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Risk Factors for Portal Vein Thrombosis Development in Patients with Cirrhosis

Filipe Gaio Nery

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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

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UNIVERSIDADE DO PORTO

Risk Factors for Portal Vein Thrombosis Development in Patients with Cirrhosis

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"Simplicity is the ultimate sophistication"

Leonardo da Vinci

1452-1519

Aos meus pais, a quem devo a minha existência e a pessoa que sou.

À minha irmã que, talvez sem saber, sempre foi um modelo.

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À Sofia: este trabalho é para ela – para que no futuro seja mais um motivo de orgulho no pai.

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To the patients and their families. The major goal of the work, which comprises this thesis, is to attain results that may clarify which risk factors are associated with portal vein thrombosis in patients with cirrhosis. It is by increasing this scientific knowledge that we can ultimately help patients to live longer and better. They helped us by participating in our studies, so we can help them in the future.

Scientific Outputs

In accordance with "Artigo 34º do Decreto-Lei nº 115/2013", this thesis contains materials and results from the following published papers. The author of this dissertation has contributed actively in the conceptualization, execution, interpretation and writing of these works (listed in order of appearance).

1. Nery F, Valla D. Splanchnic and Extrasplanchnic Thrombosis in Cirrhosis: Prophylaxis vs Treatment. Curr Hepatology Rep 2014; 13:224-234. Doi: 10.1007/s11901-014-0233-7. (Appendix 1)
2. Sarin SK, Philips CA, Kamath PS, Choudhury A, Maruyama H, Nery FG, Valla DC. Towards a comprehensive new classification of portal vein thrombosis in patents with cirrhosis. Gastroenterology 2016 Oct; 151(4):574-577.e3. doi: 10.1053/j.gastro.2016.08.033. (Appendix 2)
3. Nery F, Chevret S, Condat B, de Raucourt E, Boudaoud L, Rautou PE, Plessier A, Roulot D, Chaffaut C, Bourcier V, Trinchet JC, Valla DC. Causes and consequences of portal vein thrombosis in 1243 patients with cirrhosis: results of a longitudinal study. Hepatology 2015 Feb; 61(2):660-7, doi: 10.1002/hep.27546. (Appendix 3)
4. Nery F, Correia S, Macedo C, Gandara J, Lopes V, Valadares D, Ferreira S, Oliveira J, Gomes MT, Lucas R, Rautou PE, Miranda HP, Valla D. Nonselective beta-blockers and the risk of portal vein thrombosis in patients with cirrhosis: results of a prospective longitudinal study. Aliment Pharmacol Ther. 2019 Jan 22. Doi: 10.1111/apt.15137. [Epub ahead of print]. (Appendix 4)

This thesis includes unpublished results

Abstract

Background. Nonmalignant portal vein thrombosis is a significant event in the course of cirrhosis, known to affect the most severe patients. Its impact on liver disease progression or decompensation is not clear but it is known to decrease survival after liver transplantation. Some associated risk factors have been described but are not consensual or have not been validated to date.

Aims. To determine i) risk factors for the development of nonmalignant portal vein thrombosis in the context of cirrhosis, and ii) the impact of the thrombotic event on liver disease progression, decompensation or death (secondary aim).

Methods. Two prospective observational longitudinal studies were conducted. THROMBOCIR, a multicenter study, undertaken between June 2000 and March 2006, on 1243 Child-Pugh A and B patients, and FRTVPCir, a single-center study, undertaken between January 2014 and February 2017, on 108 patients, mostly Child-Pugh A (78%). Abdominal Doppler ultrasound study was performed in each of the studies every 3 or 6 months.

Results. Global incidences of portal vein thrombosis of 9.5% and 10.2% were found in THROMBOCIR and FRTVPCir studies, respectively. Factors found to be related to the future development of portal vein thrombosis were medium or large-sized esophageal varices at baseline in both studies (hazard ratio [HR]=2.14; 95% confidence interval [CI]: 1.27-3.60; *P*=0.004 and HR=5.67; 95% CI: 1.49-21.63; *P*=0.011 in THROMBOCIR and FRTVPCir studies, respectively), increased prothrombin time in the THROMBOCIR study (HR=0.82; 95% CI: 0.68-0.98; *P*=0.03), and the use of non-selective beta-blockers, but only in univariate analysis in THROMBOCIR (HR=1.67; 95% CI: 1.02-2.73; *P*=0.04); however in the FRTVPCir study, the use of non-selective beta-blockers was a related risk factor (HR=10.56; 95% CI: 1.35-82.73; *P*=0.025) independently of its effect over decreased heart rate or portal vein blood flow velocity. No relationship was found between decreased portal blood flow velocity and portal vein thrombosis in either study. Subanalysis of inflammatory markers in the FRTVPCir study revealed interleukin-6 above 5.5 pg/mL (HR=5.64; 95% CI: 1.21-26.33, *P*=0.028) and lymphopenia (HR=0.18; 95% CI: 0.04-0.80, *P*=0.023) at baseline as predictors for future portal vein thrombosis. Higher interleukin-6 titers were related to more severe portal hypertension (presence of esophageal varices grade ≥ 2 and collaterals). In the largest study, portal vein thrombosis shared some of the same risk factors (esophageal varices size and increased prothrombin time) with but was

not related to liver disease progression, decompensation or death.

Conclusions. In compensated cirrhosis, portal vein thrombosis is a significant event occurring in approximately 1 in every 10 patients. Associated risk factors are those related to a more severe grade of portal hypertension (presence of and more advanced grades of esophageal varices), slightly advanced liver insufficiency (increased prothrombin time, only seen in the most powerful study), and inflamed patients (increased interleukin-6). Non-selective beta-blockers act over portal vein thrombosis development by mechanisms other than their direct effect over systemic or splanchnic circulation. Portal vein thrombosis does not impact liver disease progression or induce decompensation.

Resumo

Contexto. A trombose da veia porta, na ausência de malignidade, é um evento significativo no curso da cirrose, afetando normalmente os doentes mais graves. Apesar de relacionada com uma diminuição da sobrevida após transplante, o impacto que a trombose da veia porta tem na progressão ou descompensação da doença hepática não é evidente. Alguns fatores de risco têm sido descritos. Contudo, além de não consensuais carecem, também, de validação.

Objetivos. Determinar i) fatores de risco associados ao desenvolvimento de trombose da veia porta não maligna no contexto de cirrose, e ii) o impacto do evento trombótico na progressão e descompensação da doença hepática, assim como na sobrevida dos doentes.

Métodos. Foram conduzidos dois estudos prospetivos longitudinais observacionais. O estudo THROMBOCIR, multicêntrico, conduzido entre Junho de 2000 e Março de 2006, incluiu um total de 1243 doentes com cirrose Child-Pugh A e B. O estudo FRTVPCir, unicêntrico, conduzido entre Janeiro de 2014 e Fevereiro de 2017, incluiu 108 doentes, a maioria com cirrose Child-Pugh A (78%). Ecografia abdominal com estudo Doppler foi realizada a cada 3 ou 6 meses.

Resultados. A incidência global de trombose da veia porta foi de 9.5% e de 10.2% no estudo THROMBOCIR e FRTVPCir, respetivamente. As varizes esofágicas de pelo menos grau 2 à inclusão relacionaram-se com o desenvolvimento futuro de trombose da veia porta em ambos os estudos (*hazard ratio* [HR]=2.14; 95% intervalo de confiança [IC]: 1.27-3.60; *P*=0.004 e HR=5.67; 95% IC: 1.49-21.63; *P*=0.011 no estudo THROMBOCIR e FRTVPCir, respetivamente), o aumento do tempo de protrombina no estudo THROMBOCIR (HR=0.82; 95% IC: 0.68-0.98; *P*=0.03) e o uso de beta-bloqueadores não cardio-seletivos em análise univariada no estudo THROMBOCIR (HR=1.67; 95% IC: 1.02-2.73; *P*=0.04). No estudo FRTVPCir, a utilização de beta-bloqueadores não cardio-seletivos foi identificada como fator de risco para desenvolvimento da trombose da veia porta (HR=10.56; 95% IC: 1.35-82.73; *P*=0.025) independentemente do seu efeito na diminuição da frequência cardíaca ou velocidade do fluxo a nível da veia porta. Nenhuma relação entre velocidade do fluxo da veia porta diminuída e o evento trombose foi identificada em qualquer dos estudos. A subanálise dos marcadores inflamatórios no estudo FRTVPCir mostrou que níveis de interleucina-6 superiores a 5.5 pg/mL (HR=5.64; 95% IC: 1.21-26.33, *P*=0.028) e a linfopenia presentes à inclusão eram preditores da ocorrência futura de trombose da veia porta. Níveis de interleucina-6 mais elevados foram encontrados nos doentes com

hipertensão portal mais pronunciada (presença de varizes esofágicas de pelo menos grau 2 e de colaterais porto-sistémicas). No estudo maior, a trombose da veia porta partilhou alguns dos mesmos fatores de risco (presença de varizes esofágicas e tempo de protrombina aumentado), mas não esteve relacionada com a progressão ou descompensação da doença hepática assim como com incremento da mortalidade.

Conclusões. Na cirrose compensada, a trombose da veia porta não maligna é um evento significativo e que ocorre em aproximadamente 1 em cada 10 doentes. Os fatores de risco que lhe estão associados são aqueles relacionados com maior expressão clínica de hipertensão portal (varizes esofágicas de pelo menos grau 2), marcadores de insuficiência hepática ligeiramente mais avançada (aumento do tempo de protrombina, apenas documentado no estudo com maior poder estatístico), e doentes mais inflamados (títulos elevados de interleucina-6). Os beta-bloqueadores não cardio-seletivos contribuem para o desenvolvimento de trombose da veia porta através de outros mecanismos que não pelo efeito direto sobre a circulação sistémica ou esplâncnica. A trombose da veia porta não tem impacto na progressão da doença hepática nem induz descompensação.

List of Abbreviations/ Acronyms (order of appearance)

CHUP – Centro Hospitalar Universitário do Porto

LT – Liver transplantation

PVT – Portal vein thrombosis

PBFV – Portal blood flow velocity

NSBB – Non-selective beta-blocker

FVL - Factor V Leiden

PTHR - Prothrombin G20210A

HE – Hepatic encephalopathy

PT - Prothrombin Time

INR - International Normalized Ratio

MELD – Model for end-stage liver disease

Hs-CRP – High sensitive C-reactive protein

TNF-α - Tumor necrosis factor-alpha

IL – Interleukin

GB – Gastrointestinal bleeding

HCC – Hepatocellular carcinoma

DUS – Doppler-ultrasound

CEUS – Contrast-enhanced ultrasound

MRI – Magnetic resonance

CT-scan – Computed tomography scan

UNOS – United Nation for Organ Sharing

OPTN - Organ Procurement and Transplantation Network

VWF - Von Willebrand Factor

ADAMTS-13 – A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13

MTHFR - Methylenetetrahydrofolate reductase C677T

HR – Hazard ratio

CI – Confidence interval

JAK2 - Janus Kinase-2

MPD - Myeloproliferative disorders

CALR – Calreticulin

LPS – Lipopolysaccharide

DIC - Disseminated intravascular coagulation

TLR4 - Toll-like receptor 4

TM – Thrombomodulin

SBP - Spontaneous bacterial peritonitis

ELISA – Enzyme-linked immunosorbent assay

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CHAPTER I

Introduction

CHAPTER I

INTRODUCTION

1. THESIS MOTIVATION

When I first started my residence in Internal Medicine back in 2005, at the Centro Hospitalar Universitário do Porto (CHUP), I never could have imagined where I would be or what I would be doing today. Since this tertiary health institution is one of the 3 centers in Portugal where liver transplantation (LT) is done, it seemed reasonable, as resident, to do an internship in Hepatology at an international reference center in Europe. After helpful advice from Prof. Helena Miranda, Paris would be my next stop. It was in 2008 that I was, for a period of 6 months, resident at the Liver Unit of Hôpital Beaujon – Clichy, France. By that time, I gained a particular appreciation for vascular liver diseases, probably influenced by Prof. Dominique Valla, who was responsible for the Liver Unit at the time. After that period, and back at CHUP, I ended my residency and started to work as an assistant, dedicating almost half of my time to the care of patients with liver diseases. In 2012 I was invited to go back to Paris, by that time as a hepatologist. In order to leave CHUP for Hôpital Beaujon, I was asked to start a PhD, and it seemed quite clear to me that it should be on vascular liver diseases, which would be one of the major pathologies I would come across on the ward I would be working on. Deciding on a subject and a specific area to start investigating was simple: Portal vein thrombosis (PVT) would be the main subject, due to its prevalence and eventual impact on cirrhosis, and the specific issue to be addressed would be the risk factors for its development, as they had not been clearly recognized, mainly due to the heterogeneity of the methodologies applied in the previously published papers. This would be the opportunity to study this particular issue in-depth and proceed to a critical examination while conducting an original work. Since the stay in Paris was only foreseen for one year, the research had to be undertaken in two steps, the first in Paris, with the THROMBOCIR (multicenter) study and the second one conducted in Porto, the FRTVPCir (single-center) study. Both of these prospective studies shared some common hypotheses and aims, even though particular designs were assessed.

2. THESIS HYPOTHESES

Hypothesis 1. Portal vein thrombosis is a significant event in patients with cirrhosis, with an increased incidence depending on the severity of the liver disease. Features related to portal hypertension markers and to the degree of liver failure are responsible for PVT development.

Hypothesis 2. Blood stasis is one of the pillars of Virchow's triad leading to thrombosis. A decreased portal blood flow velocity (PBFV) is a risk factor for PVT development in patients with cirrhosis.

Hypothesis 3. Non-selective beta-blockers (NSBB) decrease portal hypertension via β1 and β2 blockade. Their use in patients with cirrhosis is associated with future PVT development.

Hypothesis 4. Factor V Leiden (FVL) and G20210A prothrombin (PTHR) gene mutations are well-known risk factors for thrombosis. Their role in PVT genesis in patients with cirrhosis is not completely clear so far. Factor V Leiden and PTHR gene mutations are concurrent risk factors for PVT development in cirrhosis.

Hypothesis 5. Inflammatory response and coagulation cascade activation has been recognized as inducing vascular thrombosis in different vascular territories, but no studies have been conducted so far with reference to splanchnic vessel bed. Increased inflammatory markers exist in patients with cirrhosis before PVT development.

Hypothesis 6. Recent longitudinal data have changed the notion that PVT leads to liver decompensation and increased morbid-mortality out of the LT setting, but no prospective studies have been done so far. Portal vein thrombosis is not related to liver decompensation or progression.

3. SPECIFIC AIMS

Concerning hypotheses 1, 2 and 3, our aims were:

- To search for risk factors, commonly seen on a daily clinical basis, which could be related to portal hypertension (size of esophageal varices, low platelet count, spleen size, ascites, hepatic encephalopathy [HE]), the degree of liver failure (increased prothrombin time [PT]/ international normalized ratio [INR] and bilirubin, low albumin, increased model for end-stage liver disease [MELD] score), PBFV or the use of NSBB (THROMBOCIR and FRTVPCir studies).

Concerning hypothesis 4, our aims were:

- To evaluate the prevalence of FVL and PTHR gene mutations in a large cohort of patients, to compare to the population in general and search for the competing risk for PVT development in patients with cirrhosis (THROMBOCIR study).

Concerning hypothesis 5, our aims were:

- To search for inflammatory markers (leukocytes, high-sensitive C reactive protein [Hs-CRP], ferritin, tumor necrosis factor–alpha [TNF-α], interleukin [IL] - 6) that could be related to an increased risk for PVT development (FRTVPCir study).

Concerning hypothesis 6, our aims (secondary aim) were:

- To search for the impact of PVT on liver decompensation and progression, and mortality in a large cohort of prospectively followed patients (THROMBOCIR study).

4. THESIS OUTLINE

This thesis is divided into 7 chapters:

Chapter 1: Establishes the main motivations, hypotheses and aims of the work.

Chapter 2: Reviews the literature concerning the most important and significant works conducted so far in the field of portal vein thrombosis in patients with cirrhosis, bearing in mind that manuscripts published after our first published results and which refer our own data are not mentioned in this section but afterwards in the discussion, when applicable.

Chapter 3: Broadly outlines the methodology applied in the two prospective studies conducted (THROMBOCIR and FRTVPCir). Detailed methodologies are described in the corresponding published articles.

Chapter 4: Describes the results found in both studies in a "hypothesis-step" approach, referring to the published results (whenever applicable) presented as appendices, and in a detailed way to non-published results. Respective publishers authorized the reproduction of the published manuscripts.

Chapter 5: Provides a general discussion of the main results.

Chapter 6: Resumes the major findings and outcomes of the studies.

Chapter 7: Describes the clinical implications of our findings and addresses future fields of research.

CHAPTER II

LITERATURE REVIEW

CHAPTER II

LITERATURE REVIEW

Cirrhosis, the ultimate stage of liver fibrosis progression, histologically characterized by conversion of normal liver architecture into hepatocyte-containing nodules surrounded by bands of fibrous tissue of various breadth (1), has a heterogeneous distribution worldwide, reflecting different etiologies and diagnostic assessment tools (2). However, the real prevalence of cirrhosis is difficult to ascertain, being estimated to range, in necropsy studies, between 4.5% and 9.5% of the general population (3). After a clinically silent period, cirrhosis complications arise and are reflected by loss of hepatocellular function, portal hypertension complications (ascites, hypertensive gastrointestinal bleeding [GB], HE), hepatocellular carcinoma (HCC) or extrahepatic complications (renal impairment, hepatopulmonary syndrome, infection, acute-on-chronic liver failure, etc.) (4-6). Patients with compensated liver disease have median survival rates of more than 12 years, with survival rapidly decreasing with decompensated disease (4). Cirrhosis is the 11th most common cause of death, being responsible for 1.16 million death/year and still rising (2).

Portal vein thrombosis refers to the presence / development of a clot within the portal vein tract, along the portal vein trunk and/or one or both of its branches, which may, or may not completely occlude the vessel (7). In cirrhosis, it may course asymptomatically and be found in the context of a routine abdominal exam (for example in the setting of HCC screening) or with symptoms, namely abdominal pain, depending on the extension of the clot within the superior mesenteric vein harboring a poor prognosis, or those related to liver disease decompensation (7). Importantly, PVT must be differentiated from malignant vascular invasion, which in patients with cirrhosis is almost always related to HCC, being a clinically distinct entity harboring a different treatment and prognosis. Doppler-ultrasound (DUS) findings may aid in differential diagnosis, demonstrating an absent flow in the portal vein or one or two of its branches by color Doppler study, but contrast-enhanced ultrasound (CEUS) allows for a final diagnosis in more than 97% of the patients (8). Magnetic resonance (MRI) or contrast-tomography scan (CT-scan) confirms not only the diagnosis but also determines the extension of the clot within the splanchnic vessel bed (7). Once PVT is diagnosed, anticoagulation is usually the treatment to be offered, in order to i) avoid extension and ii) promote PVT resolution. Yet, not all the patients with PVT and cirrhosis are candidates for anticoagulation, and there is probably a subgroup of patients to which this treatment should be offered immediately (after adequate screening and respective treatment of esophageal varices), i.e. those who are candidates for or on the waiting list for LT, while other patients should be considered individually and according to local policies and experience

(*Appendix 1)*(7, 9, 10).

1. THE EPIDEMIOLOGY OF PVT

Older reports on PVT incidences and prevalences show some discrepant results reflecting different geographic regions, methodologies and study designs (most of them retrospective or cross-sectional in nature) as well as different technics / diagnostic procedures. Furthermore, the indistinct use of the terms incidence and prevalence in literature interfere with epidemiological data interpretation. In England, in 1954, in 111 patients with cirrhosis, PVT was documented intraoperatively in 11% of them (11). Even though no stratification was made, all patients presented with decompensated liver disease by the time of PVT diagnosis. In Hong-Kong, a necropsy study gathering 126 cirrhotic patients documented mural thrombi involving portal vein in 25.4% of the cases (12). By contrast, in Japan, a very low prevalence of 0.6% was reported in 708 patients followed for a 10-year period in a mixed population of Child A to C patients (most of them Child C) (13). The diagnosis was based on angiographic studies (either transhepatic or superior mesenteric arterial portography). Other ancient reports, also using invasive diagnostic tools such as surgical technics or angiography, are in line with the heterogeneity of the aforementioned results, with prevalence ranging from 5.2% to 21% (14-18). Even so, the highest prevalences of PVT are those reported among patients undergoing LT, reflecting an underlying more severe liver disease. Nonami *et al* reported a 15.7% PVT prevalence by the time of LT in patients with end-stage cirrhosis (19). Gayowski *et al*, in a cohort of 88 American veterans, found prevalence even higher of 26% by the time of LT (20). All of them were Child-Pugh C. After excluding patients with HCC, another study documented a prevalence of PVT at LT of 17.5% (21). In a cohort of patients listed for LT and longitudinally followed, a 1-year incidence of PVT of 7.4% was reported, with the diagnosis made by DUS (22). Other studies also include mostly patients with advanced liver disease, even if not on a LT waiting list. Amitrano *et al* reported PVT prevalence of 11.2% in 701 patients admitted to the hospital (90% were Child-Pugh B and C), most of them due to an acute episode of liver disease decompensation (23). Villa *et al*, in a group of Child-Pugh B7-C10 cirrhotic patients found PVT up to 16,6% per year (24). A recent prospective assigned study enrolling a mixture of 81 Child-Pugh A to C cirrhotic patients, showed a 1-year incidence of PVT of 15% (25).

As such, PVT in cirrhosis is found, today, to be a non-negligible event, with discrepant reported incidences and prevalences, but clearly more recognized in patients with more severe liver disease, such as those candidates for LT or admitted at the onset of an acute episode of decompensation,

while clear estimates of the incidence or prevalence of PVT in less severe liver disease patients is not known.

2. CLASSIFICATION SYSTEMS

Several PVT classifications have been proposed since 1991 after the work of Stieber *et al* (26). This was purely anatomical and the first classification involving the whole portal venous system. Others succeed, but most of them refer only to anatomical considerations, as the location and extension of PVT (19, 27, 28). The most known and used PVT classification is the one proposed by Yerdel *et al*, which has implications in LT decisions and techniques to be applied (29). Bauer *et al* also proposed a pure anatomic classification that is useful for therapeutic monitoring purposes (30). It was only after the recent Baveno report that parameters other than anatomy were considered, such as the time setting of the thrombotic event (recent versus chronic) or the etiology of the underlying liver disease (10). Yet, functional aspects and outcomes were not included. The main issue is to have a PVT classification that allows not only to stratify according to location and extension, time setting and underlying etiology, but also one that enables to consider the subset of patients that will most benefit from anticoagulation treatment. The proposal of a new anatomic-functional classification system gathering all these aspects has been recently undertaken, which now requires external validation (*Appendix 2)* (31).

3. NATURAL HISTORY AND CLINICAL IMPACT OF PVT

3.1. PVT outcome without anticoagulation

Six decades ago, Laws *et al*, advanced the already existing notion that PVT would start as a thrombus partially occluding the lumen that could i) evolve, extending to complete thrombosis; ii) lead to cavernomatous transformation with the formation of numerous collateral veins running alongside the portal vein or; iii) spontaneously revert with complete recanalization of the vessel lumen (32).

Still, the natural history of PVT was not known until recently, when two recent retrospective longitudinal studies showed that PVT, once established and not treated, had a remarkable potential of reversal. Luca *et al*, in a cohort of 42 patients with partial extrahepatic non-malignant PVT, observed a spontaneous decrease in the thrombi volume in 45% of the patients, while in 21% it remained unchanged. Only 17% of the patients evolved to complete PVT, and none developed

portal cavernoma (33). In another population of 150 virus-related cirrhotic patients, 42 were diagnosed with PVT, 31 of which with partial PVT. Overall, PVT improved in 48% of the patients, remained unchanged in 45% and worsened in only 7% of the cases, with no portal cavernoma development (34). Recurrence of PVT after previous spontaneous resolution occurred in 9 patients, showing that PVT may have a dynamic character (34). Also, John *et al*, in a prospective study gathering 290 patients listed for LT, found that 30% of the patients with PVT at inclusion and 35% that developed PVT while on the waiting list, recanalized at least partially without any treatment (35). Yet, others haven't found these optimistic results. Francoz *et al* found no spontaneous resolution of PVT without anticoagulation in their cohort (22), however only 10 patients didn't received anticoagulation, and even if all PVT were partial, the follow-up period (5.8 months) was short, meaning that more time may be needed for recanalization (22). Also, after 6 months of follow-up, Zocco *et al* found 2 patients with total and 3 patients with partial PVT. Six months after the diagnosis, none of them regressed and one complete thrombosis evolved to portal cavernoma (25). Once again, not only is the number of events scarce, but follow-up was too short to draw any conclusions concerning the potential reversal of PVT with time.

3.2. Impact of PVT on progression and decompensation of liver disease

The notion that PVT may lead to progression and decompensation of liver disease is well-known, being the result of data published by the time of the thrombotic event (cross-sectional) and not by prospectively conducted studies. In 1954, Hunt *et al*, after the description of 7 patients with PVT suggested that its occurrence could be related to some sort of liver decompensation (variceal hemorrhage, HE, ascites, deterioration of the clinical condition), even if no other symptoms other than those associated to portal hypertension could occur (11). In a cohort of 701 patients admitted to the hospital due to an episode of liver decompensation, with advanced liver disease, PVT was often diagnosed at the same time, in 79 (11.2%) of them (23). And by the time of LT, PVT was also more commonly found in patients with concomitant decompensated liver disease (patients with chronic HE, ascites and GB) (19). This means that PVT may be more frequent in patients with advanced or decompensated liver disease, but it is not possible to extrapolate if it is the cause of decompensation, solely based on these cross-sectional studies. However, reasonable pathophysiological explanations may corroborate the aforementioned results. A work conducted by Wanless *et al,* involving 61 explanted cirrhotic livers found, in 36% of them, some degree of intimal fibrosis within the portal vein (involving intra-hepatic segments), which is in a higher range when compared to other works with lower prevalence of (extra-hepatic) PVT, using methods other than the examination of the whole liver (36). The occlusion of portal venules lead to

adjacent tissue collapse, creating areas of microinfarcts and "parenchymal extinction" which, in turn, will be replaced by fibrous septa (36, 37). This aggravation in intrahepatic block leads to an increase in portal hypertension that could precipitate, at least in theory, a liver decompensation event. When a branch of the portal vein is selectively ligated, there is a decrease in homolateral hepatic volume, proportional to the degree of ligation with a compensatory hypertrophy of the contralateral lobule (38, 39). The degree of necrosis is also related to more severe degrees of occlusion (38). While transforming growth factor beta (an antiproliferative factor, inducer of apoptosis) overexpression in the embolized lobe leads to hepatocyte apoptosis and subsequent atrophy, in the non-embolized lobe, transforming growth factor alpha (a mitogenic polypeptide, which activates signaling for cell proliferation) overexpression leads to hepatocyte proliferation and related lobe hypertrophy (40). Extrapolation of occlusion of a portal vein branch to portal vein trunk must be seen with extreme caution.

Still, and in opposition to the aforementioned, older data, mainly based on cross-sectional studies, PVT is usually accidently found in an asymptomatic patient (7, 23, 34). Recent longitudinal studies show that PVT is not a cause for liver decompensation. Luca *et al* found that progression of PVT did not lead to more episodes of liver decompensation, death and specific portal hypertension complications, and that the severity of liver failure *ad initium* would be the precipitating factor related to liver decompensation in the future and not PVT itself (33). Also, John *et al* noted no increased GB episodes in patients with PVT (35).

Nevertheless, the liver has a particular dual afferent vessel system, leading to a hyperarterialization after a decrease in portal vein blood flow. This capacity of the hepatic artery to buffer changes in portal blood flow has been well documented (41, 42). If this mechanism is of importance and explains why in recent longitudinal studies no decompensation seems to arise after PVT, is still a field open to investigation.

In short, cross-sectional studies, documenting PVT by the time of a liver decompensation episode suggest the possibility of a cause-and-effect relationship. However, recent longitudinal studies suggest that PVT and liver decompensation are not directly related, but probably share common precipitating risk factors.

