

# Venous Thrombophilia, Platelet von Willebrand Factor Mediated Arteriolar Microvascular Thrombosis in JAK2V617F Mutated Thrombocythemia and Acquired ADAMTS13 Deficiency as Causes of Intrahepatic Obstructive Microvascular Liver Diseases in Budd-Chiari Syndrome and Splanchnic Vein Thrombosis

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## Article Info

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Thrombosis;  
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## Abstract

Congenital venous thrombophilia is associated with increased risk of venous thrombosis at adolescent and adult age, with recurrent abortions and fetal loss in females, and less frequently with splanchnic vein thrombosis, but not with arteriolar microvascular circulation disturbances. Based on original observations in view of the literature on thrombocythemia in patients with essential thrombocythemia (ET) and polycythemia vera (PV), both ET and PV are associated with increased risk of platelet-von Willebrand factor (VWF) mediated arteriolar microvascular circulation disturbances at adult age, with recurrent abortions and fetal loss in females, and less frequently with splanchnic vein thrombosis, but not with venous thromboembolism.

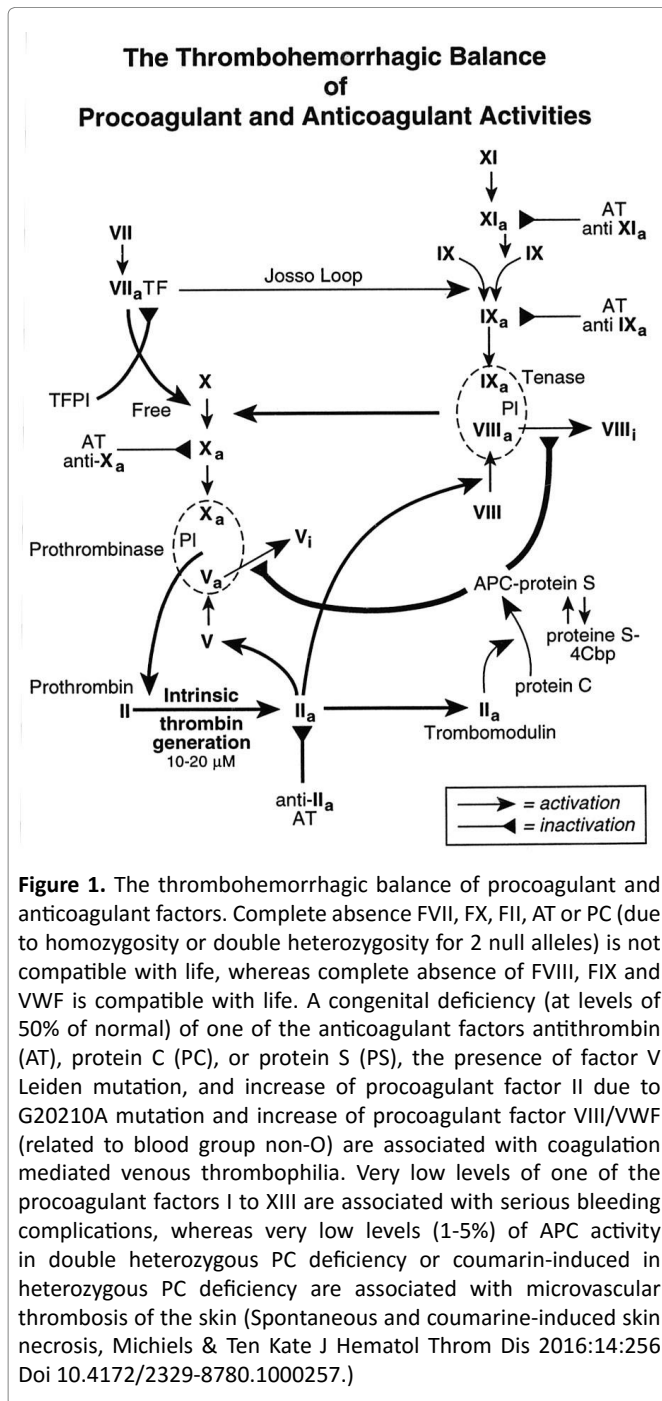
The high incidences of congenital venous thrombophilic factors including antithrombin III, protein C and S, factor V Leiden and the prothrombin G20210A mutation, acquired lupus anticoagulant as well as the presence of the JAK2<sup>V617F</sup> mutation indicative for thrombocythemia in trilinear myeloproliferative disease (MPD) are described as the underlying hypercoagulable states in patients with Budd-Chiari syndrome (BCS) and splanchnic vein thrombosis (SVT). In this editorial we propose the novel concept of coagulation and/or platelet mediated microvascular liver pathology is the primary event for the development of BCS, splanchnic vein thrombosis, and portal hypertension with portal and oesophageal varicose veins as a serious complication in patients with congenital thrombophilia and/or JAK2<sup>V617F</sup> mutated sticky platelets in clonal ET and PV. Clinical and liver pathology observations are in line with a two hit hypothesis of coagulation- and/or platelet-mediated thrombosis in the liver microcirculation as the underlying etiology of BCS and splanchnic vein thrombosis in patients with congenital venous thrombophilia and/or an acquired JAK2<sup>V617F</sup> mutated thrombocythemia in ET and PV patients. Severe ADAMTS13 deficiency in advanced liver cirrhosis is related to the severity of liver cell insufficiency due to the combined ADAMTS13 synthesis defect and autoantibodies against ADAMTS13 thereby explaining the more pronounced ADAMTS13 deficiency as compared to the degree of AT III synthesis deficiency in advanced liver cirrhosis. An imbalance between the severely decreased ADAMTS13:AC level and its substrate may indeed reflect the predisposing state for platelet thrombi in the liver microcirculation in patients with advanced liver cirrhosis similar on top of congenital venous thrombophilia and platelet-VWF mediated arteriolar microvascular thrombosis in JAK2<sup>V617F</sup> mutated thrombocythemia as etiological risk factors of intrahepatic microvascular obstructive diseases in BCS followed by splanchnic vein thrombosis.

