Endothelial and microvascular function in liver cirrhosis: an old concept that needs re-evaluation?

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Abstract

Liver cirrhosis is characterized by significant circulatory dysregulation, related to an imbalance among several vasodilating agents, mainly nitric oxide. In contrast to portal vein and macrovascular hemodynamic alterations, which have been rather well described, the peripheral microcirculatory and endothelial structure and function in this population are less well studied. Endothelial dysfunction is characterized by an imbalance between endothelium-derived relaxing and contracting factors. A number of methods have been used to assess endothelial and microvascular function in human studies. Venous occlusion plethysmography was used for many years as the gold standard for evaluating endothelial function, but flow-mediated dilatation (FMD) of the forearm is currently the most frequently used method. In patients with cirrhosis, the few existing studies have mainly used FMD, but the relevant results are largely contradictory. In recent years, several noninvasive and easily applicable methods, such as near-infrared spectroscopy, laser speckle contrast imaging, peripheral arterial tonometry, optical coherence tomography and nailfold video-capillaroscopy, have been increasingly used to assess peripheral microvascular function and have demonstrated a number of advantages. In this review, we present functional methods to evaluate peripheral microvascular and endothelial function, and we discuss the existing evidence on circulatory dysfunction in patients with liver cirrhosis.

Keywords Cirrhosis, endothelial dysfunction, microcirculation, flow-mediated dilatation, venous occlusion plethysmography

Ann Gastroenterol 2022; 35 (5): 471-482

Introduction

The endothelium is a single layer of cells lining the interior surface of blood and lymphatic vessels that plays a major role in the regulation of vascular function [1]. Among other things, it participates in the regulation of vasomotor tone and vascular permeability, the balance between procoagulant and anticoagulant activity, angiogenesis, leukocyte trafficking, inflammatory processes, and innate and acquired immunity [1]. Any impairment of endothelium, caused by physical or chemical stimuli, can lead to endothelial dysfunction, which typically includes an imbalance between vasoconstricting and vasodilating agents, procoagulant and anticoagulant factors, or growth promoting and inhibiting substances [2,3]. Nitric oxide (NO) is the vasoactive agent most commonly linked to endothelial function, since impaired NO bioavailability is the hallmark of endothelial dysfunction [4].

Liver cirrhosis is a major issue of public health, being responsible for approximately 1.2-1.3 million deaths per year worldwide, while it appears to be among the 10 most common causes of death in several countries [5-7]. The prevalence of cirrhosis is difficult to be evaluated because of the asymptomatic initial stages; thus, it was estimated at around 0.3% in surveys but at 4.5-9.5% in autopsy studies in the general adult population [6,8,9]. The most common causes of cirrhosis are chronic hepatitis B, chronic hepatitis C, alcohol-associated liver disease and non-alcoholic fatty liver disease [6]. From a circulatory standpoint, liver cirrhosis is

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Conflict of Interest: None

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characterized by significant dysregulation, related to imbalance of several vasodilating agents, including NO [10]. According to current knowledge, reduced NO bioavailability and oxidative stress in the intrahepatic vascular system results in increased intrahepatic resistance and portal hypertension [11]. On the other hand, several systemic vascular beds were shown to overproduce NO, a fact linked to arterial vasodilation and a hyperdynamic state [11]. In contrast to portal vein and macrovascular hemodynamic alterations, which have been rather well described, the peripheral microcirculatory structure and function in cirrhosis are less well studied [12,13]. As microcirculatory endothelial dysfunction is associated with increased target organ damage, cardiovascular events and mortality in the general and other populations, there is a need for a better description of the status and the impact of peripheral endothelial dysfunction in cirrhosis [13-17].

During the last decades, a number of methods have been used to assess endothelial and microvascular function in human studies [18]. Venous occlusion plethysmography (VOP) was used for many years as the gold standard for evaluating endothelial function, while, flow-mediated dilatation (FMD) of the forearm is currently the most frequently used method [18]. Recently, several noninvasive and easily applicable methods, such as near-infrared spectroscopy (NIRS), peripheral arterial tonometry (PAT), optical coherence tomography (OCT), and nailfold video-capillaroscopy, have gained more and more ground [18,19]. In this article, we aim to present the currently used functional methods to evaluate peripheral microvascular and endothelial function and discuss the existing evidence on circulatory dysfunction in patients with liver cirrhosis. For this purpose, we conducted a literature search in PubMed, Scopus and Web of Science databases up to 12 March 2021, using the following keywords: "cirrhosis", "endothelial function", "endothelial dysfunction", "venous occlusion plethysmography", "flow mediated dilatation", "near infrared spectroscopy", "capillaroscopy", "peripheral arterial tonometry", "glycocalyx", "laser doppler flowmetry", "laser speckle contrast imaging", "laser speckle contrast analysis", "optical coherence tomography".