3.3. Impact of PVT on survival

The impact of PVT on survival may be different when considering patients transplanted or not transplanted with PVT.

3.3.1. Impact of PVT on survival without LT

Maruyama *et al,* found similar cumulative 10-year survival for patients with and without PVT (34). John *et al*, found similar results in patients while on the waiting list for LT (35). Recently, in a case-control study, survival was not affected by PVT irrespective of the degree of occlusion (43). The analysis of a large LT recipient population (22291 patients) showed that those who developed PVT while on the waiting list didn't die more (44). Curiously, in an analysis made using the United Nation for Organ Sharing (UNOS) database involving 66506 patients listed for LT, patients with PVT presented a lower mortality than those without (45). There is no data concerning whether anticoagulation was given or not, which may bias the result. The heterogeneity of the different studies concerning not only the inclusion of patients with and without HCC but also the length of the follow-up may give rise to different results. A systematic review of 13 different manuscripts clearly showed this heterogeneity but also that PVT could negatively impact the short-term (at 5-day, 6-week or 1-year), and the long-term survival (3-year follow-up) (46). However, this conclusion is drawn from abstracts and not published papers (47) so no final conclusions can be drawn regarding the negative impact of developing PVT on survival in cirrhotic patients out of the LT setting.

3.3.2. Impact of PVT on survival with LT

Almost all the studies reporting survival after LT are retrospective in character (9). Gayowski *et al.*, found no impact on patient survival, but PVT correlated with worse graft survival (20). Others saw no poor outcome after LT. Dumortier *et al.* found similar 1-year post-LT survival rates for those with (83.7%) and without PVT (86.7%), but most patients transplanted with PVT (89%) had partial occlusion of the vein (48). Other groups found similar results (49-51). John *et al.*, in a prospectively conducted study, showed no impact on short-term (6 months) survival post LT (35). Yet, others report contrasting conclusions, with an increased PVT-related mortality after LT (22, 29, 44, 52). Two recent studies, both involving several hundreds of liver transplanted patients collected from the Organ Procurement and Transplantation Network (OPTN) database, but in different periods of time, clearly show a worse patient and graft early (90-days) (53) and long (up to 8 years of follow-up) post-LT survival (54). Yet, HCC was identified as being one of the risk factors for PVT development while on the waiting list in both papers and no mention has been done to if this negative impact on survival had some relationship to cancer relapse after LT or not. These conflicting results have been addressed in two recent meta-analyses. The first, showed that occlusive PVT before LT had a negative impact in the 1-year post-LT survival (55). The second, gathering more published data, showed that 30-day and 1-year post-LT survival was worse in

patients that had PVT previous to LT (56). Also, they documented that within PVT patients, 30-day survival was significantly worse according to higher degrees of portal vein occlusion and this relation, even if maintained at 1-year after LT did not met statistical meaning (56). The negative impact of PVT on LT and survival may be related to the extension and degree of the clot (29, 56), a more complex and prolonged time of surgery (29, 49, 54), higher transfusion requirements (49, 57), and longer intensive care unit and hospital stays (58).

In short, the current notion is that PVT does not impact survival outside of the LT setting and also that higher degrees of occlusion bear dismal prognosis after LT.

4. KNOWN RISK FACTORS FOR PORTAL VEIN THROMBOSIS IN CIRRHOSIS

4.1. Virchow's triad

Thrombosis, occurring at any site or blood vessel bed, is a consequence of not one but many risk factors that, acting together, induce clot formation under special circumstances. Virchow's triad is helpful in explaining this theory while addressing this multifactorial concept based on three fundamental pillars: a hypercoagulable state, blood stasis and endothelial damage (59). These fundaments may also be applied to PVT in order to systematize the already known risk factors and to aid in further investigation fields.

4.1.1. Hypercoagulable state

4.1.1.1. Hemostasis in advanced liver disease

To understand the role of hemostasis in the genesis of PVT in the particular context of cirrhosis, some notions must be cleared concerning the current knowledge on hemostasis in advanced liver disease. Patients with cirrhosis are often found to have disturbed routine laboratory tests such as PT/ INR, bleeding time and platelets. In contrast to what was previously believed these tests were suggested to be of no use to predict the risk of bleeding in cirrhosis (60).

Hemostasis is a sequential process and depends on the interaction of platelets, wall vessels and clot factors. Primary hemostasis refers to the process in which the loose platelet plug is formed on the injured vascular endothelium and secondary hemostasis refers to the cascade that allows stabilization the clot with the conversion of fibrinogen into fibrin (61).

Platelets. Thrombocytopenia (<150.000/μL) is a common (up to ¾ of the patients) and an early finding in patients with cirrhosis being, most of the time, moderate (62, 63). When severe, although rare (platelets <40.000/μL are expected to occur in about 1% of the patients), a work up

must be done in order to exclude other concurrent causes, such as infection, active alcohol consumption, immunological disorders or others (62, 64). In cirrhosis, a low platelet count may be mainly explained by: i) portal hypertension and related hypersplenism, which leads to splenic pooling and the sequestration of platelets from the circulation (65-67); ii) low thrombopoetin levels related to platelet underproduction which also seems to be dependent on the severity of liver disease (67, 68); and iii) the presence of antiplatelet antibodies (69, 70), even though their presence in cirrhotic patients are not always consistent with thrombocytopenia (71). In spite of these quantitative platelet defects that could favor a bleeding tendency, compensatory mechanisms exist that may counteract this occurrence. Von Willebrand Factor (VWF) is a multimeric protein extremely important in primary hemostasis. When binding to the exposed subendothelium collagen fibers of an injured vessel wall, VWF contributes to platelet adhesion and clot formation. In cirrhosis, VWF is elevated and rises in relation to the severity of liver disease (72-74), and may even predict decompensation episodes and mortality (74). The higher titers of VWF in this context may be explained by i) endothelial damage; ii) endotoxemia; iii) overexpression in the liver; iv) higher endothelial total surface (explained by the presence of extensive collaterals); v) higher endogenous vasoconstrictor levels; and vi) a reduced VWF clearance (72-74). Also, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS-13), which is a cleaving protease of VWF, was found to be decreased in a manner that is inversely related to liver disease severity, possibly contributing to higher circulating VWF levels (75, 76). Overall, the elevated circulating titers of VWF may compensate qualitative and quantitative platelet defects (73). Platelets are also important for thrombin generation and capable of doing so since their number does not usually fall below 60.000/μL (77). In short, severe thrombocytopenia in cirrhosis is rare (and apparently not more frequent than in chronic liver diseases without cirrhosis) (62) and mechanisms exist that seem to compensate the quantitative and qualitative platelet defect, such as higher VWF levels and a preserved capacity of thrombin generation, for an adequate adhesiveness and aggregation.

Coagulation. The liver is responsible for the production of several pro- and anticoagulant factors. Accordingly, it is expected that a more severe liver disease relate to a parallel reduction of the aforementioned factors, which would explain the prolonged conventional global coagulation tests such as PT, INR or the activated partial thromboplastin time (78). However these tests are not suitable to assess the coagulation balance in cirrhosis, since they do not express the whole amount of thrombin generated in the absence of protein C activators (79). As previously stated, thrombin generation in cirrhosis is preserved provided there is a sufficient platelet number. Also, it is now known that thrombin is generated in at least the same amount in the plasma of patients

with cirrhosis when compared to healthy subjects: this occurs according to *in vitro* conditions resembling those *in vivo*, using methods reflecting the action of both anticoagulants and procoagulants, for example with the presence of thrombomodulin or Protac® (an extract of snake venom that also activates Protein C *in vitro*) (80, 81).

A few years ago a remarkable difference between the variation of pro- and anticoagulant factors was found in the course of the liver disease, favoring the tendency of higher levels of pro- versus anticoagulants, the so called "imbalance of coagulation". A recognized reduction of the anticoagulant levels of Protein C, S and antithrombin was not accompanied in the same way and proportion by some procoagulant factors, namely factor VIII (a very important driver for thrombin generation), which is actually raised (80, 82). The ratio factor VIII: Protein C in cirrhotic patients increases according to the severity of liver disease being around 3 in Child-Pugh A and reaching values of 5.6 in the more severe Child-Pugh C patients (80, 81). While low levels of Protein C are explained by a deficit of synthesis, the elevated levels of factor VIII are due to decreased clearance mediated by VWF and low-density lipoprotein receptor-related protein (83).

Fibrinolysis. Once coagulation is activated and a clot forms, the way to prevent its extension and an increased risk of thrombosis is to degrade fibrin. This is achieved with the conversion of plasminogen to plasmin by profibrinolytic drivers, which are opposed by antiactivators avoiding hyperfibrinolysis that would otherwise potentiate bleeding, with most of the involved proteins and enzymes being produced by the liver (84). In cirrhosis, both hyperfibrinolysis and hypofibrinolysis have been described. Laboratory abnormalities commonly present in cirrhosis express a probably restored balance between pro- and antifibrinolytic factors, since findings of increased tissue plasminogen activator and plasmin activity, and decreased α-2 plasmin inhibitor, plasminogen and thrombin-activatable fibrinolysis inhibitor have been described (85-88).

4.1.1.2. Altered hemostasis and portal vein thrombosis

As previously mentioned, PVT is known to occur with more frequency in patients with more severe liver disease. The most severe liver patients are also those who will have the more profound hemostasis alterations, making it reasonable to try to establish a connection between the two.

Platelet count has been found to be inversely correlated to the risk of PVT occurrence in patients on the waiting list for LT, which might be explained by the impact of portal hypertension, which could surpass any protective role of thrombocytopenia (22), as confirmed in two other studies as a baseline finding (25, 89), thrombocytopenia being independently associated with the development of PVT in one of them in multivariate analysis (89). Thrombocytopenia has also been found to be correlated to PVT by other groups (90). Yet, these results must be approached with

caution, since laboratory data that is measured at the time of the event may not be representative of a causative relationship/risk factor, but may be an actual consequence of the event, i.e., the low platelet count may be due to platelet consumption occurring associated to thrombosis (91). Others did not find thrombocytopenia to be a risk factor for PVT development (34). Patients with cirrhosis are now known to be at risk for venous thromboembolism (92, 93), recently confirmed in a robust meta-analysis (94), and acquired coagulopathy does not protect against the thrombotic event (94, 95). While the use of venous thromboembolism prophylaxis is still low and neglected, there is consensus of the benefits of putting these patients, while hospitalized, under mechanical or medical prophylactic measures (9, 94, 95). Tripodi *et al* hypothesized that the coagulation imbalance expressed by the increased factor VIII and decreased protein C could explain the higher risk of venous thromboembolism (80). When looking specifically at what happens at the level of the portal vein axis, Kalambokis *et al* found no relation between increased factor VIII level, but an independent association of the ratio factor VIII-to-protein C with PVT development, reflecting the role of the coagulation imbalance in promoting PVT (96). These results were not replicated by others, which found no association between procoagulant imbalance and PVT (97). But prospectively conducted studies are lacking to study the role of coagulation imbalance as a risk factor in PVT development. Chen *et al* found no important differences between groups with and without PVT when considering the ratios of pro- versus anticoagulant factors, but the population studied was small and the data was collected after PVT occurrence, not before (98). Tang *et al* showed progressively decreased levels of anticoagulant factors (Protein C, Protein S and antithrombin) in relation to liver disease severity with constant levels of factor VIII, as well as a lower Protac®-induced coagulation inhibition percentage, all these findings related to a greater procoagulant imbalance (97). However, no relationship between these results and PVT was found, even when stratified for the severity of the liver disease. Once again, the study included patients already with PVT, making it difficult to extrapolate a causal relationship (97). Zocco *et al* found that in spite of being a constant in all patients with cirrhosis, when considering those with higher MELD scores (at least 13 points), Protein C, Protein S and antithrombin levels were significantly lower and correlated with PVT occurrence (25). In short, prospective studies are lacking when trying to establish a link between coagulation imbalance and PVT development in cirrhosis. Decreased Protein C (24, 25), Protein S (25) or antithrombin levels (25) and increased D-dimer levels (25) have been associated with an increased risk for subsequent PVT development. The other available studies, either of retrospective or cross-sectional character, have analyzed risk factors determined at the time of the diagnosis of the thrombotic event (97-102).

4.1.1.3. Genetic factors

Studies performed before the 2000's, enrolled a small number of patients and mixed those with and without cirrhosis, which led to conflicting results, notably in what concerns to FVL mutation and PVT occurrence in cirrhotic patients (103, 104). After that period, more studies were published enrolling only patients with cirrhosis and analyzing mainly the role of FVL, PTHR and Methylenetetrahydrofolate reductase C677T (MTHFR) mutations. Amitrano *et al* found a thrombophilic genotype in 69,5% of the 23 patients with cirrhosis and diagnosis of PVT, with all of the aforementioned mutations being of statistical significance (105). These results were almost all supported by Erkan *et al*, which found FVL mutation to be more frequent (hazard ratio [HR]=11.45; 95% confidence interval [CI]: 1.98-66.24; *P*<0.01) in patients with cirrhosis and PVT compared to those without, finding the same result for PTHR mutation (HR=11.45; 95% CI: 1.98-66.24; *P*<0.01), but not for MTHFR mutation (106). But once again a very small group of 17 patients was analyzed, with large CI found (106). With a bigger group of 701 cirrhotic patients, 79 of them with PVT, Amitrano *et al* found a five times higher risk for the development of PVT in carriers of the PTHR mutation, but not with FVL or MTHFR mutations (23). The mutation of the G20210A prothrombin gene consisting in a substitution of a G→A at nucleotide position 20210 leads to higher plasma prothrombin levels. The same group also documented the role of a heterozygote state for PTHR mutation and elevated levels of plasma factor II, as well as the ratio factor II:Protein C, reflecting the prothrombotic/antithrombotic balance favoring PVT development (107). Later on, Mangia *et al* prospectively enrolled 43 patients with cirrhosis and PVT achieving different conclusions: i) a lower prevalence of a thrombophilic genotype than previously reported and stated above, of 27,9%; ii) no relationship between the presence of any of FVL, PTHR or MTHFR mutations and PVT occurrence (108). Not even two recently published meta-analyses laid this uncertainty to rest (109, 110). They used different methodologies, considering the analysis of the two major mutations: FVL and PTHR. The first one, gathered patients with and without cirrhosis, small case series and did not exclude HCC for study selection, concluding that the presence of FVL mutation does not confer a significantly increased risk for PVT in cirrhosis (estimated risk of 1,99%), as opposed to cirrhotic patients carriers of PTHR mutation, with an estimated attributable risk for PVT of 9,37% (109). The second one excluded the previously mentioned possible bias from the analysis. The found prevalence of FVL mutation was significantly higher in patients with cirrhosis and PVT than in those without the event (HR=2.55; 95% CI: 1.29-5.07; *P*=0.007), while the prevalence of PTHR mutation was not different between both groups (HR=2.93; 95% CI: 0.94-9.07; *P*=0.06) (110). In this last case a subgroup analysis was conducted, separating 1 Asian and 4 European studies, with no differences seen among the

European studies between both groups (PVT versus no PVT) (110). When considering only the role of MTHFR mutation, it is known that in a homozygous state it leads to high homocysteine levels. Some considerations about its role in PVT in cirrhosis have previously been mentioned. Another meta-analysis found that the presence of MTHFR mutation in homozygosity increases the risk of PVT in cirrhotic patients (111). Regardless of the uncertainty, current guidelines recommend the screening of underlying inherited thrombophilic conditions (7, 10).

Janus Kinase-2 (JAK2) gene is responsible for the control of the production of blood cells from hematopoietic stem cells; its mutation, described in 2005, has been linked to myeloproliferative disorders (MPD) (112). Myeloproliferative disorders are the cause of PVT in approximately 25% of the patients, and JAK2 mutation has been found to be present in 16% to 34% of non-cirrhotic patients with PVT (113, 114). However, in patients with cirrhosis few studies have been conducted so far, but similarly as in non-cirrhotic patients, there also seems to be a relationship between JAK2 gene mutation and PVT. Saugel *et al* recently reported in a small case-control study a tendency (although with no statistical meaning) for those patients with cirrhosis harboring the mutation to develop PVT, when compared to those who did not (115). Despite the fact that these results still need to be urgently reproduced on a larger scale, the latest Baveno VI consensus suggests the addition of JAK2 mutation analysis in the systematic screening of a cirrhotic patient developing PVT, similarly as for non-cirrhotic patients (10).

More recently, an association between the calreticulin (CALR) gene mutation and MPD was found; CALR and JAK2 mutations are mutually exclusive (116). Splanchnic vein thrombosis was found to be associated with the CALR mutation in non-cirrhotic patients in a Spanish cohort, but in a frequency far lower than JAK2 mutation and, when considering only PVT, the mutation was only described in 2 out of 140 patients (1,4%) (117). This low prevalence (0,7% considering PVT and Budd-Chiari syndrome all together) was also reported in a case-control study from the EN-Vie study cohort (118). Still, when considering only non-cirrhotic patients with JAK-2 V617F negative MPD, CALR mutations may be present in up to 31% of patients with PVT (116), but corresponding data in patients with cirrhosis are still lacking and no studies have been conducted so far regarding CALR mutation and PVT relationship in cirrhosis.

4.1.1.4. Antiphospholipid antibodies

Antibodies reacting against negatively-charged phospholipids were described 30 years ago to be associated with two patients with hepatitis B virus induced cirrhosis by Violi *et al* (119). Later on, the same group, in a cohort of 20 cirrhotic patients, found a significant positivity for anticardiolipin antibodies in 9 of them (120). A significant association between antiphospholipid

antibodies, both lupus anticoagulant and anticardiolipin antibodies, and splanchnic thrombosis in patients with cirrhosis (cohort of 73 patients with cirrhosis mixed etiologies with 9 splanchnic thrombotic events, 8 of which PVT) was found a little later, also by the same group (121). A case-control (10 PVT cirrhotic patients matched with 20 cirrhotic patients without PVT) study reached the same results with positivity for anticardiolipin antibodies being significantly related to PVT (122). Notwithstanding the presence of antiphospholipid antibodies in patients with chronic liver diseases and its association to histological severity (123) and autoimmune etiology (124) (which probably represents an immunological epiphenomenon due to an hyper stimulation of the immune system) (125), their definitive role in the genesis of PVT is yet to be established. Amitrano *et al* found no relationship between the presence of antiphospholipid antibodies (anticardiolipin and beta-2-glycoprotein-I antibodies) and PVT in patients with cirrhosis in a study matching 50 patients with and without liver cirrhosis and with and without PVT (126). A meta-analysis recently reinforced the notion that there doesn't seem to be a causal effect of antiphospholipid presence on PVT occurrence (127). However there are only limited studies addressing this particular issue, with non-standardized methodologies and few patients. Importantly, there is no mention in any study concerning reevaluation of antiphospholipid antibodies titer 12 weeks after their initial measurement, not allowing any particular conclusion regarding its role in PVT genesis and related antiphospholipid syndrome to be drawn.

4.1.2. Blood stasis

In 1856, Virchow stated that "*phenomena due to the interruption of the blood-stream*" was one of the factors leading to thrombosis illustrating, in this way, one of the pillars of his triad (59). In the natural history of chronic liver diseases, hepatic stellate cell activation, dysfunction of the liver sinusoidal endothelial cells, microvascular thrombosis and progressive architectural distortion, promote an increase in the intrahepatic vascular resistance leading to portal hypertension (128). Also, collateral vessel formation, and arterial and splanchnic vasodilation are other typical findings resulting, after the application of the hydraulic derivation of Ohm's Law (Pressure = Flow x Resistance) in an increase in portal hypertension (129).

In patients with cirrhosis, PBFV has long ago been recognized to be slower than in normal individuals (18) and lowers in proportion to the severity of the disease (expressed by Child-Pugh classification) (130) and higher degrees of fibrosis (131). A PBFV of 15cm/s has been set as the best cut-off value for the detection of portal hypertension by DUS study, with sensitivity and specificity of 88% and 96%, respectively (130). Also, PBFV has been related to a worse prognosis with a shortened survival when <10cm/s (132).

4.1.2.1. Decreased portal vein blood flow velocity and PVT

It was long ago, back in 1954, that Hunt *et al* stated that "stagnation of blood in the main portal vein is probably the only constant etiological factor of real importance" for PVT development in cirrhosis (11). However many years passed until this issue came to light once again, with Amitrano *et al* hypothesizing portal blood flow stasis as the most important risk factor favoring PVT (133). Only more recently, Zocco *et al* found a significant relationship between a decreased PBFV (< 15cm/s) and PVT development (HR=44.9; 95% CI: 5.3-382.7; *P*<0.001) in a prospectively conducted study enrolling 73 patients followed for 1 year (25). Beyond the hemodynamic implication on PVT, she proposed that stagnation of portal blood flow could lead to higher levels of thrombin in portal circulation due to a deficient washout (25). This theory has not yet been confirmed. The low number of thrombotic events (twelve) and the resulting large CI means these results should be viewed with caution. Corroborating this finding, Abdel-Razik *et al*, achieved a similar result, with PBFV < 15cm/s at baseline predicting a significantly higher occurrence of PVT, with a mean basal value of 11.6±4.3cm/s for those who developed PVT versus 17.9±4.5cm/s (*P*<0.001) for those who did not (89). In a case-control study in which 50 PVT cirrhotic patients were matched with 50 cirrhotic patients without PVT, similar results were found, with a 6 times higher risk for developing PVT for each cm/s decrease in portal blood flow velocity below the cut-off of 15 cm/s (43). Chen *et al*, found no differences in PBFV between groups in a study involving 162 patients, 40 of which with documented PVT; however, this was cross-sectional study in design, not allowing a rigorous assessment of risk factors for PVT (90). A longitudinal retrospective study found same results (34). In a randomized controlled trial focusing on enoxaparin treatment in cirrhotic patients, a lower mean PBFV was not found to be a risk factor for PVT (24). Even though a decreased PBFV is considered to be a risk factor for PVT development and is an attractive hypothesis to explain PVT, there is a need for prospective studies with greater patient number and with well-standardized measurements of PBFV to be conducted.

Non-selective beta-blockers are routinely used on patients with cirrhosis in the context of primary or secondary prophylaxis of variceal bleeding (10), due to their effect on reducing portal hypertension via β1 (lowers cardiac index) and β2 receptor blockade (induces splanchnic vasoconstriction) (134). Non-selective beta-blocker use also lowers bleeding-associated mortality (135). However, despite these positive effects of NSBB, the reduction of PBFV and related effect on PVT genesis by inducing blood stasis has been hypothesized (136). Preliminary results of a small cohort of 56 patients with cirrhosis only presented in an abstract form, found NSBB to be a risk factor for PVT development (137). The role of NSBB on PVT genesis has not yet been confirmed.

4.1.2.2. Other markers of portal hypertension

As previously stated, the severity of the intrahepatic blockade is related to increased portal hypertension which, in turn, may lead to a deceleration of the PBFV, increasing local blood stasis and precipitating PVT development. It is reasonable, therefore, to search for other markers of portal hypertension and try to relate them with PVT.

Systemic collaterals. Portosystemic collaterals are vessel structures that are formed in order to bypass an occlusion or an anatomic distortion, from a high to a low-pressure vascular bed (138). They may be classified in 4 groups, one where the protector epithelium joins the absorption epithelium leading to esophageal, gastric and rectal varices; a second group with the recanalization of the falciform ligament through the umbilical vein; a third group with vascular collaterals formed in the contact zones of abdominal organs with retroperitoneal tissue or adjacent to the abdominal wall; and a fourth group with a portosystemic shunt through the renal vein (139). Studies addressing the role of systemic collaterals on future PVT occurrence are lacking. Maruyama *et al*, in a longitudinal retrospective study enrolling only virus-related cirrhotic patients, found baseline flow volume in the largest collateral vessel (left gastric vein, short gastric vein and splenorenal shunt were evaluated) as an independent risk factor for PVT development (34). This increased risk could be related to a "stolen effect" causing deviation of blood from the portal vein trunk to collateral vessels leading to local stasis. However, the authors, found no difference in PBFV in patients developing, or not, PVT. Also, they state that the presence of collaterals would lead to a deviation of active thrombin from the portal vein trunk (34), contradicting Zocco's previous theory (25). Gastroesophageal varices are collateral vessels/ portosystemic shunts. An acute upper hypertensive bleeding episode has been named as the major sign of PVT, and has been found in up to 82.4% of patients experiencing PVT (18). The concomitant presence of PVT in the acute variceal bleeding setting has long been related to a more severe bleeding episode and increased rebleeding rates in a population of cirrhotic patients that underwent portal decompressive surgery as treatment for upper hypertensive hemorrhage (17). Nonami *et al* found a statistically significant association between previous GB and PVT occurrence by the time of LT; however being a study with a retrospective character, no causal effect could be established (19). Francoz *et al* found that previous variceal bleeding in a cohort of cirrhotic patients listed for LT was a risk factor for PVT in multivariate analysis (22). In a cross-sectional study also enrolling only cirrhotic patients awaiting LT, only a past history of variceal bleeding increased 2.5 times the risk of PVT (140). However Hernandez-Conde *et al* did not find a previous upper GB episode to be a risk factor for subsequent PVT occurrence, in their longitudinal retrospective study (141), nor did Villa *et al* in their randomized trial (24). So, gastroesophageal

variceal bleeding may be a form of clinical presentation of PVT, but if there is a cause-effect relationship must still be established in future prospective studies. Variceal bleeding can, otherwise, reflect a more severe state of portal hypertension that could be expressed by the size of esophageal varices. However, no relationship between the degree of esophageal varices and PVT has been found by the majority of published papers to date (22, 24, 25, 89). Importantly, it is also necessary to establish if the presence of bigger gastroesophageal varices or their bleeding, mirroring more severe portal hypertension also reflects diminished PBFV favoring, in this way, PVT.

Hypersplenism and its consequences. Banti was the first to relate the presence of reduced peripheral blood cells to enlarged spleens, but it was Chauffard who first used the term "hypersplenism" in 1907 (65). The spleen has a particular anatomic relationship with the liver through the portal vein system, so that portal hypertension (irrespectively of the etiology) is considered one of the multiple causes leading to splenomegaly and consequent hypersplenism with thrombocytopenia and other cytopenias (65, 142). The role of portal hypertension in spleen enlargement is also reinforced in studies showing a decrease in spleen size and related hypersplenism in patients after undergoing LT (66). Nevertheless, and even though after LT an almost complete normalization of splanchnic circulatory changes is seen, spleen size does not completely normalize in most of the patients, meaning that the "hyperplasia" component does not resolve after LT (143). However, the increase in spleen size in the context of the intrahepatic blockade, such as the one occurring in cirrhosis, has not been systematically found to be directly correlated to an increase in portal pressure in most of the older studies (144-146), but to a related increase in splenic arterial inflow (145) and pulp hyperplasia (146, 147). So, splenomegaly must also be attributed to mechanisms other than an increased portal hypertension alone, which means that the term "congestive splenomegaly" is an over simplification to justify spleen enlargement in cirrhotic patients. However, studies enrolling more patients and with the current methodologies to measure portal pressure (notably the hepatic venous pressure gradient) are lacking in order to definitively clarify this issue. If the cirrhotic liver and related portal hypertension may aid in justifying splenomegaly, the opposite is also true. In cirrhotic patients, there is evidence of local splenic production of endothelin-1, and it has also been documented that higher endothelin-1 levels exist in splanchnic when compared with systemic circulation (148). This endothelial factor is now known to be involved in the pathogenesis of the intrahepatic blockade, inducing local vasoconstriction and fibrogenesis (147) and also to increase portal pressure gradient values (149). Thus, the spleen is still a piece of the portal hypertension hemodynamics puzzle which has to be completely resolved, but evidence exists that the cirrhotic liver may account for splenomegaly

and that splenomegaly may contribute to liver disease progression, both justifying an increased portal hypertension.

