

# Portal hypertension in cirrhosis: Pathophysiological mechanisms and therapy

Yasuko Iwakiri,<sup>1</sup> Jonel Trebicka<sup>2,3,\*</sup>



## Summary

**Portal hypertension, defined as increased pressure in the portal vein, develops as a consequence of increased intrahepatic vascular resistance due to the dysregulation of liver sinusoidal endothelial cells (LSECs) and hepatic stellate cells (HSCs), frequently arising from chronic liver diseases. Extrahepatic haemodynamic changes contribute to the aggravation of portal hypertension. The pathogenic complexity of portal hypertension and the unsuccessful translation of preclinical studies have impeded the development of effective therapeutics for patients with cirrhosis, while counteracting hepatic and extrahepatic mechanisms also pose a major obstacle to effective treatment. In this review article, we will discuss the following topics: i) cellular and molecular mechanisms of portal hypertension, focusing on dysregulation of LSECs, HSCs and hepatic microvascular thrombosis, as well as changes in the extrahepatic vasculature, since these are the major contributors to portal hypertension; ii) translational/clinical advances in our knowledge of portal hypertension; and iii) future directions.**

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## Introduction

Portal hypertension is defined as increased pressure within the portal vein, which is the blood vessel connecting the outflow of the gastrointestinal and spleen (splanchnic organs) and the liver. Portal hypertension develops as a consequence of increased intrahepatic vascular resistance due to impaired hepatic sinusoidal circulation, most frequently<sup>1</sup> arising from chronic liver diseases (CLDs). CLDs cause structural alterations of the liver via increased extracellular matrix (ECM) accumulation and turnover (fibrosis) and changes of the cellular phenotypes, associated with dysfunction of liver sinusoidal endothelial cells (LSECs), activated hepatic stellate cells (HSCs) and inflamed resident or infiltrating macrophages.<sup>2</sup> These changes induce increased intrahepatic resistance, which increases the pressure in the portal vein (portal hypertension) and is the initial step towards complications of CLD. As a secondary event, portal hypertension induces splanchnic and systemic arterial vasodilation,<sup>3</sup> leading to the development of a hyperdynamic circulatory syndrome and thereby aggravating and driving clinically detrimental complications.<sup>4</sup> In recent years, the basic mechanisms of LSEC and HSC dysregulation have been extensively studied and potential therapeutic targets have been proposed. However, due to the complexity of the pathogenesis of portal hypertension and unsuccessful translation of preclinical studies to the human setting, development

of effective therapeutics requires further mechanistic insight.

Portal hypertension is the driver of complications in cirrhosis, such as ascites and gastro-oesophageal varices (which can haemorrhage), as well as hepatic encephalopathy due to portosystemic shunting, hepatorenal syndrome and hypersplenism.<sup>5</sup> Patients with complications of portal hypertension show repeating readmissions in the hospital and are described as having unstable decompensated cirrhosis. Another area of active investigation in recent years is the mechanism of systemic inflammation, which is a cause and consequence of acute decompensation in cirrhosis, and closely associated with pre-acute-on-chronic liver failure (ACLF). Ultimately, ACLF develops with a dramatically high mortality rate of approximately 40% at 28 days.<sup>6</sup>

In this review article, we will discuss: i) cellular and molecular mechanisms of portal hypertension, focusing on dysregulation of LSECs, HSCs and hepatic microvascular thrombosis, as well as the changes in the extrahepatic vasculature; ii) translational/clinical advances in our knowledge of portal hypertension, and iii) future directions.

## Cellular & molecular mechanisms of portal hypertension

### Hepatic cells

Among the hepatic cells involved in the development of portal hypertension, LSECs and HSCs are

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<sup>1</sup>Section of Digestive Diseases, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA; <sup>2</sup>Translational Hepatology, Department of Internal Medicine I, University Clinic Frankfurt, Frankfurt, Germany; <sup>3</sup>European Foundation for the Study of Chronic Liver Failure-EF Clif, Barcelona, Spain

\* Corresponding author.  
Address: Translational Hepatology, Department of Internal Medicine I, Goethe University Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt – Germany; Tel.: +49 (0) 69 6301 0, fax: +49 (0) 69 6301 84441.  
E-mail address: [Jonel.Trebicka@kgu.de](mailto:Jonel.Trebicka@kgu.de) (J. Trebicka).

directly involved in the increased hepatic resistance. Thus, their modulation may both relieve the pressure and in the longer run ameliorate fibrosis.

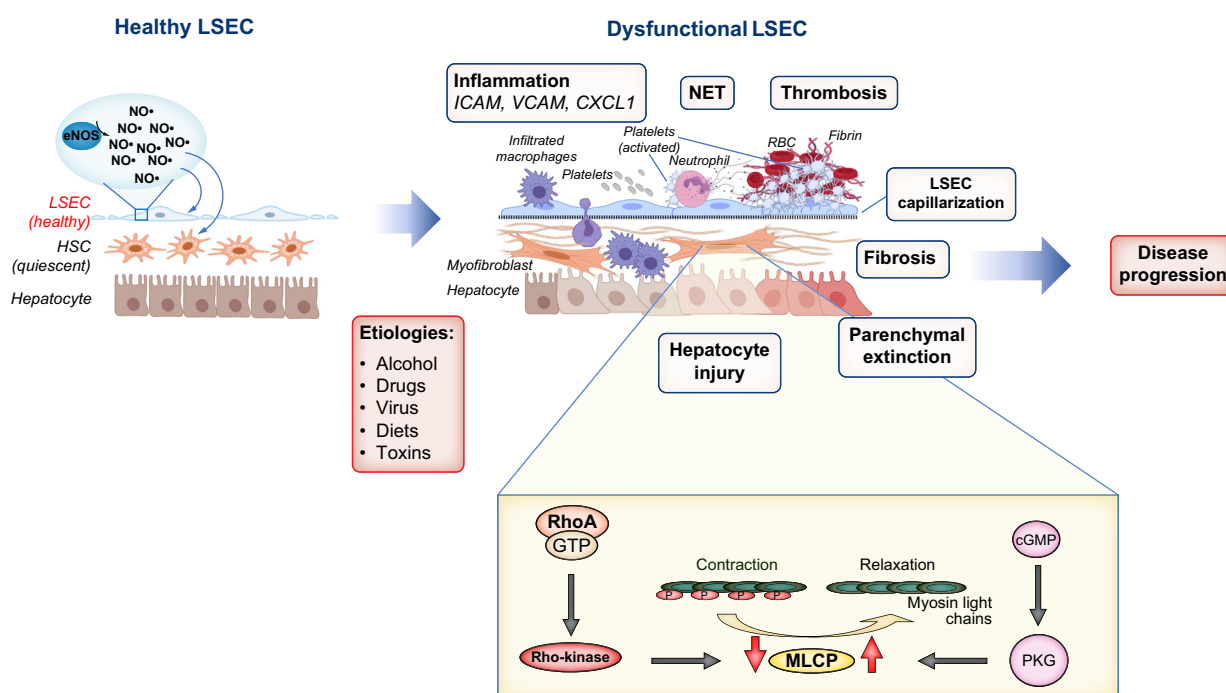
### LSEC dysfunction

Normal LSEC function is necessary for liver homeostasis (Fig. 1). This section discusses factors contributing to LSEC dysfunction and characteristics of dysfunctional LSECs, as well as the mechanisms that lead to LSEC dysfunction.

