## ARTICLE TITLE

Review article: the role of the microcirculation in liver cirrhosis


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## KEYWORDS

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## SUMMARY

### Background

Intrahepatic microvascular derangements and microcirculatory dysfunction are key in the development of liver cirrhosis and its associated complications. Whilst much has been documented relating to cirrhosis and the dysfunction of the microcirculation in the liver parenchyma, far less is known about the state of the extrahepatic microcirculation and the role this may have in the pathogenesis of multiple organ failure in end stage liver cirrhosis.

### Aim

To provide an update on the role of the microcirculation in the pathophysiology of cirrhosis and its associated complications and briefly discuss some of the imaging techniques which may be used to directly investigate the microcirculation.

### Methods

A Medline literature search was conducted using the following search terms: ‘cirrhosis’, ‘microcirculation’, ‘circulation’, ‘systemic’, ‘inflammation’, ‘peripheral’, ‘hepatorenal’ and ‘hepatopulmonary’.

### Results

Significant microvascular alterations exist in patients with cirrhosis and the heterogeneous character of these, both among organ systems and within specific organs, is demonstrated. Data suggests that the systemic inflammation associated with advanced cirrhosis, induces microcirculatory dysregulation and contributes to

haemodynamic derangement. The resultant vasoconstriction and hypoperfusion in the systemic extrahepatic microvasculature, is likely to be instrumental in the pathophysiology of organ failure in decompensated cirrhosis. The mechanistic action of vasoactive agents used to correct the circulatory disturbance of advanced cirrhosis is poorly understood, however it is likely that they involve the microcirculation.

## Conclusion

Further research into the role of the microcirculation in patients with liver cirrhosis using devices such as the sidestream- and incident-dark field imaging videomicroscopes, will improve physicians understanding of the pathophysiology of cirrhosis, and may provide a platform for real time evaluation of an individual's microcirculatory response to vasoactive mediators, thus guiding their therapy.

## INTRODUCTION

Cirrhosis is an increasing cause of morbidity and mortality in developed countries. It is now the fifth leading cause of death in the United Kingdom and the thirteenth worldwide (1, 2). The progression of cirrhosis is characterised by significant intra- and extrahepatic microvascular alterations and dysregulation. A dysfunctional microcirculation leads to regions of heterogeneous microvascular flow which causes tissue hypoxia. In turn, this creates worsening microcirculatory dysfunction and ultimately a downward spiral ensues eventually leading to organ failure and death (3). The structural and dynamic changes that occur in the microcirculation of cirrhotic patients are widespread. Within the liver, the hepatic microvasculature demonstrates increased intrahepatic resistance, and this results in portal hypertension and circulatory dysfunction (4-6). Whilst the systemic haemodynamic dysfunction of cirrhosis is well characterised, the extra hepatic microcirculation in cirrhosis has been less widely studied. Initial reports have shown heterogeneous alterations and microcirculatory dysfunction in extrahepatic vascular beds (7), however the extent of microcirculatory abnormalities and the impact this may have in the pathogenesis of organ failure in end stage liver cirrhosis is yet to be determined.

In the intensive care setting, technological advancements in hand held videomicroscopes have made feasible the monitoring and quantification of the microcirculation at the bedside. Certainly, within the context of the critically ill, derangements in the microcirculation are frequently encountered, and a direct correlation is seen between the degree of microvascular dysfunction present, and the development of multiple organ failure in sepsis (8-10). Given the importance of restoring a homogenous microvascular flow in septic patients, a number of recent

studies have focused on the effect of pharmacological agents on the microvasculature. These studies have shown that despite restoration of systemic haemodynamics with vasopressor agents there is often not a parallel improvement in the microcirculatory alterations, leading to a loss of systemic and microcirculatory coherence. Vasoactive pharmacological agents are also commonly used to treat cirrhosis and its associated complications, especially in the context of variceal bleeding and renal impairment. Unfortunately however, not all patients respond to exogenously administered vasoconstrictors, and certainly the microvascular response to these agents is poorly defined.