## Introduction

Hereditary thrombophilia caused by congenital deficiency of an anticoagulant factor or an increased procoagulant factor is associated with an increased risk of coagulation mediated venous thrombosis (figure 1)<sup>1</sup>. Deficiency of the main physiological anticoagulant factors include deficiency of antithrombin III (AT) Protein C (PC) and Protein S (PS), and the Factor V Leiden (FV Leiden). Increased Factor II levels due to prothrombin G20210A gene mutation and blood group non-O related elevated

Factor VIII levels have been identified as risk factors for venous thrombosis. The congenital thrombophilias AT, PC, PS and FV Leiden are associated with increased risk of venous thrombosis at adolescent and adult age, with recurrent abortions and fetal loss in females, and less frequently with splanchnic vein thrombosis, but not with arteriolar microvascular circulation disturbances<sup>1</sup>. The frequency of hereditary thrombophilia in patients with confirmed idiopathic thrombosis (outside the clinical setting of surgery, trauma, or cancer) is approximately 25%. The most common genetic predisposition to venous thrombosis in Caucasians is activated Protein C (APC) resistance, which is caused by the Factor V Leiden mutation in about 90%. Combined deficiencies of FV Leiden, PC, PS, AT, the prothrombin mutation G20210A or non-O blood group have been described to lead to a higher risk of venous thrombosis (figure 1)<sup>1</sup>. The liver produces the procoagulant vitamin K dependent procoagulant factors II, VII, IX, X, the vitamin K dependent anticoagulant factors protein C, protein S, and the vitamin K independent factor FV, FVIII, fibrinogen and antithrombin III<sup>2</sup>. As AT III is neither vitamin K dependent nor a reactive protein, quantitative measurement of AT III levels using a chromogenic assay best reflect the degree of liver parenchymal function in chronic liver diseases<sup>2</sup>.

The PVSG classifications used platelet counts in excess of  $600 \times 10^9/L$  as the minimum criterion for the diagnosis of ET<sup>3-5</sup>, thereby overlooking the early stages of myeloproliferative diseases (MPD). This comprises about 30% of masked MPD, which prompted Michiels & Thiele to define the European Clinical and Pathological (ECP) criteria<sup>4</sup>. The ECP criteria lowered the platelet count cut-off to  $400 \times 10^9/L$  (upper limit of normal) on top of bone marrow histology as a pathognomonic clue to the diagnosis of thrombocythemia in various MPDs<sup>4</sup>. ET according to the ECP criteria comprises three types of WHO defined prefibrotic MPD or myeloproliferative neoplasms (MPNs) including ET, thrombocythemia associated with polycythemia vera (PV), and prefibrotic primary megakaryocytic granulocytic myeloproliferation (PMGM) without features of PV. The ECP criteria in table 1 include the presence of the JAK2<sup>V617F</sup> mutation in granulocytes and large clustered megakaryocytes in a normal cellular or hypercellular bone marrow due to increased erythromegakaryopiesis or erythro-megakaryo-granulopoiesis as pathognomonic clues to the diagnoses of ET and PV (Table 1)<sup>3-6</sup>. The combined use of JAK2<sup>V617F</sup> mutation screening and BMB evaluation has the potential to differentiate early stage MPN from reactive thrombocytosis and erythrocytosis with a sensitivity and specificity near to 100% (Table 1)<sup>3-6</sup>. JAK2<sup>V617F</sup> mutated thrombocythemia in ET and PV patients is associated with increased risk of platelet-mediated arteriolar microvascular circulation disturbances at adult age, with recurrent abortions and