**Capillarisation:** LSECs have fenestrae of approximately 0.1 microns organised into groups of sieve plates, which facilitate the transport of macromolecules from the hepatic sinusoids to the space of Disse, then to HSCs and hepatocytes. Additionally, a unique feature of LSECs that distinguishes them from endothelial cells in other organs is the lack of a basement membrane, which allows efficient movement of macromolecules between the lumen of the sinusoid and the space of Disse.<sup>7</sup> Loss of fenestrae and the appearance of a basement membrane in LSECs is termed “capillarisation”.<sup>8,9</sup> Vascular endothelial growth factor (VEGF) is known to be a key factor that maintains the fenestrae in the absence of a basement membrane<sup>10</sup> through endothelial nitric oxide synthase (eNOS)-derived nitric oxide (NO) signalling.<sup>11</sup> A study showed that removal of VEGF signalling in transgenic

### Key points

- Portal hypertension, the consequence of increased hepatic resistance and splanchnic hyperperfusion, is a driver of serious complications in cirrhosis.
- The dysregulation of LSECs and HSCs is the main feature that leads to increased vascular resistance, but hepatic microvascular thrombosis also plays a significant role in the development of portal hypertension.
- Splanchnic hyperperfusion is the result of severe changes in the extrahepatic vasculature (including neoangiogenesis) and hypocontractility of the splanchnic arteries.
- The gut-liver-axis plays an important role in the development of complications of portal hypertension, although the mechanical increase of portal pressure does not directly impair the gut barrier long-term.
- Diagnosis of clinically significant portal hypertension is possible using extracellular matrix-derived biomarkers, as well as liver and spleen stiffness measurements.
- Novel endoscopic ultrasound-guided pressure measurement offers a good alternative, but hepatic venous pressure gradient measurement remains the gold standard.
- Cell-specific drug-delivery and combinatorial therapies targeting the extrahepatic and intrahepatic vascular compartments are emerging as the best treatment strategies for portal hypertension.



**Fig. 1. Liver sinusoidal cell dysfunction in liver pathogenesis.** Normal LSEC function is necessary for liver homeostasis. Various aetiologies can cause LSECs to become dysfunctional, leading to disease progression. Capillarisation is the loss of fenestrae and appearance of a basement membrane in LSECs. eNOS-derived NO plays a pivotal role in liver homeostasis by regulating vascular tone, maintaining fenestrae, maintaining HSCs in a quiescent state and blocking platelet attachments to endothelial cells among other functions. LSEC dysfunction often precedes pathological events, including inflammation, NET formation, microvascular thrombosis, parenchymal extinction (regions of tissue loss and fibrosis secondary to vascular obstruction and microvascular thrombosis), hepatocyte injury and fibrosis, leading to the development of portal hypertension. By contrast, in HSCs, calcium-independent contraction is mainly regulated by MLCP, which is inhibited by the RhoA/Rho-kinase pathway and activated by the NO/PKG-pathway. CXCL1, chemokine (C-X-C motif) ligand 1; HSCs, hepatic stellate cells; ICAM, intercellular adhesion molecule; LSECs, liver sinusoidal endothelial cells; MLCP, myosin light-chain phosphatase; NO, nitric oxide; NET, neutrophil extracellular trap; PKG, cGMP-dependent kinase; VCAM, vascular cell adhesion molecule.

mice, in which liver-specific secretion of a soluble VEGF decoy receptor inactivates endogenous VEGF, results in a loss of LSEC fenestration and portal hypertension, as well as HSC activation independent of hepatic parenchymal damage. Administration of VEGF to these mice ameliorated portal hypertension.<sup>12</sup> In addition, the composition of collagen in the space of Disse may play a role in the maintenance or loss of endothelial fenestration.<sup>13</sup> A recent study in mice showed that delta like canonical Notch ligand 4 (Dll4), a ligand of the Notch signalling pathway that is predominantly expressed in endothelial cells, promotes LSEC capillarisation by basement membrane formation.<sup>14</sup> Additionally, this study showed that DLL4 knockdown in a human LSEC cell line decreased ECM expression while its overexpression led to an increase in ECM proteins including collagens, fibronectin and laminin, confirming the role of DLL4 in LSEC capillarisation.<sup>14</sup>

**Regulation of NO production:** The ability of LSECs to generate NO is one of the most recognised indicators of LSEC function, given a wide spectrum of pivotal homeostatic functions mediated by eNOS-derived NO, including control of hepatic vascular tone, inhibition of thrombosis, and blocking HSC activation during fibrogenesis<sup>15</sup> (Fig. 1). In liver diseases, it is well known that eNOS-derived NO production and/or NO bioavailability are significantly reduced, contributing to liver disease pathogenesis. A recent study identified a novel mechanism of eNOS regulation by  $\beta$ -arrestin2 ( $\beta$ -Arr2), demonstrating that decreased  $\beta$ -Arr2 levels in cholestatic LSECs contribute to reduced eNOS activity and sinusoidal portal hypertension in mice,<sup>16</sup> while overall expression of  $\beta$ -Arr2 (at protein and mRNA levels) increases in human and experimental cirrhosis.<sup>17</sup> Overexpression of  $\beta$ -Arr2 in LSECs significantly increases NO production, suggesting a role of  $\beta$ -Arr2 in eNOS activation and NO production.

Regulation of eNOS activity can be aetiology specific, given that eNOS is regulated by interactions with several proteins, which may themselves be regulated uniquely in different disease conditions. Heat shock protein 90 (Hsp90) activates eNOS, leading to NO production.<sup>18</sup> Recently, we reported a mechanism of alcohol-driven LSEC dysfunction.<sup>19</sup> In this study, we showed that LSECs can metabolise ethanol and that ethanol can increase Hsp90 acetylation through enhanced production of acetyl-CoA, a substrate for protein acetylation, by the action of alcohol dehydrogenase 1 (ADH1) and cytochrome P450 2E1 (CYP2E1).<sup>19</sup> Further, acetylation of Hsp90 decreases its interaction with eNOS, decreasing production of eNOS-derived NO, while a deacetylation mutant of Hsp90 increases an interaction with eNOS, leading to NO production. Overexpression of histone deacetylase 6 (HDAC6), an Hsp90-specific de-acetylase enzyme,<sup>20</sup> in liver endothelial cells in mice increased the association of Hsp90 with eNOS and increased NO production, resulting in amelioration of alcohol-induced liver injury.<sup>19</sup> These observations indicate that LSEC dysfunction can be treated in an aetiology-specific manner.