This article therefore provides an update on the role of the microcirculation in the pathophysiology of cirrhosis and its associated complications. We review the literature on the study of the microcirculation in the liver parenchyma and extrahepatic regional tissue beds. Furthermore, we describe some of the microvasculature imaging techniques enabling real time monitoring of the microcirculation in vivo, knowledge of which may advance our comprehension of the mechanistic action of therapeutic agents and potentially guide future treatment modalities within this field of interest.

## THE MICROCIRCULATION

### Anatomy and regulation

At the tail end of the oxygen cascade, and ultimately regulating tissue blood flow to match micro-regional oxygen demand, lies the largest ‘organ’ in the body – the microcirculation (11). Anatomically it consists of a network of blood vessels less than

100micrometres (μm) in diameter including arterioles, capillaries, post-capillary venules, and the blood flowing within these vessels (12).

The structure (and function) of the microcirculation is highly heterogeneous between different organ systems. Arterioles, arising after the original artery has entered an organ and bifurcated up to eight times, continue to subdivide until vessel cross-sectional diameter is <20μm. Below this size, flow continues through capillaries wherein the microvessels' diameter is only marginally larger than the erythrocytes flowing within them in single file (8-10μm). Blood is drained away from capillaries through increasingly enlarging post-capillary venules.

Functionally the microcirculation serves to transport oxygen and nutrients to tissue cells, and to remove carbon dioxide and other products of metabolism. Additionally, it plays a role in immunological function and temperature regulation, in modulating the distribution of fluid between the intravascular and extravascular compartments, and in delivering therapeutic agents to target cells (3, 13).

Capillary blood flow is determined by the hydrostatic driving pressure, haemorheology, capillary patency, and arteriolar tone. Regulation of microcirculatory vascular tone, ultimately determining capillary patency, can be viewed as a three-tier hierarchy; autoregulation (lower tier), intrinsic regulation (middle tier), and extrinsic regulation (top tier) (12) (Figure 1). Autoregulation predominantly involves the myogenic response to increased blood pressure and distension of the vessel wall. Intrinsic regulation involves the release of regulatory molecules to control vessel tone; vasodilators of functional hyperaemia (carbon dioxide ($CO_2$), lactate, adenosine, Potassium ions), endothelial secretions (nitric oxide (NO), prostacyclin ($PGI_2$), endothelin (ET-1), endothelium-derived hyperpolarizing factor (EDHF)) and

local paracrine secretions (histamine, serotonin, thromboxane (Tx)). Extrinsic regulation allows the central nervous system to exert control of the microcirculation through neurohormonal mechanisms.

The hepatic microcirculation is unique due to the dual blood supply of the liver. After repeated branching, the hepatic arterioles and portal venules supply blood to the hepatic sinusoids. These correspond to the capillary bed of the liver, and are the location where transport of nutrients and removal of waste products takes place. The hepatic arterioles wind themselves around the portal venules, and it has been demonstrated that communication occurs between the vessels. In conditions of reduced portal venous flow blood is shunted via hepatic arteriolo-portal anastomoses to maintain microvascular perfusion and oxygen delivery (14). Sinusoids run straight between the liver cells, and these are lined with specialised endothelial cells characterised by flattened processes perforated with small fenestrae, and the absence of a basement membrane (15). Hepatic stellate cells (HSC) are located external to the endothelium in the space of Disse, a space between the hepatocytes and endothelium, and by acting as contractile machinery they play an important role in regulating vessel calibre and thus blood flow (16). After passing through the sinusoids, blood flows into terminal central veins which combine and reach the hepatic vein.

**Intrahepatic microcirculation**

In liver cirrhosis, marked changes occur within the intrahepatic microcirculation causing disruption to the normal regulatory mechanisms and ultimately leading to an increase in intrahepatic vascular resistance and portal hypertension (4-6). The rise in

sinusoid resistance is due to both mechanical factors relating to microvascular structural changes, and dynamic factors including endothelial dysfunction, reduced NO production, increased release of vasoconstrictor products and contraction of HSC (17-24).