Functional methods of endothelial dysfunction assessment in cirrhosis

VOP

VOP was the first method applied for the evaluation of forearm microvascular function [18]. This method provides the opportunity to assess vascular responses to several vasoactive agents without affecting other organs and provoking systemic effects [20]. However, VOP is now rarely used in clinical research because of its semi-invasive and time-consuming nature (Table 1) [21,22]. This technique is based on the use of automatically calibrated mercury-in-silastic strain gauges which are applied around the limb under examination [18]. Typically, a cuff is placed proximally around the upper arm

and inflated up to 40-50 mmHg for 10-sec intervals, followed by 5-sec intervals of deflation, while another cuff, placed around the wrist, is inflated up to 200 mmHg [22-24]. In this manner, the venous outflow from the forearm is occluded without affecting the arterial inflow and the changes in tissue volume are proportional to the arterial inflow rate [18,22]. Vascular function is assessed by measurement of changes in forearm blood flow (FBF, mL/min/100 mL tissue) induced by infusion of vasoconstricting or vasodilating substances (i.e., norepinephrine, acetylcholine, etc.) [22]. Endothelium-dependent vasodilation is evaluated by infusion of endothelial agonists, such as acetylcholine and bradykinin, and endothelium-independent vasodilation by infusion of direct smooth muscle relaxing agents (i.e., nitrates) [18,22].

In the setting of liver cirrhosis, VOP was the most commonly applied method in the first studies that aimed to assess peripheral vascular function and determine *in vivo* the factors that contribute to circulatory dysfunction. Early cross-sectional studies involving small numbers of subjects showed that there was no difference in vasoconstriction induced by N-monomethyl-L-arginine (L-NMMA), a selective inhibitor of NO synthase, between patients with cirrhosis and controls; hence, they concluded that overproduction of NO was not the sole cause of peripheral vasodilation in cirrhosis [25-27]. Furthermore, in another cross-sectional study, Ryan *et al* demonstrated that there was significant hypo-responsiveness of the peripheral vessels to vasoconstrictor agents, such as norepinephrine and angiotensin II [28]. This impaired response was due to the enhanced release of endothelium-derived relaxing factors, including NO, as it was restored after inhibition of NO synthase [23,29]. With regard to vasoconstricting agents, Newby *et al* found that angiotensin II also contributed to basal peripheral vascular tone in cirrhotic patients, because decreased forearm blood flow, induced by infusion of angiotensin II, was augmented after the administration of losartan, a selective antagonist of angiotensin II type 1 receptor [26].

Concerning the different stages of cirrhosis, Campillo *et al* demonstrated that patients with advanced cirrhosis (Child-Pugh B and C) exhibited a greater vasoconstrictor response to L-NMMA and reduced response to noradrenaline, compared to patients with mild disease, suggesting that NO has a larger contribution to peripheral vasodilation and hyperdynamic state in more advanced stages of cirrhosis [30]. Moreover, Helmy *et al* showed in a cross-sectional study of 8 pre-ascitic cirrhotic patients and 10 cirrhotic patients with refractory ascites that vascular responsiveness to angiotensin II can be different in different stages of cirrhosis, given the fact that losartan increased the forearm blood flow only in patients with refractory ascites [31]. As far as endothelin-1 (ET-1), another major vasoconstrictor agent is concerned, Helmy *et al* indicated that patients with Child-Pugh A cirrhosis exhibited a weaker vasoconstricting response to ET-1 compared to healthy individuals [32], while Vaughan *et al*, in a prospective study of 6 patients with end-stage cirrhosis, concluded that ET-1 evoked vasodilation in cirrhotic patients, in contrast to the vasoconstriction observed in controls, a response that was restored after liver transplantation [33].

Table 1 Main strengths and limitations of the functional methods used to evaluate endothelial function

Method	Vascular bed	Strengths	Limitations
VOP	Forearm vasculature	High reproducibility High validity Evaluation at rest and after local infusion of vasoactive factors	Semi-invasive Time consuming
FMD	Conduit artery	Simple, noninvasive Inexpensive Correlation with coronary endothelial function	High intra- and interobserver variability Low reproducibility Requirement of strict protocols and well-trained operators Requirement of proper preparation (i.e., medication, environmental factors)
NIRS	Muscle microcirculation	Noninvasive Inexpensive Evaluation at rest and during exercise Measure of tissue O_2 consumption	Adipose tissue thickness is a confounder Myoglobin is a potential confounder
PAT	Finger microvasculature	Simple, noninvasive High reproducibility, operator-independent	High cost Movement artifacts Influence of environment
LDF	Skin microcirculation	Simple, noninvasive Continuous measurement of blood flow	Needs well-trained operator Low reproducibility
Endothelial glycocalyx	Sublingual microcirculation	Tracer dilution method: Direct assessment of glycocalyx volume	Tracer dilution method: Invasive Time consuming
	Sublingual microcirculation	OPS and SDF: Simple, noninvasive Rapid Bedside performance	OPS and SDF Indirect assessment of glycocalyx volume
Nailfold video-capillaroscopy	Nailfold microcirculation	Simple, noninvasive Evaluation in 3 functional phases	Needs well-trained operator
LSCI	Skin microcirculation	Simple, noninvasive Continuous measurement of blood flow in real-time	High cost Needs well-trained operator Movement artifacts
OCT	Retinal microcirculation	Simple, noninvasive High resolution imaging	Needs well-trained operator

VOP, venous occlusion plethysmography; FMD, flow-mediated dilatation; NIRS, near-infrared spectroscopy; PAT, peripheral arterial tonometry; LDF, laser Doppler flowmetry; OPS, orthogonal polarization spectral; SDF, sidestream dark field; LSCI, laser speckle contrast imaging; OCT, optical coherence tomography

With regards to vasodilatory responses, in several cross-sectional studies cirrhotic patients showed enhanced endothelium-mediated vasodilation, induced by acetylcholine and methacholine, in comparison with controls (Fig. 1) [23,34,35]. This hyper-responsiveness was not observed after the infusion of sodium nitroprusside, suggesting again that endothelial disturbances were primarily involved in excess vasodilation [23,35,36]. Interestingly, prospective randomized crossover studies showed that antibiotic treatment with norfloxacin restored the hypo-responsiveness to N-LMMA and the hyper-responsiveness to acetylcholine, and also restored the initially elevated forearm blood flow in cirrhotic patients, indicating that intestinal bacteria overgrowth and migration may contribute to these individuals' endothelial vascular dysfunction [37,38].

