

# Evolution in the understanding of the pathophysiological basis of portal hypertension: How changes in paradigm are leading to successful new treatments

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## Summary

Among the common complication of cirrhosis portal hypertension witnessed a major improvement of prognosis during the past decades. Principally due to the introduction of rational treatments based on new pathophysiological paradigms (concepts of thought) developed in the 1980s. The best example being the use of **non-selective** beta-blockers and of vasopressin analogs, somatostatin, and its analogs. Further refinement in the knowledge of the molecular mechanisms involved in the regulation of both the splanchnic and hepatic circulation has led to the emergence of new treatments, which are based on evidence that show not only structural but also vasoactive components increase the hepatic vascular resistance, as well as of angiogenesis. This knowledge and future improvements will most likely result in more effective treatment of portal hypertension and effective prevention of its complications in early stages.

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## Introduction

Portal hypertension is a frequent clinical syndrome that is characterized by an increased portal venous pressure. This results in an increase of the pressure gradient between the portal vein and the inferior vena cava above the **normal values**

(**1–5 mmHg**). Portal hypertension is most commonly caused by **chronic** liver disease, followed by schistosomiasis and by **pre-hepatic portal vein occlusion**. The latter two conditions are particularly common in North Africa, Asia and Amazonia. The relevance of portal hypertension is well illustrated by the fact that in patients with cirrhosis of the liver, portal hypertension is the main cause of death and of liver transplantation. Only in Europe it is estimated that 29 million patients suffer from chronic liver disease, and that 170,000 die each year from complications of **cirrhosis**, a number **exceeding** the mortality due to **breast cancer** [1].

The knowledge of the pathophysiology of portal hypertension has changed dramatically during the past decades due to the combination of the availability of new methods for clinical evaluation, the introduction of reliable experimental models and the application of recent advances in cellular, molecular, and systems biology. Another important recent concept is that for the **complications of portal hypertension** to occur, the **portal pressure** should increase **above a critical threshold value of 10 mmHg** that defines what is known as clinically significant portal hypertension [2]. Implicit in this concept is that if we can **prevent** the **portal pressure gradient to exceed >10 mmHg**, we would be able to prevent all the complications of portal hypertension and the decompensation of cirrhosis [3]. **Variceal bleeding** and **ascites rarely**, if ever, occur **until the portal pressure gradient increases above 12 mmHg**, indicating a level of absolute protection we strive to achieve during therapy [4]; while recognizing that marked reductions of the risk of variceal bleeding/rebleeding and of spontaneous bacterial peritonitis can be achieved with less marked reductions of the portal pressure gradient [5–8]. This improved knowledge of the mechanisms of portal hypertension and of the haemodynamic targets to reach in therapy has led to the development of new treatments for portal hypertension that have resulted in a marked improvement of prognosis [2].

It is predicted that application of new concepts arising from current research will contribute to further advances in therapy. This review offers a detailed account of this changing panorama, focusing in cirrhotic portal hypertension and indicating how the advances in understanding have led to new successful therapies.

Keywords: Portal pressure; Hepatic stellate cells; Liver sinusoidal endothelial cells; Endothelial dysfunction; Hyperdynamic syndrome; Splanchnic vasodilation.

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Abbreviations: HSC, hepatic stellate cells; LSEC, liver sinusoidal endothelial cells; NO, nitric oxide; TLR, toll like receptors; L-LOX2, l-llysil-oxydase 2; VEGF, vascular endothelial growth factor; PVL, portal vein ligation.



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## Review

### Key Points

- Portal hypertension is the most common and severe complication in patients with cirrhosis, representing the first cause of death or need for liver transplantation
- Changes in the understanding of the pathophysiology of portal hypertension has allowed the introduction of new effective treatments
- The old pathophysiological view considered that increased resistance to portal flow through the cirrhotic liver resulted in reduced portal flow and congested splanchnic circulation, with porta-caval shunts being the mainstay of therapy
- Demonstration of increased portal blood inflow, splanchnic vasodilation and hyperdynamic circulation as important contributors to portal hypertension lead to the introduction of vasoconstrictors (terlipressin, somatostatin and analogs), and of nonselective beta-blockers for the treatment and prevention of variceal bleeding, with a progressive and dramatic decrease in its incidence and mortality
- Recent advances in the knowledge of the molecular mechanisms regulating hepatic vascular tone, endothelial dysfunction, and its relationship with fibrogenesis, angiogenesis and hepatic vascular occlusion/thrombosis is the basis of current emerging therapies for portal hypertension

### From past to present and its implications for the future (Fig. 1)

The first concept that needs to be stressed when considering the pathophysiology of portal hypertension is that according to the hemodynamic application of Ohm's Law, the **portal pressure gradient ( $\Delta P$ )** is directly **proportional** to the amount of blood **flow** circulating through the portal venous system (Q) and the **resistance** opposing this flow (R) ( $\Delta P = Q \times R$ ). Therefore, from a theoretical point of view, an **increase** in portal **pressure** can be considered as **secondary** to an increase in **resistance**, to an increase in blood **flow**, or to a combination of **both** factors.