Vascular structural changes include angiogenesis and pathological sinusoidal remodelling. The sinusoidal endothelial cells (SEC) lose their characteristic fenestrations and an organised basement membrane is formed, a process referred to as sinusoidal capillarization. This results in an impairment of oxygen diffusion from the sinusoidal vessels to the liver parenchyma (25). HSC are also involved in the pathological structural changes during sinusoidal remodelling. They migrate and promote coverage of sinusoidal vessels leading to vasoconstriction which contributes to the high vascular resistance (26). Deposition of collagen in the space of Disse leads to narrowing and distorting the sinusoidal lumen, further restricting microvascular flow (14). As a result of these changes, the liver tissue becomes hypoxic and an accumulation of hypoxia-inducible factors (HIF) stimulates the production of angiogenic growth factors such as vascular endothelial growth factor (VEGF) (27, 28). The up regulation of VEGF not only leads to angiogenesis, but also stimulates activation and migration of HSC which induces an inflammatory response and further enhances the process (29-31). Angiogenesis results in new vessel formation and shunting between pre-and post-sinusoidal vessels (17). A rise in portal vein pressure is seen due to inadequate dilatation of poorly compliant abnormal intrahepatic neo-angiogenic vessels (32). The new vessels themselves also further perpetuate the inflammatory response by expressing adhesion molecules and chemokines promoting the recruitment of inflammatory cells (25).

SEC normally release vasoactive substances to maintain sufficient flow. In liver cirrhosis, an imbalance occurs leading to impaired vasorelaxation - a process referred to intrahepatic endothelial dysfunction (20). NO has been recognised as the main endothelial vasodilator of the sinusoidal microcirculation (21). In cirrhosis, NO levels in the intrahepatic microcirculation have been shown to be insufficient to prevent excessive vasoconstriction and increased hepatic vascular tone (22). The reduced bioavailability of NO is the hallmark of endothelial dysfunction and is due to decreased endothelial NO synthase (eNOS) activity resulting from oxidative stress, regulatory defects and increased level of eNOS inactivators (23 ,33). Although NO has been shown to be the primary molecule involved in vasodilation, there are number of other endothelial dilators identified to be deficient in cirrhosis and an increased release of vasoconstrictive mediators such as TxA2 and ET-1 (23)**.**

**Splanchnic microcirculation**

The enhanced intrahepatic resistance leads to a rise in portal pressure and a reduction in hepatic perfusion. Maladaptive splanchnic vasodilatation occurs in an attempt to restore hepatic perfusion increasing blood inflow with a resultant rise in portal pressure. Rather than improving hepatic perfusion, this hyperaemia and portal hypertension ultimately leads to the development of a collateral circulation and porto-systemic shunting. An interesting concept relating to this, was put forth by Newby and Hayes in 2002 (34). They proposed that as splanchnic vasodilatation and porto-systemic shunting increases, progressively more of the cardiac output is diverted into the splanchnic circulation at the expense of the extrahepatic vascular beds - a splanchnic steal phenomenon. In attempt to correct these circulatory derangements,

homeostatic activation of a cascade of neurohormonal vasoconstrictor systems causes extrahepatic peripheral vasoconstriction and reduced tissue perfusion. This theory is at odds with the generally accepted view that liver cirrhosis is associated with peripheral vasodilatation and a hyperdynamic circulation. Newby and Hayes supported their claim with a number of assertions. Firstly, systemic sympathetic blockade of the sympathetic nervous system (SNS) or renin angiotensin system (RAAS) causes profound hypotension and reduction in systemic vascular resistance, for all peripheral sympathetic systems are working at their maximal. Secondly it explains the impaired pressor responses to administered vasoconstrictor agents, since the basal vascular tone of the extra-splanchnic circulations is already increased and thus cannot be increased anymore. Whilst these notions have yet to be substantiated, direct observation of the microcirculation may provide evidence to support this theory. Furthermore, sodium and water retention occurs, resulting in an expansion of the plasma volume and an increased cardiac output (35). This hyperdynamic circulation increases portal venous inflow further, further exacerbating portal hypertension and splanchnic steal of blood contributing the development of hepatorenal syndrome (HRS) and multiple organ dysfunction (36-40).