FMD

FMD is a simple, noninvasive, established technique for evaluation of endothelial function in conduit arteries (e.g., brachial artery), currently widely used in human research [39,40]. It is based on the physiological capacity of arteries to respond by dilating to an increase in blood flow induced by mechanical or chemical stimuli [39]. The procedure involves the ultrasonographic measurement of arterial diameter at rest and after reactive hyperemia (Fig. 2) [39]. Reactive hyperemia is accomplished either after 5-min arterial occlusion with an inflated cuff (endothelium-dependent vasodilation), or after administration of a NO-donor, such as nitroglycerin (endothelium-independent vasodilation), and FMD is computed as a percentage change from baseline [11,39,41].

During post-ischemic hyperemia, the change in arterial diameter depends mainly on endothelial NO synthesis and release [39,42]. It is noteworthy that FMD in the forearm is strongly correlated with coronary endothelial function, and is also considered as a reliable marker of NO bioavailability in several populations [19,43]. Despite the important advantages of this method, there are still some challenges regarding its practical application. Firstly, FMD has high intra- and interobserver variability, limiting its reproducibility [19]. As a consequence, it requires good standardization, strict protocols and well-trained, experienced operators [18,40]. Furthermore, there is a need for a proper preparation of the subjects with regard to medication, physical and environmental factors, so as to attenuate confounders that might have an effect on vascular responsiveness (Table 1) [40,44].

Table 2 presents studies that used FMD for the evaluation of peripheral endothelial function in patients with cirrhosis. The first studies to compare brachial artery FMD between patients with liver cirrhosis and controls included small numbers of patients (n<20) and suggested no difference between the group of cirrhotic patients as a whole and that of controls [45,46]. However, both in subgroup analyses of the above data, and in several studies that assessed the vascular function with FMD in different stages of cirrhosis, FMD levels were found to be significantly lower in early compared to advanced disease stages, indicating an exaggerated release of endothelial vasodilators, such as NO, with cirrhosis progression (Fig. 1) [11,41,45,47].

With regard to associations with coexisting disturbances, Sárközi *et al* showed that patients with cirrhosis exhibited

greater FMD compared to controls, and this increased FMD was inversely correlated with neural baroreflex sensitivity; such findings suggest that overproduction of NO in cirrhosis may also be involved in the baroreflex impairment that is present in these patients [48]. Moreover, FMD was found to be higher in cirrhotic patients with ascites than in pre-ascitic patients, and was directly associated with an increased interlobar renal resistive index, a finding suggesting that cirrhotic patients with higher values of FMD have impaired renal microcirculatory function and are more prone to acute kidney injury [41]. Finally, in a cohort of 54 cirrhotic patients, FMD was a shown to be a significant predictor of pulmonary edema after living donor liver transplantation, as higher pre-transplantation levels of FMD were significantly associated with a higher incidence of pulmonary edema after transplantation [49]. In a prospective case-control study, Rimbas *et al* studied cardiac function and endothelial function in 46 cirrhotic patients, demonstrating diastolic dysfunction in cirrhosis, but no difference in endothelial function between patients and controls; however, they did not correlate these parameters [50].

It must be noted, however, that not all studies using FMD found higher levels in cirrhotic patients compared to controls, or in more advanced stages of disease compared to early stages (Fig. 1) [51-53]. For example, Marcacci *et al* found that FMD decreased with worsening stages of cirrhosis, indicating the presence of impaired and not increased vasodilation in patients with advanced cirrhosis [51]. A study of 156 patients with alcohol-related liver disease showed that increasing severity of portal hypertension was associated with coincidental deterioration of endothelial function and lower values of FMD [52]. Furthermore, in a recent study of 50 cirrhotic individuals and an equal number of controls, FMD was lower in patients with cirrhosis. In the same study, treatment with rifaximin resulted in improvement in FMD, suggesting that gut microbiota contribute to peripheral endothelial dysfunction in cirrhosis [53].