**Autophagy:** Autophagy is a highly conserved and controlled intracellular process involving the degradation and recycling of cellular components in lysosomes. A recent study by Ruat *et al.*<sup>21</sup> showed that autophagy is important for LSEC homeostasis. Decreased autophagy in mice with endothelial cell-specific *Atg7* deletion was associated with increased liver fibrosis and oxidative stress, as indicated by decreased expression of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidases, as well as those regulated by the nuclear factor-erythroid 2-related factor 2 (Nrf2). In addition, a phosphorylated

(active) form of eNOS was decreased in endothelial-specific *Atg7* knockout (KO) mice with acute liver injury induced by carbon tetrachloride (CCl<sub>4</sub>). These results indicate that autophagy in liver endothelial cells has a protective role against liver injury.

Similarly, in non-alcoholic steatohepatitis, autophagy in LSECs seems protective against liver fibrosis progression and inflammation.<sup>22</sup> Endothelial-specific *Atg5* KO mice with diminished autophagic response, showed an increase in endothelial inflammation (hepatic vascular cell adhesion molecule 1 [VCAM-1], chemokine (C-C motif) ligand 2 [CCL2], and CCL5 expression), liver injury (increased alanine aminotransferase), hepatic cell death (increased cleaved caspase-3), and liver fibrosis in response to a 16-week high-fat diet, compared to a chow control diet. Deficiency in autophagic responses also resulted in increased liver fibrosis in response to intraperitoneal injection of CCl<sub>4</sub> for 6 weeks.

Enhancing autophagy in LSECs is protective against liver injury. A study in mice showed that statins help to maintain LSEC function in response to ischaemia reperfusion-induced liver injury by increasing autophagy and subsequent induction of Kruppel-like factor 2 (KLF2) activity, which is important for the maintenance of microvascular function.<sup>23</sup> However, autophagy does not seem to be protective for all liver cell types, since autophagy is involved in HSC activation by providing an energy source in pathological conditions.<sup>24</sup> Taken together, these studies indicate that autophagy in LSECs is protective against LSEC dysfunction, liver injury, and fibrosis.

**Aging:** Aging is a critical biological factor that influences LSEC function. With increases in age, there are major morphological changes in LSECs, including increased LSEC thickness and reduced fenestration, known as pseudocapillarisation.<sup>25–28</sup> These age-related changes increase susceptibility to chronic liver injury<sup>29</sup> and diabetes.<sup>30</sup> In addition, aging increases the occurrence of portal hypertension and liver fibrosis in rats, as demonstrated by comparisons of aged rats (16 months) to young rats (1 month).<sup>29</sup>

Given these insights, age-related pseudocapillarisation could represent a therapeutic target. A study by Hunt *et al.*<sup>30</sup> assessed the porosity in cultured LSECs in response to multiple drugs that act on the pathways that influence NO, and showed that treatments with nicotinamide mononucleotide, sildenafil, and 7-ketocholesterol increased fenestration porosity and frequency in LSECs isolated from young and old mice. Such drugs could potentially be used for the treatment of age-associated susceptibility to liver fibrosis and portal hypertension.

### Hepatic stellate cells

HSCs are another unique cell type in the liver. In the quiescent state these cells store vitamin A, but upon injury they are activated and transdifferentiate into a myofibroblastic phenotype.<sup>31</sup> A seminal study by the group of Robert Schwabe demonstrated that 98% of myofibroblasts populating the fibrotic septa were derived from HSCs.<sup>32</sup> There is a large amount of literature on HSCs (>4,800 citations in PubMed 2005–2020), as well as very well written reviews on how these cells are activated and their role in the development of liver fibrosis.<sup>31,33</sup> However, their role in portal hypertension has been investigated less extensively (>200 citations in PubMed 2005–2020), though fibrosis is associated with liver stiffness, angiogenesis and contraction, which all link HSCs to portal hypertension.

**Stiffness:** Liver stiffness may induce a mechanical increase in hepatic resistance and at least aggravate portal hypertension.

The physical and chemical properties of the environment, in which HSCs are embedded, are also important for their activation,<sup>34</sup> at least partly via Src and RhoA pathways.<sup>35</sup> The majority of the fibrotic material is synthesised by HSCs, and together with other cells they contribute to the perpetuated remodelling of the matrix, reviewed elsewhere.<sup>36,37</sup> The stiffness of the matrix is provided, among other mechanisms, by the crosslinking of collagens, which leads to a less reversible fibrosis.<sup>38</sup> The stiffness itself may further boost the activation of HSCs, and the activated HSCs may migrate to those fibrosis spots due to so-called durotaxis,<sup>39</sup> which has been shown for fibroblasts,<sup>40</sup> and is likely to occur in HSCs as well. Moreover, the swelling of HSCs, e.g. in the presence of hyperammonia, may activate them and induce their contraction.<sup>41</sup> This may further lead to aggravation of fibrosis and thereby increase liver stiffness, one of the main features of clinically significant portal hypertension (CSPH) in humans.<sup>42</sup>

**Angiogenesis:** HSCs are the hepatic pericytes involved in vessel formation and stabilisation.<sup>43</sup> In this role, HSCs follow the transformation of LSECs upon injury (see above) and also drive LSEC transformation by releasing VEGF, which works in a paracrine and autocrine manner on LSECs and HSCs.<sup>43</sup> Especially, platelet-derived growth factor (PDGF) is an important factor, which not only recruits HSCs but also boosts their fibrogenic potential. The boost in hepatic angiogenesis is probably meant to be a repair mechanism, but it aggravates the liver pathology and does not alleviate portal hypertension.<sup>43,44</sup> The reason is that these newly formed vessels are different from the sinusoidal vasculature, and do not increase the blood perfusion of the cirrhotic liver, but they further impair the homeostasis of hepatocytes and aggravate liver damage, further fuelling fibrosis and inflammation.<sup>45</sup> In experimental work, several anti-angiogenic strategies have been shown to be beneficial for portal hypertension, which could be partly attributed to improved fibrosis.<sup>46–49</sup> Further targets to be addressed may be Vasohibin-1<sup>50</sup> and placental growth factor (PlGF).<sup>51</sup> But in these studies, their effect on portal hypertension was at least partially due to their extrahepatic anti-angiogenic effect. Since hepatic resistance is at least partly dependent on fibrosis, it is difficult, or even impossible to separate the role of intrahepatic angiogenesis on portal hypertension from its role on fibrosis. Along with the transdifferentiation of HSCs, the fibrogenic and angiogenic potential increases with the development of a myofibroblastic apparatus enabling contraction. Contraction is the dynamic part of hepatic resistance.

