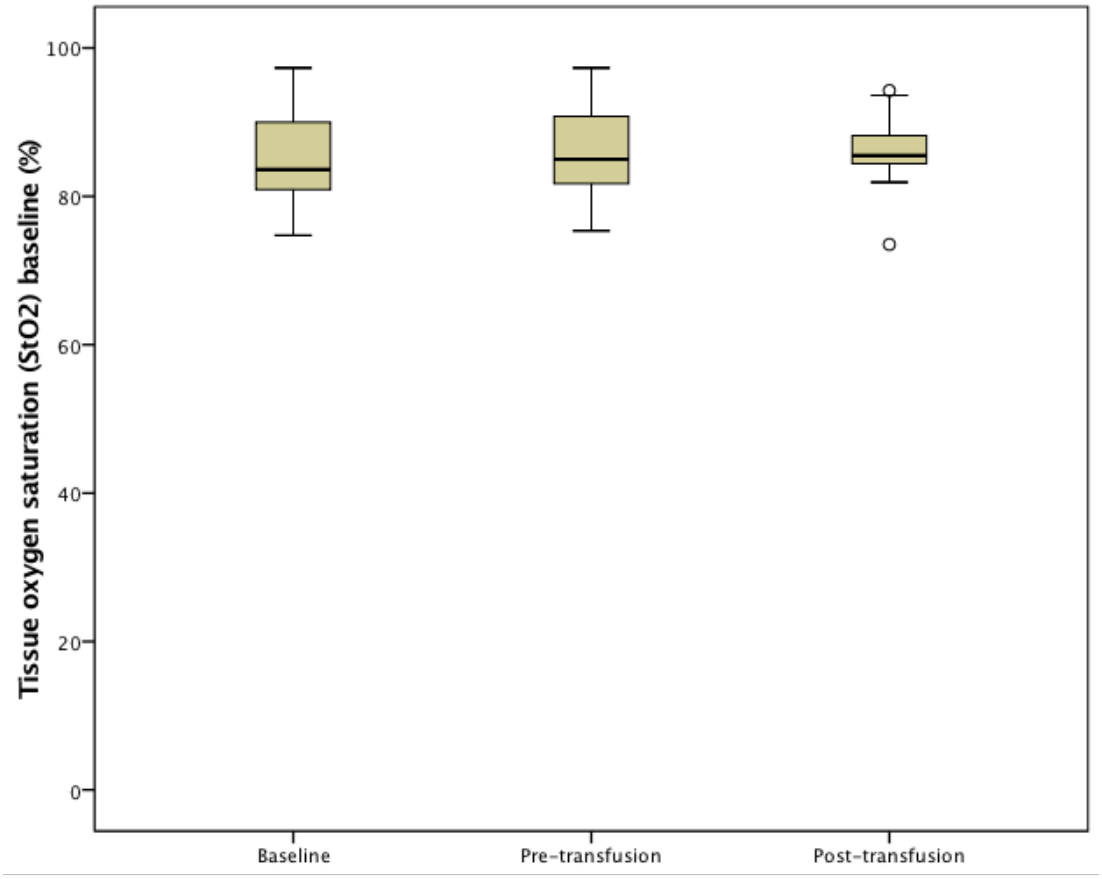
Table 17: Mean (SD) values of oxygenation

Variable	Baseline	Pre-transfusion	Post-transfusion
FiO_2 (kPa)	.50 (0.05)	.47 (0.07)	.46 (0.06)
SpO_2 (%)	98.4 (1.8)	99.1 (1.2)	99.1 (1.8)
PaO_2 (kPa)	27.51 (13.3)	26.0 (6.8)	26.85 (7.5)

9.4.1 Baseline tissue oxygen saturation

Average baseline StO_2 at baseline measurement was 82.80 % (78.67 – 90.48) (Figure 24). There was no significant difference between pre- and post- transfusion baseline average StO_2 85.00 % (81.06 – 91.14) vs 85.49 % (84.34 – 89.81) (p=.248).

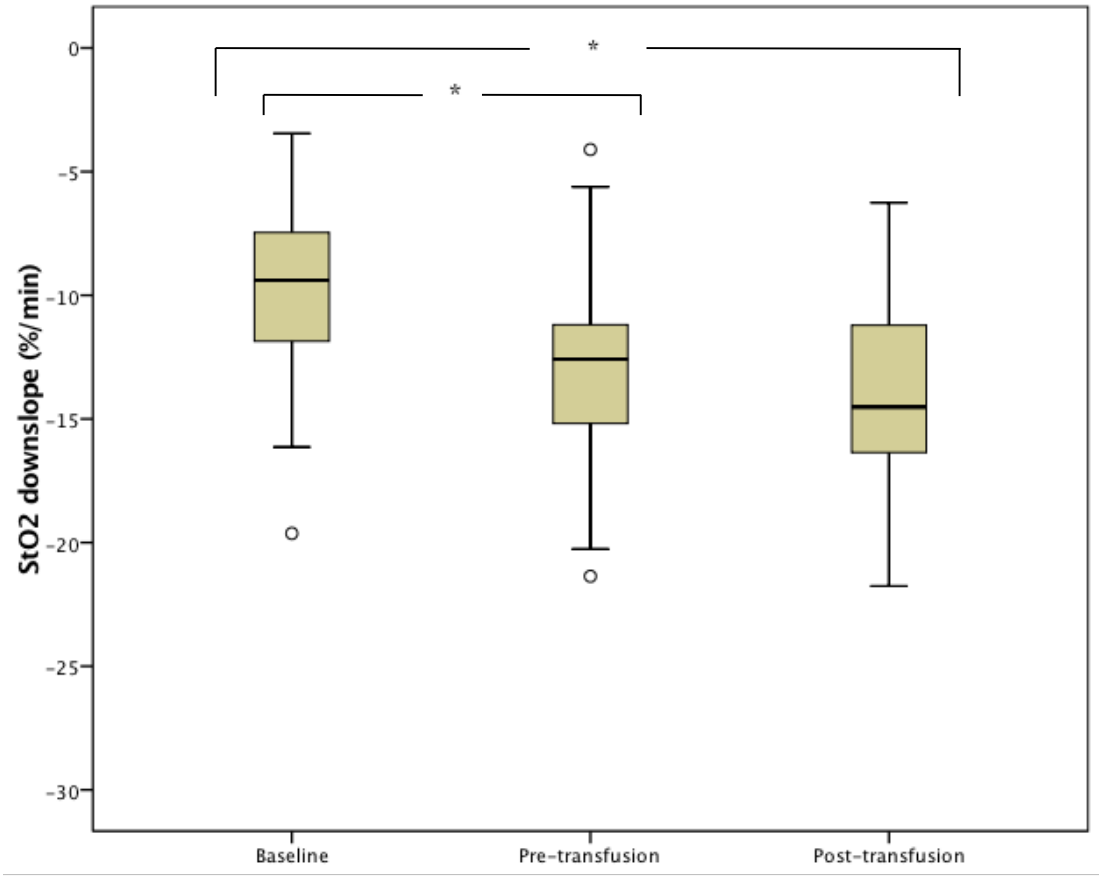
Figure 24: Baseline tissue oxygen saturation at each time point