The mechanisms responsible for splanchnic vasodilatation have been extensively investigated, and as with the sinusoidal microcirculation, NO is considered to be the pivotal factor. NO bioavailability is increased at the level of the splanchnic microvasculature in patients with cirrhosis and portal hypertension, mostly because of an increased activity of eNOS (41). This upregulation of eNOS is classically thought to be triggered by an increase in portal pressure leading to circumferential vascular stretch in the splanchnic microcirculation activating the production of VEGF and eNOS (42). VEGF promotes angiogenesis and plays an important role in the

pathogenesis of the porto-systemic collateral circulation. Interestingly, in the recent systemic inflammation hypothesis, Bernadi et al (43) propose that the overproduction of eNOS derived NO may precede splanchnic vasodilatation. Thus, the hyperdynamic circulation and enhanced mechanical shear force could represent a feed forward mechanism further increasing eNOS upregulation. The review suggests that pro-inflammatory cytokines, including tumour necrosis factor $\alpha$ (TNF-$\alpha$), contribute to NO-mediated vasodilatation. TNF-$\alpha$ induces protein kinase B (also known as Akt) mediated eNOS upregulation and anti-TNF-$\alpha$ therapies have been observed to attenuate the hyperdynamic circulation in portal hypertensive animals (44-46). Asides from eNOS and NO upregulation, splanchnic vasodilatation is also caused by several other vasoactive mediators including $PGI_2$, carbon monoxide (CO), endothelium derived hyperpolarising factors (EHF) and endocannaboids (41).

**Extrahepatic microcirculation**

In cirrhosis, the systemic circulation classically undergoes progressive generalized vasodilatation (47), although there is an increasing body of evidence refuting this. Reduced blood flow has been demonstrated in the peripheral limbs, renal and cerebral macrovasculature of patients with liver disease (48-53). In one study, McAvoy et al (54) examined differential visceral blood flow in Child Pugh B and C cirrhotics. Their findings demonstrating selective regional increases in blood flow in the splanchnic and hepatic circulations, yet diminished flow in other regional tissues despite the hyperdynamic circulation, support the splanchnic steal concept.

Whilst the haemodynamic derangements in the systemic macrocirculation have been extensively studied, albeit with contrasting findings, much less is known about the state of the extrahepatic systemic microcirculation in cirrhosis. The systemic microcirculation and its role in disease pathophysiology is an area of growing interest, in part facilitated by the recent development of medical imaging technology which allows the microcirculation to be directly visualised in vivo. Within the context of critically ill patients, derangements in the microcirculation are frequently encountered causing disruption to regulatory mechanisms (8, 55, 56). The resultant microcirculatory dysfunction is characterized by heterogeneous abnormalities in blood flow and a regional mismatch in oxygen demand and supply (10), and this has been associated with worsening clinical outcomes and organ failure (9, 56).

Multiorgan dysfunction is commonly seen in decompensated cirrhosis, acute-on-chronic liver failure (ACLF), and in sepsis, and there is increasing evidence that the underlying pathophysiological mechanisms may be similar. The systemic inflammation hypothesis proposes that decompensation of cirrhosis occurs in the setting of severe systemic inflammation and oxidative stress (43). Recent findings published by the CANONIC study group (57) strongly support the hypothesis, and indicate that systemic inflammation is the cause of decompensation in cirrhosis. The main mechanism of inflammation is translocation of bacteria, albeit without overt infection, and the spread of pathogen-associated molecular patterns (PAMPs) (58, 59). PAMPs interact with immune cells and epithelia stimulating intracellular signalling pathways, leading to the expression of genes encoding inflammatory molecules (43). Evidence from sepsis demonstrates high levels of circulating inflammatory mediators, and reactive oxygen species, cause deleterious effects on microcirculatory homeostasis, microcirculatory thrombosis, mitochondrial and cell

dysfunction, ultimately leading to cell death (60, 61). The picture of advanced cirrhosis could therefore be described as a systemic inflammatory multiorgan syndrome with associated haemodynamic disturbance, bearing a close resemblance to sepsis.

In view of the vascular abnormalities and systemic inflammation present in cirrhosis, it is logical to hypothesise that extrahepatic microvascular alterations exist, and that they play a key role in the pathogenesis of multiple organ failure. Notably, however, a dissociation between microvascular perfusion and global haemodynamic variables has been well documented (10, 62-64), as has the heterogeneity of the microvasculature within different organ systems (10, 65). Accordingly, this makes drawing conclusions about various pathophysiological states difficult to achieve, though direct visualisation of the microcirculation in vivo can aid this.