If the spleen is related to local hemodynamic disturbance, splenomegaly and its consequences may be linked to an increased risk of PVT development. An increased spleen size (34) and splenic thickness (89) was documented more frequently in patients developing PVT by some authors. A related hypersplenism translated by thrombocytopenia was also found to be an independent risk factor for PVT (22, 89). However, others did not confirm this independent effect of low platelet count on PVT development (24, 25).

Ascites. With the increase in portal hypertension secondary to liver fibrosis progression, and collateral formation, splanchnic and systemic vasodilatation occurs leading to the activation of the renin-angiotensin-aldosterone system and sympathetic nervous system ultimately promoting renal sodium and water retention supporting ascites formation (150). In the context of cirrhosis and portal hypertension, ascites also relates directly to the severity of the hepatic venous pressure gradient (151). A decreased PBFV was found in patients with ascites (152), which is ultimately linked to local blood stasis, possibly favoring PVT. However, even though ascites has been found to be a risk factor for PVT development by some authors (34, 35, 53), this has not been consistently found among studies (24, 140), mirroring different methodological approaches not only in the design of the study and patients enrolled, but also in the grading/ classification of ascites.

4.1.3. Endothelial damage

The endothelium is a major organ comprising the entire circulatory system with a vast number of functions currently recognized (153). Fluid filtration, adjustment of the vascular tone, hemostasis and endocrine functions sum up some of its purposes (153, 154). These functions may be disturbed by local or systemic inflammation or shear stress leading to endothelium dysfunction and the creation of a prothrombotic and antifibrinolytic microenvironment favoring local thrombosis (153, 155). Wanless *et al* has already proposed that intimal inflammation within the smallest veins and sinusoids of the liver could induce thrombosis (36). Even though endothelial damage and dysfunction is recognized as one of the pillars of Virchow's triad, no studies have been conducted so far studying this in relation to PVT. However, it is possible to theorize about the relationship between endothelial dysfunction and PVT.

Inflammation and infection. The luminal surface of the endothelial cell is covered by a sort of sheath, the endothelial glycocalyx layer, which comprises many macromolecules with many functions, one of them being the regulation and adhesion of platelets and leucocytes, important

in the inflammatory response, as well as in the cytokine-mediated enzymatic degradation of the layer in this context (156). Sepsis has long ago been recognized as a model for endothelial glycocalyx layer change in conformation with shedding induced by reactive oxygen species, TNF-α, heparanase, and bacterial endotoxins among others (157). This inflammatory environment is of major importance in explaining multiorgan failure with vasodilatation, increased vascular permeability and activation of the coagulation cascade (157). There are some common points that maybe be shared by the endothelial dysfunction in sepsis and cirrhosis in order to try to establish a model between inflammation and thrombosis.

Von Willebrand Factor. The multifunctional acute-phase glycoprotein VWF is synthesized by the endothelial cell, being secreted by the constitutive or the inducible pathway, this last one being activated by inflammatory stimuli via TNF-α, IL-6 and IL-8 (157, 158). The ultra large multimers of VWF formed in this context are highly thrombogenic, while inducing platelet activation and aggregation, being "dismantled" by ADAMTS-13 in order to maintain homeostasis in normal conditions (158, 159). Von Willebrand factor also has a role in promoting inflammatory cascade by contributing to leukocyte adhesion (160) and complement cascade activation (161). Increased levels of ultra large multimers of VWF and decreased levels of ADAMTS-13 have been found in association with disseminated intravascular coagulation, severe sepsis and complicated malarial infection, allowing a link to be established between inflammation/ infection and coagulation activation (159). Increased VWF levels have been consistently found to be related to venous thrombosis (162) and have already also been found to be an independent risk factor for PVT development only by a group of researchers (96). As VWF levels are upregulated in cirrhosis in proportion to liver disease severity (72-74), they can play a role in PVT development which may be related to endothelial dysfunction (163).

Endotoxin/ Lipopolysaccharide. The intestinal epithelial barrier is characterized by normal functioning tight and adherens junctions that become disrupted in the context of cirrhosis, portal hypertension, hepatotoxins as alcohol and related acetaldehyde, and local expression of pro- and anti-inflammatory interleukins (as IL-6, TNF-α, interferon gamma) (164). Lipopolysaccharide (LPS), a major component of the gram-negative bacterial wall, is an endotoxin that, together with other microbial products such as peptidoglycan, lipopetides and bacterial DNA, may translocate from the disrupted intestinal lumen to the mesenteric lymph nodes and other extraintestinal sites (165). The proximity of the gut and the liver establishes a close relationship with direct drainage to the splanchnic vessel bed comprising portal vein. Endotoxemia has long been recognized to be present in patients with liver disease in higher levels than in healthy individuals and its titer rises in proportion to the severity of the liver disease according to Child-Pugh's class (166). The

relationship between endotoxemia and thrombosis in other vessel beds other than portal vein has long been documented. In rabbit animal models, the injection of endotoxin of *Escherichia coli* immediately induced microvascular thrombosis (167). Lipopolysaccharide was also found to increment thrombus extension in arterial and venous vessel beds after its administration in a murine animal model and after the induction of initial thrombus by local ferric chloride injection (168). In humans, microvascular thrombosis and disseminated intravascular coagulation (DIC) has been associated to fatal cases of meningococcal septicemia induced by the liberation of endotoxin (169). Other cases of DIC have been well documented in severe sepsis induced by gram-negative bacteria (170). Endotoxin may promote thrombosis while inducing the expression of VWF (171) and toll-like receptor 4 (TLR4) (172), which is a primary signaling receptor for LPS. Also, LPS may induce the production of TNF-α and IL-6, both leading to tissue factor expression by endothelial cells and subsequently to DIC and eventually thrombosis (170). Downregulation of thrombomodulin (TM) is another way by which LPS may induce thrombosis. Thrombomodulin is a transmembrane glycoprotein mainly synthesized by vascular endothelial cells that serves as a receptor for thrombin, reducing its procoagulant activity and therefore having anticoagulant properties (173). In the absence of TM, thrombin activates fibrinogen to generate fibrin inducing clot formation (174). In patients with sepsis and DIC, TM is downregulated facilitating and perpetuating coagulation and inflammatory cascade (174). The inhibitory effect of TM was found to be lost in a murine model in which LPS, after being administered to mice, induced activation of coagulation confirmed by the measurement of thrombin-antithrombin complex, with an increase in endogenous thrombin potential (175). Starr *et al* also documented an increase in fibrin formation, no increase in activated protein C and a profound and sustained downregulation of TM expression after LPS administration to mice, mainly seen in the older but not the younger animals (176). This downregulation of TM during endotoxemia was also described in a group of young septic patients with severe meningococcemia (177) and in another mouse model with LPS administration in which fibrin deposition was verified in the organs, particularly in the endothelia of the liver (178). Overexpression of tissue factor and downregulation of TM have been found to be LPS-dose dependent (179). If LPS is recognized to induce microvascular thrombosis/ DIC while inducing overexpression of VWF, TLR4, tissue factor, cytokines liberation and downregulation of TM, and if LPS levels are raised in cirrhosis in relation to the degree of portal hypertension and bacterial translocation, it is reasonable to consider that LPS may play a role in PVT genesis. Violi *et al* has addressed this issue proposing that a hypercoagulable state induced by overexpression of tissue factor and VWF secondary to endotoxemia, as a consequence of endothelial dysfunction, would be determinant to splanchnic and systemic vein thrombosis in cirrhosis (180). However, no

specific prospective study has ever been made addressing this issue.

Local inflammation/ infection/ injury. The relationship between local inflammation and thrombosis was established more than a century ago (181). Portal vein thrombosis associated to local infection or in contiguous structures to the portal system characterizes pylephlebitis (182). In a recent retrospective study enrolling 95 patients from Mayo Clinic, pancreatitis, diverticulitis and peritonitis were the leading conditions associated to pylephlebitis, with bacteremia found in 44% of the patients (183). However, whether cirrhosis confers a different added risk for pylephlebitis development is yet to be determined, since no studies have ever addressed this issue.

Spontaneous bacterial peritonitis (SBP) refers to primary infection of the peritoneal fluid with cultural positivity in approximately 40% of the cases, in which *Escherichia coli* is the most commonly isolated gram-negative bacteria (5). A Spanish retrospective longitudinal study enrolling cirrhotic patients listed for LT found, in univariate analysis, SBP as being more frequent among those who developed PVT (141). However, a prospective study conducted by Villa *et al* found no relationship between previous episodes of SBP and PVT occurrence (24).

Splenectomy, colectomy and other intra-abdominal surgeries as well as abdominal trauma and portocaval shunt procedures are some of the local risk factors that have been identified as promoters of PVT while inducing direct endothelial damage, but are not specific to cirrhosis (184).

4.2. Beyond Virchow's triad

Considerations must be undertaken if a specific etiology for cirrhosis is implicated in a more prothrombotic environment, eventually related to an increased inflammatory milieu and so an increased risk for PVT development. Consensus on this subject does not exist. Amitrano *et al* found a more common hepatitis specific viral etiology among 72% of patients with PVT (23). Maruyama *et al* in a retrospective longitudinal study enrolling 150 patients with virus-related cirrhosis noted a prevalence of 28% of PVT, which is higher than other series with mixed etiologies of cirrhosis (34). Autoimmune hepatitis (21), cryptogenic cirrhosis (21, 53), nonalcoholic steatohepatitis (53, 54, 185) have been found to be related to PVT development. However, others did not find any relationship between the etiology of underlying liver disease and PVT development. (20, 25, 89, 140) These discrepant results may result from bias of selection, different methodological approaches for inclusion and regional discrepancies concerning etiologies of cirrhosis. However, if some etiology is found to be associated to PVT development, the most probable cause is the related proinflammatory environment and eventual link to endothelial damage, once again bringing up one of the pillars of Virchow's triad.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

1. THROMBOCIR STUDY (186) – *APPENDIX 3*

This study was conducted in Paris, France. It gathered 1243 Child A and B patients deviating from a multicenter cohort (43 liver referral centers in France and Belgium) of 1278 patients prospectively followed (Protocol CHC 2000), and whose primary purpose was to address the best periodicity (3- versus 6-month) for HCC screening, after exclusion of 35 patients with PVT at inclusion (187). This study was registered at clinicaltrials.gov website (http://clinicaltrials.gov/ct2/show/NCT00190385). Patients were enrolled between June 2000 and March 2006. At each visit clinical and biological parameters were recorded. All patients underwent DUS allowing registration of PBFV and occlusion of the portal vein trunk or its branches when present. A more exhaustive and detailed methodology description can be found in the published article (186).

2. FRTVPCIR STUDY (188) – *APPENDIX 4*

This study was conducted in Porto, Portugal. This was a prospective, single-center study (CHUP) in which patients with cirrhosis were enrolled between January 2014 and February 2017. Patients with cirrhosis irrespective of the etiology and degree of liver failure were included, provided they had not had a previous splanchnic or extra splanchnic vein thrombosis, HCC or were under anticoagulation or anti-aggregation treatment. At each visit, a complete follow-up protocol was filled with demographic data, health status characterization and clinical examination. Blood sample collection and abdominal DUS were also performed. When PVT was suspected by the Doppler study, confirmation by a CT-scan on the same day was required. Detailed methodology concerning patient selection and study design, follow-up and data collection, abdominal DUS and portal vein diagnosis and statistical analysis is reported in the published article (188).

2.1. Specific considerations for subanalysis of inflammatory markers and PVT development (unpublished results)

2.1.1. Patient selection and study design

Patients with active infection or hospitalization in the previous 3 months and who were under anti-TNF-α therapy were excluded from final analyses along with all the exclusion criteria reported elsewhere (188).

2.1.2. Blood collection and processing

Blood was drawn without stasis from a peripheral vein, after proper local disinfection with chlorhexidine 2% solution, in tubes containing sodium citrate 3.2%, ethylenediaminetetraacetic acid - EDTA or clot activator and immediately transported to the laboratory. Blood was centrifuged at 2500G for 15 minutes according to local laboratory protocol. Standard analyses were immediately performed at the central biochemical laboratory of CHUP. Serum and plasma were stored in aliquots of 200µL and 500µL in 1,5mL tubes and frozen and stored at -80ºC. Total blood was also stored at -20ºC.

Enzyme-linked immunosorbent assays (ELISA) were performed using Triturus ELISA instrument®. For specific analysis of TNF-α and IL-6, Citomed® commercialized reagents were used: Human TNF-α Quantikine® ELISA Immunoassay kit (Ref.[a]DTA00C) and Human IL-6 Quantikine® ELISA Immunoassay kit (Ref.[a]D6050). Tumor necrosis factor alpha and IL-6 determinations were done according to specific protocols following the manufacturer's instructions with calibrators and samples processed in duplicate. Lower cutoff values of 15.6 pg/mL and 3.13 pg/mL were used considering a population of healthy donors with TNF-α and IL-6 levels inferior to the calibrator of the lower concentration.

2.1.3. Statistical analysis

Summary statistics, namely, percentages, means or medians (normal distribution was assessed using the Kolmogorov-Smirnov test) and respective standard deviations or interquartile range were computed. Comparisons between continuous variables and the occurrence of PVT were made using independent samples t test or Mann-Whitney U test for skewed distributions. Cause-specific hazards were modeled using the Cox proportional hazards model, with the cause-specific HR as the measure of the association between covariates and outcome. Log-linear relationships and proportional hazards assumptions were checked. Multivariate models included variables significantly associated with the outcome in univariate analyses at a level of 5% as well as

variables previously reported to be associated with an increased risk of PVT in patients with cirrhosis. A step-wise selection procedure was used. Ninety-five percent CI's were computed. Time-dependent covariates were used to assess the predictive value of time-dependent measurements of PBFV on the hazard of the development of PVT. As the Kolmogorov-Smirnov test for normality (together with graphic observation of the distribution) indicated that IL-6 didn't follow a normal distribution, the median values were presented and used for comparisons. The comparison of the median IL-6 values according to patient characteristics was estimated using the non-parametric Kruskal-Wallis test.

Statistical analyses were performed using Stata version 11.2 for Windows (Stata Corp LP, College Station, TX, USA).

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

1. THESIS STUDIES OUTLINE

The results presented in this thesis are derived from the two main prospective studies – THROMBOCIR, appendix 3 (186) and FRTVPCir, appendix 4 (188). The first, the largest longitudinal study published to date, gathering information collected in 43 liver referral centers in France and Belgium and, the second, comprising data collected in a single LT center in Portugal – CHUP.

HYPOTHESIS	AIMS	RESULTS/ STUDY
1. Features related to portal hypertension markers and to the degree of liver failure are at the genesis of PVT development.	To determine PVT risk factors related to portal hypertension (size of esophageal varices, low platelet count, spleen size, ascites, HE) and the degree of liver failure (increased PT/ INR and bilirubin, low albumin, increased MELD).	THROMBOCIR and FRTVPCir Studies.
2. Decreased PBFV is a risk factor for PVT development in patients with cirrhosis.	To determine a possible cause-effect relationship between decreased PBFV and PVT development.	THROMBOCIR and FRTVPCir Studies.
3. The use of NSBB in patients with cirrhosis is related to future PVT development.	To determine the relationship between NSBB use and PVT development. To find possible ways NSBB induce PVT.	THROMBOCIR and FRTVPCir Studies. FRTVPCir study
4. Factor V Leiden and PTHR gene mutations are concurrent risk factors for PVT development in cirrhosis.	To determine FVL and PTHR gene mutations and to settle competing risk for PVT development.	THROMBOCIR study
5. Increased inflammatory markers exist in patients with cirrhosis before PVT development.	To determine inflammatory markers (leukocytes, Hs-CRP, ferritin, TNF-α, IL-6) and related risk for PVT development.	FRTVPCir study – unpublished results

HYPOTHESIS	AIMS	RESULTS/ STUDY
6. PVT is not related to liver decompensation.	(Secondary aim of the study) To determine the impact of PVT on morbidity (decompensation and progression of liver disease) and mortality.	THROMBOCIR study

Table 1. Thesis studies outline gathering general information concerning hypotheses, respective aims and the study conducted to achieve the correspondent results.

The THROMBOCIR and FRTVPCir studies are presented in Appendices 3 (186) and 4 (188), respectively.

Appendix 3

Causes and consequences of portal vein thrombosis in 1,243 patients with cirrhosis: results of a longitudinal study.

Nery F, Chevret S, Condat B, de Raucourt E, Boudaoud L, Rautou PLE, Plessier A, Roulot D, Chaffaut C, Bourcier V, Trinchet JC, Valla DC, Groupe d'Etude et de Traitment du Carcinome Hépatocellulaire. Hepatology. 2015 Feb; 61(2):660-7

doi: 10.1002/hep.27546. Epub 2015 Jan 5.

Appendix 4

Nonselective beta-blockers and risk of portal vein thrombosis in patients with cirrhosis: results of a prospective longitudinal study.

Nery F, Correia S, Macedo C, Gandara J, Lopes V, Valadares D, Ferreira S, Oliveira J, Gomes MT, Lucas R, Rautou PE, Miranda HP, Valla D. Aliment Pharmacol Ther. 2019 Jan

Doi: 10.1111/apt.15137. Epub ahead of print.

2. HYPOTHESES 1, 2 AND 3

HYPOTHESIS	AIMS	RESULTS/ STUDY
1. Features related to portal hypertension markers and to the degree of liver failure are at the genesis of PVT development.	To determine PVT risk factors related to portal hypertension (size of esophageal varices, low platelet count, spleen size, ascites, HE, etc.) and the degree of liver failure (increased PT/ INR and bilirubin, low albumin, increased MELD, etc.).	THROMBOCIR and FRTVPCir Studies.
2. Decreased PBFV is a risk factor for PVT development in patients with cirrhosis.	To determine a possible cause-effect relationship between decreased PBFV and PVT development.	THROMBOCIR and FRTVPCir Studies.
3. The use of NSBB in patients with cirrhosis is related to future PVT development.	To determine the relationship between NSBB use and PVT development. To find possible ways NSBB induce PVT.	THROMBOCIR and FRTVPCir Studies. FRTVPCir study

Table 2. General information regarding hypotheses 1 to 3, respective aims and correspondent studies.

Both cohorts mainly enrolled cirrhotic patients with a more stable liver disease. The THROMBOCIR study, involving 1243 patients, gathered 863 Child-Pugh A and 380 Child-Pugh B patients, with 118 patients (9.5%) developing PVT; while the FRTVPCir study, with 108 patients enrolled, 84, 19 and 5 Child-Pugh A, B and C patients, respectively, with 11 of them (10.2%) being diagnosed with PVT. Follow-up time was longer in the THROMBOCIR than in the FRTVPCir study (mean follow-up 47 months *versus* 19.4 months).

Only medium or large-sized esophageal varices (HR=2.14; 95% CI: 1.27-3.60, *P*=0.004 and HR=5.67; 95% CI: 1.49-21.63, *P*=0.011 in the THROMBOCIR and FRTVPCir studies, respectively), as a variable related to a more severe degree of portal hypertension, was identified as a risk factor for PVT development in both studies (Tables 3 and 4, respectively). A more severe liver disease

documented with increased prothrombin time in the THROMBOCIR study was also one of the variables linked to PVT development (Table 3).

Neither a decrease in PBFV with time in the THROMBOCIR study (Table 3) nor a lower PBFV at baseline in the FRTVPCir study (HR=1.04; 95% CI: 0.92-1.17, *P*=0.897) were associated to future PVT occurrence.

THROMBOCIR STUDY						
Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Etiology of cirrhosis						
HCV +/- alcohol	0.72	0.49-1.04	0.08			
Alcohol	1.50	1.05-2.16	0.028			
Serum bilirubin (µmol/L)	1.16	1.06-1.27	0.001			
ALT (N<40 IU/L)	0.77	0.61-0.98	0.036			
Prothrombin time (%)	0.76	0.68-0.86	<0.0001	0.82	0.68-0.98	0.03
Medium or large esophageal varices	2.15	1.43-3.23	0.0002	2.14	1.27-3.60	0.004
De novo ascites*	1.81	1.14-2.89	0.01			
Decreasing portal vein blood flow velocity*	0.98	0.95-1.01	0.19			
Non-selective beta-blocker*	1.67	1.02-2.73	0.04			

Table 3. THROMBOCIR main and significant risk factors in univariate and multivariate analysis; HCV, hepatitis C virus; ALT, alanine aminotransferase; *Predictive factors evaluated as time-dependent variables. (Adapted from Supplemental Tables 1, 2 and 3 from Nery F. *et al*, Hepatology 2015 [186]).

Despite the fact NSBB were related to PVT development in univariate but not in multivariate analysis (Table 3) in the THROMBOCIR study, they played a role as a risk factor for future PVT occurrence in FRTVPCir (Table 4), independently of their effect on lowering heart rate or on decreasing PBFV.

FRTVPCir STUDY				
Variable		HR	95% CI	*P*
Esophageal varices (Medium/ Large *vs* Null/ small)				
	Crude	5.67	1.49-21.63	0.011
	Adjusted for NSBB	2.45	0.55-10.89	0.238
NSBB (yes *vs* no)				
	Crude	10.56	1.35-82.73	0.025
	Adjusted for PBFV	12.47	1.58-98.43	0.017
	Adjusted for heart rate	13.66	1.51-123.85	0.020
	Adjusted for EV	6.15	0.63-59.96	0.118

Table 4. FRTVPCir multivariate Cox proportional models of predictive factors for portal vein thrombosis development, adjusted for potential confounders. NSBB, Non-selective beta-blocker; PBFV, Portal blood flow velocity (cm/s); EV, Esophageal varices (Adapted from Table 2 from Nery F. *et al*, Aliment Pharmacol Therap 2019 [188]).

3. HYPOTHESIS 4

HYPOTHESIS	AIMS	RESULTS/ STUDY
4. Factor V Leiden and PTHR gene mutations are concurrent risk factors for PVT development in cirrhosis.	To determine FVL and PTHR gene mutations and to settle competing risk for PVT development.	THROMBOCIR study

Table 5. General information concerning hypothesis 4, respective aims and correspondent study.

Factor V Leiden and PTHR gene mutations were searched for in the 3 most represented centers out of the 43 involved in the THROMBOCIR study: Beaujon, Jean Verdier and Avicenne Hospitals in 302 patients out of a total of 428 enrolled in the 3 centers. After excluding Child C patients, 283 patients were analyzed and FVL mutation was present in 5% of the patients (13 patients in heterozygosity and in 1 patient in homozygosity) and PTHR gene mutation in heterozygosity in 8 (3%) of them. No relationship was found between the presence of either of these mutations and PVT development (HR=1.84; 95% CI: 0.68-4.98, *P*=0.23).

4. HYPOTHESIS 5

HYPOTHESIS	AIMS	RESULTS/ STUDY
5. Increased inflammatory markers exist in patients with cirrhosis before PVT development.	To determine inflammatory markers (leukocytes, Hs-CRP, ferritin, TNF-α, IL-6) and related risk for PVT development.	FRTVPCir study – unpublished results

Table 6. General information concerning hypothesis 5, respective aims and correspondent study.

A panel of inflammatory markers usually used in the daily clinical routine setting (leucocytes, neutrophils, lymphocytes, Hs-CRP and ferritin) together with TNF-α and IL-6 were determined. For specific analysis of potential inflammatory markers as risk factors for PVT development, 107 patients were considered from the FRTVPCir study. One patient out of 108 that was under anti-TNF-α therapy (etanercept) for psoriasis was excluded from the final analysis. This specific patient did not developed PVT in the course of the follow-up. Portal vein thrombosis occurred in 11 out of 107 patients (10.3%).

Baseline clinical, laboratory and DUS findings are expressed in Table 7. No major differences exist when comparing the original cohort of patients. Tumor necrosis factor alpha levels were below the lowest limit of detection in all patients tested. Lower lymphocyte count and increased IL-6 at baseline were related to future PVT development (Table 8). As IL-6 did not follow a normal distribution, median values were taken into consideration, with a clear association to PVT development above 5.5 pg/mL (HR=5.64; 95% CI: 1.21-26.33, *P*=0.028). To determine the effect of IL-6 and lymphocytes on PVT development, adjustment to variables for potential confounders was performed (Table 9). The association between increased IL-6 values and PVT remained significant even after adjusting for all the considered variables at the same time (HR=8.79; 95% CI: 1.42-54.44). Low lymphocyte count at baseline was also a marker of future PVT occurrence (HR=0.18; 95% CI: 0.04-0.80, *P*=0.023). On average, lymphocytes decreased in both groups (PVT *versus* no PVT development) with time. The variation before the occurrence of the thrombotic event (an average decrease of 0.043 ± 0.364 x 10^9/L) was more pronounced (0.132 ± 0.163 x 10^9/L) than that which occurred in patients who did not develop PVT at the end of follow-up (0.033 ± 0.394 x 10^9/L), even if with no statistical significance (*P* = 0.413).

	Without PVT (N=96)	With PVT (N=11)
Age (years)	54.1± 11.0	57.8 ± 8.5
Male gender	69 (71.9%)	6 (54.5%)
Aetiology of cirrhosis		
Alcoholic	43 (44.8%)	4 (36.4%)
Viral[a]	14 (14.6%)	1 (9.1%)
Alcoholic + Viral[a]	11 (11.5%)	3 (27.3%)
Metabolic[b]	12 (12.5%)	0 (0.0%)
Autoimmune	12 (12.5%)	3 (27.3%)
Cryptogenic	4 (4.2%)	0 (0.0%)
Current alcohol use	7 (7.3%)	2 (18.2%)
Current NSBB use	47 (49.0%)	10 (90.9%)
Ascites	19 (19.8%)	4 (36.4%)
Esophageal varices (grade≥2)	28 (289.2%)	8 (72.7%)
Child-Pugh A/ B/ C	74 (77.1%)/ 17 (17.7%)/ 5 (5.2%)	9 (81.8%)/ 2 (18.2%)/ 0 (0%)
MELD ≥ 13	19 (19.8%)	2 (18.2%)
Albumin (g/dL)	4.2 ± 0.6	3.9 ± 0.6
TB (mg/dL)	1.5 ± 1.4	1.5 ± 0.6
AST (U/L)	44.2 ± 31.7	44.2 ± 19.8
ALT (U/L)	38.1 ± 31.6	29.5 ± 15.8
INR	1.25 ± 0.24	1.26 ± 0.19
Platelets (10^9/L)	109.3 ± 57.4	84.4 ± 37.7
Portosystemic collaterals	21 (21.9%)	3 (27.3%)
PBFV (cm/s)	20.4 ± 5.0	20.6 ± 6.1
Spleen size (cm)	15.1 ± 3.4	16.1 ± 3.3
Leucocytes (10^9/L)	5.2 ± 2.1	4.3 ± 2.1
Neutrophils (10^9/L)	3.2 ± 1.3	2.7 ± 1.8
Lymphocytes (10^9/L)	1.4 ± 0.7	0.95 ± 0.4
Hs-CRP (mg/L)	5.4 ± 8.9	8.7 ± 10.7
Ferritin (ng/mL)	290 ± 379	237 ± 302
TNF-α (pg/mL)	*	*
IL-6 (pg/mL) [Median (P25-P75)]	4.8 (1.6-9.9)	7.6 (5.8-19.3)

Table 7. Clinical, abdominal Doppler ultrasound and laboratory findings, including considered inflammatory markers (shaded rows) at baseline in patients with cirrhosis who did or did not eventually develop portal vein thrombosis (PVT). Data are expressed as mean ± SD and categorical variables as frequencies (%). [a]Hepatitis B virus and/ or hepatitis C virus; [b] Wilson's disease or hemochromatosis or non-alcoholic steatohepatitis or alpha-1 antitrypsin deficit; [c] NSBB, Non-selective beta-blockers; MELD, model for end-stage liver disease; TB, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalized ratio; PBFV, portal blood flow velocity; Hs-CRP, high-sensitive C-reactive protein; TNF-α, tumor necrosis factor alpha; IL-6, Interleukin-6; *All subjects with TNF-α levels below the lower limit of detection.

The risk of PVT development, when considering low lymphocyte count was, therefore, already present at baseline. Portal vein thrombosis developed more often in patients with lymphocyte count less than the median value of 1.2 x 10^9/L (Figure 1). Patients with lymphocyte count less than 1.2 x 10^9/L presented an almost 6 times higher risk of PVT (*P* = 0.041).