A number of factors may account for the conflicting results between early and more recent studies in the field. One of the most important confounders can be the vascular alterations evoked in patients with cirrhosis associated with preexisting or coexisting cardiovascular risk factors that can be present in these individuals, such as central obesity, hypertension, diabetes mellitus and others. A seminal study by Berzigotti *et al* studied exactly this issue by evaluating endothelial
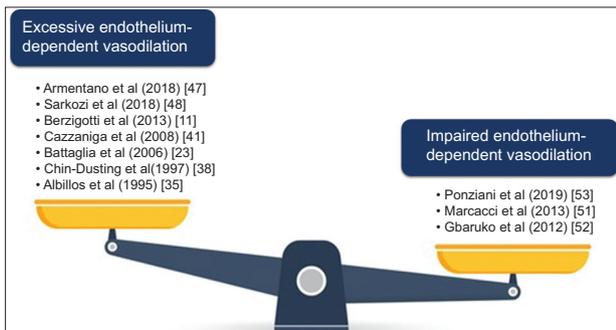
Figure 1 Mechanistic studies using venous occlusion plethysmography and flow-mediated dilatation that demonstrated excessive or impaired endothelium-dependent vasodilation in patients with liver cirrhosis
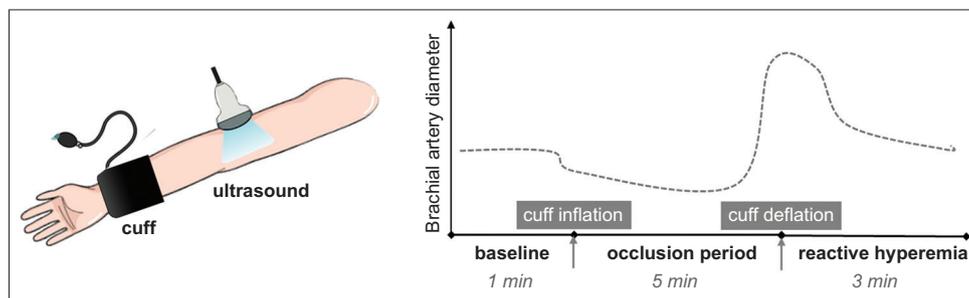
Figure 2 Assessment of endothelial function with flow-mediated dilatation (FMD). Left: the setup for FMD measurement. Right: changes in brachial artery diameter during post-occlusion reactive hyperemia

Table 2 Studies using flow-mediated dilatation to evaluate peripheral vascular function in cirrhosis

Study [ref.]	Study type	Participants	Characteristics of cirrhotic patients	Results
Ponziani *et al*, 2019 [53]	Prospective, case-control	50 cirrhotic patients and 50 HC	HCV or HBV: 52% ALC or NAFLD: 48% Child-Pugh A: 64% Child-Pugh B: 26% Child-Pugh C: 10% MHE: 40%	FMD was lower in cirrhotic patients compared to HC [6.35% (3.40-9.68) vs. 10.69% (7.78-13.24), P=0.0003]. There was no difference in FMD between patients with MHE and patients without. In patients with MHE, there was a significant increase in FMD after treatment with rifaximin [9.65% [5.41-12.57] vs. 4.76% (2.45-7.89), P=0.015]
Rimbaş *et al*, 2018 [50]	Prospective, case-control	46 cirrhotic patients and 46 age- sex- matched HC	ALC: 52.17% HCV or HBV: 41.30% PBC: 4.35% CC: 2.18% Child-Pugh A: 50% Child-Pugh B: 35% Child-Pugh C: 15%	There was no difference in FMD between cirrhotic patients and controls [22±20 vs. 20±13 P=ns]. Cirrhotic patients exhibited resting biventricular diastolic myocardial dysfunction
Armentano *et al*, 2018 [47]	Pilot cross-sectional study	12 cirrhotic patients	Child-Pugh A: 33.33% Child-Pugh B: 33.33% Child-Pugh C: 33.33%	FMD was found to be higher in Child-Pugh C than Child-Pugh B and Child-Pugh A (9.87±2.36% vs. 8.52±4.72% vs. 5.18±2.44, respectively, P<0.05).
Sárközi *et al*, 2018 [48]	Cross-sectional	24 cirrhotic patients 23 sex-, age- matched HC *FMD was estimated in 21 cirrhotic patients and 15 HC	HCV: 50% ALC: 12.5% CC: 12.5%	FMD was higher in patients with cirrhosis compared to HC (9.81±3.77% vs. 5.59±1.36%, P<0.01). Neural baroreflex sensitivity was inversely related to FMD in cirrhosis (r=-0.49, P<0.05).
Berzigotti *et al*, 2013 [11]	Cross-sectional	47 cirrhotic patients	HBV or HCV: 36.2% ALC: 51.1% NAFLD: 6.4% Child-Pugh A: 29.8% Child-Pugh B: 44.7% Child-Pugh C: 25.5%	53% of patients had endothelial dysfunction (FMD <10%). Systemic endothelial dysfunction (low FMD) increased in parallel with CV risk (linear trend P=0.039). FMD increased in parallel with Child-Pugh score. The proportion of patients with systemic endothelial dysfunction decreased with worsening of Child-Pugh class. However, within each of the Child–Pugh classes, the proportion of systemic endothelial dysfunction increased according to the CV risk.
Chen *et al*, 2013 [49]	Prospective cohort	54 cirrhotic patients	Not mentioned	Pre-transplantation values of FMD, NTD and FMD/NTD were higher in those who developed pulmonary edema after LT (P<0.01, P=0.01, P=0.02, respectively). Only FMD was found to be significant predictor of pulmonary edema after LT
Marcacci *et al*, 2013 [51]	Cross-sectional	60 cirrhotic patients and 11 HC	Compensated cirrhosis: 66.7% Decompensated cirrhosis: 33.3%	FMD was reduced in worsening of cirrhosis (HC: 9.9±1.1%, compensated: 6.1±1.8%, decompensated: 5±1.3%, P<0.01). A statistically significant correlation was observed between FMD and ET-1 (r=-0.4427), P-selectin (r=-0.477), vWF (r=-0.166).