*i. Peripheral microcirculation*

The sublingual microcirculation was evaluated in cirrhotic patients with and without sepsis using sidestream dark field imaging (SDF) by Sheikh et al (7). They found that microvascular perfusion was significantly impaired in decompensated cirrhotic patients when compared to compensated cirrhotic patients secondary to vasoconstriction. Furthermore, they also demonstrated that the sublingual microcirculation in patients with decompensated liver cirrhosis had a very similar appearance to one with sepsis, lending support to the systemic inflammation hypothesis. Activation of inflammation is key in the pathogenesis of microvascular alterations in sepsis, and high plasma levels of inflammatory cytokines such as TNF-

α, interleukin (IL)-1, IL-6 and key regulatory cytokines (G-CSF and GM-CSF) promoting activation of monocytes and neutrophils, are present in decompensated cirrhosis (58, 60, 66, 67). Acute-on-chronic liver failure (ACLF) is a recently recognised syndrome, characterised by acute decompensation of cirrhosis, multiorgan failure and high short term mortality (68). Within ACLF, cytokines and chemokines involved in the innate, rather than adaptive, immune system are upregulated. This suggests that a dysregulated immune response leading to microcirculatory dysfunction, may be the predominant pathophysiological mechanism in the progression from decompensated cirrhosis to organ failure (58). In sepsis, there is heterogeneous expression of inducible NO synthase (iNOS) (69), a substance found to be elevated in cirrhosis (70) and implicated in the development of pulmonary microvascular alterations (71), which is thought to result in pathological shunting of blood flow in the microvasculature. Impaired red cell deformability leading to enhanced aggregation has been identified in both conditions (72, 73), as has increased leucocyte and platelet rolling, and adhesion to the endothelial surface possibly impairing circulation of other cells (25, 74).

A second study utilised SDF imaging to investigate the effect of age, diabetes, cirrhosis and chronic kidney disease on sublingual microvascular flow (75). Eighteen patients with liver cirrhosis of varying aetiologies were investigated, seventeen with Child-Pugh class A and one with Childs-Pugh B. In this instance, the findings differed to Sheikh et al in that there was a non-significant trend towards increased microcirculatory flow in cirrhotic patients when compared to controls. This may represent the lower disease severity of the study participants as early in the course of the disease the decrease in systemic vascular resistance may be haemodynamically compensated by a rise in cardiac output, prior to the excessive

activation of vasoconstrictor systems. Aside from the use of SDF imaging, other technologies have been utilised to assess the dynamic response of the peripheral microcirculation in liver cirrhosis. Using near infrared spectroscopy (NIRS), Thomson et al (76) found that patients with liver cirrhosis had a significantly larger post-occlusive hyperaemic response than controls indicating microvascular dysfunction. Finally, laser Doppler spectroscopy performed on nineteen patients with liver cirrhosis, observed reduced flow in the peripheral microcirculation compared to controls (77).

*ii. Renal microcirculation*

Renal dysfunction is a frequent complication in cirrhosis resulting from inflammation, renal hypoperfusion, cardiac and circulatory dysfunction. Excessive splanchnic vasodilatation and splanchnic steal of blood causes homeostatic stimulation of the RAAS, SNS and antidiuretic hormone leading to renal vasoconstriction. Additionally, circulating inflammatory mediators may trigger endothelial dysfunction and microcirculatory dysregulation (23, 78). The end result is a marked decrease in renal perfusion and glomerular filtration rate (GFR), renal tubular damage, increased serum creatinine concentration and ultimately HRS. Vasoconstriction is also likely present at the level of the microcirculation, as experimental studies in cirrhotic rats have demonstrated heterogeneous alterations in the renal cortical microcirculation with reduced vessel diameter and impaired perfusion (79, 80). Toll-like receptor 4 (TLR4) has been identified as playing an important role in HRS, and it is overexpressed in the kidney tissue and urine in cirrhotic patients with acute kidney injury (81). TLR4 is activated by PAMPs, upregulating the release of inflammatory

mediators which induce endothelial and microcirculatory disruption (82). Endothelial dysfunction at a microvascular level is associated with a reduction in renal vasodilator production demonstrated by decreased urinary excretion of prostaglandin (PG)E2, 6-keto PGF1α (a $PGI_2$ metabolite) and kallikrein (83, 84). This imbalance between the activity of systemic vasoconstrictor systems and renal production of vasodilators favours vasoconstriction (85). Renal hypoperfusion ensues which is further amplified by the stimulation of intrarenal vasoconstrictors including ET-1 and angiotensin (86).