	HR	95% CI	*P*
Leucocytes (10^9/L)	0.74	0.50-1.09	0.127
Neutrophils (10^9/L)	0.73	0.42-1.24	0.245
Lymphocytes (10^9/L)	0.18	0.04-0.80	0.023
Log Hs-CRP (mg/L)	1.43	0.89-2.29	0.135
Ferritin (ng/mL)	1.00	1.00-1.00	0.743
IL-6 > 5.5 pg/mL (*vs* ≤ 5.5 pg/mL) *	5.64	1.21-26.33	0.028

Table 8. Time-dependent predictive factors for portal vein thrombosis from univariate Cox models on inflammatory markers. Hs-CRP, high-sensitive C-reactive protein; IL-6, Interleukin-6; * Observed IL-6 median value was 5.5 pg/mL.

		HR	95% CI	P
Interleukin-6 > 5.5 pg/mL (*vs* ≤ 5.5 pg/mL) *				
	Crude	5.64	1.21-26.33	0.028
	Adjusted for NSBB	5.00	1.05-23.65	0.043
	Adjusted for alcohol	5.55	1.18-26.00	0.030
	Adjusted for MELD ≥ 13	5.97	1.24-28.8	0.026
	Adjusted for spleen size	5.50	1.17-25.90	0.031
	Adjusted for collaterals	5.63	1.20-26.47	0.029
	Adjusted for EV	4.96	1.05-23.23	0.042
	Adjusted for ascites	4.91	1.00-24.11	0.050
Lymphocytes (10^9/L)				
	Crude	0.18	0.04-0.80	0.023
	Adjusted for spleen size	0.19	0.04-0.87	0.033

Table 9. Multivariate Cox proportional models of predictive factors for portal vein thrombosis (PVT) development, adjusted for potential confounders. NSBB, Non-selective beta-blocker; MELD, Model for end-stage liver disease; EV, Esophageal varices; * Observed Interleukin-6 median value was 5.5 pg/mL.

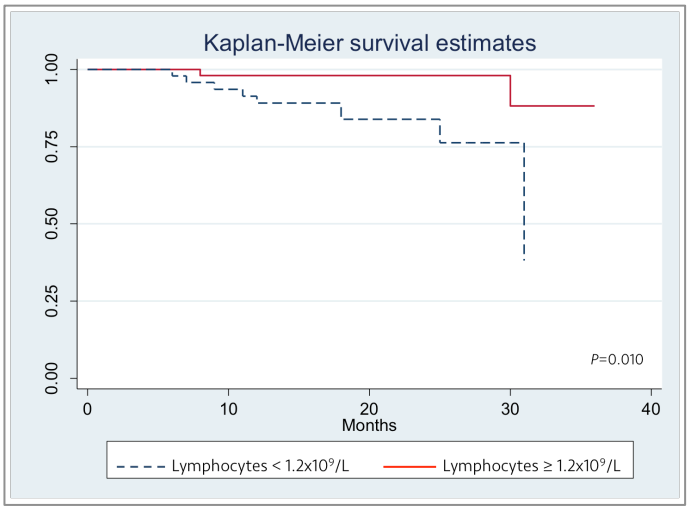

Figure 1. Incidence of portal vein thrombosis in patients with cirrhosis according to lymphocyte count (cut-off point in the median).

Even though IL-6's effect over PVT development was unrelated to the global set of the variables tested, their levels were significantly increased in patients with high-grade esophageal varices and in those with collaterals found in DUS (Table 10).

	HR	Median IL-6 value (pg/mL) 95% CI	*P*
Esophageal varices			
Grade <2	4.23	1.55-6.4	
Grade ≥2	6.96	4.94-9.27	0.048
Spleen size (cm)*			
< 15	4.41	1.55-6.19	
≥ 15	6.16	4.23-8.02	0.4694
Collaterals			
Absent	4.73	3.20-5.92	
Present	10.05	4.92-12.60	0.018
Alcohol consumption			
No	5.08	3.32-6.4	
Yes	9.73	1.55-12.8	0.127
Antibiotic prophylaxis			
No	5.27	3.32-6.81	
Yes	9.27	1.55-74.6	0.145
Hs-CRP (mg/L)*			
< 2	1.55	1.55-3.32	
≥ 2	10.2	8.02-12.6	0.0001

Table 10. Median Interleukin-6 (IL-6) values (pg/mL) according to patient characteristics. Hs-CRP, High-sensitive C-reactive protein. *Cut-offs defined according the median values observed in this sample.

5. HYPOTHESIS 6

HYPOTHESIS	AIMS	RESULTS/ STUDY
6. PVT is not related to liver decompensation.	(Secondary aim of the study) To determine the impact of PVT on morbidity (decompensation and progression of liver disease) and mortality.	THROMBOCIR study

Table 11. General information concerning hypothesis 6, respective aims and correspondent study.

In the large cohort of patients enrolled in the THROMBOCIR study, progression and decompensation of liver disease were defined in detail as expressed in the methodology section of the published article (186) (Appendix 3): liver disease decompensation as a composite including clinically detectable ascites, HE, variceal bleeding, jaundice or serum bilirubin higher than 45 μmol/L (2.5mg/dL), and liver disease progression as a composite including any of the aforementioned or any of the following laboratory findings: PT < 45%, serum albumin < 28g/L, or serum creatinine > 115 μmol/L (1.3mg/dL). Fifty two and 39 patients progressed and decompensated respectively out of the 118 who were diagnosed with PVT, while 303 and 201 patients progressed and decompensated respectively out of 1125 patients without PVT. In those who developed PVT and progressed and/or decompensated, 23, 5 and 24 progressed before, on the same day and after PVT diagnosis respectively, while 16, 5 and 19 patients decompensated before, on the same day or after PVT diagnosis respectively. In multivariate analysis, PVT did not impact liver disease progression or decompensation, irrespectively of the degree of occlusion (Table 12).
The presence of at least medium-sized esophageal varices was significantly related to progression (HR=1.70; 95% CI: 1.21-2.38, *P*=0.002), decompensation (HR=2.60; 95% CI: 1.78-3.81, *P*<0.0001) and death (HR=2.00; 1.22-3.26, *P*=0.0056). Also, an increased PT correlated well to liver disease progression (HR=0.79; 95% CI: 0.94-0.99, *P*=0.002) and decompensation (HR=0.73; 95% CI: 0.63-0.84, *P*<0.0001).

	Univariable models unadjusted estimates			Adjusted for baseline prognostic variables*		
Models	HR	95% CI	*P*	HR	95% CI	*P*
Liver disease progression						
Partial PVT	1.58	1.02-2.45	0.04	1.51	0.73-3.14	0.27
Partial or Complete PVT	1.48	0.97-2.26	0.067	1.32	0.68-2.55	0.41
Liver disease decompensation						
Partial PVT	1.77	1.07-2.92	0.027	1.60	0.69-3.74	0.28
Partial or Complete PVT	1.61	0.98-2.62	0.058	1.37	0.62-3.03	0.44

Table 12. Impact of portal vein thrombosis (PVT) on liver disease progression and decompensation. Models of the estimation of PVT effect as time-dependent variable from Cox models stratified on randomization arms. *age, esophageal varices, creatinine, bilirubin, prothrombin time, albumin and body mass index. (Adapted from Table 3 THROMBOCIR study [186]).

CHAPTER V

Discussion

CHAPTER V

DISCUSSION

1. HYPOTHESES 1, 2 AND 3

AIMS	MAIN RESULTS
1. To determine PVT risk factors related to portal hypertension and degree of liver failure.	Esophageal varices ≥ grade 2 were clearly associated with PVT in both studies and increased PT in THROMBOCIR study.
2. To determine a possible cause-effect relationship between decreased PBFV and PVT development.	Decreased PBFV was not associated with PVT development, either at baseline or its decrease with time.
3. To determine the relationship between NSBB use and PVT development. To find possible ways NSBB induce PVT.	Patients on NSBB are at risk of developing PVT, irrespectively of their effect on a decrease in heart rate or in PBFV.

Table 13. Main results regarding proposed aims for the three first hypotheses.

In contrast to the majority of the works conducted to date in which populations with more severe liver disease are usually involved, we determined risk factors for PVT development in more stable liver disease patients, since the THROMBOCIR study enrolled only Child-Pugh A (mostly) and B patients, 863 and 380, respectively, and the FRTVPCir study included a vast majority (95%) of Child-Pugh A and B patients, 84 and 19, respectively. In the FRTVPCir study, in the remaining and residual population of Child-Pugh C patients no thrombotic event was documented. This is important, as PVT may be seen as a non-negligible event even in less severe liver cirrhotic patients, since we achieved a PVT cumulative incidence of 4.6%, 8.2% and 10.7% in the 1st, 3rd and 5th years in THROMBOCIR and a global incidence of 10.2% in the FRTVPCir studies. Still, theses incidence rates are somehow lower than the ones found by Zocco *et al* (16.4% in the 1st year; PVT found in 12 patients among 73 that completed follow-up)(25) and Abdel-Razik *et al* (17.9% in the 1st year; PVT found in 17 patients among 95 that completed follow-up)(89) in a mixed Child-Pugh population of cirrhotic patients, though, more severe ones.

Both studies were clear in finding the presence of at least medium-sized esophageal varices as a risk factor for PVT, expressing a more severe state of portal hypertension. Even if some authors achieved the same results in a retrospective cohort (189), higher grades of esophageal varices were not consistently found to be particularly associated to PVT by others (25, 89). Nevertheless,

it is still necessary to try to establish an eventual link between this more severe portal hypertension state, as expressed by higher degrees of esophageal varices and related local blood stasis induced by a decrease in PBFV. This seems to be reasonable, as esophageal varices, being collaterals deviating blood from portal vein tract could, at least in theory, decrease PBFV. Curiously, both of our studies (186, 188) that identified esophageal varices as being predictors of PVT did not find PBFV as a risk factor for PVT and both other longitudinal studies that associated a decrease in PBFV to PVT, did not find esophageal varices size to be predictive of PVT (25, 89). Methodological questions must be raised, since important issues (such as standardization of timing and evaluation of esophageal varices before patient inclusion, those related to PVT evaluation, and particularities concerning the equipment used, Doppler beam incidence, interobserver variability, etc.) are still to be definitively resolved.

As stated, patients with more severe liver disease are those who may be more prone to develop PVT (19-21, 190). The severity may be explained not only by signs of portal hypertension as aforementioned, but also by a deficit in liver synthesis characterized by high levels of bilirubin, lower levels of albumin and longer PT. Also, liver insufficiency may be "quantified" by means of the Child-Pugh score evaluation in which these analytical parameters taken together with ascites and HE are evaluated (191, 192) and by the MELD score (193) which uses, besides bilirubin and INR values, creatinine levels. Both the THOMBOCIR and FRTVPCir studies failed in finding any association between advanced Child-Pugh scores or a higher MELD grade with PVT. However, both studies enrolled a vast majority of patients with a more stable and not advanced liver disease, so, with less important deficit of synthesis. Nevertheless, THROMBOCIR, with 1243 patients, being a more powerful study, found that increased PT was associated with PVT (186). High MELD score was associated with PVT development by Zocco *et al*, however it lost significance in multivariate analysis (25). Abdel-Razik *et al* even settled a MELD score cut-off of 15 in which higher punctuation was related to PVT occurrence. Higher MELD scores may imply an acquired prothrombotic associated condition favoring PVT, as increased D-dimers have been found in patients with higher MELD scores (25, 89), as well as decreased protein C and antithrombin (25). Liver insufficiency may play a role in the future development of PVT, despite being weak and only seen in powerful studies, and probably associated to related acquired prothrombotic condition.

Portal blood flow velocity decreases with the increased severity of liver failure expressed by higher Child-Pugh scores (130) and has also been associated to decreased survival (132). As PVT is expected to be more prevalent among the severest cirrhotic patients, if a decreased PBFV is expected in these patients, PVT would be therefore explained by this mechanism. Zocco *et al* (25) and Abdel Razik *et al* (89) found an association between reduced PBFV (< 15cm/s) at baseline and

future PVT development. These findings were recently supported by a retrospective case-control study matched for age, gender and MELD score, in which for each decrease of 1 cm/s in PBFV less than 15cm/s there was a 6-times higher risk of developing PVT (43). However, a decreased PBFV was not always found to be associated to PVT (90). THROMBOCIR (186) and FRTVPCir (188) studies failed in establishing a relationship between lower PBFV and increased risk for PVT development. THROMBOCIR is to date the most powerful study involving the largest cohort of patients. Different operators with different equipment in the 43 centers involved may add some fragility to the data. However, in order to diminish associated confusing factors, the operator and equipment used was always the same in the follow-up of each one of the patients and PBFV was seen as a time-dependent variable, strengthening our results (186). FRTVPCir followed a similar methodology as the one adopted by Zocco *et al* (25), with all the Doppler measurements validated by a senior (and always the same) radiologist consultant (188). We cannot conclude, based on our findings in both studies an independent relationship between PBFV and PVT, which means that this is not a settled issue and standardization of methodologies that involve same fasting periods, equipment, incidence beams and validation by other operators must be considered in future investigations. Other methods of measuring PBFV other than by DUS that can be reproduced and validated by others should be considered in future works such as, for, example, four-dimensional flow magnetic resonance (194).

Non-selective beta-blockers are commonly used for primary and secondary prophylaxis of variceal bleeding (10). Their risk for PVT development was proposed some years ago due to a possible effect on lowering PBFV (136). Pellicelli *et al*, in an unpublished study found an association between NSBB use and PVT (137). Two recent retrospective studies also achieved similar results, but no information concerning time under treatment, dose used, and hemodynamic aspects were considered (189, 195). We found that NSBB were related to PVT occurrence, this effect being more pronounced in FRTVPCir (188) than in THROMBOCIR (186). The FRTVPCir study is, to date, the only prospectively conducted study that specifically addressed this problematic and tried to find the mechanisms by which NSBB could be related to PVT development. We clearly documented, in patients under NSBB treatment, not only a reduction in PBFV but in heart rate as well. Contrary to what was expected, these effects by NSBB use did not justify PVT, thus the underlying mechanism remains unknown. The administered dose of NSBB also was not related to PVT even though a tendency towards higher doses was found (188). Nevertheless, older studies found that patients with cirrhosis had an enhanced sympathetic nervous system activity in relation to the severity of the liver disease (196, 197). Valla *et al* found that in patients injected with propranolol (a NSBB), subsequent infusion of adrenalin led to further decrease in azygos blood flow (198). This means

that in patients with cirrhosis under NSBB therapy an additive and enhanced effect of catecholamines released by an already activated sympathetic nervous system, which could be in proportion to the severity of the liver disease, could lead to further hemodynamic disturbance helping to justify the implication of NSBB therapy on PVT development and probably in identifying patients in which NSBB should not be used. This is an important issue to be addressed in future studies since NSBB are widely used in patients with cirrhosis.

2. HYPOTHESIS 4

AIMS	MAIN RESULTS
4. To determine FVL and PTHR gene mutations and to settle competing risk for PVT development.	No risk attributed to the presence of FVL, PTHR gene mutations, or both, and PVT development was found.

Table 14. Main results regarding proposed aims for the fourth hypothesis.

Current guidelines advise the screening of genetic conditions favoring thrombosis, namely the presence of FVL and PTHR gene mutations for patients with cirrhosis, diagnosed with PVT (7, 10). In the THROMBOCIR analyzed population, only 5% and 3% of the patients revealed positivity for FVL and PTHR gene mutations respectively, all but one (FVL mutation) in heterozygosity, which was not related to an incremented risk for PVT development (186). Our data is approached to the prevalence of both mutations in the general French population without any thrombotic event (199). The relationship between the presence of these two mutations and an increased risk for PVT development in cirrhosis has not been clearly settled, with even meta-analysis (109, 110), revealing conflicting results among studies. We found other risk factors to be more important than the genetic ones that, in the context of cirrhosis, predispose patients to PVT.

3. HYPOTHESIS 5

AIMS	MAIN RESULTS
5. To determine inflammatory markers (leukocytes, Hs-CRP, ferritin, TNF-α, IL-6) and related risk for PVT.	Increased IL-6 levels and decreased lymphocyte count at baseline predicted PVT development. Interleukin-6 levels were elevated in patients with some features of more severe portal hypertension (higher esophageal varices grade and presence of collaterals).

Table 15. Main results regarding proposed aims for the fifth hypothesis.

No study has specifically addressed the issue of inflammation and related PVT risk in patients with cirrhosis. The scarce data is extracted from published papers mainly as secondary outcomes.

We found that a low lymphocyte count at baseline was related to future PVT occurrence, with a 6-times higher risk when below the cut-off value of 1.2 x 10^9/L. We also found a trend to a decrease in lymphocyte count with time in all patients, more evident in those developing PVT, even if without statistical meaning. Vascular inflammation, as a result of the interaction between platelets and leucocytes on the activated endothelium (via multiple signalling pathways) may be responsible for microvascular occlusion in many vascular beds (200). In the liver, leucocyte adhesion to the hepatic sinusoidal endothelium is enhanced by platelet binding, namely of lymphocytes mediated by the secretion of CCL2 nuclear factor-κB-dependent (201). Theoretically, this lymphocyte migration/ homing may explain a reduction in their peripheral count, local sinusoidal inflammation and subsequent microvascular occlusion. Thus, lymphopenia could be a marker of a homing effect reflecting local inflammation and by this mean a propensity to thrombosis. However, a direct thrombotic effect of a lower lymphocyte count on any vascular bed is unknown. Curiously, platelet binding to the vascular endothelium has been observed in a larger extent in the portal tract than in the sinusoids (201). Lymphocyte homing to the liver may be explained by this mechanism, but they may also home directly to the spleen in a direct relation to its size reflecting, once again, a higher degree of portal hypertension. However, the action of low lymphocyte count over PVT development was unrelated, in our cohort, to an increase in spleen size. This is currently an open field research issue to be addressed.

Endotoxin levels have been found to be related to the severity of cirrhosis (166) and the severity of cirrhosis to increased circulating IL-6 (202). Also, in a recent cross-sectional study, IL-6 was found to be related to not only to poorer liver function, but also to more severe grades of esophageal

varices and even to mortality (203). Only Villa *et al* in her study addressing specifically the safety and efficacy of enoxaparin in PVT prevention in patients with Child-Pugh B7-C10 cirrhosis evaluated the immune response to bacterial translocation dosing, among others, IL-6 (24). She found increased IL-6 levels in patients with higher soluble CD14 levels (a marker of host response to microbial products, namely LPS), this last being related to PVT development (P=0.030) (24). Unfortunately no results are presented concerning IL-6 relationship to PVT. We present the first study that found a close relation between higher IL-6 levels and PVT occurrence. Even though IL-6 levels increased with the severity of esophageal varices size (validating the results achieved by Kao *et al* [203]) and other markers of portal hypertension such as the presence of collaterals, its effect over PVT is beyond the one related to these features of portal hypertension and eventual local blood stasis, which may be linked to local endothelial dysfunction. Another way to justify the association between IL-6 and PVT is by an increased synthesis of VWF by the endothelial cell which is known to be induced by IL-6, favoring, in this way, thrombosis (157, 158).

Other inflammatory markers tested were not related to future PVT occurrence. Concerning C-reactive protein, only Abdel Razik *et al* and Chen *et al* longitudinally tested this association, which was null (89, 90). We used, as Chen *et al* (90), Hs-CRP, which is more sensitive than the standard test. Even though their levels increased in proportion to IL-6 (Table 10), as expected due to the fact that it is produced after IL-6 signaling (204), they were not found to be related to PVT. This may be explained by an increased sensitivity of IL-6 as an inflammatory marker than Hs-CRP in patients with cirrhosis, already documented by Le Moine *et al* that showed a bad correlation between these both markers revealing a defective acute-phase response in cirrhosis (205). Also, while being mainly produced in the liver (204), C-reactive protein is not found to be a good marker of inflammation in the setting of cirrhosis. This is corroborated by the work of Park *et al* who disclosed that in the context of a more severe liver disease, C-reactive protein response to bacteremia is decreased (206).

4. HYPOTHESIS 6

AIMS	MAIN RESULTS
6. (Secondary aim of the study). To determine the impact of PVT on morbidity (decompensation and progression of liver disease) and mortality.	Portal vein thrombosis, either partial or occlusive is not related to increased morbid-mortality. Decompensation and PVT share some same risk factors.

Table 16. Main results regarding proposed aims for the sixth hypothesis.

Increased post-LT early-mortality is well established in those patients with PVT submitted to this surgical procedure (53). Also, historically and in cross-sectional studies, decompensation and progression of the underlying liver disease has been attributed to PVT, as both diagnoses (PVT and decompensation) were done at the same time (19, 23). In the THROMBOCIR study, there were patients with PVT that decompensated and progressed after the event, patients without PVT having the same outcome and without PVT that also decompensated and progressed. In the end, in multivariate analysis we did not find any parallel between PVT occurrence irrespectively of being partial or occlusive and decompensation or liver disease progression. We also did not find any relationship with an increased mortality. However, we found that PVT and decompensation and progression shared exactly the same risk factors as the presence of at least medium-sized esophageal varices and increased PT favoring the hypothesis that they may reflect the same expression of a more severe liver disease. Our results go in line and prospectively validate recent previous longitudinal studies (33, 35). We also found an outstanding variability on the course of PVT with time without anticoagulation, with almost 70% of the patients spontaneously resolving PVT (186). We validated in the largest cohort of patients, a tendency, previously reported, in which this dynamic characteristic of PVT without treatment was already perceived (33-35). Nevertheless these results should be viewed with caution, as anticoagulation at prophylactic doses may change the clinical course, with impact in decompensation and survival. Villa *et al* well documented that patients under enoxaparin in prophylactic doses (40mg/ day) decreased the probability not only of developing PVT but also decompensation and mortality (24). The improvement of microcirculation/ decrease in microthrombi induced by enoxaparin treatment with a decrease in bacterial translocation and improvement in endothelial function is an advanced hypothesis to justify the positive results achieved (24). Accordingly, our findings, altogether showing the potential of reversibility of PVT once diagnosed without treatment and the minor impact in

morbid-mortality out of the LT setting, together with the ones by Villa *et al* (24) are important in order to design future studies with the aim to settle which subtypes of patients benefit most from anticoagulation treatment, either in prophylactic or in therapeutic doses. It is however important to mention that a beneficial effect of anticoagulation treatment seems to exist, as recent evidence presented in a robust meta-analysis, shows that PVT resolves more often with no more bleeding events noticed, with a decline in the episodes of variceal bleeding in those patients under anticoagulation (207).

CHAPTER VI

Conclusions

CHAPTER VI

CONCLUSIONS

We have identified some new risk factors for PVT development and validated others in two independent cohorts. Markers of more severe portal hypertension (presence of esophageal varices grade ≥ 2), liver insufficiency (as expressed by prolonged PT) and inflammatory status (revealed by higher titers of IL-6) were found to be associated to future PVT development in the studies. Liver insufficiency may ultimately be related to a hypercoagulable state and increased inflammation to endothelial dysfunction, fundamental pillars of Virchow's triad to explain thrombotic phenomena. More inflamed patients were also those with more severe grades of portal hypertension. No relationship between decreased PBFV and PVT was found. Non-selective beta-blockers induced PVT independently from their effect over heart rate or PBFV. Portal vein thrombosis and liver disease progression and decompensation share of the same risk factors but there does not seem to be a causal relationship between the two. Portal vein thrombosis is more a marker than a promoter of liver disease progression or decompensation and both events may occur together in the course of the disease unrelated to each other.

CHAPTER VII

Clinical Implications And Future Perspectives

CHAPTER VII

CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

The results that we achieved with both studies allowed us to document that PVT is a non-negligible event in cirrhosis, even in more stable patients, such as those with Child-Pugh A and B liver insufficiency, affecting about 1 in every 10 patients. Regular screening programs for PVT diagnosis do not exist as for HCC out of the context of LT. We have not only found an absent relationship between PVT and liver disease decompensation, progression or death, but also a high rate of spontaneous repermeabilization of the portal vein or its branches after the thrombotic event, which is why based solely on these results, we cannot propose a regular PVT screening program. However, given the discovered incidences, there must be awareness concerning PVT diagnosis in stable cirrhotic patients among physicians. Nevertheless, we found that PVT and liver decompensation share some of the same risk factors, which may, together with the results advanced by Villa *et al (24)* allow us to determine, in the future, subgroups of patients in which risk factors, being identified, may determine which patients shall benefit most and be the target of prophylactically therapeutic measures. Parameters related to increased portal hypertension such as the ones related to the presence of at least medium-sized esophageal varices in both studies and to some degree of liver insufficiency settled by increased PT in THROMBOCIR are related both to PVT and to liver decompensation. We also found an association between NSBB use and PVT. This is of major importance, because i) their use is advised for primary and secondary prophylaxis of variceal bleeding (10); ii) they were found to negatively impact survival in patients with more advanced liver disease such as those with Child-Pugh C and refractory ascites (208). Thus, even though there is clear indication for this therapeutic class of drugs, a subgroup of patients exists in which its use is deleterious, which means that NSBB use should not be generalized. The future validation of our results will imply a change in the strategy concerning the selection of patients that undergo ligature of esophageal varices even in the setting of primary prophylaxis. We also found lymphopenia and IL-6 as markers for PVT development. For the first time, an increased inflammatory milieu has been recognized to predispose patients to PVT development, adding to the preexisting knowledge of Virchow's triad, the third pillar related to endothelial dysfunction. If measures to decontaminate gut in order to diminish bacterial translocation, anti-inflammatory therapeutic strategies, prophylactically anticoagulation, etc. are effective in decreasing inflammation and subsequently improve endothelial dysfunction in this context and prevent PVT development is yet to be determined.

One of the goals of research is not only to give new knowledge to science and current state of the art, but also to give raise to new fields of investigation in order to try to answer raised unmet questions. There are several issues that must now be clarified in future works:

I) To address if higher degrees of esophageal varices inversely relates to PBFV;
ii) To find by which means liver insufficiency, translated by increased bilirubin, extended coagulation times/ decreased coagulation factors or decreased albumin may lead to PVT, probably relating to an acquired prothrombotic condition due to failure in producing anticoagulants and procoagulants in right proportion;
iii) To find local hemodynamic aspects that may promote PVT in patients under NSBB;
iv) To find the subgroup of patients that would benefit most of NSBB treatment without an increased risk of PVT development;
v) To search for lymphocyte homing mechanisms for the liver, spleen and eventually other organs in the context of cirrhosis and if this mechanism is implicated in the enhancement of endothelial inflammation and more propensity to thrombosis;
vi) To establish a link between endotoxemia, IL-6 levels and PVT and to try to find the way IL-6 induces PVT, namely via downregulation of thrombomodulin (reflecting LPS levels) or endothelial synthesis of VWF;
vii) To find possible new therapeutic strategies in order to avoid PVT development, namely in patients awaiting LT (prophylactically anticoagulation, anti-inflammatory and gut decontaminating therapeutics, etc.);
vii) To create a predictive score for PVT development in patients with cirrhosis.

APPENDICES

Appendix 1

Splanchnic and Extrasplanchnic Thrombosis in Cirrhosis: Prophylaxis vs Treatment.