9.4.2 Ischaemic phase during vascular occlusion test (StO2 downslope)

There was a significant difference in StO_2 downslope readings between baseline (-9.39 %/min (-12.03 – -7.44) and pre-transfusion (-12.58 %/min (-15.5 - -10.85) (p=0.004)), and baseline and post-transfusion (-14.52 %/min (-16.86 - -11.05) (p=0.001)) (Figure 25). There was no significant difference between pre- and post-transfusion StO_2 downslope (p=0.198).

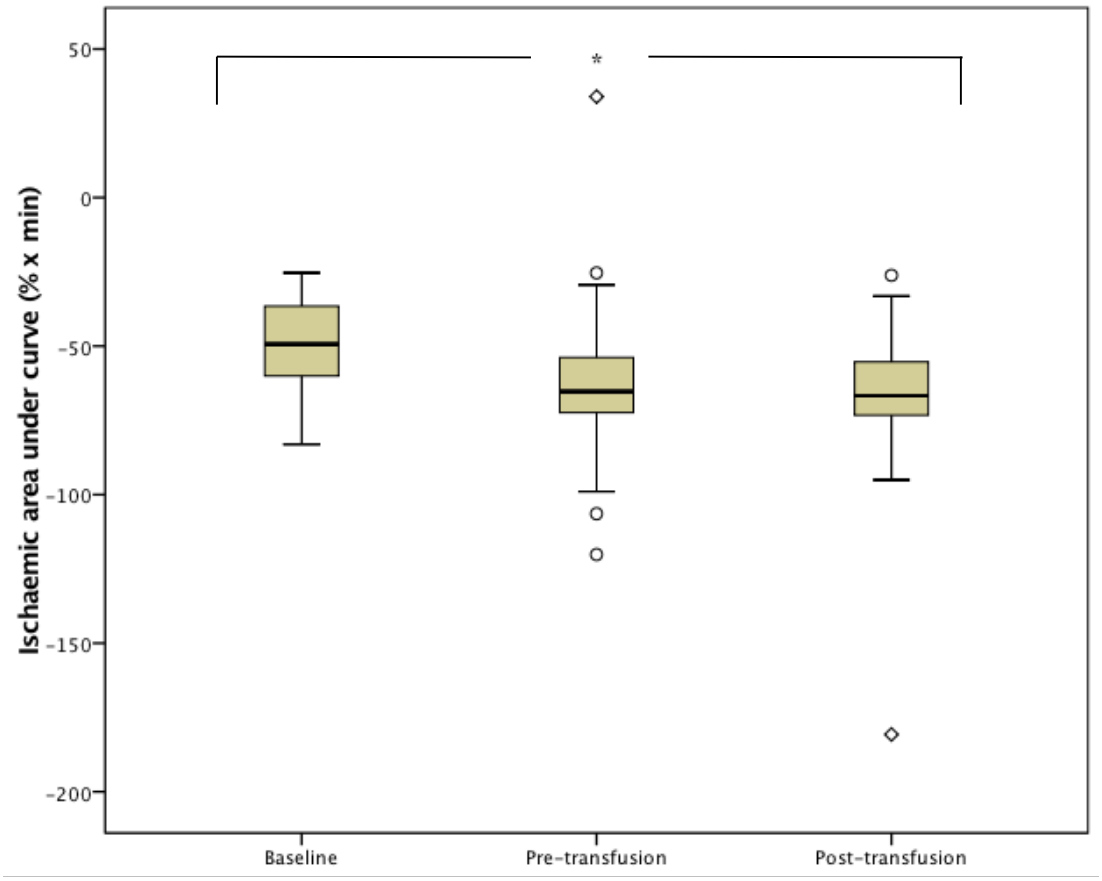
Figure 25: StO_2 downslope during vascular occlusion test at each time point



9.4.3 Ischaemic area

There was no significant difference between baseline and pre- -transfusion ischaemic AUC (baseline: -49.32 % x min (-60.60 - -35.76); pre-transfusion: -65.31 % x min (-77.27 - -48.71) (p=.05)) and between pre- and post-transfusion ischaemic AUC (AUC post-transfusion -66.69 % x min (-75.45 - -52.39) (p=.807)). However, there was a significant decrease from baseline to post-transfusion (p=0.002) (Figure 26).