Terlipressin is used in HRS as it has a much greater effect on vascular vasopressin receptors ($V_1$) than renal vasopressin receptors ($V_2$), thereby reducing the steal into the splanchnic circulation, and diverting blood to the systemic and renal circulations, improving renal function in around 50% of patients (87). The ultimate aim of restoring renal tissue perfusion is related more to microvascular perfusion than arterial blood flow (88), however, the renal microcirculatory response to Terlipressin in HRS is poorly defined. Schnieder et al (89) observed the renal microcirculation using contrast-enhanced ultrasonography in four HRS patients following Terlipressin administration demonstrating heterogeneous renal microvascular responses with wide individual variability. A dramatic increase in renal microcirculatory perfusion following Terlipressin was observed in two of the study patients, although there was a modest decrease in perfusion in the remaining two patients.

*iii. Cerebral microcirculation*

Cerebral haemodynamic derangement is well known in hepatic failure, and it is generally accepted that cerebral blood flow decreases in patients with liver cirrhosis (90). Hepatic encephalopathy (HE) is a common complication of advanced cirrhosis and carries a very poor prognosis (91). Multiple pathophysiological pathways, mostly related to ammonia metabolism, are involved in the development of HE. Mounting evidence also suggests that abnormal cerebral haemodynamics, and a loss of cerebral autoregulation, plays a key role in the pathophysiology (92-95).

Transcranial Doppler ultrasonography (TCD) can be reliably used to measure cerebral blood flow and indices of cerebral vascular resistance (CVR). Macías-Rodríguez et al (96) measured cerebral vascular resistance using TCD and showed CVR is increased in association with the severity of cirrhosis and the presence of HE. Kawakami et al (92) found higher CVR in both patients with liver cirrhosis and HE, and those with advanced liver disease secondary to other pathologies. Guevara et al (97) demonstrated increased resistance of both the renal artery and middle cerebral artery in patients with cirrhosis and ascites, and further noted it closely correlated with markers of vasoconstrictive systems (RAAS and SNS). These findings support the notion that the cerebral vascular bed is affected by tissue hypoperfusion and organ dysfunction, in a similar manner to the renal and peripheral vascular beds, and adds to the evidence that cerebral haemodynamic derangement is involved in the pathophysiology of HE.

The increase in CVR is likely to be secondary to microvascular dysregulation and structural deterioration due to high levels of inflammatory cytokines and neutrophil dysfunction, coupled with the associated activation of the RAAS and SNS (96). Endothelial disruption and microvascular damage ultimately lead to haemodynamic

derangement, and may promote astrocyte oxidative stress, consequently increasing the blood brain barrier to ammonia, worsening HE (98). Thus, it is plausible that vasopressor agents targeting the cerebral microcirculation could be beneficial in HE, as they are in HRS. That said, a study in acute liver failure with severe encephalopathy demonstrated worsening of cerebral hyperaemia following the administration of terlipressin (99). Cerebral hyperaemia and increased intracranial pressure are usually associated with acute liver failure, although interestingly some patients with chronic liver disease, who undergo a transjugular intrahepatic portosystemic stent (TIPPS) insertion, develop acute cerebral vasodilatation. This closely correlates to whole body NO production, and is thought to be driven by an iNOS dependent mechanism (100).