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PORTAL HYPERTENSION (JC GARCIA-PAGÁN, SECTION EDITOR)

Splanchnic and Extrasplanchnic Thrombosis in Cirrhosis: Prophylaxis vs Treatment

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Abstract Venous thromboembolism (deep vein thrombosis and pulmonary embolism) and portal vein thrombosis (PVT) occur in up to 6.3 % and 15.9 % of patients with cirrhosis, respectively. There is recent evidence that a procoagulable prothrombotic state is related to cirrhosis despite the reduced levels of many coagulation factors, and decreased platelet counts. Indeed, (*i*) the combination of high levels of factor VIII, with low levels of protein C and antithrombin induces a procoagulant state in vitro; while (*ii*) increased levels of von Willebrand factor and decreased ADAMTS 13 activity can compensate for decreased platelet counts. PVT is partial in a majority of patients in whom it develops and may spontaneously resolve in some of them. Although PVT is associated with features of more severe liver disease, it is uncertain whether it plays a causal role in the decompensation of cirrhosis. In patients listed for liver transplantation, PVT may make the procedure difficult or impossible. Pre-transplant PVT is associated with increased post-transplant mortality rates. Studies evaluating clinical outcome of anticoagulation therapy for splanchnic or extrasplanchnic venous thrombosis are scarce. Anticoagulation therapy, given to patients with cirrhosis of intermediate severity before PVT occurrence, in prophylactic doses, appears to decrease decompensation and mortality rate. Interestingly, this improvement is out of proportion of the prophylaxis of extrahepatic portal vein thrombosis. The risk of bleeding does not seem to be increased in patients with cirrhosis receiving anticoagulation therapy, once prophylaxis for bleeding related to portal hypertension has been implemented. Overall, the room for anticoagulation therapy is probably larger than previously recognized, and may be of particular benefit in patients without portal vein thrombosis. However, clinical trials remain to be done before the benefit risk ratio of anticoagulation therapy is properly evaluated.

Keywords Venous thromboembolism · Deep vein thrombosis · Pulmonary embolism · Portal vein thrombosis · Cirrhosis

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Introduction

Cirrhosis, a disease with a high tendency to bleed as a result of portal hypertension, is also associated with venous thromboembolism [1] and portal vein thrombosis [2]. Strategies for prophylaxis and treatment of these two clinical entities in patients with cirrhosis are still debated. There is reluctance to initiate anticoagulation therapy due to risk of bleeding related to portal hypertension. The goal of this section is to provide an overview on the data on the use of anticoagulation

for prophylaxis or treatment of splanchnic and extrasplanchnic vein thrombosis in patients with cirrhosis.

Coagulopathy in Cirrhosis

In patients with cirrhosis, routine laboratory exams show abnormalities that, in patients without cirrhosis, have been related to a higher tendency to bleed, such as prolonged prothrombin and activated partial thromboplastin times [3]. Severe bleeding at surgical portosystemic shunting or liver transplantation was initially attributed to cirrhosis related hemostatic defects, leading to the administration of large amounts of blood components and coagulation fractions [4–6]. However, with improved surgical techniques, the use of blood products was reduced to a large extent [7]. Bleeding at surgery in patients with cirrhosis is now attributed mainly to technical issues and to portal hypertension [8], not to an acquired coagulopathy. Indeed, patients with cirrhosis have a preserved capacity to generate thrombin in vitro, provided that platelet counts are above $60x10^9$/L [9]. A procoagulant state has been shown in vitro by a resistance to the anticoagulant action of thrombomodulin or Protac®, two agents that serve as PC activators [9, 10, 11••]. This resistance is likely due to increased levels of factor VIII (FVIII) and decreased levels of protein C (PC) and antithrombin, which parallels the severity of liver disease [10].

Thrombocytopenia related to hypersplenism is common in patients with portal hypertension in general and cirrhosis in particular [12]. Of note, platelet counts above $50x10^9$/L are not associated with impaired primary hemostasis when tested in conditions resembling those reigning in vivo. Compensation for thrombocytopenia appears to come from increased von Willebrand factor (VWF) levels [13]. ADAMTS 13 converts high molecular weight von Willebrand factor into smaller molecules of decreased capacity to bind platelets. ADAMTS 13 levels are reduced in patients with cirrhosis, which can explain an increased capacity of von Willebrand factor to promote adhesion of platelets to subendothelial matrix [11••, 14].

Thus, there is little ground to support the view that coagulation or primary hemostasis are impaired in cirrhosis, provided that platelet counts are above $50\text{-}60x10^9$/L . Platelet counts below this threshold have been found in less than 1 % of patients with cirrhosis, in the absence of active alcoholism or infection, which is not more frequent than among patients with chronic, non-cirrhotic liver disease [15]. Pro- and antifibrinolytic systems are also altered in patients with cirrhosis but the resulting effect on fibrinolysis remains unclear [16, 17].

Non-splanchnic Venous Thrombosis

In the general population, venous thromboembolism (VTE), the syndrome including deep vein thrombosis (DVT) and/or pulmonary embolism (PE) has an annual incidence of 1 to 3 in 1000 patients. PE is the third most common cause of death from cardiovascular diseases. The main long-term complications of VTE are chronic thromboembolic pulmonary hypertension and post-thrombotic syndrome [18, 19].

Epidemiology and risk Factors

Metabolic syndrome, immobilization, surgery, trauma, oral contraceptives, cancer, and acute medical illnesses are well known risk factors for VTE that are also found in patients with liver disease [18–21]. However, cirrhosis has not been listed among the risk factors for VTE in recent guidelines [22, 23]. A retrospective case-control study reported that patients with ill defined "severe liver disease" were 90 % less likely to develop VTE than controls [24]. However, subsequent surveys summarized in Table 1, have concurred in indicating an increased risk of VTE among patients with cirrhosis, with reported incidences of VTE in hospitalized patients with cirrhosis ranging from 0.5 to 6.3 % [25–27, 31, 32•, 33–35].

Risk factors for VTE in patients with cirrhosis have included hypoalbuminemia, and the elevation of partial thromboplastin time [27, 31]. Diabetes [29] and NAFLD [36] were found as the only independent risk factor for DVT in some studies. A limitation in the analysis of risk factors is that they were not all investigated among different studies. Immobilization, estrogen levels or inflammation, have not been specifically analyzed, although these conditions are common in hospitalized patients with cirrhosis [37, 38]. Similarly intestinal bacterial overgrowth and gut translocation may lead to a pro-inflammatory state in cirrhosis. Bacterial translocation, which increases with the severity of liver disease [40], may induce an inflammatory state [39] and coagulation activation [41, 42]. Girleanu et al. [35] found sepsis as a risk factor for thrombotic events in patients with cirrhosis. However, data on the relationship between bacterial translocation and prothrombotic changes in patients with cirrhosis are still lacking.

Prophylaxis for VTE

Thus, patients with liver disease appear not to be protected from VTE [10]. However, according to recent surveys, VTE prophylaxis was implemented in only 21-25 % of the hospitalized patients with cirrhosis [1, 24, 34]. It was recently reported that, among 235 patients with cirrhosis given prophylactic anticoagulation on 355 admissions, the incidence of gastrointestinal bleeding was 2.5 %, VTE 1.4 %, heparin induced thrombocytopenia 0.5 %, and death 3.9 % [43•]. A recent meta-analysis showed that thromboprophylaxis with heparins did not increase the risk of bleeding and did not decrease the risk of VTE [44•]. However, another meta-analysis showed heparin thromboprophylaxis to decrease

Table 1 Main characteristics and findings of epidemiological studies on VTE in patients with cirrhosis

Author	Study design	Period of inclusion	Number of patients enrolled	Incidence of VTE	Prevalence of VTE	Risk of VTE
Northup *et al.* [25]	Retrospective case/control	1993-2001	21000	0.5 %	-	-
García-Fuster *et al.* [26]	Retrospective cohort	1992-2007	2074	0.8 %	-	-
Gulley *et al.* [27]	Retrospective case-control	1995-2005	Cirrhosis 963 Controls 12 405	1.87 %	-	-
Søgaard *et al.* [28]	Retrospective case-control	1980-2005	Patients with VTE 99 444 (cirrhosis 544, non-cirrhotic liver disease 1109); Controls 496 872	-	-	Overall risk of VTE : cirrhosis RR 1.74 (95 % CI 1.54-1.95); Non-cirrhotic liver disease RR 1.87 (95%CI 1.73-2.03);
Lesmana *et al.* [29]	Cross-sectional	2004-2007	256		4.7 % (only DVT)	-
Wu *et al.* [30]	Cross-sectional	1998-2006	Decompensated cirrhosis (DC) 241 626 Compensated cirrhosis (CC) 408 253 Without liver disease (WLD) 575 057	-	DC 0.82 % CC 0.81 % WLD 0.76 %	
Lizarraga *et al.* [31]	Retrospective case-control	2004-2008	14 790	0.73 %	-	-
Dabbagh *et al.* [1]	Retrospective cohort	2000-2007	190	6.3 %	-	-
Ali *et al.* [32•]	Cross-sectional	2005	449 798	-	1.8 %	-
Saleh *et al.* [33]	Retrospective cohort	1979-2006	Alcoholic liver disease (CALD) 492 7000; Nonalcoholic liver disease (CNALD) 4 565 000		CALD 0.6 % CNALD 0.9 %	
Aldawood *et al.* [34]	Retrospective cohort	2009	226	2.7 % (only DVT, not PE)		
Gîrleanu et al. [35]	Retrospective case-control	2010-2012	Cirrhosis 3108	0.99 % (only DVT, not PE)		

Diagnosis of cirrhosis was variably diagnosed by liver biopsy or by clinical manifestations, before or by the time the diagnosis of venous thromboembolism was made. VTE–venous thromboembolism; PE–pulmonary embolism; DVT–deep vein thrombosis

VTE in unselected medical-surgical critically ill patients without increasing major bleeding or mortality. The latter situation bears many similarities with acute-on-chronic liver failure [45]. Therefore, adequately powered randomized trials are needed before general recommendations for or against VTE prophylaxis with heparins can be made in patients with cirrhosis.

Treatment Options for VTE

Data on treatment for established VTE in patients with cirrhosis are similarly limited [26, 29], particularly regarding the incidence, severity, and origin of the bleeding episodes [26].

In unselected patients with massive/high risk PE (5 % of all symptomatic patients [18]), thrombolysis or surgical or catheter embolectomy are recommended in the absence of contraindications [22, 23]. Data of systemic thrombolysis in patients with cirrhosis and PE are lacking. Systemic thrombolysis has been evaluated only in nine patients with cirrhosis and splanchnic vein thrombosis [46] and found to be safe. In the presence of older age, uncontrolled hypertension, recent stroke or surgery, or bleeding diathesis, the risk of major hemorrhage is estimated in 13 % of unselected patients receiving systemic thrombolysis [47] Therefore, additional data on systemic thrombolysis for massive/high risk PE in patients with cirrhosis are needed before making recommendations .

In unselected patients, anticoagulation is the mainstay of treatment for VTE. Recommended treatment duration, varies from 3 to 6 months (when cause is transient) to lifelong (when cause is persistent) [18, 22, 23]. Clinical scores that predict bleeding in unselected patients given anticoagulation (e.g., HEMORR2HAGES [48] and RIETE [49]) were not conceived for cirrhosis. The application of the HEMORR2HAGES score to a patient with cirrhosis, would result in at least 2 to 3 points (hepatic disease, reduced platelet count, and possibly anemia), which is theoretically associated with a major bleeding rate of 5.3 to 8.4 per 1000 patient-years.

Reports on anticoagulation for VTE in the context of cirrhosis are extremely limited. García-Fuster et al. [26] retrospectively reviewed 17 cases of VTE treated with anticoagulants, in which 14 patients developed bleeding events, including six severe, with five out of those six patients under acenocoumarol. However, information on the origin of bleeding, grade of esophageal varices, and INR surveillance was not provided. Additional data on the safety of anticoagulation, collected in patients treated for PVT, will be discussed below.

The translation of the recommendations for VTE treatment from unselected patients [18, 19] to patients with cirrhosis should be cautious [50], as specific clinical trials have not been performed; dose adjustment and monitoring are hampered by underlying alterations in anti-factor Xa activity [51] and INR [52]; and the optimal duration of anticoagulation has not been evaluated.

Splanchnic vein Thrombosis

Cirrhosis and malignancy are predominant in the etiology of splanchnic vein thrombosis, although many other conditions have been implicated, as reviewed elsewhere [53–55].

Epidemiology and risk Factors

Based on the analysis of explanted livers, thrombosis of the intrahepatic portal or hepatic venous system appears to be common in advanced cirrhosis [56]. However, thrombosis of the major hepatic veins is uncommon whereas thrombosis of the extrahepatic portal vein, thereafter referred to as PVT, is common. The prevalence of PVT in cirrhosis has varied among reports from 0.6 to 26 %, depending on definition and patients characteristics [57••, 58]. Incidence estimates go from 7.4 % for a mean time of 12 months [59] to 15.9 % per year [2] in liver transplant candidates. In a cohort of 898 patients with Child-Pugh A cirrhosis, the cumulative incidences of PVT was 11.9 % at 5-year follow-up [60].

PVT can be localized to main trunk and/or branches, causing partial or total occlusion. In candidates to liver transplantation, non-occlusive PVT was more frequently found (up to 12 %) than occlusive PVT (up to 5 %) [57••]. PVT may extend to involve the splenic or mesenteric veins. Extension to the superior mesenteric vein can be accompanied by intestinal infarction, a potentially fatal condition if not rapidly recognized and treated [58].

Several risk factors for PVT development have been suggested in patients with cirrhosis, including *(i)* an advanced stage of liver disease, as reflected by higher Child-Pugh or MELD scores, or the presence of encephalopathy, ascites or variceal bleeding [61–65]; *(ii)* underlying prothrombotic conditions [66–71]; *(iii)* and reduced baseline portal flow velocity (<15 cm/s) as shown using multivariate analysis by Zocco et al. [72] among prospectively enrolled patients, but an independent confirmation of the latter finding has not been reported yet.

PVT and Liver Transplantation

As shown in Table 2, high PVT incidence (up to 15.9 % per year [2]) and prevalence (up to 24.1 % [83••] and 26 % [65]) have been reported in liver transplant candidates substantiating a relationship between PVT and advanced liver disease.

Most surveys have been among recipients rather than candidate or listed patients, which limits extrapolation of the identified risk factors. In a cohort of 251 wait listed patients [59], only low platelet count, a past history of variceal bleeding and time between listing and liver transplantation were predictive for PVT development. Previous treatment for portal hypertension was an independent factor for PVT in several

Table 2 Epidemiological features and findings in candidates to liver transplantation or recipients with portal vein thrombosis at the time of evaluation or transplantation

First author study design	Patients enrolled	PVT frequency	Risk factors for PVT	Impact of PVT on survival
Nonami *et al.*, 1992 [63]/ Retrospective	849 recipients without, and 36 with previous PSS	13.8 % (in the absence of PSS) and 38.9 % after PSSs incidences	Lower liver weight	Not evaluated
Gayowski *et al.*, 1996 [65]/ Retrospective	88 recipients	26 % prevalence	-	No impact on post-LT survival
Yerdel *et al.*, 2000 [73]/ Retrospective	779 recipients	8.1 % incidence	Male. Treatment for portal hypertension. Child-Pugh C patients. Alcoholic liver disease	Decreased post LT survival
Manzanet *et al.*, 2001 [2]/ Retrospective	391 recipients	15.9 % incidence	Auto-immune and cryptogenic cirrhosis. TIPS	No impact on overall survival
Dumortier *et al.*, 2002 [74]/ Retrospective	468 recipients	8.1 % incidence	-	No impact on 1-year post-LT survival
Francoz *et al.*, 2005 [59]/ Retrospective	251 listed patients	8.4 % at listing 7.4 % during follow up incidences	Low platelet count. Past history of variceal bleeding. Time between listing and LT	Decreased post-LT survival
Lladó *et al.*, 2007 [75]/ Retrospective	335 recipients	12.5 % incidence	-	No impact on post-LT survival
Lendoire *et al.*, 2007 [76]/ Retrospective	323 recipients	8.7 % incidence	-	Decreased survival according to PVT extension
Tao *et al.*, 2009 [77]/ Retrospective	465 recipients	9 % incidence	Older age. Previous portal hypertension treatment	No impact on post LT survival
Englesbe *et al.*, 2010 [78]/ Restrospective	3295 patients with cirrhosis (1194 listed patients)	4.5 % prevalence		Increased overall risk of death. Lower survival after LT at 30 days.
Englesbe *et al.*, 2010 [79]/ Retrospective	46 530 listed patients	2.1 % prevalence	Older age. Caucasians. High MELD score. Low albumin. Hyponatremia. Diabetes. Previous hospitalizations	No impact on waiting list mortality
Englesbe *et al.*, 2010 [79]/ Retrospective	22 291 recipients	4.02 % (occlusive PVT) prevalence	Older age. Causacians. Higher MELD score. Low albumin. Ascites. Diabetes	No impact on waiting list mortality. Decreased 1-yr post LT survival
Ravaioli *et al.*, 2011 [80]/ Retrospective	889 recipients	10.2 % incidence	-	No impact on post LT survival
Fouzas *et al.*, 2012 [81]/ Prospective	25 LTR patients with PVT		-	PVT grade correlates with lower survival rates post-LT
Werner *et al.*, 2013 [82•]/ Retrospective	537 listed patients	13 % prevalence	-	Not evaluated
John *et al.*, 2013 [83••]/ Prospective	290 listed patients	24.1 % prevalence	Ascites. Worsening of renal function	No impact on waiting list mortality or 6-month post-LT survival

LTR–Liver transplant recipients; PSS–Porto-systemic shunt; HCC–Hepatocellular carcinoma; LT–Liver transplantation

studies [2, 73, 77] suggesting that PVT is related to the severity of portal hypertension.

Occlusive PVT, was found to have an unfavorable impact on 2-year post transplantation survival (50 % vs 83 %) respectively; (p=0.04) [59]. Although all studies were not unanimous in this respect, the largest available survey, found a PVT associated reduction in post transplantation survival [79]. Several groups found an increasing grade of PVT to be associated with a decreasing survival after transplantation [59, 73, 76, 79, 81]. Interestingly, two independent groups reported that the negative impact on survival after liver transplantation was limited to those patients having low MELD score prior to transplantation [79, 84]. These findings suggest that an underlying disease independent from liver disease, which cannot be cured with liver transplantation, negatively impacts the outcome. Whether this conclusion is specific to patients with occlusive PVT or should be expanded to patients with partial thrombosis will have to be evaluated.

Thrombectomy and new surgical techniques may change the outcome post-LT [74, 80]. The discussion of the means available to cope with portal venous occlusion at liver transplantation is beyond the scope of this section [57••]. PVT was associated with higher blood losses and blood transfusions in some [59, 73, 75, 82•], but not in all studies [77]. Two groups [59, 82•] found that 40 % of patients had partial or complete recanalization of the portal vein on anticoagulation. However, given the transient nature of PVT in patients with cirrhosis (see below), the lack of untreated controls, and the absence of hard clinical end points, the actual benefit from anticoagulation in transplant candidates remains to be demonstrated.

Longitudinal Evaluation of Patients with Cirrhosis and PVT

Four recently reported cohort surveys showed a spontaneous decrease in the thrombus in 31-71 % of patients in the absence of anticoagulation therapy [60, 83••, 85••, 86•]. An important point will be to ascertain whether the probability of recanalization varies according to the degree of portal vein occlusion. Of note, all four surveys showed that, after adjustment on initial severity of liver disease, PVT did not impact significantly on survival [60, 83••, 85••, 86•]. Therefore, extrahepatic PVT has not been clearly associated with a worsening in liver disease. This is in contrast with *(i)* the association, on explanted liver, of thrombosis of intrahepatic portal and hepatic veins with areas of parenchymal extinction [56]; and *(ii)* the induction by portal vein embolization of an atrophy of the embolized lobe and a hypertrophy of the non-embolized lobe in patients with cirrhosis [87]. The latter condition differs however from the partial obstruction of the main portal vein by many respects.

Therefore, it is likely that extrahepatic PVT is rather a marker for severe cirrhosis than a real factor inducing a worsening of liver disease. In other words, the development of extrahepatic PVT and the worsening of liver disease may share common determinants rather than being directly related to one another. Among the shared determinants could be the thrombosis of the intrahepatic hepatic and portal veins found in explanted livers at the time of transplantation.

Prophylaxis or Treatment

Prophylaxis

Villa et al. [88••] assessed in a randomized trial the efficacy and safety of low dose enoxaparin for 48 weeks in patients with Child-Pugh B7 to C10 cirrhosis and a patent portal vein at baseline. Compared to no treatment, patients receiving enoxaparin were protected from PVT, and had markedly reduced decompensation and mortality. Enoxaparin therapy was well tolerated and safe. This study raises many practical issues beyond that of the possible mechanisms for such a beneficial effect. First of all, an independent confirmation is eagerly awaited. Second, would a similar benefit be derived from enoxaparin in patients with Child A or advanced Child C cirrhosis? Third, given the variable nature of portal vein thrombosis in patients with cirrhosis, would low dose enoxaparin be as efficient for improving the outcome in patients with past but resolved PVT as in patients who never developed PVT? Before these issues are addressed, much caution should be applied to a prophylactic use of anticoagulation in patients with cirrhosis.

Anticoagulation for the Treatment of PVT

Early anticoagulation therapy has been recommended for patients with recent PVT in the absence of cirrhosis. Due to limited data, recommendations are less clear for patients with long standing PVT (cavernoma) [89]. For patients with cirrhosis, AASLD guidelines for clinical practice sate that "in the absence of robust data, recommendations for or against routine anticoagulation cannot be made" [89]. However, recent data brought some support to the use of therapeutic anticoagulation for PVT in patients with cirrhosis, as detailed in the following. Among 29 candidates to liver transplantation with PVT [59], the first ten were not treated with anticoagulants and recanalization of the portal vein did not occur, while the subsequent 19 patients received a therapeutic dose of LMWH or VKA. Eight of the 19 patients had recanalization. Among patients on anticoagulation, only one had an episode of variceal bleeding. Anticoagulation did not increase the duration of liver transplantation nor blood losses during the procedure [59]. Among 28 candidates to liver transplantation with PVT treated with VKA [82•], complete and partial recanalization was achieved in 39 % and 43 %, respectively. All patients, before starting anticoagulation had been screened for the presence of esophageal varices and, when present, treated

as the standard of care. Only one episode of bleeding was documented, from vaginal origin [82•]. Similar findings were also made in patients not on a waiting list for liver transplantation. Enoxaparin was given at therapeutic doses for 6 months to 28 patients with PVT, after endoscopic variceal eradication [90]. Recanalization was complete, partial and absent in 33.3 %, 50 %, and 16.7 % of patients, respectively. With prolongation of anticoagulation in patients not responding by 6 months, an overall complete response was obtained in three-fourths of patients. No major bleeding complication was observed [90]. Among 33 of 56 consecutive patients with PVT treated with nadroparin [91], complete recanalization of PV was documented in 36 % of treated patients and only 5 % of the untreated ones. Of those who did not receive anticoagulation 71.4 % had an extension of PVT. Before starting anticoagulation, all patients had been submitted to upper endoscopy and ligature was done whenever indicated. No upper digestive bleeding was seen. In the group on anticoagulation, three episodes of bleeding were seen: one epistaxis, one hematuria, and one symptomatic cerebral hemorrhage, with clinical amelioration after rehabilitation. No deaths were attributed to hemorrhagic events. Among 55 patients with splanchnic vein thrombosis, treated with LMWH or VKA [92•], partial or complete recanalization was achieved in 60 %. After complete recanalization and anticoagulation withdrawal, recurrent thrombosis was seen in 38.5 % of the patients, suggesting a benefit of maintaining anticoagulation. Before anticoagulation was started, all patients underwent upper digestive endoscopy and endoscopic or beta-blocker treatment was initiated whenever indicated. No deaths were attributed to treatment. Bleeding episodes were more frequent when platelet count was under 50 000/μL (p=0.018). Patients on VKA tended to bleed more commonly compared to LMWH (p=0.053) [92•].

Taken together these findings indicate that therapeutic anticoagulation in patients with cirrhosis *(i)* prevents extension and may promote regression of portal vein thrombus extension; and *(ii)* appears to be safe; provided *(iii)* prophylaxis for bleeding from ruptured gastroesophageal varices has been initiated prior to starting anticoagulation. However, in the absence of adequate controls group, recanalization directly related to anticoagulation therapy (and not to a spontaneous resolution of thrombosis) cannot be evaluated and could actually be marginal. Furthermore, an improved outcome of treated patients based on hard endpoints such as the occurrence of decompensation, the need for liver transplantation, or death remains to be shown. Coupled to the uncertainties on the role of PVT as a causal factor for worsened outcome in patients with cirrhosis, these limitations in therapeutic data should make one extremely cautious when deciding for therapeutic anticoagulation therapy in patients with established PVT. As recently reviewed, other important unanswered questions pertain to *(i)* the optimal agent for anticoagulation; *(ii)* the optimal duration of anticoagulation therapy; *(iii)* the optimal test for assessing anticoagulation and monitoring therapy [50, 93]. Due to these uncertainties alternatives to

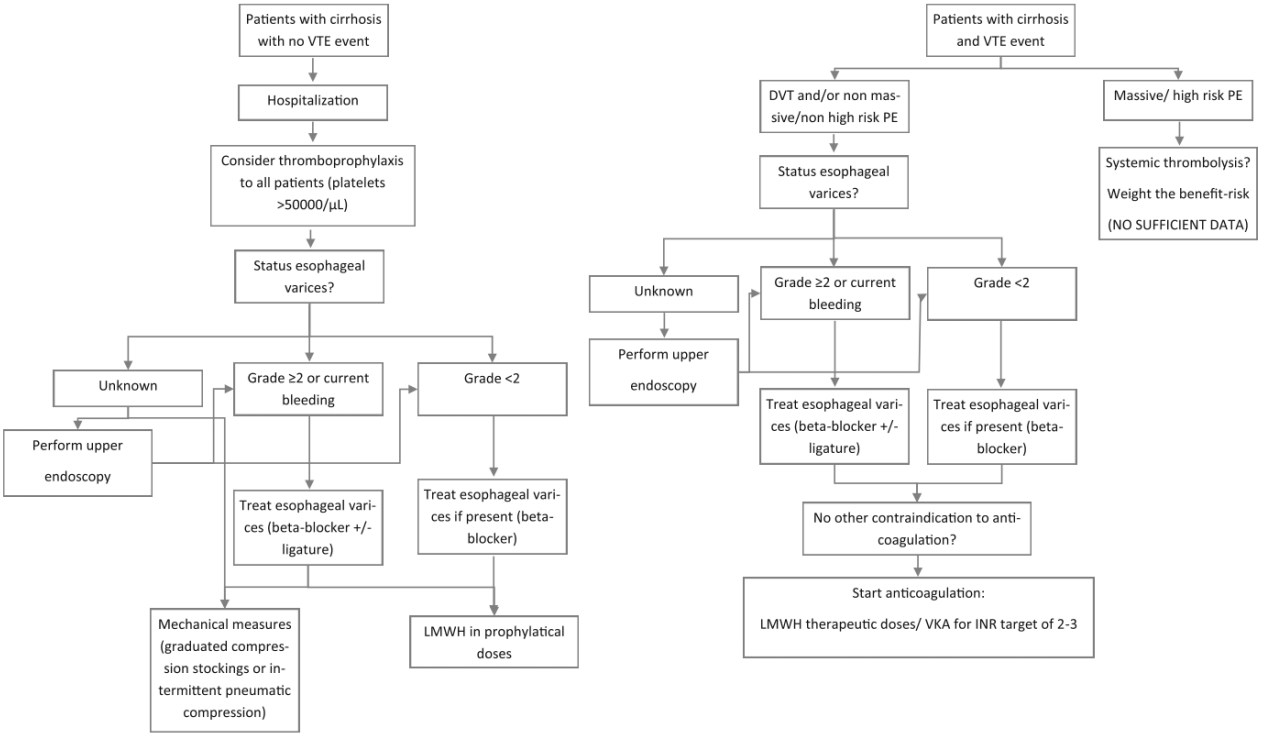

Fig. 1 Flowchart for proposed VTE prophylaxis in hospitalized patients with cirrhosis and proposed treatment of VTE events

anticoagulation should be considered, of which two have been proposed, transjugular intrahepatic portosystemic shunt (TIPS) and thrombolysis.