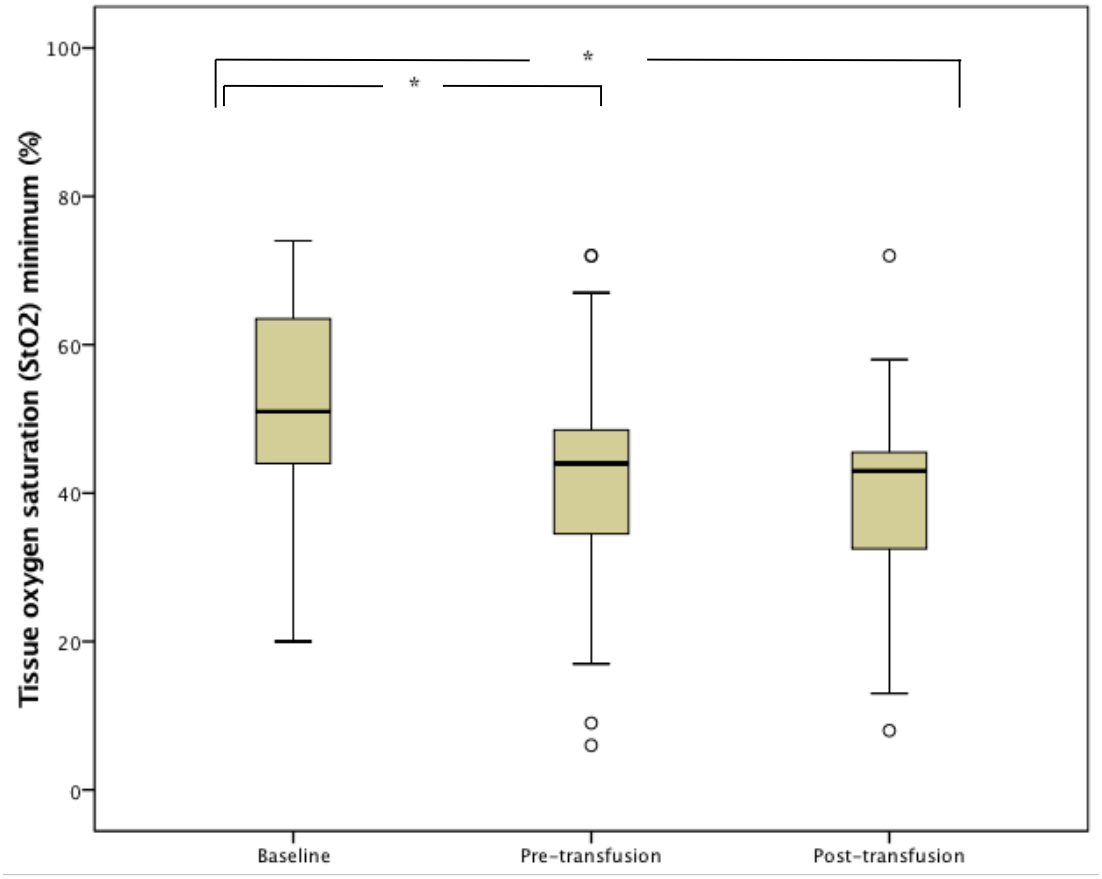
Figure 26: Ischaemic area under curve during vascular occlusive test at each time point



9.4.4 Minimum tissue oxygen saturation during VOT

There was a significant decrease in the minimum StO_2 during VOT from baseline (51 % (44 – 64.75) to pre-transfusion (44 % (34 – 49) (p=0.011)) (Figure 27). There was no significant difference seen between pre- and post-transfusion (43% (27 – 47) (p=0.807)). The decrease between baseline and post-transfusion was significant (p=0.001).

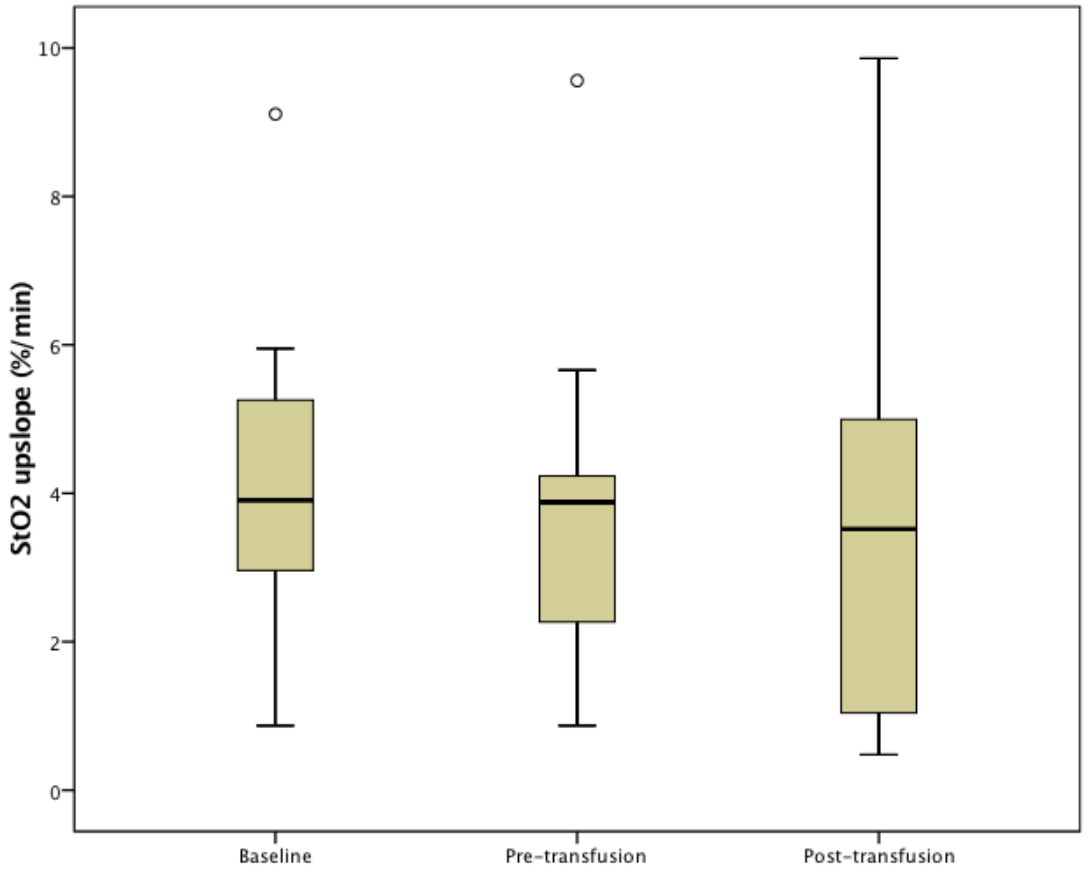
Figure 27: Minimum StO_2 during vascular occlusive test at each time point



9.4.5 Reperfusion phase during vascular occlusive test (StO2 upslope)

There was no significant difference in the tissue saturation upslope during the reperfusion phase of the vascular occlusive test between baseline (5.29 %/min (3.91 – 5.92)), pre-transfusion (3.88 %/min (2.17 – 4.24)) or post-transfusion (3.52 %/min (.91 – 5.25)) (p=.782). (Figure 28)