*iv. Pulmonary microcirculation*

Pulmonary microvascular complications are seen in liver cirrhosis in the form of hepatopulmonary syndrome (HPS) and portopulmonary hypertension (POPH). HPS is the term used for intrapulmonary microvascular dilatation resulting in impaired oxygenation in patients with liver disease and/or portal hypertension (101). Most cases occur in advanced cirrhosis although HPS has been recognized in patients with adequate hepatic synthetic function (102) and is only manifested in 10-17% of the overall cirrhotic population (103, 104), indicating that the pathogenesis of microvascular dysfunction in the lung is distinct from the cirrhosis induced systemic microvascular alterations. Studies using experimental animal models of cirrhosis have identified a number of vasoactive mediators including NO and ET-1. ET-1 is classically recognized as a potent vasoconstrictor yet in liver cirrhosis elevated levels

of ET-1 cause activation and increased expression of pulmonary $ET_B$ receptors resulting in NO-mediated vasodilatation via upregulation of pulmonary eNOS (105, 106). Human studies have observed increased exhaled NO concentrations in patients with HPS, which normalise after liver transplantation (107-109). Additionally, it has been proposed that lung endotoxaemia is responsible for increased levels of TNF-$\alpha$ and upregulation of iNOS, further enhancing NO levels (110).

POPH is best defined as pulmonary arterial hypertension associated with portal hypertension, whether or not portal hypertension is secondary to an underlying liver disease (111). It is characterised by vasoconstriction and vascular remodelling; however, the pathophysiology is poorly understood. A proposed mechanism suggests that the high cardiac output seen in the hyperdynamic circulatory syndrome causes pulmonary vascular wall shear stress. This then triggers endothelial dysfunction with the expression of vasoactive, proliferative and angiogenic mediators leading to vascular remodelling (112). An increased expression of vasoconstrictor molecules including ET-1 and TxB1 has been implicated in the pathophysiology of POPH (113-115), as has serotonin (112).

**Monitoring the microcirculation in cirrhosis**

Given the dissimilar, and at times contradictory findings detailed above, it is difficult to draw outright conclusions regarding the state of the microcirculation in cirrhotic patients. Significant microvascular alterations exist and the heterogeneous character of these, both among organ systems and within specific organs, has been demonstrated. Vasoconstriction and reduced perfusion predominate in the small

number of studies measuring blood flow in the systemic extrahepatic microvasculature, and this supports the splanchnic steal hypothesis. Nevertheless, the exact nature of these alterations, and the clinical implications of these changes remains to be determined, emphasising the need for further investigation and robust monitoring of the microcirculation in cirrhosis.

Recently it has been shown that patients with cirrhosis who recover from bacterial infections have a worse prognosis when compared to patients with similar disease severity without a history of infection (116). Changes in the microcirculation may be responsible for this observation given that microcirculatory alterations have been shown to persist even after recovery of systemic haemodynamics in septic shock (9). Treatment regimens involving vasoactive agents can be effective in correcting the circulatory disturbance of decompensated cirrhosis and hepatorenal syndrome, however, a wide variability in patient response is observed, with some demonstrating little benefit (87). Whilst our understanding of the mechanistic action of these vasoactive agents remains somewhat limited, it is likely that they involve the microcirculation. As such, direct monitoring of the microcirculation in vivo could not only aid us to better understand the heterogeneous nature of the microcirculation in cirrhosis, but also enable us to quantify the effects of therapeutic interventions upon it, and evaluate the relationship with clinical outcomes.

**Evaluation of the microcirculation**

The intrahepatic microcirculation poses several challenges to accurate measurement, including the dual blood supply of the liver, and difficult access to its

vessels. Previous methods used to evaluate hepatic perfusion, include the radioactive or fluorescent microspheres technique, inert gas clearance technique and multiple indicator dilution technique (14). These invasive techniques generally involve the introduction of a substance whose concentration is assessed following passage through the hepatic microvasculature, thus providing a surrogate measure of hepatic perfusion. Varying degrees of accuracy are seen, and their usage has been predominantly in experimental animal models or organs following post-mortem removal (117-120).

In more recent times, intravital microscopy has played a pivotal role in microvascular and hepatic physiology research. Advances in optical microscopy have enabled visualization of individual cells and capillaries in real time as they interact with their natural environment. The technique has since been used to analyse biological interactions, monitor hepatic disease and develop novel therapeutic approaches aimed at altering microvascular pathology in liver disease (121-123). That said, despite the technological advances and wide capabilities of intravital microscopy, it remains an invasive technique with which one is unable to directly monitor the human hepatic or peripheral microcirculation at the bedside.