TIPS for the Treatment of PVT

PVT was initially regarded as a relative contra-indication for TIPS procedure [94]. More recent studies, however, show a high procedural success rates of 73 to 100 %, a relatively low rate of TIPS dysfunction, with a final TIPS patency rate of 57–88 % [95–98]. Cavernomatous transformation [95–98], was found to cause increased technical difficulty. Likewise, older thrombi were associated with increased technical failure and a worse clinical outcome [99]. The incidence of encephalopathy was 25-27 % and 27-32 % at 12 and 24 months after TIPS, respectively [96, 97]. Two-year survival rates of about 80 % have been reported [96, 97] Survival was better in patients with successful rather than failed TIPS placement [97]. Most available surveys had a retrospective design on patients where the indication for TIPS was not PVT but refractory complications of portal hypertension [95–98]. In another study [91], seven patients with PVT were treated with a TIPS due to a contraindication to anticoagulation or thrombus progression. TIPS placement was successful in six patients. Thrombus size remained stable in four of the six TIPS patients and completely resolved in the other two [91]. TIPS placement in candidates to liver transplantation does not seem to jeopardize the surgical procedure [98]. None of the available surveys had a design permitting to assess the efficacy of TIPS insertion on hard endpoints such as encephalopathy or other decompensation events and mortality, as compared to the absence of TIPS placement in appropriate controls with PVT. Such clinical trials are needed before recommendations can be made regarding the use of TIPS in patients with cirrhosis and PVT.

Thrombolysis for the Treatment of PVT

Systemic thrombolysis has been recently evaluated in a pilot study, enrolling nine patients with cirrhosis and recent PVT [46]. Recombinant tissue plasminogen activator was given together with anticoagulation, for a maximum period of 7 days. There were four patients with complete regression, four with partial regression, and one without any change. No bleeding episodes were reported. There is an additional case report of successful systemic thrombolysis [100]. Thus, experience with systemic thrombolysis, although apparently encouraging, will require careful evaluation of the benefit/risk ratio.

Conclusions

Based on the limited available evidence discussed above, the following proposals can be made for practical purposes.

1. Hospitalized patients with cirrhosis should be considered for thromboprophylaxis of VTE, either with mechanical or pharmacological measures (Fig. 1). Portal hypertension should not be considered as a contraindication to prophylactic anticoagulation therapy once prevention of gastrointestinal bleeding has been implemented as usual. A platelet count of 50.000/μL can be taken as a threshold above which spontaneous hemostasis appears to be preserved and the risk of bleeding related to anticoagulation therapy appears to be acceptable.
2. Once a diagnosis of VTE has been made, in the absence of a massive or high risk PE, and once prevention of gastrointestinal bleeding has been implemented as usual, anticoagulation with LMWH and then VKA could be initiated. Experience with direct oral anticoagulants in cirrhosis is thus far too limited to draw any conclusion regarding their possible use [50] (Fig. 1). Treatment duration may depend on the causal factors identified beyond cirrhosis. In the absence of a persistent prothrombotic factor, a 3-month course of anticoagulation therapy should be given. In the presence of massive/high risk PE, systemic thrombolysis could be considered, keeping in mind the extremely limited experience in the setting of cirrhosis.
3. Anticoagulation to prevent PVT development in patients with cirrhosis should not be accepted yet as a standard of care, at least until the encouraging results have been duplicated.
4. At present, what could be the reasonable indications for a treatment targeting portal vein thrombosis? PVT occurring in candidates to liver transplantation is probably the best current indication for a specific therapy, due to the well documented negative impact of PVT on post liver transplantation outcome. Of the two available options, *(i)* TIPS might be proposed to patients with gastrointestinal bleeding or ascites refractory to medical therapy, provided the intrahepatic portal vein branches are visible; while *(ii)* anticoagulation until transplantation is performed could be proposed to liver transplant candidates once prophylaxis for gastrointestinal bleeding has been instituted. In making the decision, it should be kept in mind that anticoagulation prior to transplantation has not yet been shown to improve post-transplant outcome.
5. Patients with an extension of thrombosis to the superior mesenteric vein and manifestations of intestinal ischemia, as well as patients with strongly prothrombotic underlying conditions (e.g., myeloproliferative neoplasm or antiphospholipid syndrome) should also be considered for prolonged anticoagulation.

Compliance with Ethics Guidelines

Conflict of Interest Dominique Valla and Filipe Nery declare no conflict of interest.

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Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

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Appendix 2

Towards a Comprehensive New Classification of Portal Vein Thrombosis in Patients With Cirrhosis.

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Toward a Comprehensive New Classification of Portal Vein Thrombosis in Patients With Cirrhosis

Portal vein thrombosis (PVT) generally refers to a complete or partial obstruction of portal venous blood flow due to the presence of a thrombus in the lumen of the vein. A cavernoma that develops as a consequence of a demonstrated thrombus is evidence of a chronic thrombosis. Extrahepatic portal vein obstruction encompasses thrombotic and nonthrombotic occlusions of the portal vein as well as cavernoma formation with or without features of portal hypertension.[1] The designation "malignant" or "tumor" thrombosis for obstruction by neoplastic tissue growing into the portal venous lumen is misleading. Therefore, it is better termed *tumorous invasion* of the portal vein and described as a distinct clinical entity because the genesis, treatment, outcome, and prognosis are distinct.

The prevalence of nontumorous portal vein obstruction in the general population at necropsy is nearly 1% in Malmö, Sweden.[2] In patients with cirrhosis, cross-sectional estimates have ranged between 0.6 and 26%.[1] However, the prevalence could vary with age, presence and origin of underlying hepatic and nonhepatic diseases, velocity of portal venous blood flow, the procoagulant status and liver transplantation (LT) status of the patients. The prognosis and treatment of PVT depends on the nature, site, extent, and rapidity of development, duration of thrombosis, risk factors for thrombosis, stage of liver disease and the acute and chronic precipitating event(s) (Figure 1). Because of the large number of variables and protean presentations of PVT, there is lack of clarity in the literature and a need for early detection, a comprehensive classification, and an algorithmic approach to management. The present commentary addresses these issues in PVT in the context of cirrhosis and provides a potentially universally acceptable classification for structural and functional assessment of PVT and assess response to therapies.

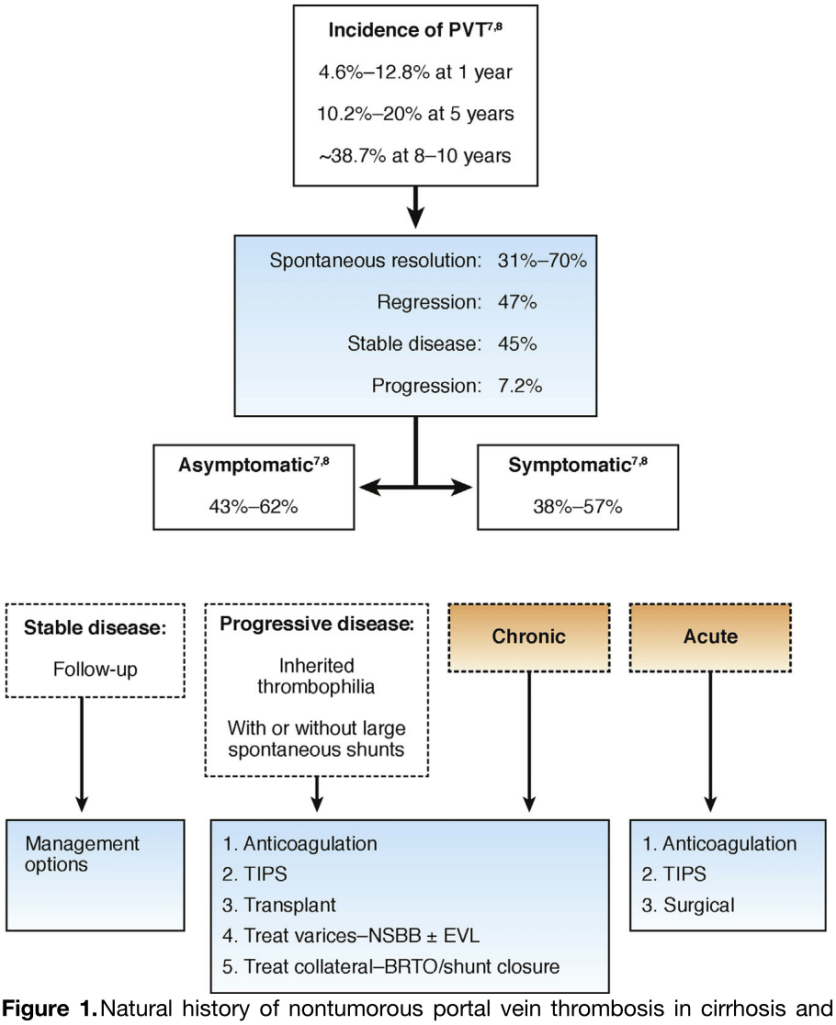

Figure 1. Natural history of nontumorous portal vein thrombosis in cirrhosis and possible therapeutic approaches.

Defining PVT

In the recent Clinical Practice Guidelines of The European Association for the Study of the Liver, acute PVT has been defined as a recent formation of a thrombus within the portal vein and/or right or left branches. In the absence of recanalization, the portal venous lumen is obliterated while portoportal collaterals develop resulting in the cavernomatous transformation of the portal vein.[3] The American Association for Study of Liver Diseases defines acute PVT as sudden formation of thrombus within the portal vein lumen, and chronic, when the obstructed portion is replaced by a network of hepatopetal collaterals bypassing the thrombosed portion of portal vein.[4] Although simple and useful, these definitions are predominantly anatomic and have limitations of only considering occlusion as a defining point. The consequence of thrombotic occlusion of the portal vein such as ascites or portal hypertension are not part of the definition. Development of acute PVT can have different functional consequences depending on whether the liver is diseased or healthy. Furthermore,

chronic thrombosis may not always be followed by cavernoma formation; moreover, cavernoma formation may be a result of congenital malformations in the absence of thrombosis.[2] Important "functional and hemodynamic aspects" and underlying pathogenic aspects, are currently not included in the definition of PVT. In fact, PVT may be best defined as a syndrome in which the presence of a thrombus in the portal vein or its branches presents either as an incidental finding on abdominal imaging; or with a myriad of abdominal signs and symptoms that represent complications of portal hypertension; or a composite of both acute abdominal and portal hypertensive manifestations in the presence or absence of cirrhosis and/or malignancy. In the present commentary, we focus only on PVT in cirrhosis.

Prevalence, Progression, and Resolution of PVT

The modality and context of assessment of PVT may influence the prevalence rate of PVT in patients with cirrhosis, being 0.6% when evaluated by percutaneous angiographic studies, 4.4% when evaluated by ultrasound Doppler, and 10% to 12% when evaluated by computed tomography and MRI.[5–7] Whereas initial studies have indicated a high prevalence of $\leq$26%, and annual yearly incidence of new PVT of 7.4% to 16% in patients with cirrhosis, however, larger studies have shown these figures to be lower.[8,9] In a prospective study of 1243 patients with Child's A and B cirrhosis, the cumulative incidence of new PVT after 1 and 5 years were 4.6% and 10.7%, respectively.[5] In a large study of 33,368 patients being evaluated for LT, 2096 (6.3%) were found to have PVT, more so in cirrhosis related to nonalcoholic steatohepatitis.[10] In patients with advanced cirrhosis and those undergoing LT, the prevalence is higher and has been reported to be from 5% to 16%.[9]

Multiple studies have shown that progression of PVT in cirrhosis is quite variable. The most common aspect in natural history is spontaneous resolution or an unchanged appearance. Nonprogression or resolution has been reported from 33% to 75% in many other studies. Maruyama et al showed that, in patients without PVT at baseline, the cumulative incidence of PVT was 12.8%, 20% and 38.7% at 1, 5, and 8 to 10 years, respectively. The natural course of PVT was improvement or regression in 47.6% of patients and stabile course in 45.2%. In another study, the cumulative rate of PVT was 4.6% at 1 year and 8.2% and 10.7% at 3 and 5 years, suggesting that the risk factors of PVT are already present at baseline.[11] Nonprogression or resolution has been reported from 33% t o 75% in many other studies,[5,9] and PVT with cirrhosis has been shown to have little influence on prognosis.[9]

PVT in patients on wait list at the time of LT and PVT posttransplant pose different challenges and the data are equivocal. In 1 study, patients with PVT at transplant have been reported to have higher 90-day mortality and graft failure than those without PVT at the time of surgery.[12] However, in another study only older age predicted development of PVT in LT recipients awaiting LT. Ninety-day patient and graft survival rates were similar at 6 months, even though survival was significantly lower at 1 year in patients with PVT.[13] PVT diagnosed during LT did not lead to inferior outcomes but transfusion rates were greater in presence of PVT, whereas duration of stay and morbidity were not.[14] Occurrence of PVT in post-LT recipients can also significantly reduce both graft and patient survival.[15]

Probability of Development or Presence of PVT

The pretest probability of developing thrombosis in deep vein thrombosis patients includes predisposing factors, risk factors, and clinical symptoms and signs.[10] Deep vein thrombosis is likely to be present when there is a high pretest probability in a given patient such as recent bed confinement in an elderly patient in last 3 days, major surgery within last 12 weeks, previous history of deep vein thrombosis, or leg swelling. A pretest probability assessment for PVT based on currently suspected or established predisposing and risk factors is presented (Supplementary Table 1). The presence of one or more of these factors could suggest a high probability of development/presence of PVT. We propose that presence of 2 major, or 1 major and 2 minor, or 4 minor criteria can give a high probability for the development or pretest probability of the presence of PVT in a cirrhotic patient. This pretest scoring system would need to be assessed in prospective clinical studies.

Proposed New Classification

To develop a new classification, a review of all the available classification systems of PVT (Supplementary Table 2)[16–23] would be worthwhile. The proposed new classification covers different variables and situations to describe the anatomic and functional aspects of PVT (Table 1 and Supplementary Figure 1). It is simple to use and helps to describe a patient with PVT precisely, including the site, degree, presentation, and functional relevance of the thrombosis. For example, a patient with cirrhosis is incidentally found to have a new and complete occlusion of the main portal vein trunk, with no evidence of bowel ischemia, would be designated as PVT type 1, occlusive, recent, and asymptomatic. The first section describes anatomic and the later 3, the functional aspects of PVT. One may depict these by using letters as well: PVT-I, ORAs. Another example is a cirrhotic patient who presents with features of acute abdominal pain, and on contrast-enhanced computed tomography examination has total occlusion of both the branches of the portal vein with the thrombus extending into the mesenteric vein along with bowel ischemia. The diagnosis is PVT type IIb, occlusive, recent, symptomatic with acute with bowel ischemia or PVT type IIb, ORSAbi with possible mesenteric extension. The other variables such as the extent and the nature of underlying liver disease could be added to the classification, if necessary. Although this classification has been developed for a population with cirrhosis, it could also be evaluated for non-cirrhotic patients.

Table 1. Anatomico-Functional Classification of PVT in Cirrhosis

Site of PVT – (Type 1, 2a, 2b, 3)
Type 1: Only trunk
Type 2: Only branch: 2a, one branch; 2b, both branches
Type 3: Trunk and branches
Degree of portal venous system occlusion (O, NO)
O: Occlusive: No flow visible in PV lumen on imaging/Doppler study
NO: Nonocclusive: Flow visible in PV lumen through imaging/Doppler study
Duration and Presentation (R, C)
R: Recent (first time detected in previously patent PV, presence of hyperdense thrombus on imaging, absent or limited collateral circulation, dilated PV at the site of occlusion)
Asymptomatic: (As)
Symptomatic: (S), Acute PVT features (with or without ABI)
Ch: Chronic (no hyperdense thrombus; previously diagnosed PVT on follow-up, portal cavernoma and clinical features of PHT)
Asymptomatic
Symptomatic: features of portal hypertension (with or without PHT)
Extent of PV system occlusion (S, M, SM)
Splenic vein, mesenteric vein or both
Type and presence of underlying liver disease:
Cirrhotic, noncirrhotic liver disease, post-liver transplant, HCC, local malignancies, and associated conditions

ABI, acute bowel ischemia; HCC, hepatocellular carcinoma; IMV, inferior mesenteric vein; PHT, portal hypertension; PVT, portal vein thrombosis; PV, portal vein; SV, splenic vein.

It can be argued why 2 terms, recent and acute have been used. A careful look at the 2 cases presented would show that both the clinical scenarios presented had recent onset PVT; one was an asymptomatic presentation whereas the other was a symptomatic presentation—acute with features of bowel ischemia. Thus, the term recent is broad and signifies the chronology, whereas asymptomatic and symptomatic describe resultant clinical scenarios. The term *acute* in clinical medicine is most often understood as a symptomatic presentation of a disease, as is also adopted in this classification for cirrhotic patients with PVT.

In a patient with cirrhosis who develops acute PVT, there could be 2 baseline clinical scenarios: presence of preexisting significant portal hypertension with varices or absence of clinically significant portal hypertension. The clinical outcome and management issues in the first situation could be challenging, with rapid development of ascites, variceal bleed and early bowel ischemia.[24] Early prevention (with beta-blockers) and endoscopic management of variceal bleed is required and needs to be tailored on a case to case basis against the need for initiating anticoagulant therapy. The role of transjugular intrahepatic portosystemic shunting to unblock the occluded portal venous system is still unclear.[25] The outcome in such patients is largely determined by the Child's status, available collateral network and the degree and rapidity of increase in hepatic venous pressure gradient. Use and choice of anticoagulants, although indicated, should be weighed in the presence of a deranged international normalized ratio and coagulation anomalies. In patients with no clinically significant PHT, close observation for development of ascites and varices needs to be monitored and low-molecular-weight heparin can be considered, although studies demonstrating its efficacy are lacking.

Areas of Uncertainty

Some issues that are not easy to include in the classification have been excluded. An asymptomatic PVT patient can become symptomatic if the thrombus later extends into the mesenteric system, whereby a diagnosis of acute PVT would be erroneously made. Moreover, acute PVT may be reported in such situations as developing a cavernoma in as short a duration as 0 to 2 days. Defining and diagnosing "acute-on-chronic PVT" is a challenge and requires careful longitudinal studies as this entity may have a different disease course.[19] PVT in pre-transplant and posttransplant settings has distinct presentations and outcomes. PVT in the posttransplant state should probably be included in a separate designation because the liver is no longer cirrhotic. As data become available, the classification may be improvised to include the etiology and comorbid conditions associated with PVT.

Future Directions

PVT is indeed a heterogeneous group of diseases that may be placed into a syndrome based on clinical presentations. The present commentary highlights the clinical presentation as that of greatest relevance in a patient with cirrhosis, and the entity of PVT should be characterized in both functional and anatomic terms and not only the latter. We believe that the proposed definition and classification of PVT provides clinicians and investigators a more uniform reporting system. The new classification should be of immense help in recruitment of a homogenous group of patients and define clear endpoints in trials of natural history of the disease and the efficacy of anticoagulant therapies in cirrhotic patients with PVT. In addition, we advocate validation and use of preset variables for segregating "high-risk" cirrhotic patients for early detection of PVT and introduction of preventive strategies.

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Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2016.08.033.

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Conflicts of interest
The authors disclose no conflicts.

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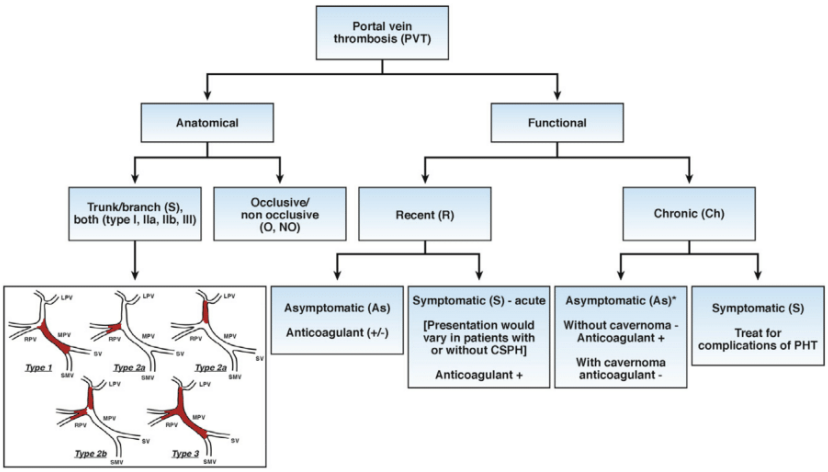

Supplementary Figure 1. Simplified Anatomico-Functional classification of portal vein thrombosis in patients with procoagulant states, anticoagulation is recommended to prevent extension of PVT.

Supplementary Table 1. Proposed Parameters for Predicting Pretest Probability of PVT in Cirrhosis

Major Characteristic	Minor Characteristic
Child's class B or C cirrhosis	Evidence of a largacte portosystemic shunt, large IGV1
Prior history of resolved PVT	Active hepatocellular malignancy
Associated prothrombotic risk factors – factor V LM, prothrombin gene mutation, MTHFR mutation.	History of/or active systemic venous thrombotic events or abortions
	Clinical symptoms and signs of acute abdomen
	New onset or worsening portal hypertension complications
	Recent abdominal interventions – endoscopic, radiological or surgical
	Portal flow velocity $<$ 15 cm per second at any time during prior Doppler evaluations

PVT, portal vein thrombosis.

Supplementary Table 2. Classification Systems of PVT

Stieber Classification (1991)[16]

Type A: segmental main PV involved
Type B: main PV and SMV
Type C: main PV and SV, main PV, SMV, SV, and IMV without considering PV branch involvement.

Importance	*Limitations*
First classification attempt	Only anatomic
Involves whole of portal venous system	No clear delineation between acute or chronic, No reference to the amount of vessel occlusion. No clinical implications

Nonami Classification (1992)[17]

Grade 1: thrombosis of Intrahepatic portal vein branches only
Grade 2: thrombosis of first branches of portal vein or at bifurcation
Grade 3: partial obstruction of PV
Grade 4: complete obstruction of PV trunk

Importance	*Limitations*
Pure consideration for portal vein	Only anatomic, Does not involve complete portal venous system
	No delineation between acute or chronic, No clinical implications

Yerdel Classification (2000)[18]

Grade 1: minimally or partially thrombosed PV where in thrombus is mild or confined to <50% of lumen with or without extension into SMV
Grade 2: >50% occlusion including total occlusion with or without minimal extension into SMV
Grade 3: complete thrombosis of both PV and proximal SMV
Grade 4: complete thrombosis of PV and both proximal and distal SMV

Importance	*Limitations*
Consideration of portal venous system involvement	Only anatomic
Management decisions in surgical procedures, including liver transplantation	No clear delineation between acute or chronic
	No clinical therapeutic decisiveness

Jingqin Ma et al (2014)[19]

Complete PVT (with or without cavernoma)
Partial PVT (with or without cavernoma)
Type I: partial PVT without cavernoma
Type II: partial PVT with cavernoma
Type III: complete PVT without cavernoma
Type IV: complete PVT with cavernoma

Importance	*Limitations*
Considers acute and chronic forms	Purely anatomic, No functional relevance
Clinically relevant, can assess progression of PVT and help therapeutic decisions	No etiology assessment

Jaimeson Classification (2000)[20]

Type 1: Thrombosis confined to the portal vein beyond the confluence of the SV and SMV
Type 2: Extension of thrombus into the SMV but with patent mesenteric vessels
Type 3: Diffuse thrombosis of splanchnic venous system but with large collaterals
Type 4: Extensive splanchnic venous thrombosis but with only fine collaterals

Importance	*Limitations*
Consideration of portal venous system involvement	No clinical, therapeutic decisiveness
Considered portal hypertension evolution and implications	Only anatomic
	Difficult to delineate acute versus chronic
	No etiology description

Bauer et al (2006)[21]

Grade 1: <25% occluded in PV, SMV, or SV
Grade 2: 26%–50% occluded in PV, SMV, or SV
Grade 3: 51%–75% occluded in PV, SMV or SV
Grade 4: 76%–100% occluded in PV, SMV, or SV

Importance	*Limitations*
Consideration of portal venous system involvement	Purely anatomic, difficult to precisely determine degree of occlusion
Gave a quantifiable grading system	Relation of degree of occlusion to flow has not been explained
Can be used for therapeutic monitoring	No etiologic description

Shie et al (2011) – for malignant PVT in cirrhosis[22]

Type I0: Tumor thrombus formation found under microscopy
Type I: Tumor thrombi involving segmental branches of portal vein or above

Supplementary Table 2. Continued

Shie et al (2011) – for malignant PVT in cirrhosis[22]	
Type II: Tumor thrombi involving right/left portal vein	
Type III: Tumor thrombi involving the main portal vein trunk	
Type IV: Tumor thrombi involving the superior mesenteric vein	
Importance	*Limitations*
Consideration of portal venous system involvement	Only for tumoral PVT
Can be utilized to see tumoral progression	No clinical or therapeutic implications
	No prognostic values between different grades

Baveno VI – Classification (2015)[23]	
Site of PVT – (Types 1, 2a, 2b, 3)	
Type 1: Only trunk	
Type 2: Only branch: 2a - One, 2b - Both branches	
Type 3: Trunk and branches	
Presentation (R, Ch)	
R: Recent (clinical presentation and presence of hyperdense thrombus on imaging)	
Ch: Chronic (with portal cavernoma and clinical features of portal hypertension, no hyperdense thrombus)	
Type of underlying liver disease: *(C, N, H, L, A)*	
C: Cirrhotic	
N: Non-cirrhotic liver disease	
H: HCC and other local malignancies	
L: Post-liver transplant	
A: Absence of underlying liver disease	
Degree of portal venous system occlusion (I,T)	
I: Incomplete: Flow visible in PV lumen through Imaging	
T: Total: No flow visible in PV lumen on imaging	
Extent of PV system occlusion (S, M)	
Splenic vein (S), mesenteric (M) vein or both (SM)	
Importance	*Limitations*
Defined acute and chronic thrombosis	Functional consequences not included
Etiology and underlying disease included	
Degree of occlusion and presence or absence of blood flow included; response to therapy can be monitored	
Useful for treating clinicians, surgeons and radiologists	

IMV, inferior mesenteric vein; PVT, portal vein thrombosis; PV, portal vein; SV, splenic vein; SMV, superior mesenteric vein.

Appendix 3

Causes and consequences of portal vein thrombosis in 1,243 patients with cirrhosis: results of a longitudinal study.

Nery F, Chevret S, Condat B, de Raucourt E, Boudaoud L, Rautou PLE, Plessier A, Roulot D, Chaffaut C, Bourcier V, Trinchet JC, Valla DC, Groupe d'Etude et de Traitment du Carcinome Hépatocellulaire. Hepatology. 2015 Feb; 61(2):660-7

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Causes and Consequences of Portal Vein Thrombosis in 1,243 Patients With Cirrhosis: Results of a Longitudinal Study

Filipe Nery,[1,2] Sylvie Chevret,[3,4] Bertrand Condat,[5] Emmanuelle de Raucourt,[6] Larbi Boudaoud,[6] Pierre-Emmanuel Rautou,[1,7,8] Aurelie Plessier,[1,7] Dominique Roulot,[9,10] Cendrine Chaffaut,[3,4] Valerie Bourcier,[11] Jean-Claude Trinchet,[11,12] and Dominique-Charles Valla,[1,7] on behalf of Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire

In cirrhosis, portal vein thrombosis (PVT) could be a cause or a consequence of the progression of liver disease. We analyzed data from a prospective trial of ultrasound screening for hepatocellular carcinoma in order to identify risk factors for and the impact of PVT in patients with cirrhosis. In all, 1,243 adults with cirrhosis without PVT were enrolled from 43 liver units in France and Belgium between June 2000 and March 2006. The mean follow-up was 47 months. Doppler ultrasonography was used to check the portal vein. Progression of liver disease was defined by the development of: ascites, hepatic encephalopathy, variceal bleeding, prothrombin <45%, serum bilirubin >45 μmol/L, albumin <28 g/L, and/or creatinine >115 μmol/L. G20210A prothrombin and factor V gene mutations were assessed in sera stored at three large centers. The 5-year cumulative incidence of PVT was 10.7%. PVT was mostly partial and varied over time. The development of PVT was independently associated with baseline esophageal varices ($P = 0.01$) and prothrombin time ($P = 0.002$), but not with disease progression before PVT, or prothrombotic mutations. Disease progression was independently associated with baseline age (hazard ratio [HR] 1.55; 95% confidence interval [CI]: 1.11-2.17), body mass index (HR 1.40; 95% CI: 1.01-1.95), prothrombin time (HR 0.79; 95% CI: 0.70-0.90), serum albumin (HR 0.97; 95% CI: 0.94-0.99), and esophageal varices (HR 1.70; 95% CI: 1.21-2.38) but not with the prior development of PVT (HR 1.32; 95% CI: 0.68-2.65). *Conclusion*: In patients with cirrhosis, the development of PVT is associated with the severity of liver disease at baseline, but does not follow a recent progression of liver disease. There is no evidence that the development of PVT is responsible for further progression of liver disease. (HEPATOLOGY 2015;61:660-667)

With the increased frequency of liver imaging thanks to accurate, noninvasive techniques, portal vein thrombosis in the absence of malignancy (so-called nonmalignant portal vein thrombosis and thereafter referred to as PVT) is increasingly identified in patients with cirrhosis. The estimated prevalence of PVT in these patients ranges from 0.6 to 26%.[1-3] Although several risk factors have been proposed

Abbreviations: FVL, Factor V Leiden; HCC, hepatocellular carcinoma; PVT, portal vein thrombosis.