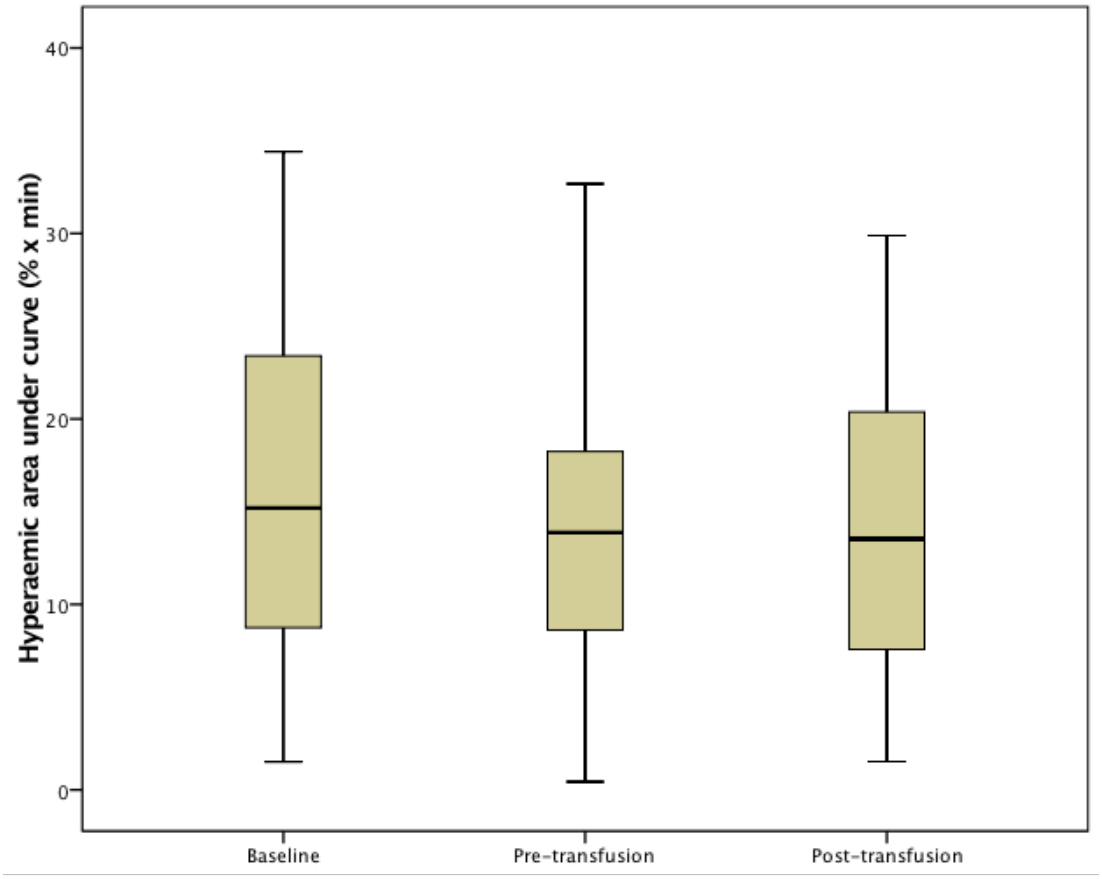
Figure 28: StO_2 upslope during vascular occlusive test at each time point



9.4.6 Post reperfusion hyperaemia

There was no difference in the AUC of post reperfusion hyperaemia following the VOT at baseline (15.20 (% x min) (8.38 – 23.85), pre-transfusion (13.87 % x min (8.01 – 18.40) and post-transfusion (13.53 % x min (6.94 – 21.93) (p=0.276). (Figure 29)

Figure 29: Post-reperfusion hyperaemia area under curve at each time point



9.4.7 Effect of haemodynamic parameters and microcirculatory flow

There were no consistent correlations at all three time points between the NIRS VOT measures, physiological parameters (SpO2, FiO2, PaO2, heart rate, Noradrenaline concentration, lactate, Hb or HCT) or IDF measures (small vessel TVD, PVD, PPV, MFI and HI).

The strongest correlations were between post-transfusion VOT ischaemic area and small vessel TVD (r= -.727 (p=0.003), small vessel PVD (r= -.754 (p= 0.002) and small vessel PPV (r= -.749 (p=0.002). At baseline the correlations coefficients for VOT ischaemic area were .104 for small vessel TVD, 0.104 for PVD and .280 for PPV. Pre-transfusion correlation coefficients for these measures were -0.279 for small vessel TVD, -0.268 for small vessel PVD and -0.044 for small vessel PPV.

9.5 Discussion

9.5.1 Statement of principle findings

Mean baseline StO_2 was not affected by either blood loss, or the transfusion of RBCs.

Blood loss led to a significant increase in the oxygen consumption rate as measured by the StO_2 downslope. Blood transfusion did not significantly subsequently change this (indeed, it was further increased from pre-transfusion). The ischaemic area was only significantly decreased from baseline to post-transfusion.

There was a significant decrease in the minimum StO_2 during ischaemia during the VOT between baseline and pre-transfusion and this difference remained post-transfusion.

The reperfusion phase of the VOT was not significantly different between the time points, although the trend was to lower values. The post-reperfusion hyperaemic phases did not alter significantly either.

There was a strong correlation between the ischaemic area of the VOT and IDF measurements of sublingual microcirculation post transfusion. There was poor correlation with other physiological variables of oxygenation, haemodynamics and indices of perfusion.

9.5.2 Interpretation of results

NIRS VOT has been used in a variety of clinical situations including surgery (Hirano et al., 2005, Vellinga et al., 2010), and the effect of blood transfusion (Donati et al., 2014, Creteur et al., 2009). Studies of septic patients have shown that oxygen consumption is decreased, possibly due to mitochondrial dysfunction or impaired microcirculatory flow depriving the tissue of the oxygen required (Gerovasili et al., 2010).

Tissue oxygenation was well maintained throughout this study, whether at baseline, after blood loss and after blood transfusion (StO2 82.80 %, 85.00 % and 85.49 respectively, suggesting balanced supply and demand throughout the operation.