Non-invasive techniques applicable to both the intra- and extrahepatic microcirculation include laser Doppler flowmetry, NIRS and video microscopy. Laser Doppler flowmetry is an established technique for the real time evaluation of microvascular red blood cell flow in tissue (124). The measurement of blood flow is based on the Doppler principle – frequency shifts in laser light by moving erythrocytes. It has been used to determine intra and extrahepatic microcirculatory perfusion, and comparison with intravital microscopy revealed significant correlation,

demonstrating its reliability in assessment of hepatic microperfusion (77, 125, 126). NIRS is able to monitor real time changes in tissue oxygen saturation using the principles of light transmission and absorption. It has been extensively validated in the measurement of hepatic oxygenation (127, 128). Intraoperatively, NIRS enables real time evaluation of perfusion and oxygenation of hepatic tissue (129), and as such, it may become valuable tool in liver transplantation and in the assessment of ischaemia-reperfusion injury. NIRS is also utilised in conjunction with vascular occlusion tests in evaluation of peripheral microcirculatory function (76).

Video microscopy techniques that allow in vivo visualisation and measurement of the microcirculation are arguably the most useful method available today (88). Based upon orthogonal polarization spectroscopy (OPS), SDF and, its most recent iteration, incident dark field (IDF) imaging, allow real time visualisation of the microvasculature at the bedside using non-invasive hand held videomicroscopes (130-132) (Figure 2). Using epi-illumination, a light source is applied to the surface of an organ whereupon the light diffuses through the tissue and is subsequently reflected back by deeper tissue layers making it translucent (134). Mucosal surfaces covered by a thin epithelial layer can also be visualised, the most common area being the easily accessible sublingual region. Numerous capillaries and venules traverse the sublingual area (135) and it has been used as a surrogate measure for the splanchnic blood flow (136-138). Admittedly, whilst the sublingual region may not be representative of the state of the microcirculation in other organ systems, the ease of access makes for feasible monitoring of the extrahepatic systemic microcirculation at the bedside (7, 75). Quantification of SDF/IDF images is standardized, reproducible and validated, allowing the functional perfusion of the microcirculation to be reliably analysed (139). Direct observation of the sublingual area allows evaluation of the

real-time microcirculatory effects of exogenous vasopressor agents, which in the future may provide a platform for individual optimization of treatment. This, in combination with the observed relationship between sublingual microvascular alterations and clinical outcome in critically ill patients (9, 56), makes it a relevant and important anatomical region to study in patients with liver cirrhosis.

## CONCLUSION

Liver cirrhosis is associated with systemic inflammation, regional microcirculatory alterations and haemodynamic derangements which may be fundamental to the pathogenesis of multiple organ dysfunction. Evidence suggests heterogeneous microvascular changes are present in extrahepatic vascular beds, and based upon previous work in critical illness, these may correlate to poor clinical outcomes. The exact mechanisms of the conventional vasoactive treatments used in the treatment of cirrhotic circulatory dysfunction remain to be determined, although it is plausible that their effect may be elicited at the level of the microcirculation. Further research into the role of the microcirculation in patients with liver cirrhosis using devices such as the SDF and IDF videomicroscopes, is therefore essential. Not only could work in this field improve physicians understanding of the pathophysiology of this disease, but it may provide a platform for real time evaluation of an individual's microcirculatory response to vasoactive mediators and thus guide their therapy, providing individualised approach to treatments.

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## FIGURE LEGENDS

### Figure 1. Regulation of microvascular tone

Figure outlining the intrinsic and extrinsic regulatory mechanisms of microvascular tone. EDHF = endothelium-derived hyperpolarizing factor.

### Figure 2. Incident dark field sublingual microcirculation image

Typical image of the sublingual microcirculation in a healthy subject taken using an incident dark field videomicroscope. A variety of sizes of blood vessel can be seen, as can individual erythrocytes seen as black dots.

## STATEMENT OF INTEREST

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## AUTHORSHIP STATEMENT

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*Author contributions:* TD and NGK participated in conception and design of manuscript. TD and SW carried out the literature search and all authors were involved in writing of the manuscript. TD was responsible for the design and drafting of the figures. All authors edited and approved the final version of the manuscript.