The history of portal hypertension can be divided in two distinct periods, during which the pathophysiology of portal hypertension has been dominated by two opposing concepts. In the past, initiated in the times of Hippocrates (Cos, 460 BC) and Galen (160 AD) and continued well into the 20th century, it was considered that portal hypertension was a result of "**congestion**" of the portal circulation due to **increased resistance to portal blood flow** through the **hard cirrhotic** liver. Present times initiated with the papers of Groszmann and Cohn [9,10] describing that **despite** the increased **resistance** to blood **flow** through the cirrhotic liver, the **splanchnic circulation in portal hypertension was not congested**, but **hyperemic**. These **contrasting** views were the basis for two radically different approaches for portal hypertension: while the **traditional "congestive"** hypothesis led to the introduction and wide use of **surgical porta-caval shunts** (and of other operations such as the meso-caval and the spleno-renal shunts), the demonstration that an **increased blood flow** plays a **critical** role in provoking advanced portal hypertension led to modern therapies, based on the use of **splanchnic vasoconstrictors** (terlipressin, somatostatin and its derivatives) [11,12] and of **non-selective beta-blockers** [13] to **decrease** portal-collateral blood **flow** and hence portal pressure.

### Evolution in the understanding of the pathophysiological basis of portal hypertension and how changes in paradigm have led to successful new treatments

Time	Paradigm	Therapeutic applications	
		Established	Non-established
<b>Past</b> (from Hippocrates to Child)	Congestion ( $\uparrow$ resistance, $\downarrow$ blood flow)	Surgical portal-systemic shunt	
<b>Present</b> 1980s	Hyperdynamic circulation	Splanchnic vasoconstrictors (vasopressin, somatostatin and its derivatives) Non-selective beta-blockers	
1985	Increased hepatic vascular tone	Vasodilating nitrates (combined with vasoconstrictors/beta-blockers)	
1998	Mechanism of sinusoidal endothelial dysfunction		NOS-gene transfer, antioxidants statins, BH <sub>4</sub>
2000	Reversal of fibrosis/cirrhosis	Etiologic treatments	Antifibrotic drugs
2004	Angiogenesis		Antiangiogenic drugs Modulation of endogenous anti-angiogenic factors
2008	Intrahepatic vascular occlusion and parenchymal extinction lesions		Anticoagulation
2000-14	Bacterial translocation/inflammation/ altered microbioma as factors worsening PH		Antibiotics, probiotics

Fig. 1. Schematic representation of the historical evolution of the understanding of portal hypertension (PH) and how changes in paradigms have led to the introduction of successful new therapies. The figure also shows new therapies under evaluation.

The next change in the way of understanding the pathophysiology of portal hypertension was shown by Bathal and Grossmann [14] that the **increased hepatic resistance to portal blood flow** through the **cirrhotic liver** was **not only a mechanical consequence** of the architectural disruption of the liver vascular anatomy caused by fibrosis and nodule formation, but that together with this structural component there was a **dynamic component** due to an **increased hepatic vascular tone**. The authors further demonstrated that the dynamic component of increased liver resistance could be due to a **deficit of vasodilatory substances** (as it was **corrected by nitroprusside**) and/or to excessive production of **vasoconstrictors**, which could account for about **one third** of the increased hepatic resistance causing portal hypertension in cirrhosis. Thus, targeting this increased resistance could allow for achieving over a **30% decrease in portal pressure in cirrhosis** [11,12]. Subsequent developments demonstrated the role of **deficient nitric oxide (NO) availability**. The cause of this dynamic component arises from **endothelial dysfunction** at the **hepatic sinusoidal cells** [15], the role of contraction of activated hepatic pericyte-like stellate [16], the implication of prostanoids and other vasoconstrictors [17,18], and the possibility of improving hepatic endothelial dysfunction by a variety of strategies [19–26].

In parallel with these developments, a better knowledge of the crosstalk between hepatic sinusoidal endothelial cells and hepatic stellate cells (HSC), and its implications in terms of activating fibrogenesis and angiogenesis [27–29] opened new perspectives, pointing out that **fibrogenesis** in cirrhosis can be **modulated** by acting on etiologic factors, collagen metabolism, on angiogenesis, and on **endothelial dysfunction** (separately or in combination) [30]. In addition, observations on pathology specimens disclosed the importance of vascular occlusion in the liver in progression of parenchymal extinction lesions [31]. Together with the emerging new concept that **cirrhosis** could be considered a **prothrombotic condition** have led to the proposal that **anticoagulants** may slow or **prevent** progression of **cirrhosis** [32].

The following sections are devoted to analysing these concepts.

### **Intrahepatic circulation and cirrhosis. Description of structural and functional changes**

A number of protean changes are manifested in the hepatic sinusoids during the process of advanced liver injury and cirrhosis development [33]. Even hepatocyte plasma membrane adjacent to the sinusoid undergoes some of these changes including loss of microvilli, but the most prominent changes occur within the sinusoidal cells themselves, including the sinusoidal endothelial cell and HSC. Sinusoidal endothelial cells normally contain fenestrae organized in sieve plates. These are thought to facilitate the transport of macromolecules from the sinusoidal lumen to the abluminal hepatocytes. In response to injury and early cirrhosis development, these fenestrae are lost. The endothelial cells themselves also undergo dramatic reprogramming in terms of their functional phenotype and angiocrine signaling pathways, which will be reviewed further below.