(Contd...)

Table 2 (*Continued*)

Study [ref.]	Study type	Participants	Characteristics of cirrhotic patients	Results
Gbaruko *et al*, 2012 [52]	Cross-sectional	31 cirrhotic patients with expressed PH 63 ASH patients with signs of initial stage of PH 62 ASH patients without signs of PH	Not mentioned	Cirrhotic patients showed decreased FMD compared to other groups (LC: 5.4±0.15%, initial stage of PH: 7.3±0.18%, no PH: 12.9±0.22%, P<0.05). 18% of cirrhotic patients exhibited paradoxical vasoconstriction response during reactive hyperemia. There was a reverse strong correlation between severity of PH and NO and a reverse moderate correlation between the severity of PH and FMD.
Cazzaniga *et al*, 2008 [41]	Cross-sectional	32 cirrhotic patients 12 HC	Ascites: 68.8% HCV: 34.4% ALC: 34.4%	FMD was higher in ascitic patients [29.5% (10.3-50)] compared to pre-ascitic patients [17.3 (2.4-48.5)] and HC [11.6 (5.1-17.8)] (P<0.001). FMD was moderately correlated to interlobar renal resistance index in cirrhotic patients (r=0.52, P=0.002).
Angeloni *et al*, 2005 [45]	Cross-sectional	19 cirrhotic patients and 20 sex- and age- matched HC	Child-Pugh A: 26.3% Child-Pugh B/C: 73.7%	FMD was similar in the 2 groups (cirrhosis: 9.4±9.5%, HC: 9.9±6.1%, NS). Child–Pugh A patients exhibited a FMD impairment while Child-Pugh B/C patients had normal FMD (3.6±11.6% vs. 11.4±8.2%, P<0.0001).
Cifkova *et al*, 2001 [46]	Prospective cohort	15 cirrhotic patients and 12 HC	Ascites 26.6% ALC: 26.6% HCV: 33.3% CC: 13.3%	FMD of cirrhotic patients did not differ from that in healthy subjects (4.2±4.0% vs. 5.2±3.8%, NS). There was no significant change in FMD before and after liver transplantation (4.2±4.0% vs. 6.3±5.4%, NS).

FMD, flow-mediated dilatation; HC, healthy controls; HCV, hepatitis C virus; HBV, hepatitis B virus; ALC, alcohol liver cirrhosis; NAFLD, non-alcoholic fatty liver disease; MHE, minimal hepatic encephalopathy; CC, cryptogenic cirrhosis; CV, cardiovascular; NTD, nitroglycerin-induced dilatation; LT, liver transplantation; ET-1, endothelin-1; vWF, von Willebrand factor; ASH, alcoholic steatohepatitis; PH, portal hypertension

function by FMD in cirrhosis and simultaneously taking into account the cardiovascular risk of their patients, estimated using standard risk calculators [11]. The authors observed that, while FMD increased with the deterioration of liver disease, FMD decreased and the proportion of patients with endothelial dysfunction increased with greater cardiovascular risk. Within each Child-Pugh class, the proportion of patients with endothelial dysfunction increased in parallel with the cardiovascular risk. Although these preliminary findings need to be confirmed by future studies, the authors concluded that liver failure is a confounding factor in the relation between endothelial function measured with FMD and cardiovascular risk, while on the other hand, FMD may not be uniformly increased in cirrhosis [11].

NIRS

NIRS is a relatively novel, non-invasive method that allows the assessment of tissue oxygenation and microvascular function in peripheral muscles and the brain [54]. It provides information on local oxygen consumption and blood flow using the near-infrared region of the electromagnetic spectrum and is based on the modified Beer-Lambert law (Fig. 3) [55]. Hemoglobin and myoglobin carry oxygen and their absorbance of near-infrared light depends on their oxygenation state [55]. Using a near-infrared light-source and an appropriate detector, the NIRS device offers an assessment of microvascular reactivity and muscle oxygenation via continuous monitoring of functional changes in oxygenated and deoxygenated hemoglobin separation [55,56]. NIRS can be used at rest, during exercise or during various vascular tests, such as post-occlusion hyperemia, to evaluate muscular blood flow, microvascular function, or the oxidative capacity or oxygen consumption of muscle [55,56]. In addition, NIRS can evaluate cerebral oxygenation status by evaluating relative changes from baseline in oxygenated, deoxygenated and total hemoglobin [57].

