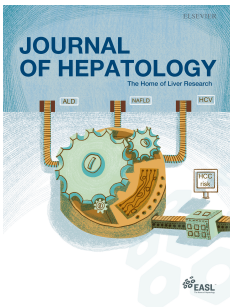

Incidence and factors predictive of recurrent thrombosis in patients with non-cirrhotic portal vein thrombosis

Anna Baiges, Bogdan Procopet, Gilberto Silva-Junior, Elba Llop, Luis Tellez, Anna Darnell, Ángeles Garcia-Criado, Fanny Turon, Oana Nicoara-Farcau, Carlos González-Alayón, Hélène Larrue, Marta Magaz, Pol Olivas, Valeria Perez-Campuzano, Jose Luis Calleja, Agustin Albillos, Juan Carlos Reverter, Christophe Bureau, Jaime Bosch, Virginia Hernández-Gea, Juan Carlos Garcia-Pagán, on behalf of REHEVASC and VALDIG, an EASL consortium

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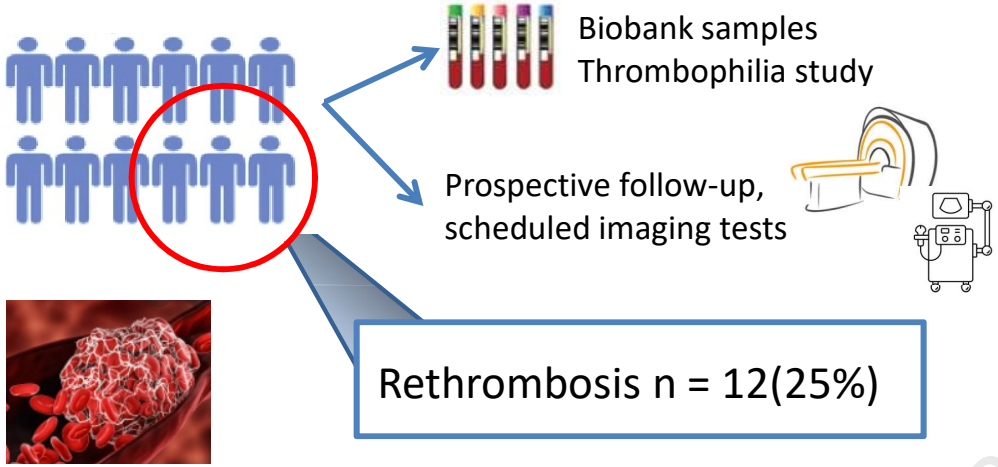
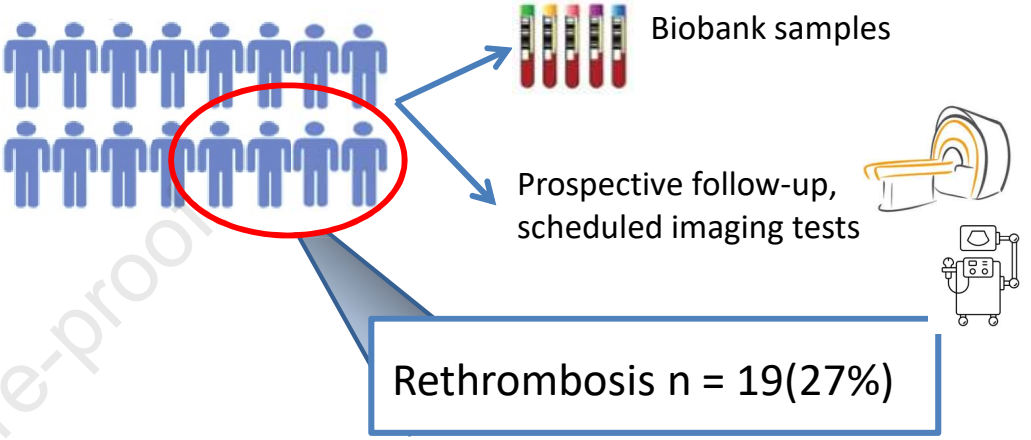
48 not anticoagulated patients with idiopathic or local factor NC-SVT