The HSC also undergo changes. Under basal conditions, the cell resides largely as a vitamin A, lipid storing cell. It is controversial whether it has contractile properties under basal conditions, but in view of the low resistance sinusoids under normal

conditions, the contractile effects of stellate cells under normal conditions are probably not required to be prominent [34]. However, in response to liver injury, a number of changes occur which have been referred to broadly as HSC “activation” [16]. This includes alterations in almost every phenotype of the cell, and can be summarized as an increase in contractility, migratory capacity, matrix deposition, and profile change in the release of paracrine factors. Some new aspects of the activation process are also discussed later in the review.

These changes in the molecular phenotypes of the sinusoidal cells result in dramatic pathophysiological alterations in the structure and function of the hepatic sinusoids. These changes also lead to the increased deposition of matrix proteins within the hepatic sinusoids and increased contractility of the perisinusoidal cells and ensuing constriction of the sinusoids themselves. While earlier concepts focus on the effects of the matrix on portal hypertension through pure mechanical phenomenon, evolution in thought has led to the understanding that the **increase in vascular resistance also contributes importantly to increased intrahepatic resistance in portal hypertension** [33]. Varying studies show that this component could be in the range of **30% to 40%** of increased intrahepatic resistance. However, in reality, there is probably an important **interplay** between these **mechanical** and **vascular** components, since many of the matrix proteins have very important effects on sinusoidal cells that ultimately regulate the vascular phenotype as well, most notably including matrix proteins such as fibronectin, discussed below. This combination of changes to both mechanical and vascular components in the sinusoids has been referred to as **pathological sinusoidal remodeling** [35]. Thus, ultimately, the changes that occur in the hepatic sinusoids in response to advanced liver injury and cirrhosis result in increased intrahepatic vascular resistance from a combination of effects of **matrix changes** and **tonicity changes**. Of which, have been described in detail in prior reviews, and the remainder of this review will focus on new ideas in the molecular pathobiology of hepatic sinusoids.

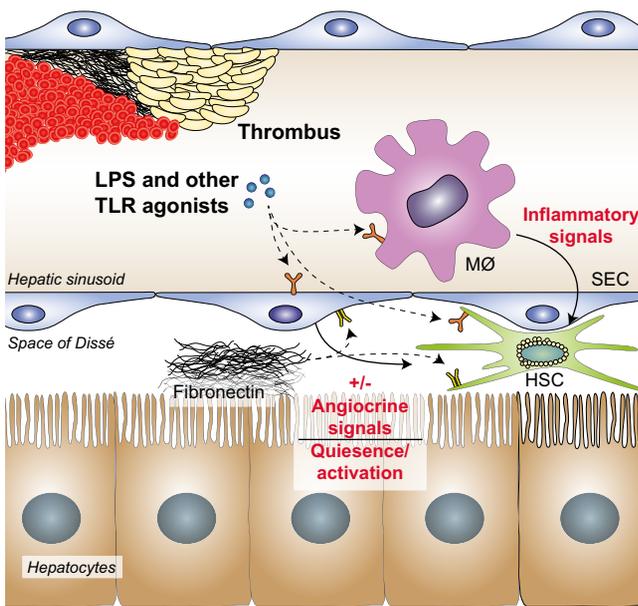
### **New paradigms in the molecular pathobiology of the hepatic sinusoids (Fig. 2)**

A number of new concepts are emerging in the molecular pathobiology of the hepatic sinusoids and how the changes affect development of portal hypertension. While all of these cannot be reviewed comprehensively here, some examples are outlined below.

#### *Sinusoidal endothelial cell*

The role of the sinusoidal endothelial cell in portal hypertension development continues to evolve with increasing evidence supporting its importance in this process. Studies from the last two decades now clearly show that the **sinusoidal endothelial cell contributes considerably to increased intrahepatic resistance in portal hypertension development** [33]. This is an important observation since prior to that, the cells were viewed largely as serving a passive role for macromolecular transport across the sinusoids. Since these initial observations, the role of the endothelial cell in portal hypertension development continues to grow [34]. The concept of **endothelial dysfunction** highlights the **impaired release of vasodilatory molecules** from sinusoidal

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**Fig. 2. Newer molecular paradigms in the sinusoids in cirrhosis.** Boxed area is a higher magnification cartoon depiction of potential molecular events within the sinusoids during cirrhosis. The cartoon shows crosstalk between sinusoidal endothelial cells (SEC), macrophage (M), and hepatic stellate cells (HSC). Selected signal pathways under active investigation are depicted including innate immunity through toll like receptors (TLR), traditional inflammatory molecules, angiocrine signals, and matrix such as fibronectin. Potential effects of thrombus are also shown.

endothelial cells during liver injury progression. This is most notably characterized by **diminished production** of the potent vasodilator, **NO**, occurring through a number of post-translational actions produced by endothelial NO synthase (eNOS) [36,37]. This is also accompanied by an **increase in vasoconstrictive** molecular release, such as **endothelin** proteins. A number of other vasoregulatory molecules have also been implicated in this process. While several therapeutic modalities to alleviate endothelial dysfunction have been evaluated in animal models and in humans including various NO donor compounds, the compounds receiving **most attention** recently for potential therapeutic benefits are the **statin** class of drugs [19,38,39].