However, this merely represents an average of tissue oxygenation, predominantly reflecting the saturation blood in the of haemoglobin after oxygen delivery and extraction since the majority of this blood is venous.

In a study of 25 cirrhotic patients, Thomson et al demonstrated baseline StO_2 variables of 75%, with a StO_2 downslope of -11.3 (-13.6 - -9.7) and ischaemic AUC (-51.6 (-64.4 - -44.7); which were not significantly different from normal controls (Thomson et al., 2010). However, the cirrhotic patients had a significantly higher post-reperfusion hyperaemia (17% (15-19)) than controls (p=0.009). There were no significant differences in the reperfusion and hyperaemic phases of the VOT over the course of the study from baseline to post-transfusion, suggesting that the integrity and function of the vascular endothelium was unchanged during surgery, in keeping with the findings from the microcirculatory study in the previous chapter. This again contrasts the findings of my study and Thomson's of cirrhotic patients to those who are septic or critically ill. This suggests that whilst cirrhosis can lead to decompensation and multi-organ failure, stable patients with cirrhosis have normally functioning microcirculation and tissue oxygenation. Studies of other groups have identified adaptation to physiological states or the environment. Studies of Sherpa's versus lowlanders at altitude that Sherpas can maintain greater microcirculatory flow, as part of a long-term adaptation to hypoxia. Thus, patients with stable cirrhosis may develop adaptations to their vascular endothelium, but with little reserve for decompensation when triggered, leading to impairments in vascular reactivity.

It has been suggested that comparing chronic anaemia with acute onset anaemia – as would be seen in surgical patients with acute bleeding - may not be equivalent due to the compensatory mechanisms that may develop, including higher levels of 2,3-DPG. 2,3-DPG is an allosteric modifier of Hb and increased concentrations increase the release of oxygen by shifting the oxyhaemoglobin dissociation curve to the right. Loss of 2,3-DPG causes a left shift of the oxyhaemoglobin dissociation curve, increasing the affinity of Hb for oxygen which reduces its offloading at the metabolising tissue by shifting p50 (the oxygen tension at which haemoglobin is 50% saturated) from 29 to 20mmHg (Raat and Ince, 2007). However studies in rats show that even when 2,3-DPG had been reduced by 50% during storage, crucial

oxygen delivery was unchanged and cardiac index and oxygen extraction was unchanged, with only a minimal effect on oxygen reserve (d'Almeida et al., 2001).

In a study of anaemic outpatients who received blood transfusion, Yuruk et al demonstrated that the rise in Hb from 82 (76 – 89) g/L to 110 (98 – 118) g/L was accompanied by a significant increase in thenar StO2 (increasing from 81% (80-84%) to 86% (81-89% (p=0.002)) after blood was transfused (Yuruk et al., 2012). Those patients were transfused 3 units of blood in comparison to the 1 unit transfusion comparisons in this study, which led to the observed Hb rise from 74.94 (10.0) (g/l) to 82.63 (8.9) (g/l) post transfusion. A greater volume of transfused blood may be required to demonstrate a significant response in NIRS variables tissue oxygenation.

In healthy volunteers thenar eminence StO2 measurements were 87% +/-3% (Bezemer et al., 2009), suggesting that the participants of this study had adequate tissue oxygenation by the time of transfusion and thus limited capacity for improvement. It may be the case that a greater rise in Hb would be required in order to see an effect upon tissue oxygenation. A study of critically ill patients who had NIRS VOT pre- and post-blood transfusion failed to show any significant change in tissue oxygenation; although demonstrated significant inter-individual variability (Creteur et al., 2009). Similar results were shown in another study of severely septic patients who were given blood transfusions (Sadaka et al., 2011).

An explanation for this could be the failure of transfused red blood cells to efficiently transport oxygen in the immediate post transfusion phase, since the storage lesion can reduce 2,3- DTP and ATP levels, thus reducing the oxygen carrying capacity. As has been discussed previously, RBCs can also act as an oxygen sensor to titrate oxygen demand and delivery by the release of local NO and ATP, resulting in vasodilatation (Crawford et al., 2006). The storage of RBC could thus reduce the cell's ability to mediate local vasodilatation despite normal microvascular reactivity, nullifying the effect of the transfused RBCs on measured tissue oxygenation.

In the present study, the StO_2 downslope measured by the VOT between baseline and pre-transfusion was significant increased. The results suggest that the oxygen

consumption rate had increased, whereby a greater rate of desaturation of haemoglobin was occurring, or microvascular reactivity had altered the delivery of oxygen to the tissues. As shown by the previous study in chapter 7 and 8, microvascular perfusion, density and flow heterogeneity was well maintained between baseline, pre-transfusion and post transfusion. The failure of blood transfusion to then improve this is in keeping with the observations from other studies whereby transfused RBCs do not have the same effect upon oxygen carrying capacity as native, non-stored RBCs. However, it may be entirely unrelated to the haemoglobin and oxygen carrying content of the blood, but due increased oxygen demand from the tissues due to the stress of surgery or effects of anaesthesia upon autoregulatory function of the vasculature.

Studies have shown that the tissue oxygen extraction rate measured by the VOT downslope is significantly reduced in critically ill and septic patients (Donati et al., 2016).This can be attributed either to lower oxygen consumption by muscle tissues due to mitochondrial dysfunction or impaired microcirculatory flow failing to adequately deliver oxygen to the tissue (Gerovasili et al., 2010). In a study of patients with severe post-partum haemorrhage, VOT downslope recorded as $S_{Occlusion}$ was impaired when patients were haemodynamically unstable and the slope increased at discharge – suggestion that oxygen consumption improved as the bleeding and haemodynamics were controlled (Heyer et al., 2009).

In a study of healthy volunteers donating 500ml of blood, which was associated with a significant reduction in systolic blood pressure, there a no significant difference in the measures of tissue oxygenation using a NIRS VOT (Jeger et al., 2010). This was contrary to the findings of other studies of blood loss after blood donation and the authors postulated that they had not caused a significant enough alteration in blood volume to create an effect in the healthy patient It may be that this blood loss was not significant enough to outstrip the compensatory mechanisms of hypovolaemia in the healthy circulation.