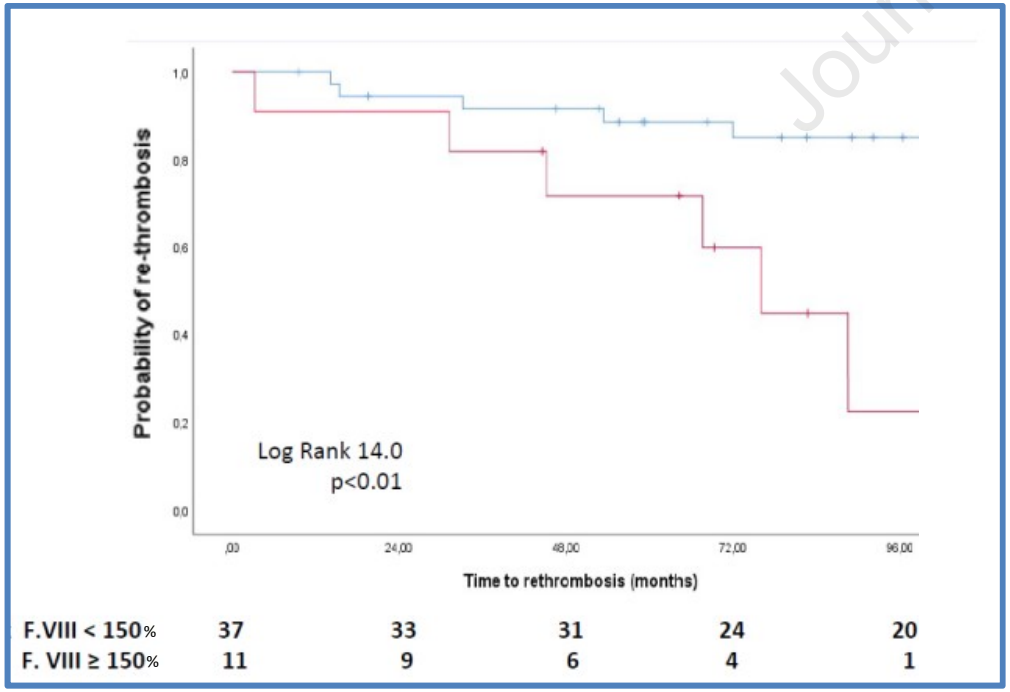


VALIDATION COHORT

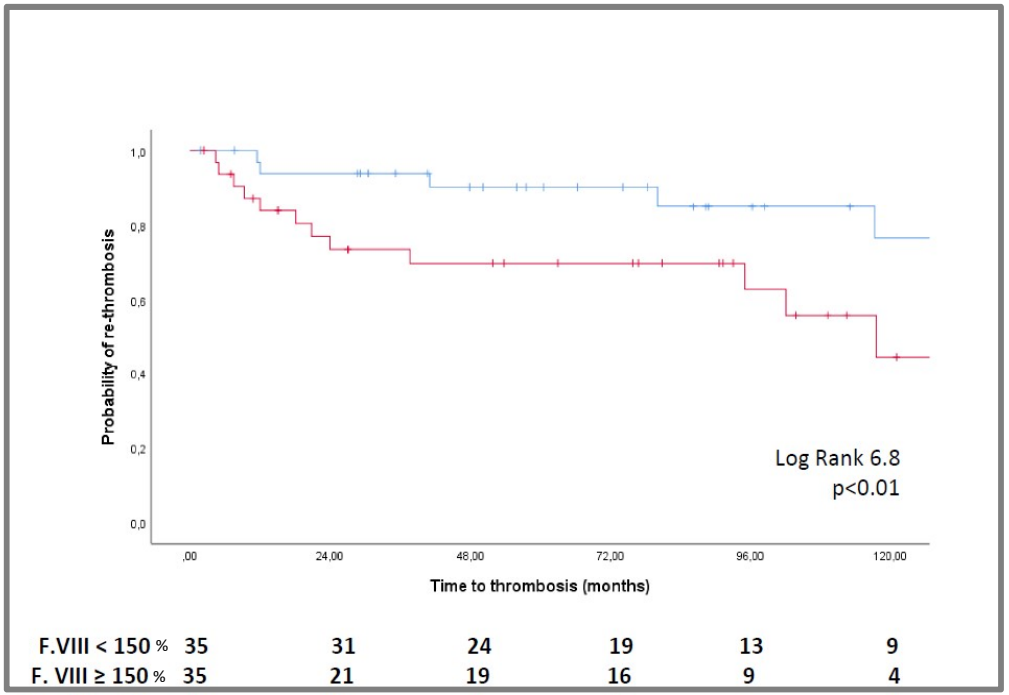
N= 70 not anticoagulated patients with idiopathic or local factor NC-SVT



Role of factor VIII



Factor VIII validation



Incidence and factors predictive of recurrent thrombosis in patients with non-cirrhotic portal vein thrombosis

Anna Baiges[1,2¥*], Bogdan Procopet[1,3,¥] Gilberto Silva-Junior[1,¥] , Elba Llop[2,4], Luis Tellez[2,5], Anna Darnell[6], Ángeles Garcia-Criado[6], Fanny Turon[1,2], Oana Nicoara-Farcau[1,3], Carlos González-Alayón[7], Hélène Larrue[8], Marta Magaz[1] , Pol Olivas[1], Valeria Perez-Campuzano[1], Jose Luis Calleja[2,4], Agustin Albillos[2,5], Juan Carlos Reverter[9], Christophe Bureau[8], Jaime Bosch[1,2,10], Virginia Hernández-Gea[1,2], Juan Carlos Garcia-Pagán[1,2*] on behalf of REHEVASC and VALDIG, an EASL consortium.

1 Barcelona Hepatic Hemodynamic Laboratory, Liver Unit, Hospital Clínic, Institut de Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain. Health Care Provider of the ERN-Liver.

2 Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Spain

3 University of Medicine and Pharmacy "Iuliu Hatieganu", 3rd Medical Clinic, Regional Institute of Gastroenterology and Hepatology "O. Fodor", Gastroenterology Department, Cluj-Napoca, Romania

4 Gastroenterology and Hepatology Service, Hospital Universitario de Puerta de Hierro-Majadahonda, Universidad Autónoma de Madrid, Madrid, Spain

5 Department of Gastroenterology, Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria, University of Alcalá, Madrid, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Spain

6 Centre de Diagnostic per l'Imatge, Hospital Clínic, Barcelona, Spain

7 Liver Unit, Hospital Universitario de Canarias (HUC), La Laguna, Spain

8 Service d'Hépatologie, Hôpital Rangueil, Fédération Hospitalo-Universitaire IMPACT, CHU de Toulouse, et Université Toulouse 3 - Paul Sabatier, 1 Av. du Professeur Jean Poulhès, 31400 Toulouse, France.

9 Hemotherapy and Hemostasis Department, Hospital Clínic, Institut de Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Spain

10 Hepatology, University Clinic for Visceral Surgery and Medicine, Inselspital, Bern University, Switzerland

REHEVASC: Registro Español de enfermedades hepáticas vasculares

VALDIG: Vascular liver diseases interest group

¥ Sharing first authorship: Anna Baiges; Bogdan Procopet; Gilberto Silva-Junior

*Sharing Corresponding Author:

Anna Baiges

Barcelona Hepatic Hemodynamic Lab

Liver Unit, Hospital Clínic, Villarroel 170, Barcelona (08036) Spain

abaigesa@clinic.cat

phone number (+34)93 2275753

Juan Carlos Garcia-Pagán, MD, PhD.

Head Barcelona Hepatic Hemodynamic Lab.

Senior Consultant in Hepatology

Associate Professor University of Barcelona

Liver Unit, Hospital Clínic. Villarroel 170, Barcelona (08036), Spain.

jcgarcia@clinic.cat

Phone number: (+34)93 2275753

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Authors contributions

AB, BP, GSJ , VHG and JCGP contributed to the study design, statistical analysis and drafting of the manuscript. AB, BP, GSJ, EL, LT, FT, OF, CA, HL, MM, PO and VPC contributed to acquisition of data. AD and AGC contributed to interpretation of imaging studies and revision of the manuscript. JLC, AA, JCR, CB, JB, VHG, JCGP contributed to critical revision of the manuscript for important intellectual content.

Abstract:

Background and aims: Clinical guidelines do not recommend long-term anticoagulation in non-cirrhotic splanchnic vein thrombosis (NC-SVT) without underlying thrombophilia because it is assumed that there is a very low risk of recurrent thrombosis (RT). Our first aim was to describe the incidence of RT in patients with NC-SVT without indication for long-term anticoagulation. The second aim was to identify RT risk factors and afterwards verify them in a validation cohort.

Methods: Multicenter retrospective observational study evaluating risk factors for RT in 64 patients with NC-SVT of idiopathic/local etiology. In a subgroup of 48 patients the potential value of additional thrombophilic parameters to predict RT was analyzed. Findings were validated in 70 independent patients with idiopathic/local NC-SVT.

Results: Of the 64 patients, 17 (26%) presented splanchnic and/or extra-splanchnic RT (overall-RT) during follow-up (cumulative incidence: 2%, 10%, 19% and 34% at 1, 2, 5 and 10 years). 53% of splanchnic RT were asymptomatic. No clinical or biochemical parameters predicted overall-RT. However, in the 48 patients with additional comprehensive thrombophilic study, factor VIII ≥ 150% was the only independent factor predicting overall-RT (HR 7.10 (CI 2.17 – 23.17) p< 0.01). In the validation cohort 19 patients (27%) presented overall-RT, and it was also independently predicted by factor VIII > 150% (HR 3.71 (1.31 – 10.5), p < 0.01). The predictive value of factor VIII was confirmed both in patients with idiopathic and with local etiology.

Conclusions: Patients with idiopathic/local NC-SVT are at risk of overall-RT. Splanchnic RT can be asymptomatic and requires screening for its detection. Values of factor VIII ≥ 150%

may help identify patients at high risk of overall-RT who could benefit from long-term anticoagulation.

Lay Summary

Patients with idiopathic/isolated local factor non cirrhotic splanchnic vein thrombosis (NC-SVT) were previously thought to be at minimal risk of rethrombosis. Our results show a 25% incidence of rethrombosis and support the indication of close follow-up to identify new thrombotic events, specially in patients with factor VIII >150%.

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Number of tables: 3 tables + 1 supplementary table

Data availability statement: Due to the confidentiality agreements, the datasets generated and analyzed for this study are only available from the corresponding author upon reasonable request.