**Table 1.** Diagnosis of the early stage prefibrotic JAK2<sup>V617F</sup> mutated myeloproliferative disorders essential thrombocythemia (ET), early thrombocytopenic prodromal PV and prefibrotic classical PV with trilinear erythrocytic, megakaryocytic and granulocytic myeloproliferation (EMGM) according to a new set of simplified European Clinical Molecular Pathological (ECMP) criteria<sup>4-6</sup>

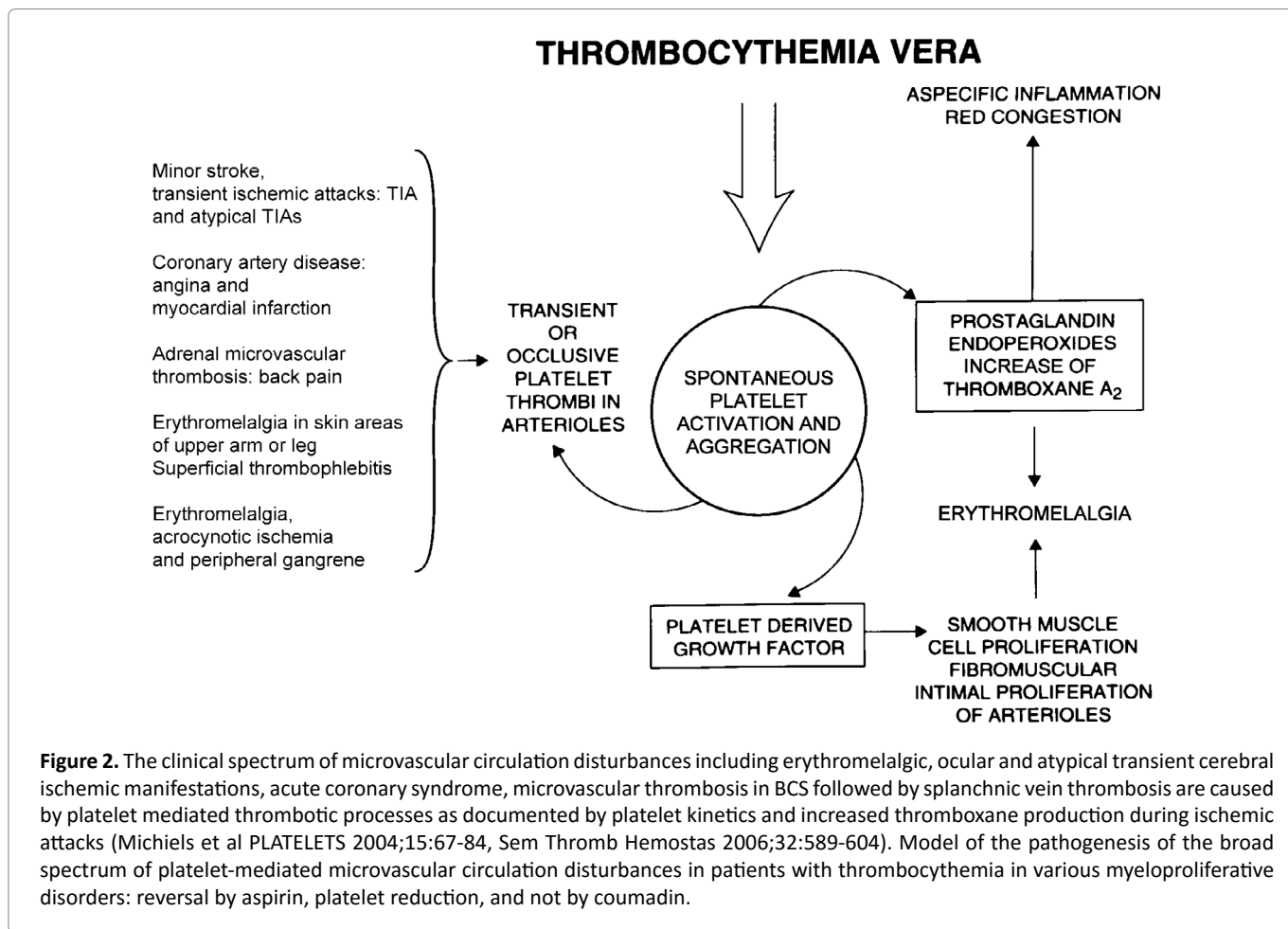
Clinical and molecular criteria	WHO bone marrow criteria
<b>ET</b>	<b>ET</b>
<ol style="list-style-type: none"> <li>1. Platelet count of &gt;400 x10<sup>9</sup>/l and the presence of large platelets in a blood smear</li> <li>2. Presence of JAK2<sup>V617F</sup> mutation</li> <li>3. Hematocrit: male &lt;0.51, female &lt;0.48,</li> <li>4. Erythrocytes less than 6.0x10<sup>12</sup>/L</li> </ol>	<p>Predominant proliferation of enlarged megakaryocytes with hyperlobulated nuclei and mature cytoplasm, lacking conspicuous morphological abnormalities. No increase, proliferation or immaturity of granulopoiesis or erythropoiesis. No or only borderline increase in reticulin.</p>
<b>Early prodromal and classical PV</b>	<b>Prodromal and classical PV</b>
<ol style="list-style-type: none"> <li>1. Platelet count of ≥400 x10<sup>9</sup>/l</li> <li>2. Early PV: normal hematocrit: male &lt;0.51, female &lt;0.48, and erythrocytes less than 6.0x10<sup>12</sup>/L</li> <li>3. Classical PV: increased hematocrit and erythrocytes above 6.0x10<sup>12</sup>/L</li> <li>5. Presence of JAK2<sup>V617F</sup> mutation</li> <li>6. Low serum EPO level, increased score for leukocyte alkaline phosphatase (LAP)</li> <li>7. No or minor splenomegaly</li> </ol>	<p>Increased cellularity due to trilinear erythrocytic, megakaryocytic, granulocytic myeloproliferation (EMGM = panmyelosis). Proliferation and clustering of small to giant (pleomorphic) megakaryocytes. No pronounced inflammatory reaction (plasmacytosis, cellular debris). Absence bone marrow features consistent with congenital polycythemia and secondary erythrocytosis. No or only borderline increase in reticulin. Spontaneous EEC</p>
<b>Prefibrotic EMG trilinear MPN</b>	<b>Prefibrotic EMG (masked PV) MPN</b>
<ol style="list-style-type: none"> <li>1. Platelet count of ≥400 x10<sup>9</sup>/l and no signs of leuko-erythroblastosis</li> <li>8. Slight splenomegaly on ultrasound and no anemia Hb &gt;12g/dl</li> <li>9. Presence of JAK2<sup>V617F</sup> mutation</li> <li>10. No preceding or allied of CML, PV, RARS-T or MDS .</li> </ol>	<p>Erythrocytic, megakaryocytic and granulocytic trilinear myeloproliferation (EMGM) and relative reduction of erythroid precursors. Dense clustering and increase in atypical giant to medium sized megakaryocytes containing clumsy lobulated nuclei and definitive maturation defects. No or only borderline increase in reticulin.</p>