The area under the curve (AUC) during the ischaemic phase was significantly lower after transfusion than at baseline (-66.69 % x min (-75.45 - -52.39) vs -49.32 % x min (-60.60 - -35.76) (p=0.002) respectively). This is in keeping with the StO2

downslope data, demonstrating a likely increase in oxygen consumption over the course of the operation within the tissues. This was further demonstrated by the lower minimum StO2 readings after baseline (51 % (44 – 64.75) to pre-transfusion (44 % (34 – 49) (p=0.011)). However, this may have been a consequence of the lower oxygen carrying capacity of the blood due to blood loss, which failed to recuperate after blood transfusion (minimum StO2 43% (27 – 47)) (p=0.807).

Reperfusion upslope and post ischaemic hyperaemic AUC values were not significantly altered at the different stages, demonstrating a properly functioning vascular endothelium.

Interestingly there were no consistent correlations with the normal physiological measures of intraoperative monitoring, nor noradrenaline or correlation with markers of tissue perfusion including lactate and Hb concentration or HCT, despite the Hb changes occurring intraoperatively which fit the pattern seen during the NIRS VOT tests.

When comparing the IDF findings from the previous chapter with the NIRS VOT data a strong correlation was seen after blood transfusion between small vessel TVD and PVD and the VOT ischaemic area (r= -.727 (p=0.003) and r= -.754 (p= 0.002) respectively for TVD and PVD). This suggests that at this point, once blood has been transfused, that oxygen consumption is more strongly related to the functional capillary density than the oxygen carriage of the blood. This is congruent with the NIRS VOT findings already discussed whereby the newly transfused RBCs can act to increase blood viscosity and improve the FCD without a significant effect upon the oxygen carriage of the haemoglobin.

This hypothesis requires further testing in a more controlled setting, with greater numbers of patients and ongoing measurements after the initial transfusion in which it could be ascertained whether this association receded as the RBCs regained their function with replenishment of 2,3-DPG and ATP.

9.6 Limitations

This study contained a small number of participants and in addition, no healthy controls were used to compare these results against. In the absence of this, results from studies of healthy volunteers or similar cohorts have been used for comparison. Care must be taken in this respect since different NIRS monitors are available with different algorithms for slope analysis that can produce different variables for the same measure of tissue oxygenation.

The biggest limitation of any study of patients having liver transplantation is the numerous confounding factors that influence the patient's physiology. These include the condition of the donor graft, the surgical expertise and technique, operative time and complexity, and the presence or absence of intraoperative coagulopathy.

All of the results of this study have been gained under the influence of anaesthesia, which whilst providing an immobile subject, is in itself is associated with multiple alterations in the circulation, vasodilatation and reduced cardiac output as well as alterations to microvascular flow and vascular reactivity. Ideally longer follow up of patients to see whether variables related to blood transfusion had longer term effects. However, in patients who have just had a liver transplant their physiology is often so significantly deranged that any signal could easily be lost, given the huge influence of the graft liver function, the commencement of immunosuppression and use of inotropes.

The experiment was conducted under conditions of hyperoxia with an FiO_2 between 0.46 and 0.50. It would be interesting to see whether greater variation in tissue perfusion was noted, in the same way that studies under conditions of systemic hypoxia have demonstrate tissue hypoxia (Martin et al., 2013).

The NIRS VOT technique has been extensively utilised within medical and research literature and its performance is not technically challenging. The NIRS probe and blood pressure cuff had to be sited prior to the draping of the patient and were inaccessible throughout the course of the operation which meant that any equipment problems which occurred were challenging to rectify. The InSpectra analysis

programme is not fully automated. Before the calculations of slope and area under curve are performed, the operator has to select the precise time of cuff inflation and deflation on the graph, or enter that data numerically. Thus, there is a potential for error if the correct points on the graphs are not selected, which can easily occur with the limited functionality of the software package. Future advances of the software will improve this – possibly by integrating the data capture with analysis.

As with other microcirculatory monitoring tools, the utility of NIRS at the bedside beyond the simple measurement of tissue oxygenation is limited by the need to perform the VOT test then separately analyse the data using computer software.

9.7 Conclusions

This study demonstrated for the first time during liver transplantation that cirrhotic patients maintain their circulatory competence intraoperatively to maintain tissue oxygenation. Intraoperative blood loss that led to blood transfusion was associated with an increased rate of oxygen consumption which was not significantly altered by blood transfusion.

As was the case when measuring the sublingual microcirculation, there was little correlation with the conventional monitoring of the macro-circulation intraoperatively. However, the strong correlation between the IDF measures of sublingual small vessel microcirculatory density and oxygen consumption provide an area for further investigation for elucidating the actual impact of transfused RBCs upon microcirculatory perfusion and tissue oxygenation.

Chapter 10 Conclusions and Further Work

10.1 Aims and Hypothesis

The aim of this thesis was to explore the theory that microcirculatory blood flow is affected by bleeding and blood transfusion during liver transplantation. A retrospective cohort study was performed to analyse the correlation between pre-operative anaemia and blood transfusion rates during liver transplantation. This was followed by a prospective study assessing sublingual microcirculatory blood flow and tissue oxygenation and vascular reactivity during liver transplantation to examine the effect of blood transfusion. I hypothesised that the vessel density of the microcirculation would decrease as the haemoglobin decreased due to the de-recruitment of capillary networks and that increasing anaemia would lead to a fall in tissue oxygenation and that the effect of the transfusion of packed red cells would have a smaller effect upon microcirculatory flow than native circulating red blood cells.

10.2 Statement of Principal Findings

Significant numbers of patients having liver transplant surgery were anaemic and iron deficiency was prevalent within this cohort. Over a quarter of patients received no intraoperative blood transfusions during this operation, for which the risk of bleeding is significant. Pre-operative anaemia and severity of liver disease was strongly correlated with the risk of blood transfusion. The odds of being able to have a transfusion free transplant in the absence of massive haemorrhage were significantly higher if the patient was not-anaemic preoperatively.

During the liver transplant operation, however, neither blood transfusions nor the measured haemoglobin concentration, had any significant impact upon sublingual microcirculatory flow density or perfusion in small vessels. Vascular reactivity was similarly unaffected by blood loss and the transfusion of RBCs. A significant increase in oxygen consumption rate on the StO_2 downslope on NIRS VOT testing was observed from baseline to pre-transfusion.