**Contraction:** Contraction of HSCs narrows the sinusoids and thereby further increases hepatic resistance, causing and aggravating portal hypertension. Activated HSCs may show increased contraction stimulated by different vasoconstrictors. The contraction of myofibroblasts in general may occur in 2 ways (Fig. 1). One is the short-term, strong and calcium-dependent contraction, which is induced by myosin light-chain kinase (MLCK), which is usually mediated by G-protein coupled receptors.<sup>52</sup> In particular, endothelin and catecholamines are known to induce such contractions. But myofibroblastic cells can also control vascular tone, which requires a steady and slow mechanism that is mainly regulated by myosin light-chain phosphatase (MLCP). The regulation of MLCP is crucial for determining vascular tone and thereby hepatic resistance.<sup>52</sup>

The activity of MLCP is inhibited by Rho-kinase, and thereby the contractile tone is increased.<sup>52</sup> Rho-kinase seems to be crucial for HSC function, not only in contraction but also in fibrosis, as shown by the effect of its direct inhibition.<sup>53,54</sup> Moreover, this

master regulator of vascular function is so far downstream that many receptors and their downstream pathways regulate its function.<sup>55</sup> Rho-kinase activity is regulated by many receptors, such as angiotensin II type I receptor (AT1R),<sup>56</sup>  $\beta$ -adrenergic receptor ( $\beta$ -AR),<sup>57</sup> urotensin-receptors,<sup>58</sup> sphingosine-1-phosphate receptor 2 (S1P-R2),<sup>59,60</sup> neuropeptide Y receptor (NPYR),<sup>61</sup> and Mas-receptor,<sup>62,63</sup> among others. But the intracellular pathways are also important and may reveal potential therapeutic targets, such as RhoA-activation or Gs/Adenylyl-cyclase.

On the other side, MLCP activity is enhanced by cGMP-dependent kinase (PKG), which is induced by cGMP.<sup>64</sup> The main pathway of PKG activation is by the diffusion of NO supplied by LSECs.<sup>64</sup> But cGMP can be regulated even in HSCs by different mechanisms. Phosphodiesterase (PDE), especially PDE5, can degrade cGMP, while soluble guanylate cyclase can increase its concentration. Subsequently, changes in cGMP level affect PKG activity, HSC contractility and thereby modulate hepatic resistance.<sup>65–68</sup>

These processes represent the current understanding of the role of HSCs in the development of portal hypertension, but this may be biased by current knowledge and technology, with HSCs being far more complex. One example is the role of Rev-erb-alpha which is a gene regulator of the circadian rhythm. Indeed, Rev-erb-alpha also determines the phenotype and behaviour of HSCs. However, a direct manipulation of this mechanism also decreases portal hypertension *in vivo*.<sup>69</sup> To illustrate the complexity, Fig. 2 shows the predicted processes (Fig. 2A, Table S1) and functions (Fig. 2B, Table S2) of HSCs, based on gene expression analysed by RNA-sequencing of murine-activated HSCs. These plots highlight the representative magnitude of specific processes and functions according to the number of genes expressed and underline the necessity of further research in this specific and clinically relevant field.

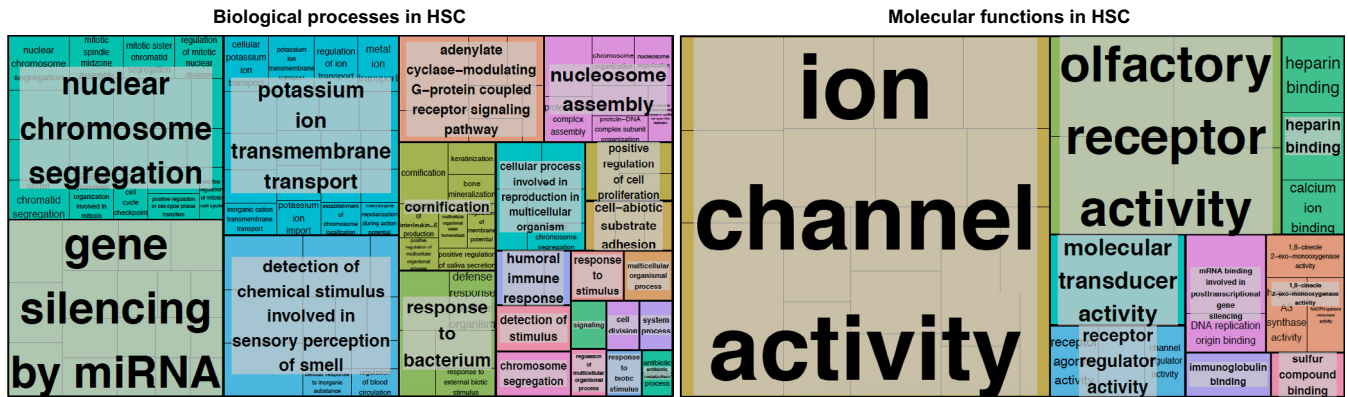
### Hepatic microvascular thrombosis

A growing body of recent studies has suggested that thrombosis is one of the key pathological factors mediating portal hypertension. Previously, cirrhosis was thought to be an inherently anti-coagulated state because of decreased coagulation factor production by damaged hepatocytes and decreased platelet counts (*i.e.* thrombocytopenia). However, an accumulating body of evidence indicates that cirrhosis represents a re-balanced or even a procoagulant state.

### Hepatic vascular thrombosis

What is the consequence of thrombi formation in the liver? A growing number of studies suggest the importance of intrahepatic microvascular thrombosis for fibrosis progression and portal hypertension.<sup>70</sup> This observation connecting thrombosis and liver fibrosis was first described as “parenchymal extinction” in pathological specimens of human liver cirrhosis.<sup>71</sup> Subsequently, a study in mice with CCl<sub>4</sub>-induced liver injury suggested that blood clotting is involved in the fibrotic response of the liver, given the observations of sinusoidal deposition of fibrin/fibrinogen and fibronectin in the damaged liver in the short-term, and deposition in fibrous septa during long-term liver damage.<sup>72</sup> The critical role of blood clotting in the process of fibrogenesis was also shown in a study with rats, in which administration of a thrombin antagonist (SR182289) significantly decreased CCl<sub>4</sub>-induced liver fibrosis.<sup>73</sup> Additionally, mice deficient in the prothrombinase fgl2/fibrinolytic, an enzyme responsible for the





**Fig. 2. Biological processes and molecular functions in primary human HSCs.** Treemap representation of biological processes (A) and molecular functions (B) revealed by RNA-sequencing (transcriptome analysis) of human primary HSCs. The Treemap plot shows a two-level hierarchy of GO terms. Each rectangle is a GO term cluster representative. The representatives are joined together to loosely related GO terms and visualised. The size of the rectangles reflects the significance (*p* value) of the respective GO term. GoRilla<sup>207</sup> was used in combination with REVIGO<sup>208</sup> and R-studio (V1.3.1093) with packages TmPlot and TreeMap (R-Studio Team (2020). <http://www.rstudio.com/>) for the visualisation and GO term determination. The details of the pathways involved in the biological processes are found in Table S1 and the molecular functions in Table S2. GO, gene ontology; HSCs, hepatic stellate cells.