Introduction

Non-cirrhotic non-malignant splanchnic vein thrombosis (NC-SVT) is the second most frequent cause of portal hypertension (PHT) in the western world (5-10%) [1]. Once the diagnosis of NC-SVT is established and a chronic liver disease is ruled out, an extensive etiological work-up should be performed with the aim of identifying potential underlying associated diseases. The etiology of NC-SVT can be classified as associated to a systemic factor, associated to a local factor or idiopathic: a myeloproliferative neoplasm (MPN) or a prothrombotic disorder can be identified in around 40% of patients, while up to 30% patients have an identifiable preceding local factor (previous abdominal surgery and/or abdominal infection or inflammation). Remarkably, one third of patients with a local factor also associate a systemic prothrombotic disorder, which supports the need of performing an exhaustive study of thrombophilia even in these cases. On the other hand, despite recent efforts to identify new thrombophilic mutations [2] and detect occult MPN [3,4], in up to 30% of cases no etiological factor can be identified despite a complete examination and have to be labelled as idiopathic NC-SVT [1].

Progression or recurrence of thrombosis (also called rethrombosis, RT) together with portal hypertension-related bleeding are the main threats of NC-SVT [5], and both have a negative impact in survival [6,7]. Presence of an underlying prothrombotic disorder, previous episodes of thrombosis elsewhere and intestinal infarction during the acute SVT episode increase the risk of RT and therefore current guidelines recommend to maintain long-term anticoagulation in these situations [8,9]. In the setting of NC-SVT of idiopathic etiology or exclusively associated to a local factor, the risk of recurrence is unknown and assumed to be low and therefore, due to the lack of robust data long-term anticoagulation is not recommended. However, clinical experience highlights that there are also cases of RT in this subgroup of non-treated patients. In this regard, recently the Baveno VII consensus has pointed out the possible role of D-dimer < 500 ng/mL in stablishing a low risk of thrombosis recurrence in patients without thrombophilia or with only low-risk prothrombotic disorders [10].

Previous studies have shown that, similarly to patients with cirrhosis, patients with NC-SVT exhibit an hemostatic profile with decreased levels of both procoagulant and anticoagulant factors together with increased levels of factor VIII and von Willebrand, all-together resulting in an elevated generation of endogenous thrombin potential [11–13]. This altered hemostatic profile is common to all NC-SVT regardless of the etiology of the thrombosis but its clinical significance is unknown. In other clinical scenarios (lower limb deep vein thrombosis and pulmonary embolism) increased levels of factor VIII [14,15] or Von

Willebrand Factor [16], have been associated with a higher risk of venous thrombosis. Indeed, factor VIII was shown to predict thrombosis recurrence in a concentration related manner [14,15]. In another study, factor VIII values above 150% were associated with venous rethrombosis [17].

The aims of the present study were to 1) describe the rate of RT in patients with NC-SVT and specifically in those with idiopathic or with a local factor not receiving long-term anticoagulation and 2) to identify factors predicting splanchnic and extra-splanchnic RT.

Patients and methods

This is a multicenter, retrospective observational study including patients with chronic non-cirrhotic non- malignant SVT (NC-SVT). All participating centers belong to REHEVASC consortia (Registro Español de Enfermedades Hepáticas Vasculares) and/or to the EASL-endorsed consortium VALDIG (Vascular liver diseases interest group). Inclusion criteria to participate in the study were to prospectively register all consecutive patients with the diagnosis of NC-SVT and manage them according to a pre-established protocol: 1) Evaluation of the extension of the splanchnic thrombosis by angio-CT scan or angio-MRI at diagnosis; 2) Performance of an exhaustive thrombophilic study as previously described (5, 13) even in patients with a recognized local factor; 3) Ruling out an underlying chronic liver disease; 4) Use of long-term anticoagulation in patients with prothrombotic conditions (MPN, congenital or acquired thrombophilia), severe initial thrombotic event (PVT with intestinal ischemia) or previous thrombotic events; and 5) Perform scheduled image follow-up studies to specifically evaluate recurrent splanchnic thrombosis (6). All patients included in this study signed a written informed consent to be registered and, a large proportion of them, consented as well to provide a blood sample for DNA, plasma and serum storage at the local Biobank. The protocol was reviewed and approved by the ethical committee at each participating institution.

Cohorts

From 2003 to 2015, 114 patients with chronic NC-SVT of different etiologies were included in the study. Among the 114 patients, 48 patients with idiopathic / local factor NC-SVT not receiving long-term anticoagulation and with a baseline biobank sample were considered the training cohort (participating centers Hospital Clínic de Barcelona, Hospital Ramon y Cajal, Hospital Puerta de Hierro) **(Figure 1)**. Patients with NC-SVT of idiopathic / local etiology not receiving anticoagulation that had a baseline biobank blood sample diagnosed after 2015 were allocated to the validation cohort (n = 70, participating hospitals Hospital Clínic de Barcelona, Hospital Ramon y Cajal, Hospital Puerta de Hierro, Regional Institute of Gastroenterology and Hepatology “O. Fodor; Hôpital Rangueil). Patients in the training

cohort were followed-up a median of 88 months (range 5 -156) and in the validation cohort 66 months (range 2 – 80).

Definitions

Baseline time of inclusion was the time of the first imaging study that allowed an accurate evaluation of the extension of NC-SVT in the splanchnic venous system (angio-CT-scan or angio-MRI). Radiological images were evaluated by each participating center. Splanchnic rethrombosis (RT) was defined as the development of a thrombus in a segment of the splanchnic venous axis not previously involved or progression from an incomplete to complete thrombosis proved by angio-CT scan, angio-MRI or by Doppler-US examination. For risk analysis, thrombosis extension at baseline was classified in three groups according to the territories involved (independently of the occlusion grade – complete or partial). Group 1: thrombosis involving a single-segment of the portal venous axis (intrahepatic portal vein branches, portal vein trunk, superior mesenteric vein or splenic vein); Group 2: affecting two of those previously mentioned segments and Group 3: affecting either three or more segments. In patients that had portal cavernoma at baseline, an accurate evaluation of the patency of collateral circulation, as well as patency of the splenic and mesenteric vein, was performed. Re-thrombosis in the portal cavernoma collateral circulation was considered as re-thrombosis. Extra-splanchnic thrombotic events (eRT) were defined as occurrence of any thrombotic event (i.e. myocardial infarction, stroke, deep vein thrombosis, etc) out of splanchnic territory. All re-thrombotic events (splanchnic + extra-splanchnic) will be referred as overall-RT.

Follow-up

Patients were censored at the time of splanchnic/extra-splanchnic RT or when the last imaging study able to evaluate splanchnic RT was performed. Patients were followed-up with imaging tests every six months with ultrasound (earlier if a clinical event occurred) or with angioCT/angioMRI every two to four years (the mean number of CT/MRI per patients was 9.2 ± 4 in the training cohort, and 8.9 ± 5 in the validation cohort). During the follow-up, the occurrence of overall-RT events was registered and patients were initiated on long-term anticoagulation and/or antiagregation upon clinical criteria.

Hemostatic Tests in Biobank blood samples

All patients included in the study had undergone a baseline thrombophilia study including: protein C, protein S, antithrombin, factor V Leiden, prothrombine gene mutation, lupus anticoagulant and anticardiolipin antibodies, JAK2 mutation, Calreticulin mutation (if JAK2 and all the other tests were negative).