This thesis demonstrated for the first time during liver transplantation that cirrhotic patients maintain their circulatory competence intraoperatively to maintain tissue perfusion and oxygenation. It suggests that anaesthetic management based upon standard macrocirculatory parameters of haemodynamics maintained good microcirculatory flow. There was no correlation between those usual intraoperative physiological measurements and the microcirculatory findings. Intraoperative blood loss that led to blood transfusion was associated with an increased rate of oxygen consumption which was not significantly altered by blood transfusion. Whilst blood transfusion improved microcirculatory flow, these differences were not significant.

10.3 Interpretation of Results

Cirrhosis is associated with a wide range of intra- and extra-hepatic microvascular derangements, with altered autoregulation and abnormal vasodilatation and vasoconstriction within different organ beds. It represents a state of chronic inflammation that can easily decompensate, causing multi-organ dysfunction – in a similar manner to severe sepsis and septic shock. Further investigation is needed to direct treatment strategies including vasoactive drugs and fluid management strategies whilst evaluating their effect upon the microcirculation in cirrhosis.

Outcome after liver transplantation is subject to the influence of a diverse range of factors within both the donor and the recipient. A triad of risk of major bleeding, transfusion and anaemia is apparent in surgery and all are independently linked to adverse outcomes. Pre-operative anaemia has been associated with adverse outcomes after surgery within many specialities, including liver transplantation. It is a significant risk factor for blood transfusion by lowering the patient's reserve before meeting a transfusion thresholds in the event of bleeding. Nonetheless, even after adjustment for the risk of transfusion, anaemic patients have a higher relative risk of adverse outcomes compared to non-anaemic controls (Musallam et al., 2011). Anaemia should therefore be interpreted not only as a reduction in circulating red cell mass, but also as a marker for risk.

Cardiopulmonary exercise testing (CPET) is routinely utilised to assess fitness for surgery and to stratify risk (Moran et al., 2016). CPET can measure peak oxygen

consumption and determine oxygen uptake at anaerobic threshold as quantifiable measures of the ability of the body to meet increased oxygen demands. Patients with poor CPET results measured by reduced aerobic capacity and anaerobic threshold are at an increased risk of averse outcome after surgery (Snowden et al., 2010). In liver transplant patients, impaired aerobic capacity and anaerobic threshold are predictive of morbidity and mortality (Bernal et al., 2014). Cardiac output is a key parameter limiting exertional oxygen uptake (Levine, 2008). However, anaemia is also associated with a reduced exertion oxygen uptake and impaired exercise performance (Otto et al., 2013b). Therefore anaemia may be a marker of fitness for surgery, marking its risk for adverse outcomes (Otto et al., 2013a).

The oxygen demand of the body is increased during surgery, whilst the effects of anaesthesia lead to a reduction in cardiac output. This increases the patient's risk of reaching the critical DO_2 at which oxygen demand outstrips delivery, resulting in anaerobic metabolism and potentially tissue hypoxia. RBC mediated vasodilation through NO release can help to offset these changes (Raat and Ince, 2007). As the severity of anaemia increases, this ability may reduce, leading to a critical DO_2 at an earlier threshold. Meanwhile the reduction in plasma viscosity due to reduced circulating RBCs can reduce the functional capillary density, leading to supply limited oxygen consumption, further increasing the risk of inadequate tissue perfusion and oxygenation.

Even with a low haemoglobin concentration (mean baseline Hb was 94 g/L), the sublingual microcirculation intraoperatively showed high total vessel density and very few unperfused vessels. The flow was homogenous and almost entirely continuous, and subjectively appeared fast moving. As yet, the ability of the software to accurately quantify blood velocity is inadequate. Total vessel density and perfusion was well maintained in the face of intraoperative bleeding and haemodilution. This is an important finding in confirming that microcirculatory flow and perfusion was maintained at the transfusion thresholds utilised.

A concern that arises from the use of restrictive transfusion thresholds is the potential to delay transfusion until the oxygen carrying capacity of the blood is reduced below the critical DO_2, risking the precipitation of tissue ischaemia. Given that in healthy,

non-active subjects, a haemoglobin concentration of 50 g/L was tolerated, yet concern arose regarding myocardial ischaemia (Weiskopf et al., 1998); now that guideline transfusion thresholds are 70 g/L or, in some centres 60 g/L (Massicotte et al., 2012), the therapeutic window at which critical DO_2 is reached is close to these limits. Using microcirculatory monitoring in patients with both cirrhosis and during liver transplant, the effect of blood transfusion at a capillary level can be further elucidated and guide transfusion thresholds in a real-time manner in the operating theatre or at the bedside.

Once blood was transfused there was a non-significant increase in TVD and PVD. TVD is a measure of how many capillaries are open with red blood cells within them. Given that many of the small capillaries are smaller than the RBC itself, in order for flow to occur, the natural deformability of the RBC is vital. This allows the RBC to pass through. Since the IDF image of a vessel is actually only made up as a silhouette of the RBCs within, vessels that do not have RBCs flowing through them are not detected. Thus, TVD will decrease if there are insufficient RBCs to flow through all of the vessels within the tissue, or if they are unable to flow through the vessel – as would be the case if they could not modulate their shape to fit into the vessel. Since transfused RBCs have reduced deformability (Hess, 2014), an individual stored RBC might not be able to hold open a capillary – limiting its influence on TVD. Nonetheless, the presence of added numbers of RBCs within the plasma, each that can enter a vessel will be detected by the IDF video microscope and should result in an increased TVD.