fetal loss in females, and less frequently with splanchnic vein thrombosis, but not with venous thromboembolism<sup>7-11</sup>. Erythromelalgic microvascular circulation disturbances are caused by aspirin responsive and coumarin resistant platelet-von Willebrand factor (VWF) mediated arteriolar fibromuscular intimal proliferation in JAK2<sup>V617F</sup> mutated thrombocythemia complicated by VWF-rich arteriolar thrombotic occlusion if left untreated with aspirin<sup>7-9</sup>. The broad clinical spectrum of microvascular arteriolar circulation disturbances of thrombocythemia in patients with prefibrotic ET and PV (trilinear MPN) include atypical transient ischemic cerebral and ocular manifestations and/or coronary artery disease, and erythromelalgia and its peripheral ischemic complications (Figure 2)<sup>7,8</sup>. These microvascular disturbances are caused by JAK2<sup>V617F</sup> mutated hypersensitive platelets, which at high shear stress in arterioles induce von Willebrand factor (VWF) mediated ischemic and thrombotic processes in the arteriolar peripheral, cerebral and coronary circulation and reflect a novel distinct disease entity of aspirin-responsive arterial thrombophilia occurring in about two thirds of ET and PV patients (Figure 2)<sup>7-11</sup>. Michiels et al discovered in the late 1970s and early 1980s that platelet-VWF mediated arteriolar microvascular, ischemic, and thrombotic complications of JAK2<sup>V617F</sup> mutated thrombocythemia are causally related thrombocythemia in patients with ET and PV associated thrombocythemia. The change of ET into PV during follow-up do aggravate the erythromelalgic and cerebral arteriolar microvascular circulation disturbances into major arterial