In plasma samples stored at Biobank from patients with NC-SVT of idiopathic or isolated local factor etiology not receiving long-term anticoagulation, a centralized additional analysis of the following parameters was performed: prothrombin time (PT) and activated partial thromboplastin time (aPTT), fibrinogen, factors II, V, VII, VIII, IX, X, XI, and XII, Protein C activity, Free and total protein S, antithrombin activity, von Willebrand factor (vWF) antigen, vWF ristocetin cofactor, disintegrin and metalloprotease with thrombospondin type 1 motifs 13 antigen (ADAMTS-13), the plasma capacity to generate thrombin with and without thrombomodulin, prothrombin fragment 1+2 (F1+2), activated factor VII (FVIIa), plasmin-antiplasmin complexes (PAP), D-dimer and plasminogen as previously described [11]. A possible inherited deficiency of antithrombin, protein C, or protein S was excluded as previously described (14) by establishing a ratio of protein C, protein S, or antithrombin with (factor II + factor X)/2 greater than 0.7 and by the study of first degree relatives whenever possible (14, 15).

Statistical analysis

Continuous variables are reported as median and IQR and categorical variables are reported as absolute and relative frequencies. Groups were compared using the T test or the Mann-Whitney test for continuous variables when appropriate, and the Fisher exact test was used for categorical variables. Cox regression models and Kaplan Meyer analysis were used to study predictors of RT. Variables with < 0.10 significance on univariate analysis were included in the multivariate analysis. Significance was established as a 2-sided P value of 0.05. Statistical analyses were performed using SPSS version 20.0 IBM (IBM Corp., Armonk, NY) and Stata version 14.0 (StataCorp, USA).

Results

From 2003 to 2015, 114 patients were included in the study and were followed-up a median of 88 months (range 5 – 156) (**Figure 1**). **Table 1** summarizes baseline characteristics of these patients. Summing up, 72 (63%) were male, and the median age at inclusion was 48 years (IQR 36-58). Ninety-one patients (80%) had symptoms at diagnosis of the index thrombosis while 23 (20%) were asymptomatic at the index event. The extent of thrombosis at inclusion is described in **Table 1**. Forty-one patients (36%) had an underlying prothrombotic disorder (MPN in 29 and a thrombophilic disorder in 12), 36 were associated exclusively to a local factor (32%: abdominal surgery in 17, acute or chronic pancreatitis in 5, cholecystitis in 3 and other intraabdominal inflammatory lesions in 11) and 37 (32%) were considered idiopathic after an exhaustive negative etiological study. Fourteen patients had both an underlying prothrombotic disorders and an associated local factor. These patients were included in the prothrombotic disorder group.

In agreement with current guidelines (5), in patients with NC-SVT and an underlying thrombophilia long-term anticoagulation was recommended. However, among the 41 patients with underlying prothrombotic condition, seven patients (5 with MPN and 2 with other thrombophilia) were not anticoagulated because of patient refusal (n = 3) or due to history of severe bleeding complications (n=4). On the contrary, among the 73 patients with idiopathic or isolated local factor only 9 received long-term anticoagulation and it was due to a severe initial thrombotic event with intestinal ischemia (n = 4), previous non-splanchnic thrombotic episode (n = 3) or history of other non-thrombotic conditions requiring anticoagulation (n = 2). Thus, 64 patients with idiopathic or isolated local factor etiology did not receive long-term anticoagulation.

Splanchnic and extra-splanchnic thrombosis recurrence

Median imaging follow-up to evaluate the patency of the splanchnic venous axis was 88 months (range 5-156). During follow-up, imaging tests were performed every six months and hence a median of 8.5 imaging studies per patient was performed (range 2-24). Overall, in 71% of the 114 patients the last imaging follow-up study was an angio-CT scan or an angio-MRI. In the remaining 29%, the last follow-up imaging study was a Doppler-US.

Twenty-seven of the 114 patients presented overall-RT during follow-up with a cumulative incidence of 2.6%, 7.4%, 12.9% and 19.2 % at 1, 2, 5 and 10 years respectively: 17 (15%) presented splanchnic RT after a median of 31 months (range 5-88), and 13 extra-splanchnic RT after a median of 72.5 months (range 1 – 296) (7 deep vein thrombosis, 5 arterial thromboembolic events and 1 venous + arterial event) (**Supplementary Table 1)**. Most splanchnic RT were asymptomatic (n = 9, 53%) and detected at a scheduled imaging study. Long-term anticoagulation was initiated in all patients as soon as RT was identified. No further thrombotic events were observed once treatment was initiated, but recanalization of the new splanchnic thrombosis was only achieved in five of the seventeen patients.

With the exception of eight anticoagulated patients with MPN that presented new thrombotic events probably due to a difficult management of the underlying disease, overall-RT predominantly occurred in untreated patients: 17 out of the 27 overall-RT events occurred in the group of 64 patients with NC-SVT of idiopathic/local etiology not receiving long-term anticoagulation (17 overall RT, of which 14 splanchnic RT and 6 extra-splanchnic - 4 venous and 2 arterial events, with some patients presenting more than one event) **(Figure 1)**. None of the RT episodes was associated with a new local intraabdominal insult. The cumulative probability of overall-RT in this group was 2%, 10%, 19% and 34% at 1, 2, 5 and 10 years (**Figure 2**).

Having stablished that overall-RT mostly occurred in the 64 non-anticoagulated patients with idiopathic/local etiology group (**Figure 1; Supplementary Figure 1**), we subsequently decided to focus and study predicting factors of overall-RT in this subgroup of patients. Clinical and imaging data of the subgroup of 64 non-anticoagulated patients with idiopathic/local etiology were evaluated, including extension of the thrombosis – number of veins involved and partial or complete thrombosis– and symptomatic presentation of baseline thrombosis. At univariate analysis, none of these clinical and imaging parameters were associated with an increased risk of either splanchnic RT alone (data not shown) or of overall-RT **(Supplementary Table 2)**. In 48 out of the 64 patients (75%), a baseline (at diagnosis of NC-SVT) biobank sample was available and allowed to perform an additional exhaustive analysis of hemostasis as described in the methods section. The proportion of overall-RT in this subgroup of 48 patients was similar to that of the overall 64 patients with idiopathic/local etiology (12 patients with overall RT, of which 10 were splanchnic and 4 extra-splanchnic – 3 venous and 1 arterial), supporting that the 48 patients adequately represent the local/idiopathic thrombosis group (**Figure 1)**. In the analysis to identify risk factors predicting rethrombosis, we considered only venous thrombosis and excluded one patient with arterial thrombosis. As shown in **Table 2**, factor V, factor VIII - either as a continuous variable or using the **≥** 150 cut-off - , factor VIII ratio to protein C and to protein S, and von Willebrand factor were significantly associated to overall-RT at univariate analysis.

At multivariate analysis (**Table 3**), including variables with a $p < 0.1$ at univariate analysis, the only independent factor associated with overall-RT was factor VIII, both as a continuous variable and using the **≥** 150 cut-off. As the ratio events per variable was higher than desirable, we did an additional analysis adjusting the impact of FVIII with each single variable associated with RT at univariate analysis, and we still found that FVIII was the only independent factor associated with overall-RT. As shown in **Figure 3 panel A,** the 1, 5 and 7 years cumulative incidence of overall-RT in patients with a factor VIII ≥ 150 was of 10%, 29% and 56%, respectively. Similar results were obtained when analyzing separately splanchnic and extrasplanchnic rethrombosis (data not shown).

Validation cohort

As shown in **Table 1,** baseline characteristics of the 70 patients included in the validation cohort were similar to those of the training cohort**.** Patients were followed-up for a median of 66 months (range 2–80). During follow-up, 19 patients (27%) presented overall-RT at a median of 18 months (range 3–80) (14 patients splanchnic venous RT and 5 patients extra-

splanchnic: 4 in the venous territory and 1 in arterial territory; one patient presented both an extrasplanchnic and a splanchnic RT). Eight (57%) of the splanchnic RT were asymptomatic and diagnosed incidentally during a scheduled imaging test. Factor VIII, either as a continuous value or using the **≥** 150 cut-off, confirmed its independent value predicting overall-venous RT. As shown in **Figure 3 panel B,** cumulative incidence of overall-RT in the validation cohort of patients with a factor VIII ≥ 150 was of 18%, 28% and 36% at 1, 5 and 7 years respectively.