conversion of prothrombin to thrombin, exhibited decreased fibrin deposition and necrosis in a model of viral hepatitis, again linking thrombosis to liver fibrogenesis.<sup>74</sup> Further, treatment with anticoagulant drugs such as nadroparin and enoxaparin (low-molecular-weight heparins) led to a decrease in liver fibrosis in rats after bile duct ligation,<sup>75,76</sup> thioacetamide administration (in which aspirin was also shown to reduce fibrosis), and CCl<sub>4</sub>-administration (in which another low-molecular-weight heparin, dalteparin, was shown to decrease fibrosis).<sup>77</sup> A mechanistic study in rat models of liver injury/fibrosis with CCl<sub>4</sub> or thioacetamide, with and without enoxaparin, demonstrated reduced portal pressure, reduced HSC activation, reduced fibrosis, and reduced fibrin deposition in enoxaparin-treated rats compared to control rats, possibly through a reduction of thrombosis.<sup>78</sup> However, enoxaparin may not be effective in the late stages of cirrhosis, as suggested by a study showing no amelioration or improvement in liver fibrosis, liver function, and portal pressure in cirrhotic rats.<sup>79</sup> Rivaroxaban, an anticoagulant drug that inhibits an active form of factor X, thereby inhibiting the generation of thrombin, has also been shown to reduce portal pressure in cirrhotic rats (induced by thioacetamide and CCl<sub>4</sub>), likely by reducing HSC activation, endothelial dysfunction, and microvascular thrombosis rather than via a direct effect on fibrosis.<sup>80</sup> Collectively, anticoagulation therapy seems beneficial for the treatment of liver fibrosis and portal hypertension, although it is dependent on the stage of cirrhosis.

**Congestive hepatopathy**

Chronic hepatic congestion, or congestive hepatopathy, is a cause of portal hypertension that occurs in conditions that disturb efficient blood flow in the liver, such as congestive heart failure and Budd-Chiari syndrome. Mice with a partial inferior vena cava ligation develop post-hepatic portal hypertension and can be used as a model to mimic congestive hepatopathy. Using this model, Simonetto *et al.* demonstrated that chronic congestion induces liver fibrosis through sinusoidal thrombosis and mechanical forces.<sup>81</sup> Pharmacologic treatment with warfarin, an anticoagulant agent that blocks vitamin K epoxide reductase, as well as genetic inhibition of the clotting cascade (transgenic mice

overexpressing tissue factor pathway inhibitor [SM22a-TFPI]), decreased hepatic thrombosis and liver fibrosis. Mechanistically, the authors showed that increased fibrin deposition together with sinusoidal mechanical stretch of HSCs facilitates fibrin-fibronectin complex formation at HSCs, leading to ECM deposition. Although warfarin treatment can decrease fibrosis, it did not significantly reduce portal hypertension in congestive hepatopathy. Chronic hepatic congestion also mechanically stimulates LSECs to release angiocrine factors, such as the chemokine (C-X-C motif) ligand 1 (CXCL1) and the neutrophil chemotactic chemokines; it also facilitates neutrophil recruitment at the site of the thrombus, leading to the formation of neutrophil extracellular traps (NETs). Inhibition of NET formation by knocking out neutrophil elastase, which is essential for NET formation, significantly decreased liver fibrosis and portal hypertension in mice, again supporting the role of hepatic thrombosis in liver fibrosis and portal hypertension.<sup>82</sup>

**Extrahepatic circulation**

In portal hypertension, increased portal blood inflow from the splanchnic circulation augments portal pressure and thereby contributes to the maintenance and exacerbation of portal hypertension.<sup>83</sup> Portosystemic collaterals contribute to the development of varices and the delivery of noxious substances from the portal circulation directly to the cerebral vasculature without hepatic detoxification, thus contributing to hepatic encephalopathy.<sup>84</sup> These changes together with the arterial vasodilation in the splanchnic circulation play a critical role in increasing blood flow to the portal vein.

**Pathological angiogenesis**

It was thought that portosystemic collaterals are an opening of pre-existing vessels, but a study by Sieber and colleagues was the first to indicate the evidence of angiogenesis-driven collateral vessel formation in portal hypertensive rats using a device consisting of collagen type I-filled teflon ring, which was implanted into the mesenteric bed for the assessment of angiogenesis.<sup>85</sup> Although initial collateral formation likely develops due to the opening of pre-existing vessels, accumulating evidence has

resulted in a consensus that most portosystemic collateral vessels are formed through angiogenesis.

It is thought that these portosystemic collateral vessels are “pathological” because they further increase portal vein inflow and increase the likelihood and the incidence of variceal haemorrhage.<sup>43,70,86</sup> Thus, numerous studies have attempted to target pathological angiogenesis in experimental models of portal hypertension and cirrhosis through a variety of approaches, such as targeting VEGF (with anti-VEGFR2 or a combination of anti-VEGF [rapamycin]/anti-PDGFR [imatinib]), PIGF,<sup>51</sup> apelin,<sup>87</sup> cannabinoid,<sup>88</sup> peroxisome proliferator-activated receptor (PPAR) $\alpha$  and hedgehog signalling, or using sorafenib.<sup>89,90</sup> However, the reduction of collateral vessels does not always result in a decrease in portal pressure to the normal level, since this reduction does not significantly change the portal blood flow.<sup>51,91</sup> In addition, clinical studies of an anti-angiogenic approach such as anti-VEGF treatment have given unsatisfactory results because this approach also blocks the homeostatic activity of VEGF, such as wound healing angiogenesis, and other non-specific effects of VEGF that are important for vascular homeostasis. Thus, targeting only “pathological angiogenic activity” may be a novel approach to ameliorate portal hypertension.

A recent study by De Gottardi and colleagues<sup>92</sup> showed that Paneth cells in the lining of the small intestine regulate mesenteric angiogenesis and portal hypertension. Paneth cells secrete antimicrobial peptides that mediate host-microbe interactions, keeping a balance between intestinal microflora and enteric pathogens. Using a mouse model of conditional ablation of Paneth cells (Math1Lox/LoxVilcreERT2 mice with tamoxifen), it was shown that Paneth cell-ablation resulted in a significant reduction in portosystemic collateral vessels, portal pressure, and CD31-positive vessels in mice following partial portal vein ligation. Thus, in response to intestinal flora and microbiota-driven factors, Paneth cells secrete not only antimicrobial peptides, but also pro-angiogenic signalling molecules, promoting intestinal and mesenteric angiogenesis and regulating portal hypertension.