and venous thrombotic events as the consequence of increased hematocrit, red cell mass (hypervolemia) and blood hyperviscosity<sup>7-11</sup>. Correction of the hematocrit to normal (below 0.45) is associated with the relief of major thrombosis but with the persistence of thrombocythemia and its arteriolar microvascular complications, which can best be treated and prevented by low dose aspirin (80 mg once daily) but not by coumadin<sup>9-12</sup>. The incidence of deep vein thrombosis including splanchnic vein thrombosis in 809 ET patients from 11 retrospective studies reviewed by Griesshammer et al was low, 4% (n=33)<sup>11</sup>. The European Collaboration on Low-dose Aspirin in PV (ECLAP) study initiated by Landolfi, Michiels and Patrono revealed that in routine daily practice 633 (38.6%) of 1638 PV patients at time of diagnosis already had a history of arterial thrombotic events in 27% and of venous thrombosis in 9%<sup>12</sup>.

### Congenital Thrombophilia Budd Chiari Syndrome and Splanchnic Vein Thrombosis

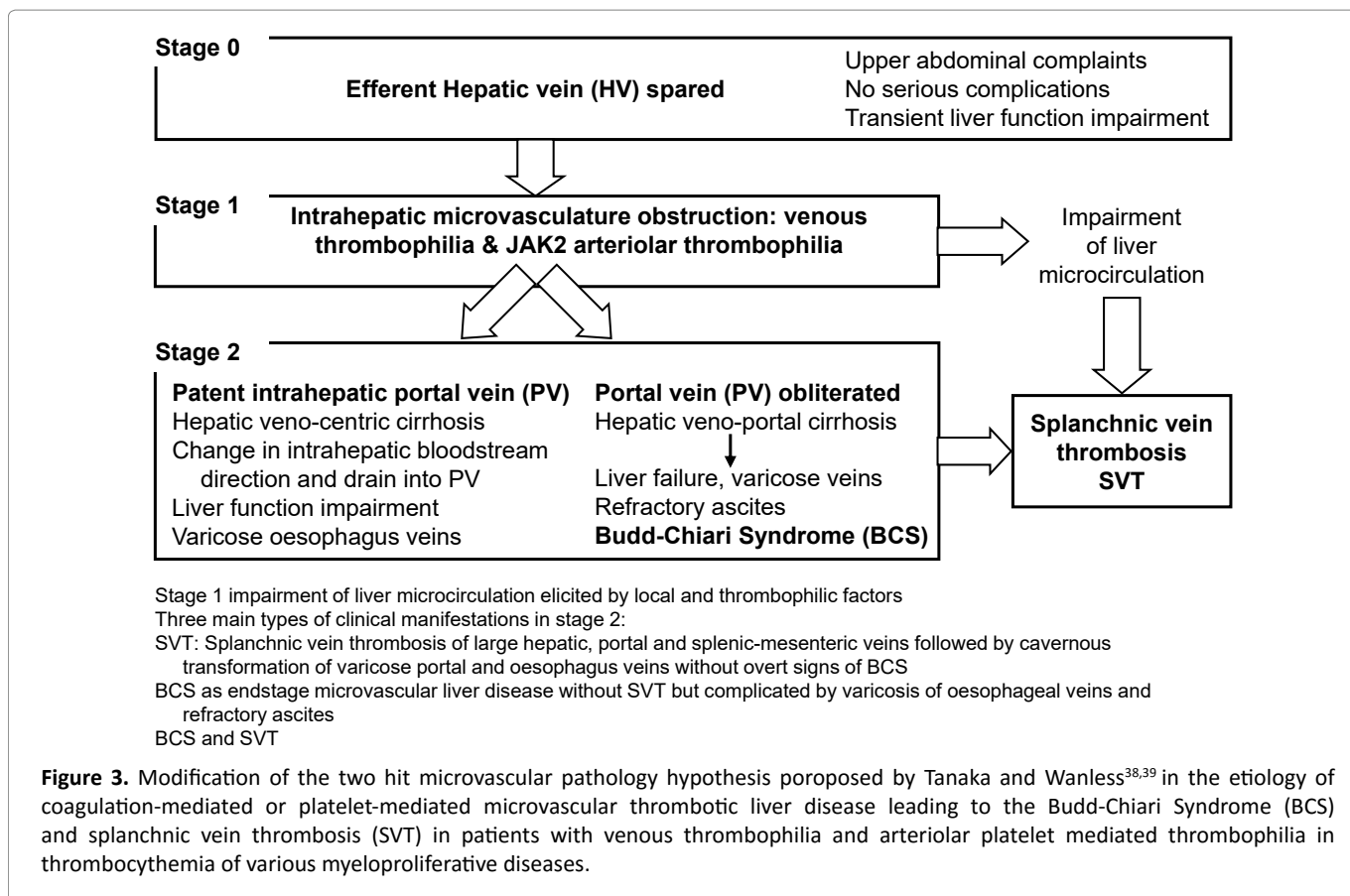
Budd-Chiari syndrome (BCS, hepatic vein thrombosis) is a rare disorder, the cause of which remains undetermined in the majority of cases. It is caused by the occlusion of hepatic outflow either at the level of hepatic veins or inferior vena cava (Figure 3 stage 1). BCS has been associated with a variety of conditions like malignancy, polycythemia rubra vera, paroxysmal nocturnal hemoglobinuria, trauma, pregnancy, oral contraceptives, and infection. Deficiencies of congenital anticoagulant factors including antithrombin,