Training and validation cohort

Considering the 118 patients with idiopathic/local factor NC-SVT included in the training and validation cohorts, 72 patients had idiopathic and 46 had local factor NC-SVT. Seventeen patients (24%) with idiopathic NC-SVT and 14 (30%) with exclusively local NC-SVT developed overall-RT. No clinical or imaging data were able to predict rethrombosis neither in the idiopathic thrombosis group nor in the local factor thrombosis group. However, the predictive value of Factor VIII was confirmed in both idiopathic and local factor NC-SVT. Indeed, as shown in **Figure 4,** in both subgroups factor VIII ≥ 150 was independently associated to overall-RT (idiopathic: HR 5.13 [1.83 – 14.34], $p < 0.01$; local: HR 5.04 (1.5 – 16.6) $p < 0.01$).

Clinical decompensation

Combining the training and validation cohorts, 13 patients presented with liver decompensation during follow-up: 10 variceal bleeding (of which 5 had had overall-RT) and 3 ascites (of which only one had had overall-RT). We analyzed if RT had an impact on liver decompensation but we did not find any statistically significant association. However, the low number of overall-RT events and the initiation of anticoagulation once RT was identified could have influenced these results and do not make it possible to draw strong conclusions on this subject.

Discussion

Recurrence of thrombosis is a recognized risk in patients with a previous thrombotic event. It is known that patients with an underlying prothrombotic disorder have a moderate/high risk of rethrombosis and therefore life-long anticoagulation is recommended [8,18]. However, the risk of RT in patients with a negative prothrombotic study is not well characterized and the benefit of long-term anticoagulation is uncertain and consequently not currently endorsed [8]. Indeed, none of the available studies addressing the risk of rethrombosis is focused on idiopathic or isolated local factor NC-SVT [5–7]. The current

study addressed this subject carefully differentiating all known NC-SVT etiologies. In addition, patients underwent scheduled imaging screening intended to evaluate patency of the splanchnic territory with the aim of also detecting asymptomatic RT. Indeed, more than 50% of patients developing splanchnic RT were asymptomatic, suggesting that they would probably have been missed if not specifically explored. In this scenario, one of the most relevant findings in our study is the previously unreported and unexpectedly high incidence of recurrent splanchnic thrombosis in patients with idiopathic/local etiology that, in agreement with current guidelines [8,9] were not under anticoagulant treatment. In the same line, preliminary data from a clinical trial assessing the need of prophylactic anticoagulation in NC-SVT patients with low prothrombotic risk also suggests that the incidence of rethrombosis in non-treated patients is non negligible [19]. Accordingly, we intended to identify predictive risk factors for developing RT in this population of patients that usually represent more than one-half of patients with chronic NC-SVT. D-dimer levels (either as a continuous value or using the proposed low risk value of < 500 ng/mL [19]) were not useful to predict recurrent thrombosis in our population. Similarly, thrombin generation was not associated with the occurrence of rethrombosis, which highlights the limitations of this test in clinical practice. The present study identified that patients with NC-SVT without associated thrombophilia but with factor VIII ≥ 150% have a significantly higher incidence of recurrent thrombosis, both in the portal venous system and in extra-splanchnic territories, than patients with factor VIII < 150 (findings confirmed for splanchnic rethrombosis alone as well as for overall-RT). The predictive value of factor VIII levels in the setting of rethrombosis had already been described in other clinical scenarios. Indeed, it has already been shown that in patients with previous deep vein thrombosis or pulmonary embolism the risk of recurrent thromboembolic events increases progressively in a dose-dependent manner with higher factor VIII activity [15,20]. Moreover, factor VIII ≥ 150% was associated with primary deep venous thrombosis in comparison with matched healthy population [20], while other studies found that the risk of recurrent thromboembolic events is significantly higher when factor VIII level is above the 75^{th} to 95^{th} percentile [14,21,22]. The mechanism by which increased factor VIII promotes thrombosis is not completely understood , but it could lead to an increased thrombin formation rate and induced resistance to activated protein C, which normally inactivates factor V and factor VIII [16]. It is important to remark that mean factor VIII levels in patients with idiopathic/local etiology included the current study (134 ± 45%) is in the same range of values than those found in a previous study comparing the hemostatic profile of patients with NC-SVT of different etiologies with healthy controls (128 ± 40%) [11], thus supporting that patients included in our study actually are a good representation of the

NC-SVT general population.

Another concern derived from our study regards the best follow-up strategy in these patients: systematic screening versus symptoms-driven imaging. In the light of our findings, it seems justified to perform regular follow-up imaging tests in patients with NC-SVT not receiving long-term anticoagulation, since most episodes are asymptomatic. Nevertheless, the best interval between examinations and its cost-effectiveness still needs to be established. As US-Doppler examination has frequently insufficient accuracy to completely evaluate the total extension of splanchnic venous axis, periodical CT / MRI scans might be needed. Selecting the patients at high risk of rethrombosis may limit the cost and/or irradiation-risk, increasing the cost-effectiveness and safety of such strategy.

As expected, patients under long-term anticoagulation due to MPN/thrombophilia had a low risk of rethrombosis in the splanchnic territory. However, in agreement with a previous work from Hoekstra et al. [23], our study also showed that patients with MPN had a relatively high risk (25% of patients) of extra-splanchnic thromboembolic events despite long-term anticoagulation, probably due to a difficult management of their underlying condition. Whether these patients may benefit from a more aggressive treatment (i.e. anticoagulation combined with antiagregation) should be further investigated and is out of the scope of the current study.

The restrictive inclusion criteria based on the participant hospitals' management policies could confer a certain selection bias risk, given that the participant hospitals had to necessarily perform systematic follow-up imaging examinations in patients with NC-SVT regardless of the underlying condition. However, we consider that the risk of selection bias is low as the majority of the centers belonging to the REHEVASC and VALDIG network have adopted this policy, as well as prospectively and consecutively registering all patients with NC-SVT. Indeed, this is what enabled us to identify patients with asymptomatic recurrence, which was more than half of the RT events.

There are certain limitations that have to be taken into consideration. First, we have to acknowledge that we have not explored the mechanisms through which factor VIII facilitates rethrombosis, and therefore it remains to be elucidated if the increased levels of FVIII are what indeed lead to RT or if, on the contrary, FVIII is just a biomarker of an underlying not identified thrombophilic condition. However, given that we have validated these results in two different independent cohorts, we consider that the possibility that factor VIII levels are just a confounder factor is unlikely. Second, despite we are confident in our radiologist's accurate evaluation of thrombosis extension, we recognize that MRI does not have the same sensitivity for the splanchnic vessels assessment as CT scan

and it was used in several patients for the baseline thrombosis evaluation. Third, death has not been analyzed as a competitive event. It should be noted however that in this cohort the number of deaths during follow-up was very low and that it was equally balanced between patients with FVIII < or ≥ 150 and, therefore, probably it should not be considered a major limitation. Finally, in this study baseline evaluation of NC-SVT did not include liver biopsy in all patients and thus we cannot completely rule out portosinusoidal vascular liver disease (PSVD) which is a risk factor for NC-SVT and rethrombosis. Still, the development of PVT is not the only distinctive feature of PSVD. Liver elastography, imaging tests and a detailed medical history looking for concomitant diseases and/or treatment with certain medications can strongly suggest the presence of PSVD. Thus, even if it is true that we cannot assure that we have not included any PSVD in our cohort, we are certain that most of our cases are not PSVD-related and, in any case, the role of factor VIII in RT would still be significant.