Tissue oxygenation also was well maintained throughout the study. The oxygen consumption rate as measured by the StO_2 ischaemic downslope increased from baseline to the pre-transfusion reading, and the blood transfusion did not improve this. This suggests that increased oxygen was being utilised by the tissue, or oxygen delivery had been altered – possibly due to decreased oxygen carrying capacity of the blood. In human studies measuring the impact of RBC transfusions on markers of DO_2 tissue oxygenation are ongoing and have shown varied results in different patient groups. Walsh et al were unable to detect differences in indices of tissue hypoxia using gastric tonometry in anaemic critically ill patients (Walsh et al., 2004). Meanwhile in patients undergoing cardiac surgery, Yuruk et al demonstrated a

significant rise in microcirculatory Hb content, yet without a statistically significant rise in microcirculatory Hb oxygen saturation after blood transfusion. Oxygen saturation within microcirculatory Hb measured by spectrophotometry increased from 65.6 +/- 8.3% to 68.6 +/- 8.4% (p=0.06) (Yuruk et al., 2011). In sepsis, this may be due to oxygen uptake not being supply dependent, or because oxygen is not distributed to the tissues that need it. The failure of transfused RBCs to improve tissues oxygenation may be the microcirculation, or due to the RBC itself. Given that RBCs are transfused to immediately increase oxygen delivery to the tissues in acute blood loss, their time to function optimally due to low ATP, 2,3-DPG and NO may prove this management strategy unsound.

In liver transplantation, detection and treatment of pre-operative anaemia should be combined with other components of a prehabilitation package, including guided exercise and nutritional supplementation. In this manner anaemia should be viewed in the same way as frailty as a marker of risk, which although not removed entirely by resolution of the reduced red cell mass, provides a modifiable risk for another possibly greater risk – allogenic blood transfusion. Meanwhile further research into the microcirculation in cirrhosis and during liver transplantation will improve the understanding of the patient's response to transplantation and may help to predict outcomes.

10.4 Strengths and Weaknesses of the Thesis

This thesis contains the first reports of the use of IDF imaging and the NIRS VOT test during liver transplantation to quantify microcirculatory flow and vascular reactivity during the operation. The focus upon the effect of blood transfusion is timely and clinically important, as well as being an area of current research priority.

However, these studies contained only a small number of participants and in addition, no healthy controls were used to compare these results against. Whilst a standardised protocol for recording data was used, the observational nature of this data meant that it is not impossible that other factors that may have influenced these variables during the conduct of surgery.

Whilst the intraoperative changes were measured, no post-operative data was obtained to investigate the persistence of findings in the microcirculation in the intensive care unit. Additionally, no post-operative outcome data was analysed. Follow up data could be collected and added to the data gained intraoperatively.

The retrospective analysis in this thesis showed a strong association between anaemia, transfusion and outcome. Nonetheless, its findings were based only upon a single centre's data – which may not be applicable to other centres – and is at risk from confounding by unmeasured variables and missing data.

10.5 Conclusions and Proposed Future Work

The concept of liver transplantation without blood transfusion has grown from initial experience of transplanting patients who are Jehovah's Witnesses – and therefore unable to receive blood products due to religious beliefs. This provided evidence of the benefit of pre-operative management to reduce blood transfusion. Strategies implemented included the use of rEPO, iron and folic acid to increase red cell mass.

The complex interplay between anaemia, bleeding, blood transfusion and outcome in major surgery, including liver transplantation provides several therapeutic targets. The risk however remains as yet undefined in terms of the absolute effect on patient outcomes.

As yet, the optimal transfusion thresholds for blood remain undetermined. These have become progressively lower over the past two decades. It may be that further reductions in transfusion threshold are possible by being able to quantify the absolute effect upon tissue oxygen delivery and microvascular perfusion and the point at which it becomes compromised by anaemia.

Prospective interventional trials comparing differing transfusion thresholds upon parameters of microvascular perfusion during liver transplantation and other major surgeries should be undertaken.

Anaesthesia remains an art in which each individual anaesthetist synthesises the information available to them from their monitors of the patient at that time, the

patient's medical history and their previous experience and training, to then decide on a course of action. The complexity of this synthesis of variables mean that different responses will be found for every anaesthetist with every patient to a single number in a single variable at a different point during any operation. The addition of the additional information gained by each monitor, whether that be of haemodynamics, depth of anaesthesia, tissue oxygenation or microcirculatory flow adds more information to the complex decision-making process that we undertake continuously. The fact that conventional macrocirculatory parameters of monitoring did not correlate with the novel monitoring methods tested in this thesis raises questions as to their utility in the intraoperative setting.

IDF imaging remains a largely research based tool due to its technical challenges for image acquisition and analysis. As the manufacturers and software developers improve the automated image analysis systems, they may be able to play a more active role at the bedside in guiding management decision for the individual patient. Prospective interventional trials comparing differing transfusion thresholds upon parameters of microvascular perfusion during liver transplantation and other major surgeries should be undertaken. These should then include robust follow up of quality of life, mortality and morbidity to aid the decision making for future transfusion triggers.

The modifiability of pre-operative anaemia as a risk factor for adverse outcome is as yet unproven. There is an argument that pre-operative anaemia is a marker for patients at higher risk of adverse post-operative outcomes, in the same way as frailty, impaired renal function or protein-energy malnutrition. Nonetheless, attempts to ameliorate it should be attempted since this can at least modify the patient's risk from blood transfusion, and its association with adverse post-operative outcome.

Further research should include the prospective randomised treatment of pre-operative anaemia in patients awaiting liver transplant. This is mandated by the association of poor outcome after transplant in patients with iron overload as well as the association between iron and infection in the context of immunosuppressed patients.

In the meantime, robust implementation of evidence based patient blood management strategies can help to reduce unnecessary blood loss and transfusion; and their impact should be studied and assessed for both clinical efficacy and cost effectiveness.

Peer reviewed manuscripts related to this thesis

1. Clevenger B, Mallett SV, '*Transfusion and coagulation management in liver transplantation'*, World J Gastroenterol 2014 May 28;20(20):6416-6158.
2. Abeysundara L, Mallett SV, Clevenger B, *Point of Care Testing in Liver Disease and Liver Surgery*, Seminars in Thrombosis and Hemostasis 2017, Jun: 43(4):407-415

Abstracts related to this thesis

1. Clevenger B, Richards T, Mallett SV, Martin D *'Association between haemoglobin concentration and sublingual microcirculation in anaesthetized patients undergoing liver transplantation'* EJA 2016, Vol 33, e-Supplement 54; 48
2. Clevenger B, Henley M, Mallett SV *'Anaemia and Blood Transfusion in Liver Transplantation: Time for Action?'* Liver Transplantation 2014, Vol 20, S227

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