### Extrahepatic vasodilation

Besides the pathological angiogenesis and impaired gut-vascular barrier, the tone of arterial vessels preceding the portal circulation is decreased.<sup>3</sup> The changes occurring in the contractile pathway oppose those in HSCs, which renders the management

of portal hypertension very difficult.<sup>1,3</sup> In particular, the circulating and local levels of NO are increased in these vessels,<sup>93</sup> which – among other mechanisms – are related to shear stress in the endothelium secondary to portal congestion and bacterial translocation.<sup>94–97</sup> Recent data demonstrating increased levels of NO in the portal vein and its strong correlation with lipopolysaccharide in human cirrhosis may underline this hypothesis,<sup>97</sup> along with experimental evidence published by different groups.<sup>97–99</sup>

Indeed, in parallel to the increased NO synthesis and bioavailability in these vessels, the vasocontractile pathways are also impaired.<sup>3</sup> It has been demonstrated that the RhoA/Rho-kinase pathway is defective and less active.<sup>100</sup> This is at least partly due to changes in the post-receptor regulation of vasoconstrictor receptors, such as AT1R, which is desensitised by increased  $\beta$ -Arr2 binding.<sup>56</sup> Moreover, in the renin-angiotensin-system there is a shift towards the activated angiotensin-converting enzyme 2 (ACE2)-/masR axis in the splanchnic vessels in parallel to the desensitised AT1R.<sup>62,101</sup> It could be shown that neuropeptide Y, as a co-neurotransmitter of catecholamines and angiotensin-2, could cause vascular contraction.<sup>61</sup> These data suggest that this vascular dysfunction is not structural and is to a large extent reversible.<sup>61</sup> Similarly, the shift towards the ACE2-/masR axis was reduced in patients after liver transplantation, demonstrating that the diseased liver plays a causative role in these changes.<sup>101</sup>

Taking into the account the contrary regulation of the extrahepatic and intrahepatic mechanisms, cell-specific and hepatic delivery of vasodilators is extremely important. There have been attempts at targeting Rho-kinase in HSCs, which do not elicit severe systemic complications.<sup>102,103</sup> Yet these are still experimental.

### Gut-liver axis and portal hypertension

The gut-liver axis has been increasingly investigated in recent years, partly thanks to technological advances in the methods used to investigate the microbiota.<sup>104</sup> Hence, we performed an analysis of the published literature on the gut-liver axis and portal hypertension from the last 15 years and plotted the key words (after removing the random words) in order to highlight the main findings or opinions (Fig. 3). Besides the general mechanisms of bacterial intestinal translocation, a wide



**Fig. 3.** Word cloud representing the most frequent keywords of abstracts with the keyword gut-liver axis. The size of the words within the word cloud correlates with the frequency of the respective word in the analysed abstract text.

spectrum of words appeared, such as inflammation, endotoxemia, hepatic encephalopathy, small bowel dysbiosis, increased permeability, mesenteric neovascularization, bacterial overgrowth, peritonitis, non-selective beta blockers, Apelin and so on (Fig. 3). Interestingly, NAFLD is mentioned in a large number of patients, although alcohol-related cirrhosis is much more closely linked to the gut-liver-axis. This calls for the clear prioritisation of this aetiology in future research.

### Gut barrier function in portal hypertension

As outlined above, gut barrier function is impaired in the presence of portal hypertension in cirrhosis. There are several reasons for this. First the aetiology of cirrhosis (alcohol, diet, decreased bile flux) or of the non-cirrhotic portal hypertension (splanchnic or hepatic inflammatory processes) may disrupt the gut epithelium directly or via increased inflammation.<sup>105–107</sup> The venous congestion associated with splanchnic vasodilation and splanchnic neoangiogenesis may impair the gut-vascular barrier and increase permeability in the gut. The complications of portal hypertension in cirrhosis, e.g. renal and immune cell dysfunction, may also facilitate bacterial translocation.<sup>107</sup> It is widely accepted that bacterial translocation exists and is of major relevance in cirrhosis.<sup>107</sup> Yet it is important to emphasise that a mechanical increase in portal pressure, in the absence of inflammatory processes or cirrhosis, is not associated with increased disruption in the gut barrier.<sup>108</sup>

Secondary to this disruption of the gut barrier, the translocation of bacterial components or bacteria into the circulation continuously activate the immune system. This may lead to chronic systemic inflammation, which may take place at multiple levels: in the portal circulation, predisposing to splanchnic thrombosis; in the liver, promoting inflammation and fibrosis; and in other organs, inducing organ dysfunction.<sup>97,109–111</sup> Translocation into the lymphatic system is also important, as it may be associated with spontaneous bacterial peritonitis and other spontaneous infections.<sup>112,113</sup>

The gut barrier impairment is, in addition to the aforementioned pathophysiological mechanisms, related to changes in the gut microbiota itself.

### Effects of microbiota on the liver

The gut microbiota harbours the largest pool of genetic material in the human body with more than  $10^{13}$  microbes with an extremely high metabolic activity.<sup>105,114</sup> Nowadays, there is no doubt that changes in the microbiota influence cirrhosis (summarised in several recent reviews) and that cirrhosis induces profound changes in the microbiota.<sup>107,115,116</sup> However, the role of portal hypertension in this setting is not yet well understood. Indeed, it is difficult to clearly attribute specific changes in microbiota to portal hypertension. By contrast, there is evidence showing that the microbiota and the bile acid pool, which can also target the farnesoid X receptor (FXR) on liver cells, may aggravate or alleviate portal hypertension and its complications.<sup>110,117–121</sup> Secreted bile acids are modified by the intestinal microbiota leading to decreased FXR stimulation which may increase the contractility of HSCs and thereby portal hypertension.<sup>117,119</sup> This is evident when FXR agonists are administered.<sup>110,117–121</sup> In addition, the microbiome is present in other compartments besides the gut in the absence of infection. In recent studies, blood, liver, bile and ascitic fluid have been analysed in patients with cirrhosis.<sup>122</sup> Indeed, in patients with complications of portal hypertension such as ascites, the

composition of the circulating microbiome in the portal vein compared to the hepatic vein and the systemic circulation varies, while the DNA of specific species was correlated with inflammatory markers, which was not the case for ascites.<sup>123,124</sup> In ascites, only the evident infection or bacterascites, but not bacterial DNA, seems to be relevant for outcome.<sup>124</sup> But in the blood circulation, the specific microbial members may aggravate hepatic inflammation,<sup>123</sup> and thereby probably hepatic resistance. This is supported by patient data showing that liver stiffness (a surrogate marker of portal hypertension and inflammation) and levels of inflammatory markers were higher in the hepatic vein than in the portal vein. These patients more often developed organ failure and had worse outcomes, even after an adequate decrease of portal pressure using transjugular intrahepatic portosystemic shunt (TIPS).<sup>125</sup> The existing evidence linking the gut microbiota and microbiome to portal hypertension is still scarce and substantial work is required to catch up with the available technological advances.