Despite these limitations, our results are strongly supported by the use of a training and validation cohort. Being aware that with only 48 patients with a complete hemostatic work-up and only 12 overall-RT we could not draw any strong conclusions, we made an important collaborative effort to gather a validation cohort that enabled robust analysis and conclusive results. Moreover, despite the apparently relatively low number of events recorded, it must be taken into account that splanchnic vein thromboses are a rare disease with a prevalence of less than 5 cases every 10.000 inhabitants. In this context, the finding of 24-27% recurrence rate is remarkable and of clinical relevance.

Summing up, this is the first study focused on patients with idiopathic/isolated local factor NC-SVT, which were previously thought to be at minimal risk of overall-RT. As abovementioned before, our results show that contrarily to what current guidelines recommend these patients require a close follow-up to identify new thrombotic events. Recurrence of NC-SVT in patients with idiopathic or isolated local etiology is a real threat that should not be ignored, and further studies are needed to confirm if patients with factor VIII >150 would probably benefit from long-term anticoagulation.

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Figure's legend:

Figure 1 Flowchart detailing the thrombosis etiology and the treatment received of the first 114 patients enrolled, including the training cohort.
OA oral anticoagulation; RT recurrent thrombosis

Figure 2: Cumulative incidence by Kaplan Meier analysis of recurrent thrombosis in patients with idiopathic/local factor non-cirrhotic splanchnic vein thrombosis not receiving anticoagulation (training cohort).

Figure 3. Panel A Cumulative incidence by Kaplan Meier analysis of recurrent thrombosis in the training cohort according to the levels of baseline factor VIII. **Panel B** Cumulative incidence by Kaplan Meier analysis of recurrent thrombosis in the validation cohort according to the levels of baseline factor VIII

Figure 4. Cumulative incidence by Kaplan Meier analysis of recurrent thrombosis in training + validation cohort according to the levels of baseline factor VIII in patients with idiopathic non-cirrhotic splanchnic vein thrombosis (panel A) and in patients with associated to local factor non cirrhotic splanchnic vein thrombosis (panel B).

Supplementary Figure 1. Cumulative incidence by Kaplan Meier analysis of splanchnic rethrombosis in the 114 patients according to NC-SVT etiologogy

Table 1. Baseline characteristics of 114 patients with NC-PVT of different etiologies, of the training cohort (n = 48 patients with idiopathic / local factor non—cirrhotic portal vein thrombosis selected from the original 114 patients) and of the validation cohort (n=70)

Variable	**n = 114 n (%); Median (IQR)**	**TRAINING COHORT N =48 N(%); Median (IQR)**	**VALIDATION COHORT n = 70 n (%); Median (IQR)**
Gender, Male	72 (63%)	30 (63%)	49 (70%)
Age, Years	48 (36-58)	45 (36 – 56)	48 (29 – 59)
BMI > 30 Kg/m^2	13 (11%)	8 (17%)	9 (13%)
Etiology			
Idiopathic	37 (32%)	27 (56%)	45 (64%)
Local Factor	36 (32%)	21 (44%)	25 (36%)
MPN	29 (25%)	0	0
Thrombophilic	12 (11%)	0	0
Clinical manifestation at NC-PVT diagnosis*			
Asymptomatic	23 (20%)		41 (58%)
Abdominal pain	59 (52%)		22 (31%)
Ascites	29 (25%)		4 (6%)
Gastro Intestinal Bleeding	20 (18%)		14 (20%)
Fever	16 (14%)		6 (8%)
NC-PVT extension at inclusion			
1 main vein	22 (20%)	8 (17%)	28 (40%)
≥ 2 main veins	71 (62%)	26 (54%)	30 (43%)
entire portal vein axis	21 (18%)	14 (29%)	12 (17%)
Rethrombosis			
Overall - rethrombosis	27 (15%)	12 (25%)	19 (27%)
Splanchnic rethrombosis	17 (15%)	10 (21%)	14 (20%)
Extra-splanchnic rethrombosis	13 (11%)	4 (8%)	5 (7%)
Baseline Blood tests			
Hemoglobin (g/L)	12.8 (11.3-14.1)	12.9 (11.8 – 14.3)	13.0 (12.0 – 14.3)
Platelet count (*10^6/L)	180 (129-263)	151 (99 – 211)	141 (100- 255)
Leucocytes (*10^6/L)	5.8 (4.2-7.6)	5.4 (3.7 – 6.9)	5.3 (4.1 – 8.17)
INR	1.1 (1.0-1.4)	1.1 (1.0 – 1.2)	1.1 (1.0 – 1.2)
ALT (U/L)	28 (20-46)	28 (21 – 48)	26 (19 – 44)
AST (U/L)	28 (23-37)	28 (19 – 40)	28 (19 – 40)
GGT, U/L	42 (20-103)	28 (18-100)	60 (28 – 200)
Albumin, g/L	41 (36-44)	43 (37 – 45)	42 (39 – 44)
Bilirubin, mg/gL	0.85 (0.6-1.3)	0.9 (0.62 – 1.30)	2 (0.70 – 8.0)
Creatinine, mg/dL	0.89 (0.75-1)	0.8 (0.8- 1.0)	0.85 (0.70 – 0.90)
Sodium, mEq/L	140 (138-142)	140 (138 – 142)	141(139 – 142)

NC-PVT: non-cirrhotic and non-tumoral portal vein thrombosis; BMI- body mass index; MPN - myeloproliferative neoplasm, Thrombophilia disorders: protein C, protein S, antithrombin, factor V Leiden, prothrombine gene mutation, lupus anticoagulant and anticardiolipin antibodies, JAK2 mutation and Calreticulin mutation (if JAK2 and all the other tests are negative); INR -international normalized ratio; ALT. alanine aminotransferase; AST-