Over the last several years, the role of **angiogenesis** in the hepatic sinusoids has also been elucidated [35,40,41]. Angiogenesis is defined as the growth and proliferation of existing endothelial cells and has been implicated not only in increased intrahepatic resistance in the sinusoids but also in the process of fibrosis development. The precise relationship between angiogenesis and increased intrahepatic resistance and fibrogenesis remain ill-defined, but may well relate to angiocrine signaling that is associated with angiogenic endothelial cells [42,43]. This angiocrine signaling likely regulates the phenotype of a number of other cells in the sinusoid, most notably the hepatic stellate cell, and it has been postulated that angiogenic endothelial cells may stimulate HSC activation. However, there are a number of complexities to this concept that may ultimately require consideration in terms of potential therapies. For example, some angiocrine signals also maintain HSC quiescence as discussed further below [28]. Additionally, while angiogenesis and angiogenic

molecules appear to be important in the development of increased intrahepatic resistance and early fibrosis, these same molecules may also be important in resolution of liver injury and resolution of early fibrosis. One example is **vascular endothelial growth factor (VEGF)**, a potent **angiogenic molecule** that may also contribute to HSC activation [44]. However, VEGF may conversely facilitate resolution of early liver injury through the recruitment of macrophages, which are required for resolution steps in early cirrhosis [45].

Another interesting and evolving concept pertaining to sinusoidal endothelial cells relates to **capillarization**. Although capillarization was described by Fenton and Schaffner several decades ago, its role continues to be revisited [33]. Capillarization refers to the deposition of basement membrane and other matrix proteins as well as the changes in sinusoidal endothelial structure that occur during early fibrosis. Recent work suggests that these changes may play a very important pathogenic role in the subsequent steps of liver fibrosis and highlight the potential role of the sinusoidal endothelial cell as a very early player, not only in increased intrahepatic resistance, but in liver injury and fibrosis itself [28,46].

#### Hepatic stellate cell

A number of important ideas are also evolving relating to the HSC in portal hypertension development. While the molecular biology of the HSC activation process continues to grow at a rapid pace [16,47], one of the notable evolutions pertaining to the HSC has been the identification of these cells as innate immune cells. A number of seminal studies have outlined that agonists of innate immune receptors including toll-like receptor 4 (TLR4) lead to hepatic stellate cell activation, which is key for sinusoidal constriction as well as deposition of matrix proteins [48,49]. This is particularly important since lipopolysaccharide is a canonical ligand for TLR4, and the lipopolysaccharide derived from the gut lumen has long been implicated in the processes of liver injury, fibrosis, and portal hypertension development [50,51]. Although not reviewed extensively here, the involvement of the traditional innate immune cell in the hepatic sinusoid, the Kupfer cell, continues to grow in its role in portal hypertension. Interestingly, a number of other ligands for the TLRs reside within the hepatic sinusoids and are released by injured cells within the liver. One such ligand example is HMGB1, which is released by injured cells in response to various insults including alcoholic liver injury [52]. Thus, the innate immune function of the HSC can contribute to the portal hypertension phenotype through not only gut-derived ligands of TLR4 but also through ligands that are released by injured cells within the liver itself.

It should be noted that the expanding role of **innate immune signaling in HSC** is only one example of a broader recognition of the **role of inflammation in portal hypertension** (as well as in acute on chronic liver failure) which involves multiple cell types, molecules, and processes. This concept is leading to changes in therapy. For example, specific antibiotics are already used to combat bacterial translocation and newly developed TLR antagonists are working their way through early phase development. The broader link of inflammation with portal hypertension was recently reviewed by Mehta *et al.* [53] and therefore not expanded in more detail here.

Signaling pathways in HSC continue to be an area of active investigation and are important not only for academic interest

but more importantly because specificity of potential therapies will be determined by the targeting of signals in a specific manner. In this regard, the renin-angiotensin system continues to be actively investigated not only in HSC but also in the splanchnic circulation [74,38]. Calcium signaling also continues to be of interest in HSC with calcium independent pathways being involved in both contraction and matrix deposition by HSC [38].

#### Matrix

As mentioned earlier, the release of matrix proteins by HSC is likely to contribute to increased intrahepatic resistance not only through the mechanical effects of the matrix but also through signaling actions. One prototypical protein that fits this idea is fibronectin [54,55]. Fibronectin is released by a number of cells within the liver and requires fibrillation of soluble fibronectin in order for this molecule to develop into deposited matrix protein. Upon its deposition, it has very important effects on a number of cell types including HSC and sinusoidal endothelial cells, and consequently leads to what could be described as an activation phenotype of both of these cells. Further study is required into the potential signaling effects of a number of other matrix proteins, especially early matrix proteins that are deposited by injured cells during early cirrhosis.