protein C, protein S, factor V Leiden, factor II mutation and less frequently acquired lupus anticoagulans or anticardiolipine (ACL) antibodies are well documented underlying causes in about 60% of patients with hepatic vein thrombosis (BCS) and in about 1/3 of patients with portal vein thrombosis (PVT)<sup>13-15</sup>. Mohanty et al studied the frequency of congenital thrombophilia in 53 BCS and 33 PVT patients in whom MPD was excluded by bone marrow biopsy and in 223 age-matched controls (Figure 4)<sup>14</sup>. FV Leiden was present in 26% of BCS cases, in 6% of PVT cases and in 2.3% of controls. Antiphospholipid syndrome was present in 21% of BCS and in 18% of PVT cases. The age and gender distribution of BCS and PVT cases show that both BCS and PVT occur between 21 and 50 years with women outnumbering men in that age group 31 to 40 years (Figure 4). Hereditary deficiency of PC, PS or AT was present in 29% of BCS and 13% of PVT patients. Other acquired risk factors like pregnancy, surgery and oral contraceptives were present in 15% of BCS and 9% of PVT patients. In the study of Mohanty 59% of the BCS and 30% of the PVT could be explained by the presence of at least one of the etiologic venous thrombophilic factor. Janssen et al compared 43 BCS and 92 PVT patients with 474 population-based controls in the Leiden Thrombophilia Study<sup>15</sup>. Among 43 BCS patients

MPD was present in 28%, FV Leiden in 26%, hereditary PC deficiency in 9.3%, prothrombin mutation in 4.7% and PS in 0%. Among 92 PVT patients MPD was present in 17%, FV Leiden in 7.6%, hereditary PC deficiency in 6.5%, and prothrombin mutation in 3.2%. The relative risk of BCS was 11.3 for FV Leiden, 6.8 for PC deficiency and 2.1 for prothrombin mutation. The relative risk of PVT was 2.7 for FV Leiden, 4.0 for PC deficiency and 1.4 for prothrombin mutation. Concurrence of either acquired or inherited thrombotic risk factors was observed in 26% of the BCS and in 37% of the PVT patients in the study of Janssen et al<sup>15</sup>.

A retrospective study of 304 ET/PV patients found Factor V Leiden in 14/304 (4.6%) and MPD was associated with VTE in 5 of 27 (16%) evaluable cases<sup>16</sup>. The prevalence of Factor V Leiden in these ET/PV patients with and without arterial thrombosis was similar - 5/78 (6%) and 9/211 (4%)<sup>16</sup>. The prevalence of the allele for Factor V Leiden in another study of 50 MPD patients (17 PV, 15 ET and 18 myelofibrosis (MF) and 30 controls) was 9% in the MPD patients and 3.4% in controls<sup>17</sup>. These two studies suggest that both venous thrombophilia and arterial platelet thrombophilia very likely contribute to increased venous thrombotic risk in MPD (Figures 2 and 3).



### BCS and Splanchnic Vein Thrombosis in Myeloproliferative Disease (MPD)

Thrombosis in splanchnic veins (hepatic or portal) is rare at time of diagnosis of MPD, but may develop during long-term follow-up<sup>18</sup>. Thrombosis in splanchnic veins is reported in 19 cases of 460 (4%) consecutive patients with ET<sup>19</sup>. In one large series of 140 PV patients, thrombosis of major abdominal vessels was observed in 14 (10%)<sup>20</sup>. A retrospective study of 187 ET patients reported that 60% of all venous thrombosis occurred in either an abdominal vein (n=10) or cerebral sinus (n=2)<sup>21</sup>. Lengfelder et al reported portal/hepatic/splenic vein thrombosis in 10 of 143 (7%) consecutive ET patients<sup>22</sup>. In contrast, a chronic MPD of ET, PV or MF can be diagnosed in up to 30% of patients with hepatic vein thrombosis (Budd-Chiari syndrome), and in about 15% to 20% of patients with portal vein thrombosis<sup>13,15</sup>. Valla et al demonstrated that nearly 40% of patients with splanchnic vein thrombosis (either hepatic or portal) had spontaneous EEC of cultured bone marrow cells as a diagnostic clue for the presence of an underlying overt or latent myeloproliferative disease state<sup>23,24</sup>. Teofili et al<sup>25</sup> assessed spontaneous endogenous erythroid colony (EEC) formation in 43 patients with venous thrombosis before the age of 45 and found that EEC was positive allowing