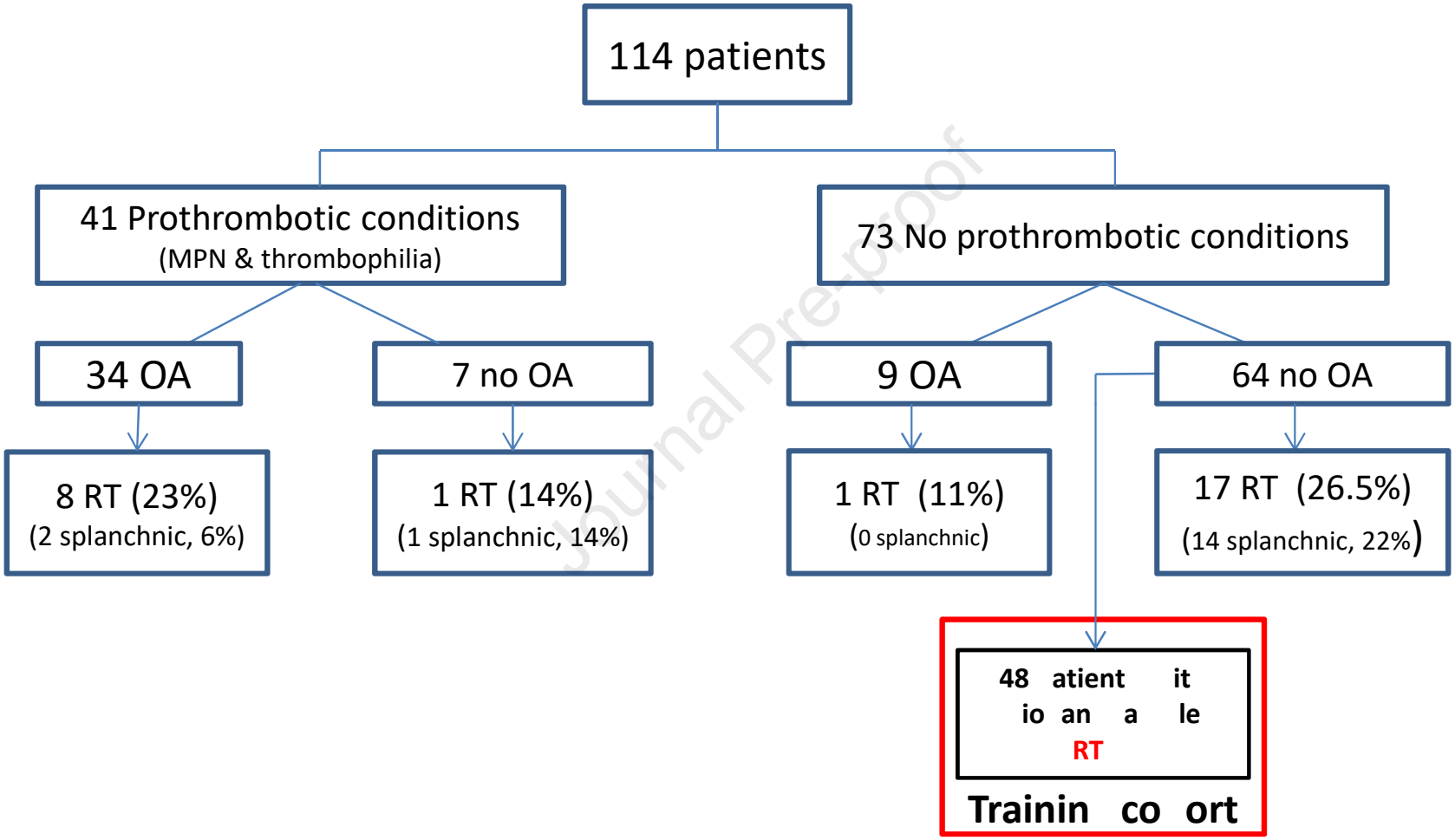
aspartate aminotransferase GGT. Gamma-glutamyl transferase, * more than one symptom.

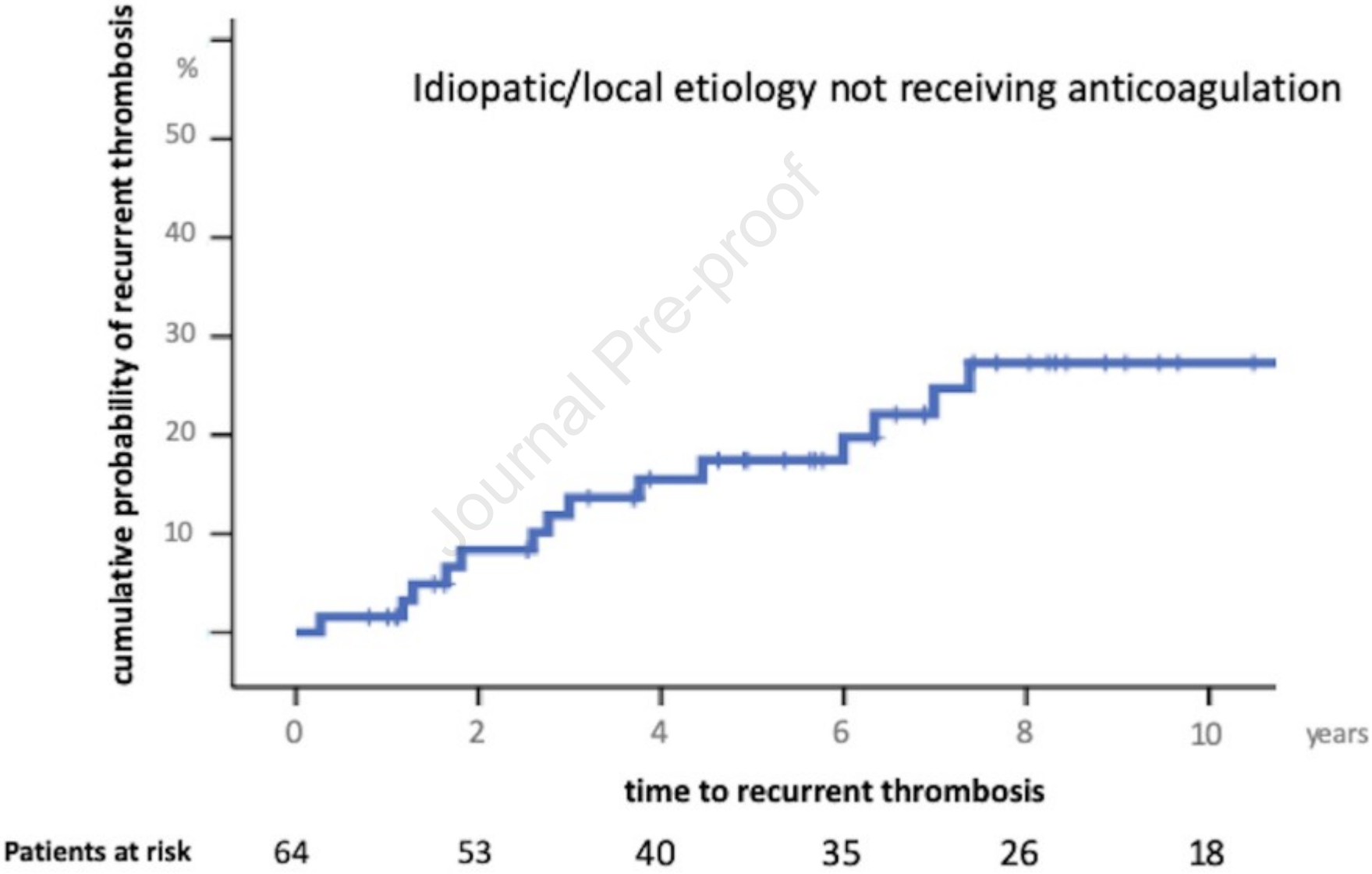
Table 2. Univariate Cox regression analysis of hemostatic factors for splanchnic and extra-splanchnic recurrent venous thrombosis (n= 11) in the 48 patients with non-cirrhotic portal vein thrombosis and baseline biobank sample (training cohort).