#### Thrombus

The role of thrombosis is undergoing important changes in thought as well in terms of its effects on portal hypertension development. This includes the increased recognition that **cirrhosis in portal hypertension** patients may actually show **evidence of increased thrombosis rather than increased bleeding** as was initially thought [56]. This is important within the intrahepatic sinusoids because increasing evidence shows that **microthrombi** within the sinusoids could propagate increased intrahepatic **resistance** liver injury and fibrosis development [57]. While this is most notable in forms of **intrahepatic congestion** such as **Budd-Chiari Syndrome** and **cardiac cirrhosis**, it may also be relevant in other forms of cirrhosis and portal hypertension as well. Indeed, this was postulated a while back by studies of human pathology specimens that showed parenchymal extinction due to microthrombi [31]. Much more work is needed in this area, but a number of pharmacological changes to management could be implied if thrombus plays an affect in the role of fibrosis development. A clinical paper showing **potential beneficial** effects of **enoxaparin** in subgroups of patients with cirrhosis supports this concept [32].

#### Extracellular vesicles

Another evolving concept in sinusoidal structure and phenotype in cirrhosis and portal hypertension development relates to mechanisms by which cells signal with each other. While traditional concepts have focused on the paracrine release of molecules that act on adjacent cells in the sinusoid, increasing evidence shows important roles of extracellular vesicles in the signaling models. Extracellular vesicles include exosomes, microparticles, and apoptotic bodies. While this has been reviewed previously, a number of recent studies show that these extracellular vesicles may be quite important in portal hypertension pathobiology [58–60]. The reasons for this include the

opportunity for released proteins to maintain stability within the vasculature for longer distances due to their protection within the extracellular vesicle. Ongoing studies aim to understand which proteins may signal through traditional paracrine pathways vs. pathways involving extracellular vesicles and the specific mechanisms by which extracellular vesicles could achieve their signaling actions.

Thus, a strong blueprint has been developed as to how the sinusoids contribute to portal hypertension through an increase of hepatic resistance. While **carvedilol** is one compound that exemplifies this concept owing to its **alpha antagonistic** properties that **vasodilate sinusoids** and thus partially contribute to the mechanism of action of this beta blocker [61–63], other compounds such as the **statin** class of drugs is undergoing further evaluation as well. This is an important **shift** in concepts from initial **thinking** that the **sinusoids** were simply an **inert vascular bed** that **lacked vasoregulatory** function. Many more molecular elucidations have occurred over the past decades and a number of new and evolving concepts continue to move these ideas forward.

### The splanchnic and systemic circulation in portal hypertension

#### Pathophysiology

In 1953, based on the clinical observation that patients with cirrhosis frequently showed **“warm extremities, cutaneous vascular spiders, wide pulse pressure, and capillary pulsations in the nail beds,”** Kowalski and Abelmann [64] first showed that cirrhosis is associated with a **hyperdynamic circulatory syndrome** characterized by an increase in cardiac output and a decrease in peripheral vascular resistance. This syndrome, although commonly recognized as a complication of cirrhosis, could be **better conceptualized as a complication of portal hypertension** since it has been observed in **all forms of portal hypertension** caused by conditions **other than cirrhosis** and confirmed in different experimental models of portal hypertension.

These findings were reproduced in subsequent studies; however, the recognition of the harmful effect of this syndrome on multiple organs was only recognized years later [65]. We now know that the multi-organ failure observed in terminal chronic liver diseases is in part attributable to this progressive vasodilatation. The harmful effects observed in the systemic circulation and several other vital organs always **originated via the vasodilatory state**. Whereas in the heart, the splanchnic, the pulmonary, and the cerebral circulation, these deleterious effects are mediated by the **vasodilatation** itself, in **other organs** such as the **kidneys** it is a **response to vasodilatation** in the other circulatory beds. Over the years we have learned that patients are **hyperdynamic before the syndrome** becomes **clinically** evident.

The systemic circulatory hyperdynamic syndrome seems **secondary to changes occurring in regional vascular beds**. Any change in peripheral vascular resistance is rapidly compensated by changes in cardiac output [66]. We believe that the **initial vasodilatation occurs in the splanchnic circulation** and that the heart response is directly related to a combination of splanchnic **vasodilatation** and **expansion of the plasma volume**, together with an **increased venous return to the heart**, in large part, through portal-systemic shunts. Despite these findings, for many years the dominant theory to explain portal hypertension in

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cirrhosis still was the 'backward flow' theory, which postulated that increased resistance was the only cause for the increase in portal pressure and predicted a **splanchnic hypodynamic** circulation, with increased mesenteric vascular resistance. This theory was supported by the finding of a decreased portal flow at the hepatic hilum [67]. However, these data were **misleading**, since it did **not** take into **account** the **flow** that is diverted through the **collateral** circulation. In the **1970s** a series of studies by Groszmann and Cohn in patients with liver cirrhosis suggested that the **splanchnic circulation was hyperdynamic** [68]. However, in the portal vein constricted model (PVL) before getting hyperdynamic a hypodynamic period is observed early in the evolution of portal hypertension [69]. This **hypodynamic** splanchnic circulation, most likely is observed also in **early cirrhosis** when portal hypertension is mainly attributable to an increase in intrahepatic vascular resistance, and portal-systemic collaterals have not yet developed. It is probable that during this stage, hypoxia develops in the splanchnic bed triggering an angiogenic response [70] that leads to the development of portal-systemic collaterals and the hyperdynamic circulation.