### Translational/clinical advances in our knowledge of portal hypertension

Portal hypertension represents a hallmark in the course of chronic liver disease, as the aforementioned changes and mechanisms lead to the perpetuation of injury, guaranteeing the progression and worsening of prognosis in cirrhosis. For this reason, for more than a century, cirrhosis was believed to be irreversible. Yet, advances in treatment, particularly for viral hepatitis, have shown that cirrhosis and portal hypertension may be reversible if the aetiology of CLD is treated. This has been shown for the treatment of autoimmune hepatitis, HBV and HCV, and alongside weight loss,<sup>126–128</sup> and abstinence. Yet, the development of portal hypertension above a certain threshold is a predictor of complications, especially in the presence of risk factors (e.g. obesity, alcohol intake, etc.). Indeed, recently it was shown in a large prospective series of patients that severe portal hypertension induces an unstable course of disease after an acute decompensating event,<sup>129</sup> at least partly due to bacterial infections,<sup>6</sup> and secondary to variceal bleeding episodes in at least 20% of cases.<sup>130</sup>

A pressure gradient of more than 10 mmHg between the portal and hepatic vein is characterised as CSPH, which marks the point at which portal hypertension is likely to cause clinical complications.<sup>131</sup> Therefore, it is extremely important to diagnose CSPH and to monitor it upon treatment.

### Diagnosis of clinically significant portal hypertension Invasive tools

The gold standard for the diagnosis of CSPH is the invasive (using venous access) measurement of the gradient between the free and so-called wedged hepatic vein – associated practical issues are discussed elsewhere.<sup>131</sup> Briefly, the wedge-pressure represents the conducted pressure of the sinusoids and therefore the portal pressure. Using this approach to measure the pressure gradient has advantages as it allows for a thorough assessment of systemic haemodynamics (Swan-Ganz) and even for a liver biopsy (if indicated).

Yet, the most reliable measurement is obtained by puncturing the portal vein using the transjugular-transhepatic approach.<sup>132</sup> This approach is often used in the TIPS setting, but very rarely for diagnostic purposes. One potential reason to use a direct puncture is if the measurement of hepatic venous pressure



gradient (HVPG) is not possible due to shunts and if it is not possible to reach a wedge-position.<sup>132</sup>

In recent reports the direct puncture of the portal vein using endoscopic ultrasound has been reported for sampling and a direct measurement of portal pressure, which seems to be safe and technically feasible.<sup>133–135</sup> This technique could aid patient management, especially when varices are present at endoscopy, although more research is needed.

Despite improvements, invasive tools are associated with higher costs and possible morbidity, which are particular drawbacks of liver biopsy. Therefore, significant efforts have been made to develop non-invasive tools.

### Non-invasive tools

Besides HVPG as the gold standard, liver stiffness measurement using different elastographic techniques seems to play a central role in the diagnosis.<sup>136</sup> Owing to technical advances, elastography can now be performed routinely using most ultrasound machines. The best validated for portal hypertension is Fibroscan, which is recommended by Baveno to guide screening endoscopy for varices, followed by 2D-Shear-wave elastography of Aixplorer, the last being superior in patients with ascites.<sup>42,137–139</sup> The combination of liver and spleen stiffness measurement seems to be the most accurate to assess CSPH and the effect of portal pressure-lowering strategies.<sup>42,125,138</sup> One reason is that liver stiffness also depends on other mechanisms, such as inflammation, cholestasis or right heart congestion, of which the latter 2 can be ruled out using an ultrasound-based elastographic measurement.

Since patients with cirrhosis undergo mandatory hepatocellular carcinoma screening, ultrasound-based elastography may be convenient as a one-stop-shop diagnostic. Yet in many patients, MRI is also performed. Besides MR-elastography, there are novel protocols, such as the measurement of extracellular volume or the liver inflammation and fibrosis score, that do not require a specific device to measure portal pressure.<sup>140–142</sup> The measurement of extracellular volume is mainly validated for fibrosis, but it also correlates with portal pressure as shown in animals.<sup>141–143</sup> Still, significant efforts are required to make it ready for widespread clinical use, but it may be interesting as a one-stop-shop diagnostic approach.

The easiest approach is to use soluble biomarkers. In particular, ECM markers may be useful for the detection of CSPH and complications,<sup>36,144–146</sup> while inflammatory and haemodynamic markers,<sup>147–150</sup> or even circulating miRNA are rather unspecific.<sup>151–153</sup> Maybe in the near future, signature molecules based on omics-techniques may help to improve the diagnosis of CSPH.

### Treatment strategies for clinically significant portal hypertension

Despite the large numbers of patients affected by portal hypertension, there are only few therapeutic options for them.<sup>154</sup> Moreover, the treatment strategies for portal hypertension are stratified according to the complications of portal hypertension rather than the portal hypertension itself. There are basically 2 larger groups of complications directly linked to the magnitude of portal hypertension, varices and ascites development. The established strategies are tested in multiple studies with hard endpoints (mortality) and thereby are better investigated than most treatments in other indications.

### Established strategies

The current treatment strategies for varices development and bleeding include non-selective beta blockers (NSBBs), which were introduced for this indication 40 years ago by the group of Didier Lebec.<sup>155,156</sup> The rationale for NSBB use is to decrease splanchnic venous inflow and cardiac output, by blocking the  $\beta_1$ -adrenergic receptor ( $\beta_1$ -AR), and to induce vasoconstriction in the splanchnic region by blocking  $\beta_2$ -AR. In the last decade, the use of carvedilol, brought into the field by the group of Peter Hayes,<sup>157,158</sup> seems to be more effective, leading to higher haemodynamic response rates<sup>159</sup> than the classical NSBBs.<sup>159,160</sup> NSBBs are indicated for primary prophylaxis (to prevent bleeding once varices are present) and for secondary prophylaxis (to prevent re-bleeding). Baveno conferences have established very useful guidelines for the use of NSBBs over the last 30 years. While NSBBs may not be useful in the prevention of varices development, if CSPH is absent, as shown by the seminal paper of R. Groszmann,<sup>161</sup> they have important benefits in the prevention of the variety of consequences of portal hypertension in patients with CSPH.<sup>160</sup> This study is complex and showed a positive effect of NSBBs, because it was not designed as a “one size fits all” strategy. The authors first tested the haemodynamic response of patients to propranolol and in case of non-response patients received carvedilol. This is very important since it demonstrates that the use of drugs should be very differentiated (almost individualised) in portal hypertension. Yet, the effect of NSBBs on portal pressure is limited to an approximately 15% decrease; thus, they may not be sufficient for the prevention of bleeding of very large varices. Therefore, the local treatment of varices, using band ligation, may be as effective as NSBBs for the prevention of first bleeding.<sup>4</sup>