	HR (CI 95%)	p-value
Functional tests		
PT ratio	0.52 (0.018-14.95)	0.70
aPTT (s)	0.95 (0.88 – 1.01)	0.15
Procoagulant factors		
Fibrinogen (g/L)	1.13 (0.66 – 1.95)	0.64
Factor II (%)	0.99 (0.96 – 1.02)	0.81
Factor V (%)	**1.02 (1.00- 1.04)**	**0.02**
Factor VII (%)	1.01 (0.98 - 1.04)	0.50
Factor IX (%)	1.01 (0.99 – 1.03)	0.15
Factor X (%)	1.00 (0.97 – 1.04)	0.64
Factor XI (%)	1.02 (0.99 – 1.06)	0.16
Factor XII (%)	0.98 (00.95 – 1.02)	0.53
Anticoagulant factors		
AT III (%)	0.99 (0.96 – 1.04)	0.97
Antigen Protein C (%)	1.01 (0.97 – 1.05)	0.52
Protein C functional(%)	1.00 (0.97 – 1.03)	0.95
Protein S total (%)	1.01 (097 – 1.05)	0.54
Protein S free (%)	1.01 (0.97 – 1.05)	0.46
Markers of endothelial activation and regulator		
Factor VIII (%)	**1.01 (1.00- 1.03)**	**<0.01**
Factor VIII ≥ 150%	**6.6 (1.9 – 22.4)**	**<0.01**
Factor VIII/prot C	2.25 (1.07 – 4.72)	0.03
Factor VIII/prot S	4.63 (1.44 – 14.83)	0.01
vW factor (u/dl)	**1.01 (1.0 – 1.03)**	**0.04**
Von Wilebrand Rco (%)	1.00 (0.992 – 1.03)	0.30
vWF ratio	**0.15 (0.00 – 1.31)**	**0.06**
ADAMTS-13 (%)	1.01 (0.97 -1.04)	0.56
ETP without TM (nM x min))	0.99 (0.97 – 1.01)	0.61
ETP with TM ((nM x min)	0.99 (0.98 – 1.00)	0.13
Markers of coagulation activation		
Factor VIIa (ng/ml)	0.88 (0.65 – 1.21)	0.44
F1+2 (nmol/L)	0.49 (0.14 – 1.76)	0.27
Markers of fibrinolysis		
Plasminogen (%)	0.99 (0.96 – 1.03)	0.79
Dimer D (ng /ml)	**1.0 (1.00 – 1.01)**	**0.10**
Dimer D > 500 ng/ml	1.81 (0.54 – 6.14)	0.35
PAP (ug/L)	1.0 (0.99 – 1.00)	0.23

Table 3. Multivariate Cox-regression analysis for recurrent splanchnic and extra-splanchnic venous thrombosis in the training cohort (n = 48, RT n = 11).

	HR (CI 95%)	p-value
Model 1		
Factor VIII	1.01 (1.00 – 1.03)	<0.01
Factor V		0.09
vW factor (u/dL)		0.95
Ddimer		0.23
Model 2		
Factor VIII ≥ 150	7.10 (2.17 – 23.17)	<0.01
Factor V		0.24
vWa (u/dL)		0.99
Ddimer		0.22





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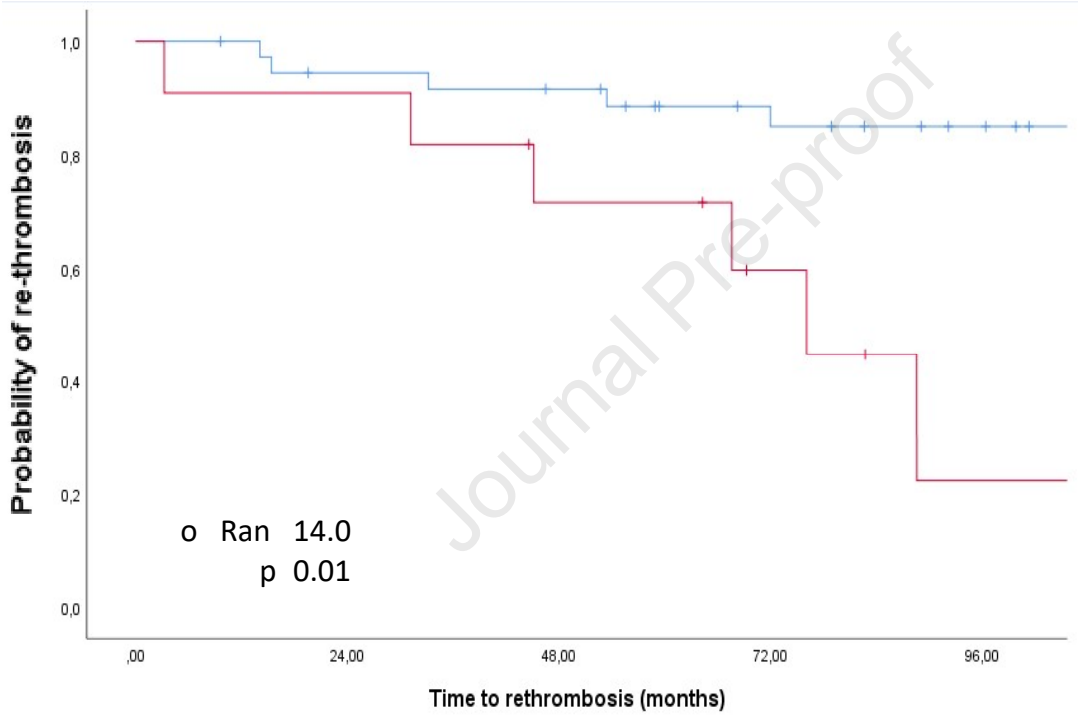

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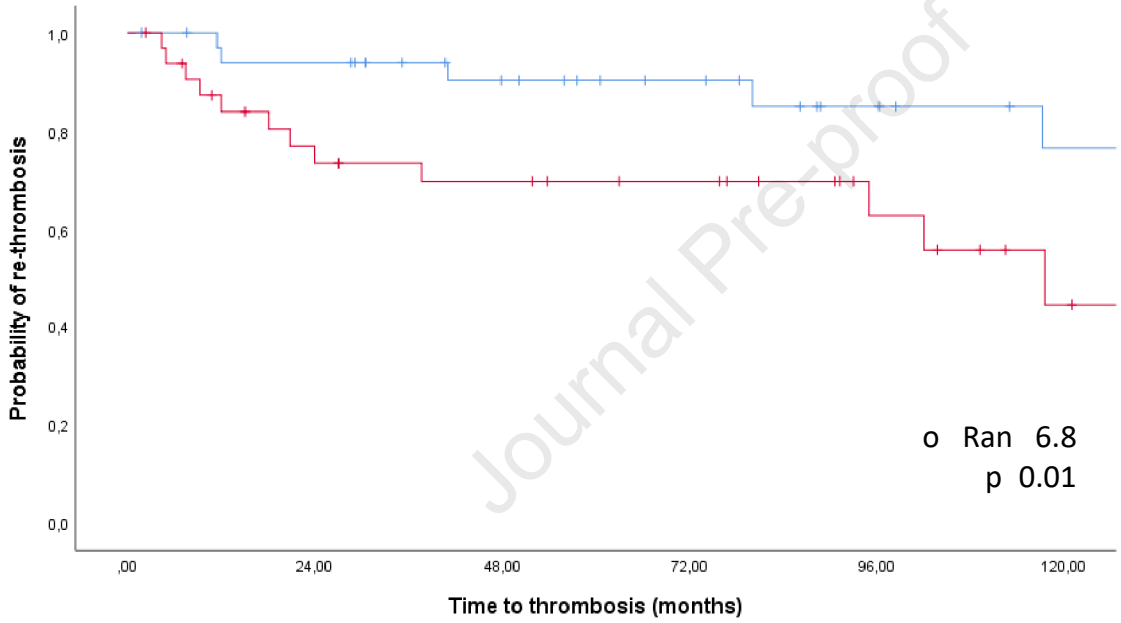

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Highlights

- Patients with non-cirrhotic splanchnic thrombosis without thrombophilia are at risk of rethrombosis
- Splanchnic rethrombosis can be asymptomatic and requires screening for its detection.
- Factor VIII ≥ 150% may help identify patients at higher risk of rethrombosis