In the 1980s a series of studies in animal models of portal hypertension allowed us to fully characterize the hemodynamic events that follow the induction of portal hypertension. These studies allowed unequivocal demonstration that, **after a short initial period of a hypodynamic splanchnic** circulation, portal hypertension **transforms** the circulatory **splanchnic** bed into a **hyperdynamic circulatory state**, which contributes to the severity of portal hypertension [69]. This has been called the **'forward flow' theory** and set the rationale for the use of splanchnic vasoconstrictors in patients with portal hypertension.

The PVL model develops massive portal-systemic shunting and a hyperdynamic circulatory state as early as 4 days after the induction of portal hypertension [69]. This model proved extremely useful to study the hyperdynamic syndrome, since most of the findings in the PVL model were subsequently reproduced in models of cirrhosis [70]. In patients with the **hyperdynamic syndrome** the **cardiac index** is usually higher than normal ( $>4 \text{ L/min/m}^2$ ). However, it is obviously insufficient to maintain arterial pressure on the face of progressive vasodilation.

### Molecular pathophysiology

#### Vasodilatation

Vasodilatation is a key component of the hyperdynamic syndrome. A wide variety of **vasodilatory molecules** play a role in inducing the vasodilatory state. However, of all these molecules **NO is the most important** [71]. The initial mechanisms and the magnitude of increase in portal pressure required to trigger NO production was defined by studying systemic and splanchnic hemodynamics, **eNOS** and **VEGF** expression in rats with different degrees of portal hypertension (PVL) and portal-systemic shunting [72]. Compared with sham rats, all PVL rats exhibited features of hyperdynamic circulation. Rats with minimal portal hypertension showed an early increase in VEGF and eNOS expression selectively at the jejunum. Inhibition of VEGF signaling markedly attenuated the increase in eNOS expression. In conclusion, mild increases in portal pressure are enough to upregulate eNOS at the intestinal microcirculation, and this occurs, at least in part, through VEGF upregulation. Thus, **unlike the intrahepatic** circulation, there is an **excess of local NO production**, which is associated with decreased responsiveness of the mesenteric circulation to

vasoconstrictors. **NO** causes vasodilatation through stimulation of soluble guanylyl cyclase to generate **cGMP** in vascular smooth muscle cells. NO also contributes to increase splanchnic blood flow by facilitating **angiogenesis** [73]. The eNOS signaling pathway is activated in portal hypertension by numerous endothelial cell stimuli. They include **VEGF**, angiotensin (1–7) (through a Mas Receptor) [74] **shear stress** and inflammatory **cytokines** [75,76], and regulated through a variety of mechanisms, including the **bacterial translocation** mediated upregulation of GTP-cyclohydrolase I, which generates eNOS through increases in the cofactor tetrahydrobiopterin [75]. The participation of inducible NOS has also been reported during decompensated cirrhosis [76]. Other vasodilators that have been also associated with the hyperdynamic syndrome are **carbon monoxide**, **prostacyclins**, anandamide, endocannabinoids, neuropeptides, endothelium-derived hyperpolarizing factor, glucagon, vaso-intestinal peptide and others [75].

#### Angiogenesis

In addition to **VEGF** several angiogenic factors are upregulated in the splanchnic vascular bed of portal hypertensive subjects, among these are the placental growth factor and the platelet derived growth factor [75]. **Angiogenesis** and **vasodilatation** go **"hand in hand"** feeding each other. The same **VEGF** that regulates the development of portal-systemic **collaterals** [77] (see section on formation of collaterals and varices below) modulates the increase in eNOS that leads to splanchnic vasodilatation [77]. It is most likely a combination of circulatory hypoxia [78] and circumferential **wall stress**, which is initiated by early increase in portal pressure, and leads to sequential events that induces the hyperdynamic state.

An endogenous **inhibitor** of **angiogenesis**, **Vasohibin-1**, was recently described to be selectively induced by the pro-angiogenic growth factor VEGF as a consequence of a specific negative feedback regulatory mechanism of pathological angiogenesis. Vasohibin-1 reduces VEGF production to an intermediate steady-state level sufficient to maintain vascular homeostasis or physiological angiogenesis associated with wound healing, but not to drive pathological angiogenesis, diminishing the hemodynamic abnormalities and portal pressure in a model of liver cirrhosis [79].