the diagnosis of overt MPD in 4 (2 PV, 1 ET and 1 IMF) and a latent MPD in peripheral blood and bone marrow in 6 patients. All 10 EEC positive suffered from splanchnic vein thrombosis (hepatic 3, portal 2, mesenteric and portal 4, mesenteric 1), but no EEC positivity was found in 25 patients with other sites of deep vein thrombosis (DVT)<sup>25</sup>. In a review of 120 cases with splanchnic vein thrombosis (Budd-Chiari syndrome 51, and portal/splenic and/or mesenteric vein thrombosis in 69) MPD was diagnosed in 80 by the presence of spontaneous EEC as a clue to MPN in 73 (61%)<sup>26</sup>. Patients with splanchnic vein thrombosis associated with latent or overt MPD were predominantly females younger than 45 years. The diagnoses according the PVSG criteria in the 80 MPN patients were overt PV in 37 (31%), ET in 2, MF in 2, and latent (masked) MPN in 39 (32.5%)<sup>26</sup>. From this analysis in 1997 De Stefano, Teofili, Leone & Michiels concluded that both spontaneous EEC and histopathology from bone marrow biopsy provide specific information as sensitive clues for the diagnosis of all variants of latent and overt myeloproliferative disorders<sup>26</sup>. Using the presence of large clustered megakaryocytes in bone marrow biopsy specimens (Table 1), a subsequent French study could diagnose MPN in 46 out of 128 patients with splanchnic thrombosis either hepatic vein or portal vein thrombosis<sup>27,28</sup>. The sensitivity and specificity of the

ECP criteria including bone marrow histopathology to detect masked, early and overt stages of MPN is near to 100% as the underlying etiology in patients with idiopathic splanchnic vein thrombosis (Table 1)<sup>4-6</sup>. However, the accuracy to diagnose of MPD by the PVSG (1975) and WHO (2001) criteria without the use of bone marrow histology but using clinical criteria such increased red cell mass, low serum EPO levels, EEC, and splenomegaly are insensitive ranging from 52% to 74% to pick up early, masked and latent stages of MPD or MPN<sup>4-6,27,28</sup>.

Patel et al described 41 cases with idiopathic Budd-Chiari syndrome (BCS with absence of congenital thrombophilia in 38), of which 24 carried the JAK2<sup>V617F</sup> mutation and 17 not, but myeloproliferative neoplasms (MPN) was detected in only 17 cases by clinical criteria without the use of bone marrow histology<sup>29</sup>. Out of 24 BCS cases positive for the JAK2 mutation, only 10 developed overt PVSG-defined clinical criteria for MPN (7 ET, 3 PV, table 2). Another 4 had evidence of masked MPN (3 ET, 1 PV) at time of presentation. Six of nine presented with splenomegaly and/or a hypercellular bone marrow suspicious for advanced stage of MF. Out of the 17 BCS patients negative for the JAK2<sup>V617F</sup> mutation, five cases showed evidence for masked MPN on bone marrow histology evaluation (1 developed ET, 1 was polycythemic associated with EEC positivity, 1 showed the combination of EEC positivity, splenomegaly and a hypercellular bone marrow, 2 had splenomegaly and a hypercellular bone marrow<sup>29</sup>. In four recent studies, the presence of JAK2<sup>V617F</sup> mutation appeared to be a specific diagnostic clue to myeloproliferative disease in 31% of patients with idiopathic Budd-Chiari syndrome and splanchnic vein thrombosis (table 2)<sup>29-32</sup>. In two additional reports<sup>33,34</sup>, patients with splanchnic vein thrombosis, but without signs of overt MPN, the laboratory markers EEC and low serum EPO were less sensitive whereas the combination of JAK2<sup>V617F</sup> mutation and bone marrow histology assessment was highly sensitive and specific for diagnosis of MPN. Splanchnic vein thrombosis associated with masked or

early stage pre-fibrotic ET was more common in females (table 2)<sup>29-34</sup>. Kiladjian *et al* assessed the diagnostic and prognostic value of JAK2 and MPL<sup>515</sup> mutations in 241 SVT patients (104 BCS, 137 PVT). JAK2<sup>V617F</sup> was found in 45% of BCS and 34% of PVT, while JAK2 exon 12 and MPL<sup>515</sup> mutations were not detected (figure 5)<sup>35</sup>. JAK2<sup>V617F</sup> was found in 96.5% of patients with BM changes specific for MPD and EEC, but also in 58% of those with one feature (BM or EEC), and in 7% of those with neither feature indicating the superiority of JAK2 screening for detection of MPN in SVT patients<sup>35</sup>. In the meta-analysis of Smalberg *et al*<sup>36</sup>, JAK2<sup>V617F</sup> screening in SVT patients identified underlying MPN disease in 17.1% and 15.4% as could be confirmed by bone marrow histology evaluation<sup>36</sup>.