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for this entity, they have not been clearly validated.[3] PVT has been associated with a prior decrease in portal blood flow velocity,[4] which is known to occur as liver disease progresses.[5] Common thrombophilia may also play a role, although available data are conflicting.[4,6-9]

Although PVT has been associated with the features of advanced cirrhosis in cross-sectional studies,[6,10] it is unclear if PVT is associated with a poor outcome independent of the severity of liver disease.[11-13] Interpretation of available data is limited by an overrepresentation of transplant candidates.[2] Theoretically, the association of PVT with advanced liver disease could be explained in several ways. 1) Advanced cirrhosis could be a risk factor for PVT. 2) By obstructing portal venous flow, PVT could cause the progression of liver disease, which would not exclude 1). 3) The factors causing the development of PVT and the progression of liver disease could be shared but the two may not be directly related the one another.

The identification of risk factors for the development of PVT in patients with cirrhosis and an accurate description of the impact of PVT on the course of cirrhosis could markedly modify the management of these patients by clarifying the rationale for anticoagulation therapy.[14] Therefore, we performed this preplanned satellite study in patients with Child A and B cirrhosis who participated in a multicenter prospective randomized trial on Doppler ultrasonography for the screening of hepatocellular carcinoma (HCC)[15] to identify risk factors for and the impact of the development of PVT in patients with cirrhosis.

Patients and Methods

Patient Selection and Study Design. Thrombocir is a satellite study of a multicenter randomized trial (CHC 2000) comparing 3- versus 6-month interval strategies for HCC ultrasound (US) screening, reported elsewhere.[15] Patients were included in Thrombocir if they fulfilled the following criteria: patients older than 18; with histologically proven cirrhosis; cirrhosis related to excessive alcohol consumption (80 g per day in males and 60 g per day in females for at least 10 years), chronic infection with hepatitis C virus (HCV), hepatitis B virus (HBV), or hereditary hemochromatosis; the absence of portal vein thrombosis, HCC, extrahepatic disease resulting in an estimated life expectancy of less than 1 year at inclusion, and coinfection with human immunodeficiency virus (HIV) at inclusion; and written informed consent.

Factor V Leiden and G20210A prothrombin gene mutations were tested in available serum samples stored in the three centers that included the largest number of patients (Hôpital Beaujon, Clichy; Hôpital Jean Verdier, Bondy; and Hôpital Avicenne, Bobigny), according to previously reported methods.[16,17] DNA was concentrated and purified from stored sera (QIAamp Circulating Nucleic Acid Kit, Quiagen, Hilden, Germany).

The Assistance Publique-Hôpitaux de Paris was the CHC 2000 trial sponsor. Approval was obtained from the Ethics Committee (CCPPRB, Aulnay-sous-Bois, France) for the original study, and also separately (May 2001) for testing genetic risk factors. All patients gave written informed consent to participate in the study.

Follow-up. Patients were regularly seen by physicians based on randomization for US screening. The usual clinical and biological data were recorded at every visit to the institution according to the randomization arm. Recommendations for the prophylaxis and the management of the complications of portal hypertension or cirrhosis were based on international guidelines, if any.[18-20] In case of death, the circumstances and likely cause(s) were recorded.

Ultrasound Screening. A Doppler US examination was performed every 3 or 6 months according to randomization. Individual patients were advised to undergo US in the same center by the same experienced operator.[15] The presence or absence of focal liver lesions, thrombosis of the portal vasculature, hepatic veins, and vena cava were reported on a standardized form. Portal vein flow velocity was recorded. Because of observer and equipment variability,[21,22] portal venous blood flow velocity was not considered among baseline characteristics, although changes in velocity over time were used in individual patients as a time-dependent variable.

Diagnosis of PVT. PVT was suspected when solid endoluminal material was detected in the main trunk of the portal vein and/or its branches, and confirmed by a filling defect on a Doppler study. Patients with suspected PVT underwent triphasic abdominal

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Potential conflict of interest: Nothing to report.

computed tomography (CT) or magnetic resonance imaging (MRI) to confirm the diagnosis. Occlusive PVT was defined by a thrombus leaving no channel for blood flow. Otherwise PVT was considered nonocclusive. Solid endoluminal material detected within 6 months before or after the detection of HCC was considered to be related to HCC and was not considered to be PVT in the subsequent analyses, because the differentiation between a bland thrombus and endoluminal tumoral invasion is difficult. The date that PVT first occurred was used to assess the incidence of PVT, whatever its later outcome (persistence or disappearance).

Decompensation and Liver Disease Progression. Decompensation was defined *a priori* as a composite outcome including the appearance of one or several of the following features: clinically detectable ascites, hepatic encephalopathy, variceal bleeding, jaundice, or serum bilirubin >45 μmol/L. Liver disease progression was defined *a priori* as a composite outcome including decompensation or the appearance of any of the following laboratory anomalies: prothrombin time <45%, serum albumin <28 g/L, or serum creatinine >115 μmol/L.

Statistical Analysis. Summary statistics, namely, medians with interquartile range (IQR) or percentages, were computed. Cumulative incidences, first of PVT then of progression or decompensation, were estimated in a competing risks setting, where the first death competed with the event of interest. Cause-specific hazards were modeled using the Cox proportional hazards model, stratified on the randomization arm with the cause-specific hazard ratio (HR) as the measure of the association between covariates and outcome. Log-linear relationships and proportional hazards assumptions were checked. Ninety-five percent confidence intervals (95% CI) were computed. Multivariate models included variables significantly associated with the outcome in univariate analyses at a level of 10%. A stepwise selection procedure was used.

Time-dependent covariates were used to assess the predictive value of the progression of liver disease and time-dependent measurements of ascites and of portal vein blood flow on the hazard of the development of PVT. Similar time-dependent covariates encoding the occurrence of PVT were used to assess the impact of such an event on the subsequent hazard of the progression of liver disease.

All statistical analyses were performed on SAS 9.3 (SAS, Cary, NC) or R 2.13.0 (http://www.R-project.org/) software packages. *P* values of 0.05 or less were considered statistically significant.

Results

From June 2000 to March 2006, a total of 1,278 patients with Child A and B cirrhosis were included in the CHC 2000 trial with a final analysis set for April 1, 2008 (mean follow-up 47 months). Of these, 35 patients with PVT at baseline (10 in the 3-month randomization arm and 25 in the 6-month randomization arm) were excluded. Thus, 1,243 patients (863 Child A patients and 380 Child B patients) were analyzed according to the flow chart presented in Fig. 1.

Clinical and laboratory characteristics and US findings at baseline are presented in Table 1 and Supporting Table 1, respectively. PVT developed in 118 patients, with only partial obstruction in 87 patients, only complete obstruction in 17, and obstruction that varied over time from partial to complete in 14 patients. Nine other patients developed portal vein obstruction related to HCC, and were not considered having PVT in later analyses. Overall, 1-, 3-, and 5-year cumulative incidence rates of PVT were 4.6% (95% CI: 3.4-5.7), 8.2% (95% CI: 6.7-9.9), and 10.7% (95% CI: 8.8-12.7), respectively. There was no difference between the incidence of nonocclusive PVT in the two randomization arms ($P = 0.15$; Fig. 2). Nonocclusive PVT varied over time, because the thrombus was not visible on later Doppler US examinations in 70/101 patients (70%), while the thrombus disappeared and then reappeared in 19/101 (19%) patients (Supporting Fig. 1).

Anticoagulation therapy was administered to 6 and 16 patients with and without PVT, respectively, for various indications. Nonspecific beta-adrenergic blockade was given prior to the development of PVT in 40/118 (34%) patients who developed PVT, and in 242/1125 (22%) patients who did not.

The variables associated with PVT in occlusive and nonocclusive thrombosis combined included alcohol (versus no alcohol), serum bilirubin level, alanine aminotransferase (ALT) level, prothrombin time, and medium- or large-sized (grade ≥2) esophageal varices at baseline. In the multivariate model, only prothrombin time (HR 0.82 [95% CI: 0.68-0.98] $P = 0.03$) and grade ≥2 esophageal varices (HR 2.14 [95% CI: 1.27-3.60] $P = 0.004$) remained statistically significant (Supporting Table 2). When occurring prior to PVT, *de novo* ascites, decreasing portal-blood flow velocity, nonselective beta-adrenergic blockade, liver disease progression, and decompensation were introduced into the model as time-dependent variables (Supporting Table 3). None of these time-dependent variables were found to be independent risk factors for PVT in a multivariate model stratified

Table 1. Characteristics of Patients at Inclusion in the Trial According to Portal Vein Thrombosis (PVT) Development

	Without PVT (N = 1,125)	With PVT (N = 118)	Total (N = 1,243)
Randomization			
3-monthly US arm	551 (49%)	67 (56.8%)	618 (49.7%)
6-monthly arm	574 (51%)	51 (43.2%)	625 (50.3%)
Male gender	778 (71.1%)	82 (70.1%)	860 (71%)
Age (<60 years)	765 (68.4%)	76 (65%)	598 (68%)
Etiology of cirrhosis			
HCV ± Alcohol	506 (45%)	45 (38.1%)	551 (44.3%)
Alcohol	432 (38.4%)	55 (46.6%)	487 (39.2%)
Current alcohol use	200 (17.8%)	16 (14.6%)	216 (17.4%)
Body-mass index (kg/m^2)	25.9 (23.1-29.4)	27 (23.6-29.4)	26 (23.1-29.4)
Ascites	30 (2.7%)	6 (5.1%)	36 (2.9%)
Splenomegaly	359 (31.9%)	39 (33%)	398 (32%)
Esophageal varices (grade ≥2)	183 (16.3%)	37 (31.5%)	220 (17.7%)
Platelet count (10^3/mm^3)	131 (92-175)	119 (89-164)	130 (91-174)
Serum sodium (mmol/L)	140 (138-142)	139 (137-141)	140 (138-142)
Serum creatinine (μmol/L)	77 (66-88)	76 (66-84)	77 (66-87)
Serum bilirubin (μmol/L)	15 (10.5-22)	19 (13-28)	15 (11-22)
AST (N <40 IU/L)	43 (29-72)	39 (29-55)	42 (29-70)
ALT (N <40 IU/L)	39 (24-74)	34 (22-52)	38 (23-70)
Prothrombin time (%)	80 (70-91)	76 (62-87)	80 (69-90)
Serum albumin (g/L)	40 (37-44)	40 (36-44)	41 (38-44)
Alkaline phosphatase (N <110 IU/L)	77 (57-108)	86 (64-124)	79 (58-109)

Data expressed as median (IQR) or N (%). Percent are computed in reference to the number of available determinations. N, upper limit of normal range.

shows that developing PVT (prior to progression of liver disease) was associated with a borderline significant increase in the risks of later progression and decompensation in univariate analyses, but not in multivariate analyses adjusting for baseline characteristics ($P = 0.41$ and 0.44, respectively).

Discussion

This preplanned study of prospective data has helped decipher the complex relationship between the development of PVT and the progression of liver disease. The parent study, involving two randomization arms of US screening every 3 and 6 months, did not show any benefit from more regular monitoring, although an increased number of nonmalignant nodules were identified in the 3-month arm.[15] The present findings show that PVT is not a direct consequence of the progression of liver disease. Furthermore, evidence of a direct impact of the development of PVT on the progression of liver disease was not found. The study population differs from that in previous studies who have mostly been liver transplant candidates. Including a majority of patients in good condition at baseline is at the same time a limitation and an advantage: a limitation because extrapolation of the data to patients with severe liver disease should be cautious; and an advantage because it has allowed evaluating the respective impacts of progression and PVT which would not have been possible at a stage of severe liver disease. Furthermore, the fact that 355 patients experienced progression allowed assessing this sizeable group of patients with advanced liver disease through time-dependent analyses.

The cumulative incidence of PVT was 4.6% at 1 year and 8.2% and 10.7% at 3 and 5 years, respectively, suggesting that the risk factors of PVT are already present at baseline and do not accumulate over time. There are only a few studies that have provided a longitudinal assessment of the development of PVT. The estimated incidence of PVT in three previous longitudinal surveys was higher (7.4%, 12.8%, and 16.4% per year); however, they included patients with more severe cirrhosis at baseline than the present study.[2,4,23]

Thrombosis was nonocclusive in 101/118 patients with PVT. This proportion is in the high range of estimates from retrospective studies in patients with more severe liver disease.[2] Because there is no validated method to measure occlusion of the venous lumen by the thrombus, the present study only separated occlusive from nonocclusive PVT of any degree. PVT was varied over time because it was no longer detected in later screening in 70% of patients, while it reappeared again in 19%. These findings are especially significant because anticoagulation therapy was only given to a handful of patients. These findings prospectively validate in a large group of patients the results of two retrospective cohort studies which showed that the degree of portal vein occlusion decreased in about 45% of patients with cirrhosis of various etiologies and severities.[23,24] Two conclusions can be drawn from these observations. First, obstruction of portal venous inflow is only partial in many patients with cirrhosis who develop PVT, at least according to routine imaging techniques; therefore, the real impact on liver blood perfusion requires a precise assessment of portal and total hepatic blood flow. Second, due to a high spontaneous variability in the extent of thrombosis over time, the actual efficacy of anticoagulation or transjugular intrahepatic portosystemic shunt (TIPS) in restoring full portal vein patency can only be evaluated in comparison to an untreated group.

The nonstatistically significant difference between the incidences of PVT according to screening intervals in Fig. 2 is probably related in part to our case definition. Indeed, patients were considered to have developed PVT on the date it was first identified, whatever the later outcome of the thrombus. Because of this, patients included in the 3-month screening arm would

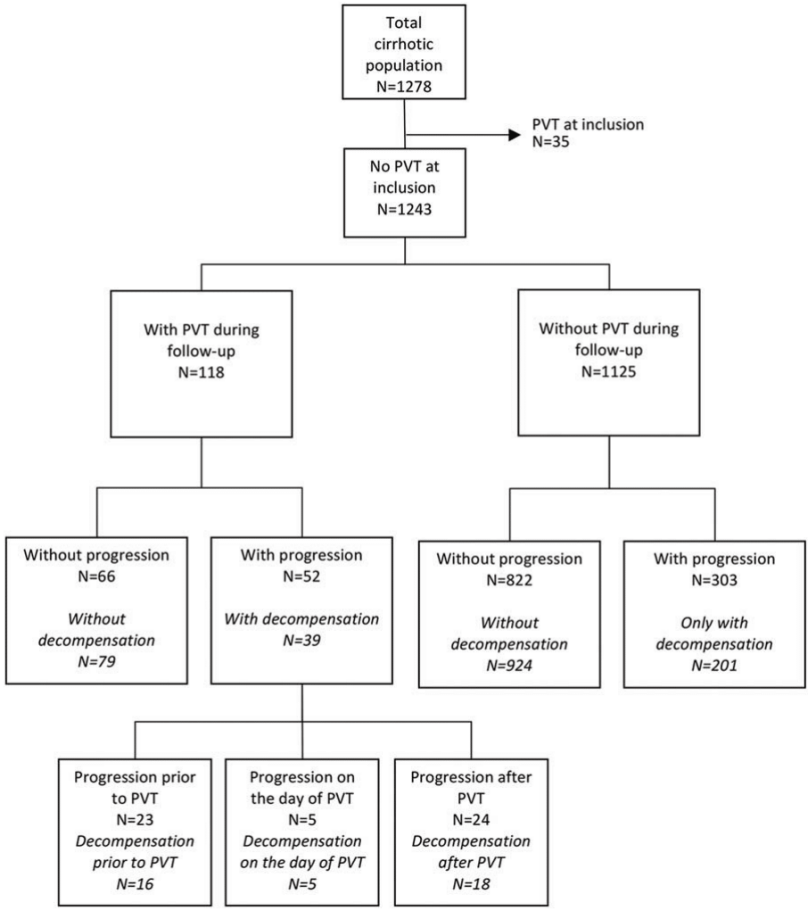

Fig. 1. Flow chart showing the distribution of patients according to the development of PVT and the progression of liver disease (defined as the development of one or more of the following features: ascites, variceal bleeding, hepatic encephalopathy, serum bilirubin >45 μmol/L, prothrombin time <45%, serum albumin <28 g/L, or serum creatinine >115 μmol/L) or decompensation defined as ascites, variceal bleeding, hepatic encephalopathy, or jaundice or serum bilirubin >45 μmol/L.

by randomization arm and adjusted for baseline variables (Supporting Table 4).

Factor V Leiden (FVL) and G20210A prothrombin gene mutations were investigated in 283 patients. Of these 283 patients, 25, 11, and 31 developed nonocclusive, occlusive, and variable (nonocclusive at certain points and occlusive at others) PVT, respectively. The G20210A prothrombin gene mutation was identified in 8 (3%) and FVL in 14 (5%) patients, all but one (FVL carrier) in a heterozygous state. The presence of the FVL or G20210A prothrombin gene mutation was not associated with PVT (HR 1.84 [95% CI: 0.68-4.98] $P = 0.23$), or the progression of liver disease (HR 0.64 [95% CI: 0.27-1.56] $P = 0.33$).

Overall, 150 patients died, 70 and 80 in the 3-month and 6-month randomization arms, respectively ($P = 0.38$). Factors contributing to death were hepatic failure in 30, renal failure in 9, spontaneous bacterial peritonitis in 11, gastrointestinal bleeding in 15, and various other factors in 86. In 20 patients, the cause of death was not determined. Both PVT and the progression of liver disease occurred in 52 patients (Fig. 1). Baseline factors associated with disease progression are reported in Supporting Table 5. As shown in Table 2, multivariate analysis identified age ($P = 0.01$), body-mass index ($P = 0.046$), grade of esophageal varices ≥2 ($P = 0.002$), prothrombin time ($P = 0.0002$), and serum albumin level ($P = 0.002$) as variables independently associated with progression. When decompensation instead of liver disease progression was analyzed, results of univariate analyses were similar (data not shown), but multivariate analyses identified only prothrombin time ($P < 0.0001$) and esophageal varices ($P < 0.0001$) as independent factors (Table 2). When death was analyzed, results of univariate analyses were also similar to those of liver disease progression (data not shown), but multivariate analysis identified only esophageal varices ($P = 0.0056$), serum albumin ($P = 0.038$), and serum bilirubin ($P = 0.02$) as independent factors.

The impact of the development of PVT on the progression of liver disease or decompensation was further tested using PVT as a time-dependent variable. Table 3

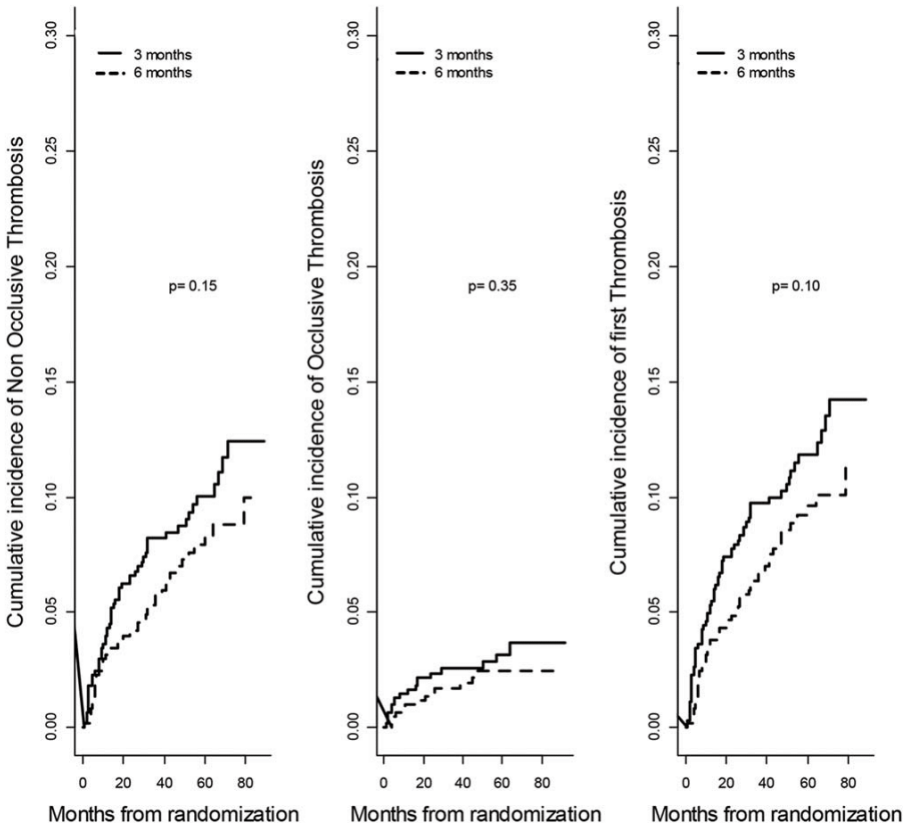

Fig. 2. Cumulative incidence of PVT by randomization arm. Left panel, nonocclusive thrombosis; middle panel, occlusive thrombosis; right panel, occlusive or nonocclusive thrombosis. By definition, occlusive thrombosis is characterized by the absence of any remaining channel for blood flow; thrombosis is considered nonocclusive otherwise.

be twice as likely to have temporary PVT identified as those in 6-month arm if the thrombus disappears within 3 months. To take into account this bias, we stratified all prognostic models on randomization arms. While thrombus development was confirmed with CT or MRI, thrombus disappearance was not requested by protocol to be confirmed by an independent imaging technique. However, repeated Doppler-US showing disappearance (presented in Supporting Fig. 1) provides solid evidence for the transient nature of a majority of these thrombi given the high sensitivity and specificity of Doppler-US for PVT diagnosis in patients with cirrhosis.[25]

This follow-up study provides an evaluation of possible causal factors of PVT in cirrhosis through time-dependent analysis. Although cross-sectional studies have shown the association of advanced cirrhosis with PVT they did not provide information on a causal relationship between advanced cirrhosis and PVT.[6,10,26] In the only existing prospective study on this topic, PVT developed at 1 year in 12/73 patients and, of the two baseline factors identified (Model for Endstage Liver Disease [MELD] and portal vein blood flow velocity), only the latter had an independent predictive value for the development of PVT.[4] There are important limitations in the reproducibility of portal vein blood flow velocity measurement according to the equipment and the operator that makes uncertain the generalization of specific thresholds or absolute values. Still, one possible explanation for these results could be that PVT is merely a consequence of more severe liver disease which causes more severe occlusion of portal venous inflow. The results of our study support this because features of more marked liver dysfunction (prothrombin levels) and portal hypertension (medium to large esophageal varices) at baseline were associated with the later development of PVT (Supporting Table 2). However, the present results also show that the development of PVT was not independently related to the worsening of liver disease over time whatever the definition of worsening: in particular, ascites, reduced portal blood flow velocity, or the composite outcome variables—progression of liver disease or decompensation (Supporting Tables 3, 4). Theoretically, nonselective beta adrenergic blockade could cause portal vein thrombosis by decreasing portal venous blood flow. However, the administration of these agents was not independently associated with the development of PVT after adjustment for the size of esophageal varices. Therefore, our findings show that

Table 2. Liver Disease Progression and Decompensation

	HR	95% Confidence Interval	P
Liver disease progression			
Age (≥60 years)	1.55	1.11-2.17	0.01
Body-mass index (kg/m^2)	1.40	1.01-1.95	0.046
Esophageal varices (≥ grade2)	1.70	1.21-2.38	0.002
Prothrombin time (%)	0.79	0.94-0.99	0.002
Serum albumin (g/L)	1.70	1.21-2.38	0.002
Decompensation			
Prothrombin time (%)	0.73	0.63-0.84	<0.0001
Esophageal varices (≥ grade2)	2.60	1.78-3.81	<0.0001
Death			
Esophageal varices (grade ≥2)	2.00	1.22-3.26	0.0056
Serum bilirubin (μmol/L)	1.15	1.01-1.31	0.038
Serum albumin (g/L)	0.96	0.93-0.99	0.02

Multivariate analysis on baseline predictive factors from Cox models stratified on randomization arm. Liver disease progression was defined as a composite outcome including the development of ascites, variceal bleeding, hepatic encephalopathy, serum bilirubin >45 μmol/L, prothrombin time <45%, albumin <28 g/L, or creatinine >115 μmol/L, Decompensation was defined as a composite outcome including the development of ascites, variceal bleeding, hepatic encephalopathy, or jaundice/serum bilirubin >45 μmol/L. HR hazard ratio. N, upper limit of normal range.

patients with more severe liver disease are at a higher risk of developing PVT, but the progression of liver disease *per se* is not a direct cause of PVT.

The notion that inherited risk factors of venous thrombosis could play a role in the development of PVT in patients with cirrhosis has been proposed but the results are contradictory.[4,6-9] The prevalence of G20210A prothrombin and FVL gene mutations found in 283 patients in our study (3% and 5%, respectively) is close to the estimated prevalence of 3.17% and 3.8%, respectively, in a large group of French subjects with no history of thromboembolism.[27] Furthermore, the results did not show any relationship between these two mutations and the development of PVT during follow-up. Although this is the largest existing study of patients with cirrhosis thus far, the power of the study is still a problem. Nevertheless, this follow-up study and previous cross-sectional studies show that these two underlying factors do not play a major causal role in PVT associated with cirrhosis.

The influence of the causal factors for cirrhosis on PVT incidence has not been comprehensively studied yet. After adjustment for other confounders, alcohol, or viral hepatitis B or C, were not found to be independent predictors for PVT. Furthermore, body mass index was not associated with PVT incidence in this study. Still, the possible impact of insulin resistance requires further assessment.

A major concern about the development of PVT in patients with cirrhosis is its role in the progression of liver disease. There are very few studies that have addressed this issue. A recent retrospective analysis in a group of patients with hepatitis-related cirrhosis who were selected because of available US data found a similar survival rate in 150 patients with (n = 42) and without (n = 108) PVT.[23] Another study found no relationship between survival and the degree of obstruction or the progression of the thrombosis in 42 patients with cirrhosis and PVT.[24] The present study, in a cohort where most patients initially had compensated cirrhosis, identified well-known independent predictive factors for the progression of liver disease, in particular, serum albumin, prothrombin, and the size of esophageal varices (Table 2). PVT, however, was not independently associated with the subsequent progression of liver disease or death. This result is confirmed by the fact that complete and permanent portal venous occlusion was shown only in a small minority of patients with PVT. Thus, the present findings suggest that the development of PVT is a marker, but not a direct cause of the progression of liver disease.

Table 3. Impact of Portal Vein Thrombosis (PVT) on Liver Disease Progression and Decompensation

	Univariate Models Unadjusted Estimates			Multivariate Models Adjusted for the Baseline Prognostic Variables*		
Models	HR	95% CI	P	HR	95% CI	P
Liver disease progression						
- Partial PVT	1.58	1.02-2.45	0.04	1.51	0.73-3.14	0.27
- Partial or Complete PVT	1.48	0.97-2.26	0.067	1.32	0.68-2.55	0.41
Decompensation						
- Partial PVT	1.77	1.07-2.92	0.027	1.60	0.69-3.74	0.28
- Partial or Complete PVT	1.61	0.98-2.62	0.058	1.37	0.62-3.03	0.44

Models of the estimation of PVT effect as time-dependent variable from Cox models stratified on randomization arms. Liver disease progression was defined as a composite outcome including the development of ascites, variceal bleeding, hepatic encephalopathy, serum bilirubin >45 μmol/L, prothrombin time <45%, albumin <28 g/L, or creatinine >115 μmol/l, Decompensation was defined as a composite outcome including the development of ascites, variceal bleeding, hepatic encephalopathy, or jaundice/serum bilirubin >45 μmol/L. HR, hazard ratio; 95% CI 95% confidence interval.