### Formation and rupture of varices

**Oesophageal** and **gastric varices** are the more relevant portal-systemic collaterals that develop in portal hypertension, as they are responsible for one of its more representative and severe complications, massive acute gastrointestinal bleeding. Other consequences of portal-systemic collaterals are linked to the **shunting of portal blood to the systemic circulation**, and include portal-systemic or hepatic encephalopathy, exacerbation of liver failure, **loss of first pass effect** for **orally administered drugs**, abnormal metabolism of endo and **xenobiotics**, **increased susceptibility to sepsis**, and exacerbation of the hyperkinetic circulation and its consequences. We will focus here on the mechanisms leading to the formation and bleeding of varices.

Oesophageal and gastric varices are formed by a conjunction of anatomical, physical and biological factors. The traditional concept was that collaterals develop by the opening and dilatation of pre-existent vessels at sites of embryonic connection between the portal and systemic circulations due to the increased portal

pressure. Some of these collaterals evolve into varicose veins through peculiar anatomical conditions of the esophageal venous circulation. Portal hypertension triggers the dilatation of submucosal veins, which become varicose, due to the incompetency in the perforating communicating veins connecting the submucosal and peri-esophageal veins. The lack of external tissue support and the negative intra-esophageal pressure during inspiration would further favour dilatation of the dilated submucosal veins and contribute to the progressive increase in size of the oesophageal varices [80]. This traditional view was challenged when Fernández *et al.* [77] reported for the first time that formation of portal-systemic collaterals in portal hypertension was modulated by active angiogenesis. Specifically they were under the control of the VEGF signaling cascade, to the point that interfering with VEGF, either by means of monoclonal antibodies anti-VEGF receptor 2, or upstream in VEGF production, resulted in >50% inhibition of collateral formation, independently from modifying portal pressure. These authors have since documented not only inhibition of collateral formation, but also regression of already formed collaterals under the combined inhibition of VEGF and platelet derived growth factor or using the multi-kinase inhibitor sorafenib [81,82]. Other implications of angiogenesis in portal hypertension, including its effects on mesenteric hyperemia, liver fibrosis, inflammation and angiogenesis are reviewed in the preceding section.

This new way of conceiving collateral formation/regression has led to proposing angiogenesis as a new target for the treatment of portal hypertension.

### Rupture of varices

The mechanism of variceal bleeding was thought to be due to the erosion of the thin wall of the varices as a consequence of gastroesophageal reflux, facilitated by the trauma from swallowing solid food. However, this hypothesis was abandoned almost 30 years ago due to the lack of objective supportive evidence, either from physiological studies or from the pathological examination of the oesophageal mucosa in patients dying from variceal bleeding. This was substituted by the so called “explosion” theory that proposes that the varices bleed due to rupturing of the thin walls caused by an exertion of tension beyond the vessels elastic limit. According to Laplace’s law, variceal wall tension ( $Wt$ ) is defined by the equation:

$$Wt = (Pv - Po) \times r/t$$

In this equation,  $Pv$  is the intravariceal pressure (determined by the increased portal pressure),  $Po$  is the pressure in the oesophageal lumen,  $r$  is the radius of the varix, and  $t$  the thickness of its wall. Therefore, wall tension increases with increased portal pressure and this effect is multiplied by increased variceal size and decreased variceal wall thickness [80,83]. This view explains the prognostic value of the variceal risk factors: Child-Pugh C class correlates well with a higher portal pressure; big variceal size implies a greater diameter, and endoscopic red colour signs probably reflect areas of decreased variceal wall thickness. Finally, the oesophageal luminal pressure is lower than intra-abdominal pressure and becomes negative during inspiration, together with the lack of external tissue support explains why oesophageal varices are more likely to bleed than other portal-systemic collaterals.

### The hepatic arterial system

Under normal circumstances approximately 25% of the cardiac output perfuse the liver. A third of this flow is contributed by the hepatic artery and the remainder by the portal vein [84]. The portal system is thought to be a passive vascular bed. Whereas decreased flow in the hepatic artery leads to a compensatory reduction in vascular resistance, decreased portal flow leads to a reduction in the cross-sectional area of the portal vasculature and a corresponding increase in portal venous resistance. Conversely, increased portal flow, by inducing passive dilatation of the vessel, leads to a passive decrease in portal vein resistance [85]. Moreover, there is no portal venous hyperaemia in response to decreased hepatic arterial flow [85]. This contrasts with the behaviour of the hepatic artery in response to decreased portal flow.

It has been known for many years that a reduction in portal flow results in an increase in hepatic arterial flow [85]. Following porta-caval anastomosis, the prognosis for recovery appears to correlate with the increase in arterial flow which follows diversion of portal blood flow [86]. The capacity of the hepatic artery to increase its flow in response to decreased portal flow ranges from 22% to 100%. In situations of hemodynamic compromise such as haemorrhage, total hepatic blood flow is not restored to normal by hepatic arterial hyperemia. However this phenomenon nevertheless has physiological importance, it tends to compensate total hepatic blood flow [85]. Arterial flow to the liver is not determined by oxygen demand. Under normal conditions, the liver extracts less than 50% of the supplied oxygen. Under conditions of increased oxygen demand, the liver augments its oxygen extraction rather than increasing arterial flow. In chronic alcohol-fed rats, oxygen requirement by the liver increases dramatically (45%) without increased hepatic arterial flow [87].