Due to its non-invasive nature and the ability to assess microvascular function, NIRS has recently been used to study patient populations with features of microvascular impairment, such as hypertensive and diabetic patients [56,58]. In patients with cirrhosis, however, NIRS has mostly been used to investigate cerebral oxygenation status [59-62]. Only
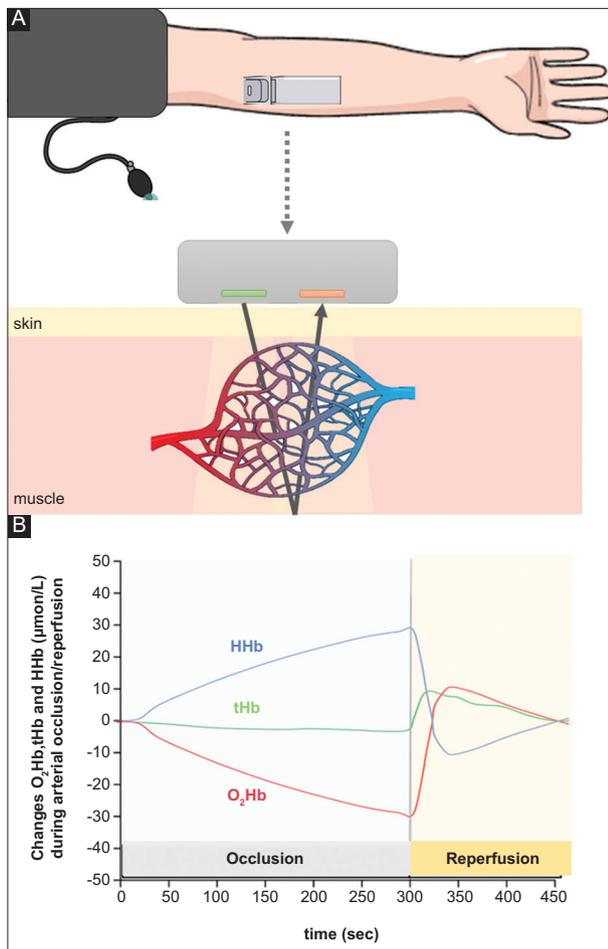
Figure 3 Evaluation of muscle oxygenation via near-infrared spectroscopy (NIRS). (A) Schematic representation of the NIRS portable device. (B) Typical NIRS recording during arterial occlusion–reperfusion maneuver. Relative changes in oxygenated (O_2Hb), deoxygenated (HHb), and total (tHb) hemoglobin (μmol/L) are depicted. During the occlusion period, the magnitude and slope of reduction in O_2Hb and HHb are measured. Stable tHb levels ensure that regional blood flow remains stable during the occlusion. During reperfusion, the magnitude and slope of rise in O_2Hb and tHb are measured

a few studies have used NIRS to evaluate muscle oxygenation and microcirculation in patients with cirrhosis. Among these, preliminary cross-sectional studies suggest that skeletal muscle deoxyhemoglobin, and total hemoglobin increase during exercise, while in the recovery phase deoxyhemoglobin returns to a pre-exercise level and hemoglobin and oxyhemoglobin increase further, suggesting abnormalities in aerobic metabolism and in extraction of O2 during exercise [63,64]. In a cross-sectional study of 25 cirrhotic patients and 10 healthy controls, baseline muscle tissue oxygenation (StO2) was lower in the former [12]. After vascular occlusion, overshoot, area under recovery curve and recovery time were increased in cirrhotic subjects, demonstrating a vasodilatory state; hyperemia was more pronounced as Child-Pugh score increased. Moreover, after serial vascular occlusion, controls exhibited an ischemic adaptability: deoxygenation rates decreased and reactive

parameters increased. The former changes were not seen in cirrhotic patients [12]. In contrast to the above, in another cross-sectional study, Wythe *et al* investigated 40 patients with cirrhosis and found no association between vasodilatory responses assessed with NIRS parameters at baseline and after vascular occlusion, with the severity of cirrhosis assessed using the Child-Pugh score [65].

Laser Doppler flowmetry (LDF)

LDF is a valid test that allows the evaluation of skin microvascular function [18]. With the assumption that changes observed in the cutaneous microcirculation reflect equivalent changes in other vascular beds, LDF is used to evaluate peripheral microvascular function [18]. This technique is based on the Doppler effect; after an initial beam of coherent light contacts moving red blood cells, its direction is altered and the emerging beam is measured by the photodiode [18,66]. The change of wavelength represents the average blood cell velocity and concentration in vessels [66]. Detailed evaluation of endothelial function can be achieved by application of post-occlusion hyperemia, thermal hyperemia, and iontophoresis combined with administration of vasoactive agents and microdialysis of pharmacological factors [18]. Although LDF is a simple and valid method, its poor reproducibility and the high cost limit its use (Table 1) [67].

As of this writing, several studies have used LDF to evaluate gastric mucosal hemodynamics in cirrhosis [68-71]; however, studies assessing peripheral endothelial function in cirrhotic patients using this method are scarce. In a cross-sectional study that included 19 cirrhotic patients and 20 healthy subjects, cutaneous blood flow was lower in cirrhotic patients than controls, while reactive hyperemia caused a greater percentage increase in cutaneous flow in the former group [72]. In another cross-sectional study, skin blood flow was significantly lower in cirrhotic patients with ascites compared to those without ascites and to controls [73]. Moreover, miconazole, an inhibitor of epoxyeicosatrieonoic acid (EET) synthesis, provoked a significant reduction in baseline skin flow in all participants, but this effect was more pronounced in patients with cirrhosis, indicating a potential role of EET in the excess peripheral vasodilation observed in these individuals [73].

Laser speckle contrast imaging (LSCI)

LSCI is another novel, non-invasive method that measures skin microvascular blood flow (Table 1) [74]. In contrast to LDF, LSCI tracks superficial microvessels in the skin and has shown better reproducibility when coupled with functional tests (i.e., reactive post-occlusion hyperemia and local thermal hyperemia) [75]. As of this writing, LSCI has been used for evaluation of endothelium-depended vasodilation in various populations, including patients with diabetes [76-79], but to our knowledge, there no study has applied this method in cirrhosis.