A similar dissociation between the progression of liver disease and PVT has been shown in a recent clinical trial of enoxaparin therapy given for 48 weeks to prevent PVT in patients with Child class B-C cirrhosis.[14] Prevention of disease progression seemed to be much more marked than the prevention of PVT alone. Furthermore, the results showed a decrease in bacterial translocation in patients receiving enoxaparin. The findings of that trial and the present study are highly consistent. They indicate that the development of PVT and the progression of liver disease are two separate consequences of a common mechanism that is targeted by enoxaparin. Coagulation factors act not only on clotting but also on platelets, endothelial cells, and stellate cells to stimulate fibrogenesis.[28] Therefore, it is tempting to hypothesize that the activation of coagulation factors in the cirrhotic liver or the portal venous system is the common mechanism for the progression of liver disease, on the one hand, and the development of PVT on the other. If this hypothesis is confirmed in further studies, the administration of anticoagulation therapy before, rather than after, the development of PVT could be indicated to improve the outcome of liver disease.

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.27546/suppinfo.

Appendix 4

Nonselective beta-blockers and risk of portal vein thrombosis in patients with cirrhosis: results of a prospective longitudinal study.

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Nonselective beta-blockers and the risk of portal vein thrombosis in patients with cirrhosis: results of a prospective longitudinal study

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Summary

Background: Nonmalignant portal vein thrombosis is a significant event in the course of cirrhosis that can contraindicate liver transplantation and even impact survival after the surgical procedure. Risk factors are not completely known or validated and are still debated.

Aim: To identify in patients with cirrhosis the risk factors for portal vein thrombosis that are assessable in clinical practice.

Methods: Between January 2014 and February 2017, 108 outpatients with cirrhosis and no portal vein thrombosis (78% Child A) were enrolled. Doppler ultrasound was performed every 3 or 6 months, for a median follow up of 19 months.

Results: Portal vein thrombosis developed in 11 patients. Nonselective beta-blockade (hazard ratio [HR] 10.56; 95% confidence interval [CI]: 1.35-82.73; $P = 0.025$), and medium or large-sized oesophageal varices (HR 5.67; 95% CI: 1.49-21.63; $P = 0.011$) at baseline were associated with portal vein thrombosis development. Although heart rate ($P < 0.001$) and portal blood flow velocity at baseline ($P = 0.005$) were significantly reduced by nonselective beta-blockers, they were not related to portal vein thrombosis development.

Conclusions: Our findings confirm an association between portal vein thrombosis development and oesophageal varices at baseline, but suggest that the association could be explained by exposure to nonselective beta-blockers, independently from effects on heart rate and portal blood flow velocity. The mechanisms that explain portal vein thrombosis development in patients on nonselective beta-blockers require elucidation in order to optimise targeting of nonselective beta-blockade in patients with cirrhosis.

Authors' complete affiliations are listed in Appendix section.

The Handling Editor for this article was Professor Peter Hayes, and it was accepted for publication after full peer-review.

1 | INTRODUCTION

In the general population, the prevalence of cirrhosis is estimated to be around 1% and in necropsy studies, 4.5%-9.5%, meaning that the disease affects several hundred million patients worldwide.[1,2] Portal vein thrombosis (PVT), causing various degrees of occlusion of the portal vein trunk and/or one or both of its branches, is a significant event in cirrhosis and, in particular, once established, PVT may become a technical contraindication for liver transplantation (LT), or negatively impact the post-transplant survival.[3,4] In Child-Pugh A and B patients, cumulative 1-, 3- and 5-year incidences have been estimated 4.6%, 8.2% and 10.7% respectively.[5] In patients with advanced cirrhosis listed for liver transplantation, 1-year incidence estimates have varied between 7.4% and 16.4%.[6,7] Even higher incidences have been reported in Egypt and China (17.9% per year and 24.7% per year respectively), suggesting that geographical factors may play a role.[8,9]

Contrasting with such a high incidence in patients with cirrhosis, causes and consequences of PVT in this setting remain disputed, as inconsistent results have been reported among surveys. These surveys actually differ by designs and populations analysed, which may explain the variability in their findings. Nonmalignant PVT in cirrhosis is generally considered a multifactorial event. The risk of PVT may vary according to the cause for chronic liver disease.[5,7,8,10] The degree of liver dysfunction and hereto-related hypercoagulable state[7,11] may be implicated. The severity of portal hypertension also appears to be involved as indicated by an increased risk of PVT in patients with low platelet counts,[6,7] large-sized oesophageal varices,[5] previous upper hypertensive haemorrhage episode,[6] large portosystemic collaterals[12] or ascites.[12] Importantly, an increased risk of PVT has been reported in patients with a portal flow velocity <15 cm/s,[7,8,13] but these findings have not been uniform.[5,9] In this context, the relationship with nonselective beta-blockade (NSBB) is of particular interest as nonselective beta-blockers may induce a slowing in portal blood flow that could precipitate thrombosis.[13] However, this point has not been investigated in depth.[14]

Therefore, a clarification of the risk factors for PVT that are assessable in clinical practice for prevention, or early recognition and prompt management is needed. This single-centre prospective and longitudinal study aims to revisit the risk factors for nonmalignant PVT development in cirrhosis, among the clinical, laboratory and radiological characteristics that are used in routine clinical practice.

2 | PATIENTS AND METHODS

2.1 | Patient selection and study design

The study *Fatores de Risco para Trombose da Veia Porta na Cirrose* (FRTVPCir) is a prospective observational study conducted from January 2014 to February 2017 at a tertiary referral liver transplantation centre (Centro Hospitalar Universitário do Porto - CHUP, Portugal). Eligibility criteria for inclusion were: age 18 years or older; the presence of cirrhosis (histologically proven and/or with unequivocal radiological or liver stiffness features) irrespective of the aetiology; the absence of an overt infection or hospitalisation in the previous 3 months; the absence of hepatocellular carcinoma (HCC) or any other malignancy (past or present), as well as the absence of PVT or any other previous splanchnic or extrasplanchnic thrombotic event, of human immunodeficiency virus (HIV) infection, of known haemostatic disorders, and of previous or current treatment with anticoagulation or anti-platelet agents. An alcoholic origin for cirrhosis was considered when alcohol consumption was over 80 g per day in males and 60 g per day in females for 10 years or more. Upper endoscopy within the last 6 months for the evaluation of features of portal hypertension was required. Patients having undergone endoscopic therapy for large varices were considered as having a history of large varices, regardless of the actual size of the varices at inclusion. Therefore, for the purpose of this study, a history of medium or large varices corresponded either to their presence at baseline or to their absence in patients with prior endoscopic therapy. In order to standardise estimates of nonselective beta-blockade exposure, a dose of 25 mg of carvedilol was considered equivalent to 80 mg of propranolol. Compensated cirrhosis was defined as a composite including patients without previous episodes of ascites, variceal bleeding, hepatic encephalopathy or jaundice (or bilirubin above 2.5 mg/dL). All the selected patients had simultaneous clinical, laboratory and radiological evaluations every 3 or 6 months, depending on whether they were listed for liver transplantation or not respectively. Patients were followed until diagnosis of PVT, liver transplantation, death or end of the period of the study. The local ethics committee approved the study protocol and personal data were encrypted as previewed by the national committee for data protection (local reference 209-DEFI/242-CES). All patients provided written informed consent to participate in the study after receiving complete oral and written information.

2.2 | Follow up and data collection

At the beginning of the study and every 3 or 6 months, a form was filled out with demographic, health status characterisation and clinical examination data. At each visit, blood was collected and immediately processed. By definition, the last observation was defined as the observation taking place before PVT occurrence or the last observation in patients not developing PVT, either at the end of follow up, or before liver transplantation, death or loss to follow up.

2.3 | Abdominal Doppler ultrasound (US) follow up and diagnosis of PVT

At each visit, an experienced sonographer (CM or JO) performed an abdominal Doppler US, the results of which were validated by a senior consultant in liver radiology (MTG). The operators were blinded to laboratory and anamnestic features. Doppler US was performed always using the same equipment (Toshiba Xario^{TM}), with a 2.4 MHz convex probe, on patients in supine position after a 12-hour fasting period and at least 15 minutes rest. For the analysis of portal flow rate, the

probe was fixed in a 30° to 60° angle between the Doppler beam and the long axis of portal vein, with portal blood flow velocity calculated automatically by the equipment and given as time averaged maximal velocity. Three portal blood flow velocity measurements with tracings of at least 5 seconds each were required and the average of them was taken as the final result. Portosystemic collaterals were considered to be present if mild (small collaterals near the spleen) or marked (splenic or gastric varices, splenorenal shunt or umbilical vein repermeation). Ascites was considered to be present or absent irrespective of its grade when present.

Portal vein thrombosis was suspected when solid hyperechoic material and a filling defect on Doppler study were found within the main trunk of the portal vein and/or its branches. Once PVT at abdomen Doppler US was suspected, a 3 phase abdominal multi-detector computed tomography scan was performed the same day in order to confirm the diagnosis, and determine the degree of the occlusion and the extension. Occlusion was considered complete when no remaining channel was seen, and partial occlusion otherwise.

2.4 | Statistical analysis

Summary statistics, namely, percentages, means or medians (after testing normality using the Kolmogorov-Smirnov test) and respective standard deviations or interquartile range (IQR) were computed. Comparisons between continuous variables and the occurrence of PVT were made using independent samples *t* test or Mann-Whitney *U* test for skewed distributions. Cause-specific hazards were modelled using the Cox proportional hazards model, with the cause-specific hazard ratio (HR) as the measure of the association between covariates and outcome. Log-linear relationships and proportional hazards assumptions were checked. Multivariate models included variables significantly associated with the outcome in univariate analyses at a level of 5% as well as variables previously reported to be associated with an increased risk of PVT in patients with cirrhosis. A step-wise selection procedure was used. Ninety-five percent confidence intervals (95% CI) were computed.

Time-dependent covariates were used to assess the predictive value of time-dependent measurements of portal vein blood flow on the hazard of the development of PVT.

For the specific analysis of the effect of nonselective beta-blockade on PVT, patients in whom nonselective beta-blocker was withdrawn before the last observation and in those in which nonselective beta-blocker was started in the course of the follow up were excluded. Differences in the mean portal flow rate velocity and heart rate according to the existence of PVT were estimated at baseline and at the end of follow up.

Statistical analyses were performed using Stata version 11.2 for Windows (Stata Corp LP, College Station, TX, USA).

3 | RESULTS

During the 3-year study period, 127 patients were considered for enrolment. As shown in Figure 1, 19 patients were excluded from final analysis. One hundred and eight patients were included. In two of these patients, acetylsalicylic acid was started during follow up, and in two others hepatocellular carcinoma was diagnosed; these four patients were censored at the date of the corresponding event. Median follow up was 19 months [IQR 17-24 months] and 12 months [IQR 8-25 months] for patients not developing and developing PVT respectively (P = 0.173).

Clinical and laboratory characteristics and ultrasound findings at baseline are presented in Table 1. Overall, most of the patients were males with Child-Pugh A cirrhosis. Alcohol was a causal factor in half of them. Fifty percent of patients were on nonselective beta-blockers. Eleven patients (10.2%) developed PVT during the follow up, as detected with Doppler US and confirmed by CT scan in all. The occlusion of the portal vein was complete in two patients and partial in nine. Variables related to PVT development by univariate analyses were nonselective beta-blockade (HR 10.56; 95% CI: 1.35-82.73; P = 0.025), and a history of medium or large-sized oesophageal varices at baseline (HR 5.67; 95% CI: 1.49-21.63; P = 0.011) (Table S1). PVT development was not related with baseline or end-of-follow up portal blood flow velocity (Table S2).

Fifty-seven patients were receiving nonselective beta-blockers at baseline. Ten of the 11 patients who developed PVT were under

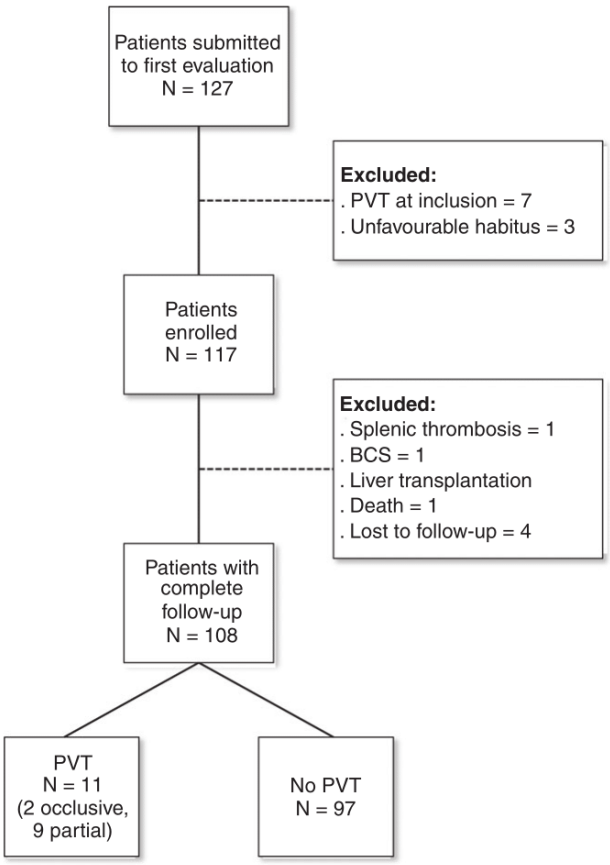

FIGURE 1 Flow diagram showing derivation of the studied cohort. PVT, portal vein thrombosis; BCS, Budd-chiari syndrome

TABLE 1 Clinical, laboratory and abdominal Doppler ultrasound findings at baseline in patients with cirrhosis who did or did not eventually develop portal vein thrombosis (PVT)

	Without PVT (N = 97)	With PVT (N = 11)
Age (years)	54.1 ± 10.9	57.8 ± 8.5
Male gender	69 (71.1%)	6 (54.6%)
Aetiology of cirrhosis		
Alcoholic	44 (45.4%)	4 (36.4%)
Viral[a]	14 (14.4%)	1 (9.1%)
Alcoholic + Viral[a]	11 (11.3%)	3 (27.3%)
Metabolic[b]	12 (12.4%)	0 (0.0%)
Autoimmune	12 (12.4%)	3 (27.3%)
Cryptogenic	4 (4.1%)	0 (0.0%)
Current alcohol use	7 (7.2%)	2 (18.2%)
Diabetes mellitus	29 (29.9%)	3 (27.3%)
Arterial hypertension	27 (27.8%)	2 (18.2%)
Dyslipidaemia	17 (17.5%)	3 (27.3%)
Body mass index (Kg/m^2)	27.7 ± 5.2	27.1 ± 5.0
Current NSBB use	47 (48.4%)	10 (90.9%)
Heart rate (bpm)	71 ± 10	67 ± 13
Current diuretics use	45 (46.4%)	6 (54.6%)
Ascites	19 (19.6%)	4 (36.4%)
Hepatic encephalopathy	8 (8.2%)	0 (0.0%)
Oesophageal varices (grade≥2)	28 (28.9%)	8 (72.7%)
OV previous rupture	16 (16.5%)	2 (18.2%)
Child-Pugh		
A	75 (77.3)	9 (81.8)
B	17 (17.5)	2 (18.2)
C	5 (5.2)	0 (0.0)
MELD ≥13	19 (19.6%)	2 (18.2%)
Compensated cirrhosis	51 (52.6%)	6 (54.6%)
Albumin (g/dL)	4.2 ± 0.6	3.9 ± 0.6
TB (mg/dL)	1.4 ± 1.4	1.5 ± 0.6
AST (U/L)	43.9 ± 31.7	44.2 ± 19.8
ALT (U/L)	37.8 ± 31.5	29.4 ± 15.8
INR	1.24 ± 0.24	1.26 ± 0.19
Platelets (10^9/L)	110.1 ± 57.6	84.4 ± 37.7
Portosystemic collaterals	21 (21.6%)	3 (27.3%)
PBFV (cm/s)	20.4 ± 5.0	20.6 ± 6.1
Spleen size (cm)	15.1 ± 3.4	16.1 ± 3.3

NSBB, nonselective beta-blockers; OV, oesophageal varices; TB, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; INR, international normalised ratio; PBFV, portal blood flow velocity.
Data are expressed as mean ± SD and categorical variables as frequencies (%).
[a]Hepatitis B virus and/or hepatitis C virus.
[b]Wilson's disease or haemochromatosis or non-alcoholic steatohepatitis or alpha-1 antitrypsin deficit.

nonselective beta-blockers during the entire follow up (Figure S1). Daily dose of nonselective beta-blockers was not significantly higher in patients developing PVT (47.5 ± 47.4 mg/d vs 34.6 ± 23.6 mg/d; $P = 0.212$). Nonselective beta-blockade decreased heart rate ($P < 0.001$) irrespectively of PVT development (Table S3 and S4). Portal blood flow velocity was significantly decreased in patients receiving nonselective beta-blockers either at baseline ($P = 0.031$) or at the end of follow up or before PVT diagnosis ($P = 0.005$) (Table S5). The effect of nonselective beta-blockade on PVT development was independent of decrease in heart rate or in portal blood flow velocity (Table 2 and Figure 2).

A history of medium- or large-sized oesophageal varices was associated with an increased risk of PVT (HR = 5.67; 95% CI: 1.49-21.63). No other markers of portal hypertension were related to subsequent PVT development, including ascites irrespective of its grade, thrombocytopenia, portosystemic collaterals or spleen size (Table S1). After adjustment for nonselective beta-blockers use however, a history of medium or large varices was not associated with a significant increase in the risk of PVT (Table 2).

4 | DISCUSSION

Thus far, the available data in patients with cirrhosis did not allow for risk factors of PVT development to be unequivocally identified. To the best of our knowledge, only four studies including the present one had a prospective and longitudinal design allowing to address this issue.[5,7,8] An additional randomised therapeutic trial enrolled only 36 patients in the control group with six patients developing PVT after 1 year of follow up, which precluded an analysis of the risk factors.[15] Of these four studies, only three, including the present one have been designed to assess the impact of baseline portal vein flow velocity by controlling for inter-observer variabil-

TABLE 2 Multivariate Cox proportional models of predictive factors for portal vein thrombosis (PVT) development, adjusted for potential confounders

	PVT hazard ratio	95% confidence interval	P
Oesophageal varices (Medium/Large vs Null/small)			
Crude	5.67	1.49-21.63	0.011
Adjusted for NSBB	2.45	0.55-10.89	0.238
NSBB (yes vs no)			
Crude	10.56	1.35-82.73	0.025
Adjusted for PBFV	12.47	1.58-98.43	0.017
Adjusted for heart rate	13.66	1.51-123.85	0.020
Adjusted for OV	6.15	0.63-59.96	0.118

NSBB, nonselective beta-blocker; PBFV, portal blood flow velocity (cm/s); OV, oesophageal varices.

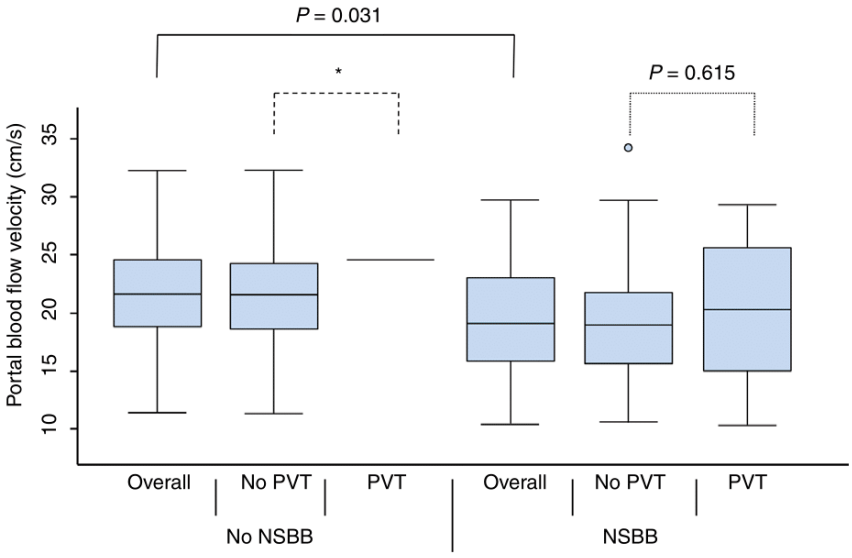

FIGURE 2 Box plot of baseline portal blood flow velocity in patients with cirrhosis developing or not developing portal vein thrombosis, according to the use of nonspecific beta-blockers use. PVT, portal vein thrombosis; NSBB, nonselective beta-blocker. * Only one patient not using nonselective beta-blocker and experiencing portal vein thrombosis

ity[7,8]; and only two, including this one have taken into account nonselective beta-blockade.[5] As to the characteristics of the study population, all the above prospective studies enrolled patients mostly without advanced cirrhosis, which contrasts with retrospective studies that mostly enrolled candidates to liver transplantation without hepatocellular carcinoma and, therefore, a more advanced liver disease.

Portal vein thrombosis developed in 10.2% (95% CI 4.6-15.7) of patients included in the present cohort. This incidence rate is intermediate between those previously reported in similarly designed surveys: 4.6%,[5] 16.4%[7] and 17.9%.[8] Only five patients (4.6%) in the present study had Child-Pugh C cirrhosis, none of whom developed PVT. Univariate analysis did not disclose any relationship between PVT development and the severity of liver dysfunction at baseline. Indeed, the distribution of Child class, mean serum bilirubin and albumin levels and mean INR were almost identical in patients with and without PVT development. In line with our findings, in the study by Zocco et al, including 25% of patients with Child C cirrhosis, liver dysfunction did not come out as a variable independently associated with PVT development.[7] However, Thrombocir on 1243 patients was powerful enough to detect the impact of subtle differences in liver function tests (serum bilirubin, serum albumin and INR) among patients eventually developing PVT .[5] Therefore, the independent impact of liver dysfunction on the risk of developing PVT can be regarded as of low magnitude and only detectable in powerful studies.[5,7,8]

Zocco et al[7] and Abdel-Razik et al[8] have documented prospectively an independent relationship between baseline portal blood flow velocity and 1-year incidence of PVT in patients with cirrhosis after adjustment for MELD score. A recently reported retrospective study based on 50 cases with PVT and their controls matched for age and MELD score comforted the association of reduced baseline portal blood flow velocity with subsequent PVT development.[13] Our findings do not confirm these results despite using a similar approach to that of Zocco et al[7] to address the issue of reproducibility in the assessment of portal blood flow velocity. Of note, average baseline portal blood flow velocity in patients developing PVT differed across studies: 11.8 ± 2.6 cm/s,[7] 11.6 ± 4.3 cm/s,[8] 16.9 cm/s (95% CI 13.9-20.0)[13] and 20.6 ± 6.1 cm/s (present study). For comparison, resting portal blood flow velocity in patients not developing PVT was 19.6 ± 5.7 cm/s,[7] 17.9 ± 4.5 cm/s,[8] 25.0 cm/s (95% CI 21.8-28.8)[13] and 20.4 ± 5.0 cm/s (present study) respectively. We do not have explanation to propose for these apparent differences besides well-established technical aspects. Therefore, conclusions on an independent link between resting portal flow velocity and the risk of PVT might have to wait for improved standardisation and reproducibility in blood flow velocity assessment. In addition, compared with baseline values, changes or absolute values for portal blood flow velocity taken during follow up may show increased predictive values for subsequent PVT.

The size of oesophageal varices was significantly associated with the risk of PVT in Thrombocir (HR 2.14, 95% CI 1.27-3.60)[5] and the present study (HR 5.67, 95% CI 1.49-21.63), not in the study by Zocco et al.[7] Yet, it is important to state that a history of oesophageal varices and nonselective beta-blocker use, is strongly related one to the other, reflecting current recommendations for clinical practice.[16] As a result, after adjustment for nonselective beta-blockade, a history of gastro-oesophageal varices failed to be independently associated with the development of PVT (Table 2). In addition, the variable 'size of oesophageal varices at baseline', as tested in Thrombocir, differs from the variable 'history of medium or large oesophageal varices', as tested in this study, because it does not take into account varices that were present but have been eradicated by endoscopic therapy at the time of enrolment.

Nonselective beta-blocker is the mainstay of therapy for the prevention of first and recurrent variceal bleeding.[16] The rationale is to lower portal hypertension through β1 receptor blockade—which reduces cardiac output—and β2 receptor blockade—which induces splanchnic vasoconstriction.[17] It has been hypothesised that through

β1 and β2 blockade, nonselective beta-blocker reduces portal vein inflow and portal flow velocity, leading to PVT development.[14] Actually, Pellicelli et al reported in a preliminary form that the use of nonselective beta-blockers was a risk factor for PVT in a cohort of 56 cirrhotic patients.[18] In Thrombocir, nonselective beta-blockade was also identified as a risk factor by univariate analysis, although multivariate analysis dismissed an independent effect after adjustment for liver dysfunction and baseline size of oesophageal varices.[5] Recently, nonselective beta-blockade was found to be an independent risk factor for PVT in a retrospective case-control study.[19] However, treatment duration, nonselective beta-blocker dose and effect on, or relationships with heart rate or portal vein blood flow were not reported.[19] In the present study, nonselective beta-blocker use was a risk factor for subsequent PVT development (HR 10.56; 95% CI: 1.35-82.73; $P = 0.025$). Patients taking nonselective beta-blockers had lower heart rates and portal flow velocities compared to their counterpart without nonselective beta-blockers (Table S3 and S5). Still, the effect of nonselective beta-blockade on PVT development persisted after adjustment for resting heart rate and portal blood flow velocity (Table 2). Thus, the effect of nonselective beta-blockade on PVT development appears to be mediated in part by different mechanisms than decreased resting heart rate, and resting portal blood flow velocity. In patients with cirrhosis on nonselective beta-blockers, the decrease in splanchnic blood flow is amplified during stress-associated liberation of adrenergic catecholamines.[20] The latter mechanism could be a unifying concept to explain the impact of nonspecific beta-blockade independent of resting portal blood flow velocity.

The limitations of this study are the relatively small sample size and follow-up period resulting in a limited power. Also, even though the effect of nonselective beta-blockade during entire follow up was taken into account, the duration of nonselective beta-blocker use before enrolment was not recorded, which may limit the interpretation of a time-dependent effect of nonselective beta-blockers on PVT development. However, this is the first prospective study specifically addressing the long debated issue of nonselective beta-blockers use and PVT development, using a robust designed way with standardisation of data collection.

In conclusion, this prospective study points to a role of nonselective beta-blockade as a risk factor for PVT development in patients with cirrhosis, independently of baseline resting portal blood flow velocity and heart rate. The mechanisms, and risk factors, that explain PVT development in patients on nonselective beta-blockers require characterisation if one wants to optimise targeting of nonselective beta-blockers in patients with cirrhosis.

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Declaration of personal interests: None.

AUTHORSHIP

Guarantor of the article: None.

Authors' contributions: FN, PER, HPM and DoV were involved in the study concept and design; FN, JG, VL, DiV, SF, HPM, CM, JO and MTG were involved in data acquisition; FN, SC, RL, PER, HPM and DoV were involved in interpretation and data analysis; FN, SC, and DoV were involved in manuscript draft; FN, SC, PER, HPM and DoV gave critical revision of the manuscript for important intellectual content; SC and RL were involved in statistical analysis; All authors revised the manuscript, data analysis and data interpretation

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section at the end of the article.

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APPENDIX

AUTHORS' COMPLETE AFFILIATIONS

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