The hepatic artery, not part of the portal system, but also seems to play an important role in the development and maintenance of portal hypertension, was shown in the study by Zipprich *et al.* [88]. The liver has a dual blood supply and the drainage of the hepatic arterial blood into the sinusoids is at the beginning of the sinusoidal network (zone 1). In cirrhosis a hyperdynamic syndrome also develops in the hepatic arterial circulatory bed. In response to the progressive loss in portal flow caused by the development of portal-systemic collaterals, the hepatic artery vasodilates, increases its flow and its participation in the maintenance of portal hypertension [88]. This hyperdynamic hepatic arterial syndrome is accentuated with the progression of chronic liver diseases. This hepatic arterial response to the progressive decrease in portal flow is initiated and perpetuated by a complex interaction of anatomical and functional factors and, contrary to what is observed in the intrahepatic portal system, an excess production of NO seems to play a role in the vasodilatation observed [89].

The vascular resistance of the hepatic artery is determined in the presinusoidal area, i.e. in the small hepatic branches before draining into the sinusoids. Studies in an isolated rat liver perfusion model of hepatic artery and portal vein observed that changes in the hepatic artery perfusion lead to changes in the sinusoidal and subsequently in the portal venous vascular resistance [88]. The mechanisms involved in this decreased vascular resistance in cirrhosis are not completely understood. It has been postulated to be due to increased levels of NO in the presinusoidal area where the final branches of the hepatic artery are

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located [89]. The local increase of vasodilators, together with the low arterial pressure, leads to a lower vascular resistance of the hepatic artery. On the other hand, it has been shown that the regulation of the hepatic arterial flow, and especially the hepatic arterial buffer response, is regulated by adenosine [90]. The vasodilatory response to adenosine is increased in cirrhosis due to a higher expression of the adenosine A1 receptor in hepatic arteries of cirrhotic livers [91]. Since this receptor leads to an increased production of NO it can be postulated that **both, adenosine and NO are involved in the reduced vascular resistance of the hepatic arteries in the cirrhotic livers** [91].

On the other hand, the higher amount of pro-angiogenic factors present in cirrhosis lead also to a neo-angiogenesis of arterial vessels. Indeed, the presence of neo-angiogenesis of arterial vessels in cirrhosis has been demonstrated in two different animal models [89]. However, the mechanisms that are involved in this arterial neo-angiogenesis, so far, have not been investigated. Furthermore, it has been shown that the vessel wall of the hepatic artery undergoes morphological changes in cirrhosis [89]. This process is called remodelling and the main anatomical change is the decrease of the amount of smooth muscle cells [89]. This results in a smaller vessel wall and an increase in the diameter of the vessel. Furthermore, due to the smaller amount of smooth muscle cells the vasoconstrictive property of the vessel is decreased.

### Areas for the future and new avenues for research

There are several areas where new advances are required, and to some extent foreseen. With regards to therapeutic applications it is likely that we will witness the advent of new treatment modalities based on the use of agents reducing and/or preventing the increase in hepatic vascular resistance by acting on hepatic vascular tone [12], etiological agents, fibrogenesis [92], angiogenesis [35,40], or in all of these. Most likely, these concepts will find their best application in early phases of portal hypertension, where prevention of clinical decompensation (i.e., preventing ascites, variceal bleeding, and encephalopathy) is a real possibility. We have new and highly effective etiological treatments to prevent or reverse the development of portal hypertension in cirrhosis due to hepatitis B or C virus infection. Early treatment also offers better possibilities for antifibrotic treatments acting either on the cause of cirrhosis (antivirals) or directly on fibrosis (anti-lysyl oxidase 2 (L-LOX2) monoclonal antibodies, anti-oxidants, obeticholic acid) will probably have a greater prospect for effectively achieving regression of fibrosis in early phases of cirrhosis, rather than in advanced stages when a lot of collagen cross-linking and avascular scar tissue have developed [93,94]. **Therapies modifying hepatic vascular tone by acting on NO signaling (i.e., by means of simvastatin) [19,39]** are already under clinical evaluation. Statins may further improve liver fibrogenesis in cirrhosis [38]. Preliminary results from a double-blind randomized controlled trial suggest a **survival benefit in advanced stages when simvastatin** is administered on top of currently available treatments [95]. The possibility of improving portal hypertension by modifying the microbioma, endotoxaemia, and its consequences is also close to clinical application.

Another area where we anticipate a marked progress is the application of new concepts on disturbances in liver microcirculation as a driving force for portal hypertension. In this regard the role of the hepatic artery in maintaining high portal pressure during a

progressive hepatic portal flow decrease should be investigated, as well as new development of non-invasive tools to assess the hepatic microcirculation in patients with cirrhosis [96].

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### Conflict of interest

JB is a consultant for Gilead, Connatus, ONO-UK, Exallenz, Intercept and Falk. RJG is a consultant for Gilead. VS has nothing to disclose.

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