Endothelial glycocalyx

Glycocalyx is a layer of proteoglycans and glycolipids that covers the endothelial lining and protects the endothelial cells from direct contact with blood cells [18,80]. Among several other functions, it has an important role in converting shear stress into shear-dependent endothelial responses, leading to NO release [18]. There are both invasive (i.e., tracer dilution technique) and non-invasive methods for the assessment of glycocalycal function: i.e., orthogonal polarization spectral and sidestream dark field. These methods image sublingual capillaries and estimate the thickness of the glycocalyx by measuring the perfused boundary region (PBR) [18,81]. The PBR is inversely correlated with glycocalycal thickness, and higher PBR values represent thinner and impaired glycocalyx that corresponds to vascular dysfunction [82]. A few research efforts have applied sidestream dark field to image the sublingual microcirculation and describe its quantitative and semi-quantitative characteristics in cirrhosis [65,83-85].

OCT

OCT is a novel, non-invasive technique that enables assessment of chorioretinal microvascular structures [86]. In recent years, OCT has proved to be a useful tool for the assessment of microcirculation function, and retinal microvascular abnormalities have been associated with increased cardiovascular risk in various populations [86-88]. To the best of our knowledge, only one prospective pilot study used this method in cirrhosis [89]. In this study, Gifford *et al* demonstrated that cirrhotic patients exhibit lower values of retinal thickness, macular volume and choroidal thickness compared to controls, while these parameters did not differ from those of patients with chronic kidney disease. Moreover, the investigators demonstrated a positive correlation between choroidal thinning and markers of endothelial dysfunction. Interestingly, retinal abnormalities are resolved after transplantation [89].

PAT

PAT is a non-invasive, operator-independent technique that evaluates the peripheral microvascular function regulated by NO bioavailability [18,19,90]. The procedure consists of 3 phases: baseline, occlusion, and reactive hyperemia after occlusion [90]. The main parameter generated by PAT, the reactive hyperemia index, is a marker of microvascular endothelial function but is also closely associated with coronary endothelial function [90,91] and is considered as an independent predictor of cardiovascular events [92]. Despite the important advantages, PAT has also some potential limitations, such as the influence of environmental factors (Table 1) [90].

So far, PAT has been used for assessment of vascular endothelial function in several populations [93-97], but to our knowledge there is only one cross-sectional study to date that used PAT in cirrhosis. Berzigotti *et al* conducted a cross-sectional

study to compare FMD and PAT in cirrhosis [98] and found that PAT could overcome the limitations of FMD, and was correlated with cardiovascular risk factors, in contrast to FMD [98].

Nailfold capillaroscopy

In recent years, the use of nailfold capillaroscopy for the assessment of microcirculation has grown because of its simple and non-invasive nature [99]. This technique provides important information about different parameters of microcirculation, such as capillary density, capillary morphology and flow velocity [99,100]. A development of capillaroscopy is the method of video-capillaroscopy, which offers the advantage of functional evaluation of capillary density during different vascular phases: i.e., baseline, during reactive post-occlusion hyperemia and after venous congestion [99]. Assessment of capillary density during venous congestion is considered the most reliable method, as it detects non-perfused capillaries that can be missed with simple capillaroscopy [101].

To the best of our knowledge, there is only one longitudinal pilot study that assessed microcirculation using the video-capillaroscopy technique in patients with cirrhosis [102]. In this study, Brito-Azevedo *et al* demonstrated that baseline capillary density is lower in cirrhotic patients compared to controls [102]. However, the capillary density during the post-occlusive hyperemic phase and the venous congestion phase was not assessed [102]; thus, details of the structural and functional microcirculation integrity in cirrhosis need to be assessed in future studies.

Biochemical markers

Through the years, numerous serum biomarkers have been used to assess endothelial function in human studies (Supplementary Table 1) [79]. A detailed description of them is beyond the aim of this review; thus, in the following lines, we briefly discuss the most important biomarkers used to evaluate endothelial function in cirrhosis.

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO synthase that is strongly associated with atherosclerosis [103]. Elevated plasma levels of ADMA have been associated with endothelial dysfunction and higher cardiovascular risk in several populations, including cirrhotic patients [104]. Moreover, ADMA plasma levels were higher in patients with decompensated cirrhosis compared to those with earlier stages of disease and healthy controls; accordingly, it was suggested that an increase in ADMA may represent a compensatory mechanism to attenuate NO overproduction [104].