In addition to the case of large varices, NSBBs may be deleterious in the presence of refractory ascites, especially in patients with severe arterial hypotension, active infection or acute kidney injury, and at least a dose reduction is recommended.<sup>137</sup> Therefore, the window of opportunity to use NSBBs for the treatment of complications of portal hypertension may be rather narrow.<sup>162</sup> Generally speaking, NSBBs are contraindicated for acute events since the required portal venous decompression needs to be achieved fast at a large magnitude. Such effects are elicited by terlipressin and shunting procedures.<sup>163</sup> Therefore, both are recommended to achieve haemostasis in patients with variceal bleeding. Terlipressin or other vasoconstrictors such as sandostatin are used in the acute setting of variceal haemorrhage, but also in the treatment of hepato-renal syndrome (HRS).<sup>137,164</sup> HRS is the maximal form of kidney dysfunction in cirrhosis and is basically a functional failure caused by intrarenal vasoconstriction, but also at least partly by the decreased effective arterial blood volume following long-standing portal hypertension and hyperdynamic circulation.<sup>165</sup> In addition, infections occur frequently as a result of complications of portal hypertension, such as bleeding<sup>130</sup> and HRS,<sup>164</sup> and conversely may further aggravate portal hypertension.<sup>166</sup>

Shunting procedures, mainly TIPS insertion, are the most effective measure to decrease portal pressure.<sup>167</sup> TIPS was introduced by the group of Martin Rössl. Besides the effective decompression of the portal vascular compartment,<sup>167</sup> a TIPS increases the effective arterial blood volume and thereby improves renal function. Although this effect is not immediate, it has been clearly demonstrated to improve renal function, increase sodium excretion and ameliorate ascites.<sup>168–170</sup>



This is a very short list of therapeutic options for the large number of patients affected by portal hypertension.<sup>154</sup> There are many explanations for the imbalance between clinical need and available therapies, including issues of a health-economic, social and political nature. The consequence is that strategies to improve patient care in portal hypertension are based mainly on repurposing existing drugs or exploration of orphan drugs.<sup>154</sup>

There have recently been many disappointing studies in humans, despite very promising experimental data. There may be several reasons for this, ranging from strong differences between animal models and human disease to non-stringent experimental and clinical study designs. These unfortunate stories have used up huge resources, and the community should learn from them, starting with the NCX-1000,<sup>171,172</sup> anti-angiogenic drugs,<sup>43</sup> rifaximin,<sup>173,174</sup> caspase-inhibitors,<sup>175–179</sup> and several more cases that have not yet been fully published.

### Single drug treatments

Statins are a success story in cirrhosis, with experimental and clinical evidence now more than 10 years old.<sup>179–182</sup> However, statins are entering clinical practice slowly, partly because of potential side effects,<sup>183,184</sup> which can be bypassed by compound modifications.<sup>185</sup> FXR agonists are another drug class that has shown efficacy for experimental portal hypertension<sup>117–120,186,187</sup>, while genetic predisposition may further suggest a beneficial effect<sup>188</sup> and the first unpublished clinical attempts have been positive (e.g. PESTO). Anticoagulants may indirectly decrease portal pressure – by resolving thrombosis, preventing re-thrombosis and thereby preventing complications of portal hypertension – and even improve survival.<sup>78–80,189</sup> This was recently confirmed in a large cohort of patients receiving anticoagulation for atrial fibrillation.<sup>190</sup> Finally, serelaxin is another promising approach, for which results are pending.<sup>191–193</sup>

Further possible strategies, which are already in the clinics are peroxisome proliferator-activated receptor agonists (fenofibrate, aligletazar, pioglitazone), which seem to have an effect on the hepatic (alpha) or collateral (gamma) circulation.<sup>194–196</sup> Soluble guanylate cyclase stimulators may also find a way into clinical practice, yet further validation is required.<sup>66</sup> Urotensin-receptor antagonists have a very interesting profile, since they decrease hepatic resistance and increase splanchnic resistance, probably due to the differential effects of the

receptor depending on its location.<sup>58,197,198</sup> Janus kinase 2 (JAK2)-inhibition is another strategy of potential interest, yet more investigations are needed, since it may aggravate steatosis if not targeted to HSCs.<sup>199–204</sup>

Taking into the account the contrary regulation of the extrahepatic and intrahepatic circulation, cell-specific and hepatic delivery of vasodilators is extremely important. There have been attempts at targeting Rho-kinase in HSCs, which do not induce extrahepatic effects.<sup>102,103</sup> Yet these are still experimental and need to be confirmed in the human setting.

### Combinatorial therapies

A possible strategy to improve efficacy is to combine drugs. This has already been well-implemented for secondary prophylaxis of variceal bleeding, combining NSBBs with local treatment of varices.<sup>137</sup> Even in patients receiving TIPS, older data from the Sauerbruch group show that NSBBs improve portal hypertension.<sup>205</sup>  $\beta$ -adrenoceptor agonists are very attractive, since  $\beta$ -adrenoceptor expression is increased only in activated HSCs and  $\beta$ -adrenoceptor agonists show additive effects in combination with NSBBs.<sup>57,206</sup> The concept of combinatorial therapies has been investigated in several Horizon 2020 projects (funded by the European Commission) such as LIVERHOPE (simvastatin and rifaximin) and in Decision (combination will be identified using omics-approach).

### Conclusion and future directions

The main reasons for the lack of treatment strategies in the field of portal hypertension may be the complexity of the pathophysiological processes and the counterplay of the intrahepatic and extrahepatic systems. Therefore, there is a clear role for microbiome-targeted and omics-guided diagnosis and therapy for portal hypertension. In the era of minimally invasive approaches, personalised medicine and artificial intelligence, we need to open the field to scientists and experts from a wide range of backgrounds and liaise with them to develop effective systems medicine and personalised approaches. The introduction of advanced technologies, such as endoscopic ultrasound and cell-specific therapies that use drug-carriers to target either LSECs or HSCs, will significantly improve patient care.

### Abbreviations

ACE2, angiogenesis-converting enzyme 2; ACLF, acute-on-chronic liver failure; AT1R, angiotensin II type I receptor;  $\beta$ 1-AR,  $\beta$ 1-adrenergic receptor;  $\beta$ 2-AR,  $\beta$ 2-adrenergic receptor;  $\beta$ -Arr2,  $\beta$ -arrestin 2; CCL<sub>4</sub>, carbon tetrachloride; CCL2, chemokine (C-C motif) ligand 2; CLD, chronic liver disease; CSPH, clinically significant portal hypertension; DII4, delta like canonical Notch ligand 4; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; EUS, endoscopic ultrasound; FXR, farnesoid X receptor; HCC, hepatocellular carcinoma; HRS, hepatorenal syndrome; HSCs, hepatic stellate cells; Hsp90, heat shock protein 90; HVP, hepatic venous pressure gradient; JAK2, Janus kinase 2; KO, knockout; LSEC, liver sinusoidal endothelial cells; MLCP, myosin light-chain phosphatase; NET, neutrophil extracellular trap; NO, nitric oxide; NSBBs, non-selective beta blockers; PDE, phosphodiesterase; PDGF, platelet-derived growth factor; PKG, cGMP-dependent protein kinase; PlGF, placental growth factor; TIPS, transjugular intrahepatic portosystemic shunt; VCAM1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

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Please refer to the accompanying ICMJE disclosure forms for further details.

### Authors' contributions

YI and JT both wrote the manuscript.

## Supplementary data

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Author names in bold designate shared co-first authorship

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