Cell-adhesion molecules—i.e., vascular adhesion molecule 1 (VCAM-1), intracellular adhesion molecule 1 (ICAM-1) and endothelial selectin (E-selectin)—are considered endothelial-specific biomarkers. Substances involved in the coagulation pathway, such as von Willebrand factor (vWF), are considered related, but not specific markers of endothelial dysfunction [79].
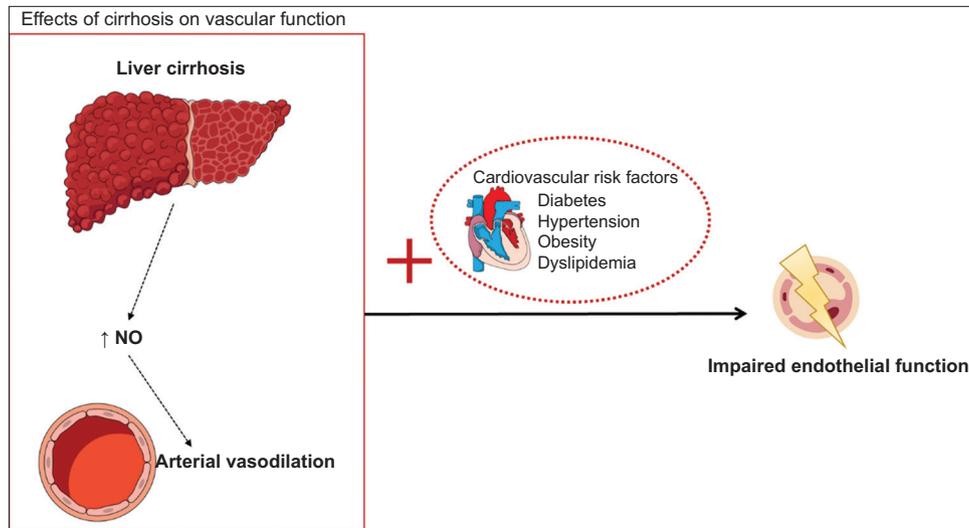
Figure 4 Hypothesis concerning the additional effects of cardiovascular risk factors on endothelial function of patients with cirrhosis. Cirrhosis is characterized by nitric oxide (NO) overproduction, resulting in peripheral arterial vasodilation and hyperdynamic circulation. The coexistence of traditional cardiovascular risk factors (e.g., hypertension, diabetes, dyslipidemia, obesity) may alter the above well-described associations, with predominance of an adverse vascular profile leading ultimately to impaired endothelial function

VCAM-1 and ICAM-1 levels are higher in cirrhotic patients than in healthy subjects and increase in parallel with disease severity [105,106]; it is notable that higher ICAM-1 levels have been associated with increased mortality in this population [106]. In addition, increased concentrations of E-selectin have also been detected in cirrhotic subjects, but in contrast to VCAM-1 and ICAM-1, E-selectin levels were inversely related with disease progression [107]. Finally, with regard to coagulation pathway molecules, several studies demonstrated a positive correlation between vWF levels and cirrhosis severity, as well as a significant association with clinical events (i.e., variceal bleeding, bacterial infection) and mortality [108-111].

Endocan is a molecule associated with systemic inflammation that has been previously suggested as a strong indicator of cardiovascular disease in several populations [112]. In a previous prospective cohort study in 68 cirrhotic patients, Toshikuni *et al* showed that elevated endocan levels were associated with increased mortality [113]. Moreover, another prospective cohort study that included 32 patients with cirrhotic cardiomyopathy and 51 cirrhotic patients without heart disease, showed that patients with cirrhotic cardiomyopathy exhibited lower levels of serum endocan. In the total cohort population, higher levels of endocan were associated with disease severity and adverse outcomes, suggesting that endocan may be a promising predictive marker in this population [114].

Concluding remarks

Liver cirrhosis is characterized by significant circulatory dysregulation, including both intrahepatic and extrahepatic vascular beds. Endothelial dysfunction is a hallmark of intrahepatic circulation leading to portal hypertension development. Moreover, several studies have also proposed that endothelial dysfunction may be a significant contributor to the peripheral microvascular changes in patients with cirrhosis. Evidence from initial studies that assessed peripheral endothelial function *in vivo* with FMD suggested an enhanced vasodilatory response with cirrhosis progression [11,41,47]. However, more recently, a number of studies using FMD demonstrated that patients with cirrhosis exhibit impaired endothelium-dependent vasodilation compared with healthy controls, which deteriorates progressively with advanced stages of the disease [51-53]. The factors accounting for these conflicting results are not known; one could have hypothesized that the presence of cardiovascular risk factors (i.e., obesity, hypertension, diabetes and others) or even established cardiovascular disease may confound the associations of cirrhosis with vasodilatory responses (Fig. 4). Based on the above, there is a need for properly designed studies to delineate the above inconsistencies. To this end, it could be extremely helpful if such studies employ novel methods (e.g., NIRS, LSCI, PAT, and nailfold capillaroscopy) for the assessment of peripheral microcirculatory structure and function that have been used in other populations, but less so in patients with cirrhosis.

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Supplementary material

Supplementary Table 1 Most commonly used biomarkers for assessment of endothelial function in human studies

NOS inhibitors	ADMA, SDMA
Coagulation pathway molecules	vWF, ADAMTS-13, t-PA, PAI-1, fibrinogen, thrombomodulin
Cell adhesion molecules	VCAM-1, ICAM-1, E-selectin, P-selectin
Arterial glycocalyx	Syndecan-1, heparin sulfate, hyaluronan
Microparticles	EMPs, PMPs
Inflammatory markers	CRP, ILs, TNF-a, TLRs, CD40 ligand, PTX3, MCP-1, Ang
Oxidative stress	Oxidized LDL
MicroRNA	MiR-126
Endocan	
EPC	

NOS, nitric oxide synthase; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; vWF, von Willebrand factor; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor 1; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intracellular adhesion molecule 1; EMPs, endothelial microparticles; PMPs, platelet microparticles; CRP, C-reactive protein; ILs, interleukins; TNF-α, tumor necrosis factor-alpha; TLRs, toll-like receptors; PTX3, pentraxin 3; MCP-1, monocyte chemoattractant protein-1; Ang, angiopoietin; EPC, endothelial progenitor cell