### Non-neoplastic Portal Vein Thrombosis in Liver Cirrhosis

Amitrano et al performed a cross sectional study in a total 701 liver cirrhotic patients diagnosed between 1998 and 2002 with Doppler ultrasonography and found 79 cases (11.2%) with portal vein thrombosis (PTV) usually associated with advanced liver disease<sup>37</sup>. Of these 79 PVT patients, 34 (43%) were asymptomatic and 45 (57%) were symptomatic and presented with portal hypertensive bleed in 31 (39%), abdominal pain in 14 (18%), and intestinal ischemia or infarction in 10 (12.6%) mainly due to mesenteric vein thrombosis (MVT). Wanless et al analysed 61 cirrhotic livers removed at transplantation to clarify the prevalence, distribution, and pathogenesis of venous lesions, as well as the association of these lesions with other morphological features and clinical morbidity. Wanless et al studied the histopathology of obliterative lesions in intrahepatic portal veins (IPV) and intrahepatic hepatic veins (IHV) of all sizes, which are known to occur in cirrhotic livers<sup>38</sup>. IPV lesions have generally been attributed to thrombosis, but the etiological pathogenesis of the intrahepatic veno-occlusive lesions is unknown. Intimal fibrosis that is highly suggestive of healed IHV

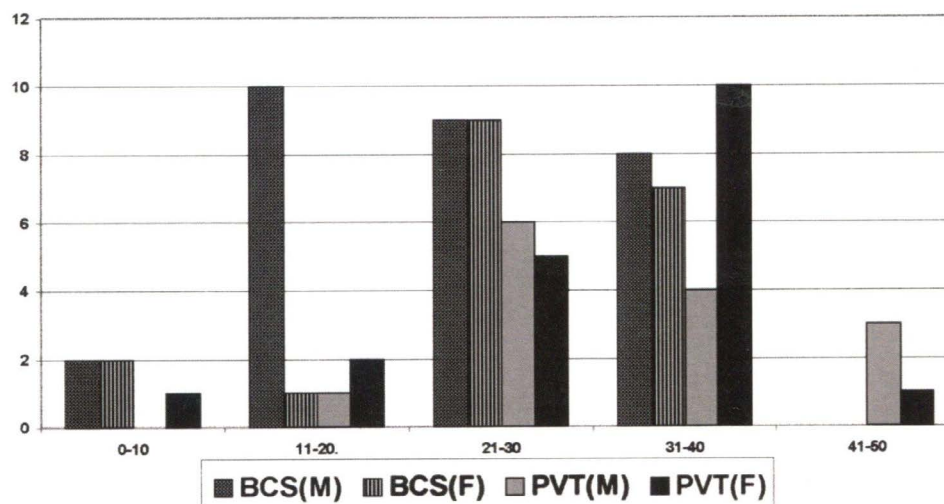
**Table 2.** Detection of JAK2<sup>V617F</sup> mutation indicative for latent (masked) or overt myeloproliferative disease (MPD) in patients with Budd-Chiari syndrome (BCS, hepatic vein thrombosis) and splanchnic vein thrombosis (SVT) including portal vein thrombosis (PVT) or isolated mesenteric vein thrombosis (MVT)

Reference Year	Patients N	JAK2 <sup>V617F</sup>		Overt MPD	Masked MPD**
		N	F/M		
Patel et al <sup>29</sup> 2006	41 BCS	24	16/8	0/10*	13**
Smalberg et al <sup>30</sup> 2006	40 BCS	13	9/5	13 (WHO)	
Collazzo et al <sup>31</sup> 2007	99 PVT	17	11/5	7	10
De Stefano et al <sup>32</sup> 2007	78 SVT HVT 15- PVT 63	32	20/12	4/15*	17
<b>Total</b>	274	86	56/30	37	48
<b>BCS/PVT</b>	100%	31%	2 : 1	14%	17%

\* MPD according to WHO criteria became overt during follow-up of SVT

\*\* Masked MPD according to WHO criteria at diagnosis and during follow-up of SVT patients

N = number of patients, F = female, M = male.



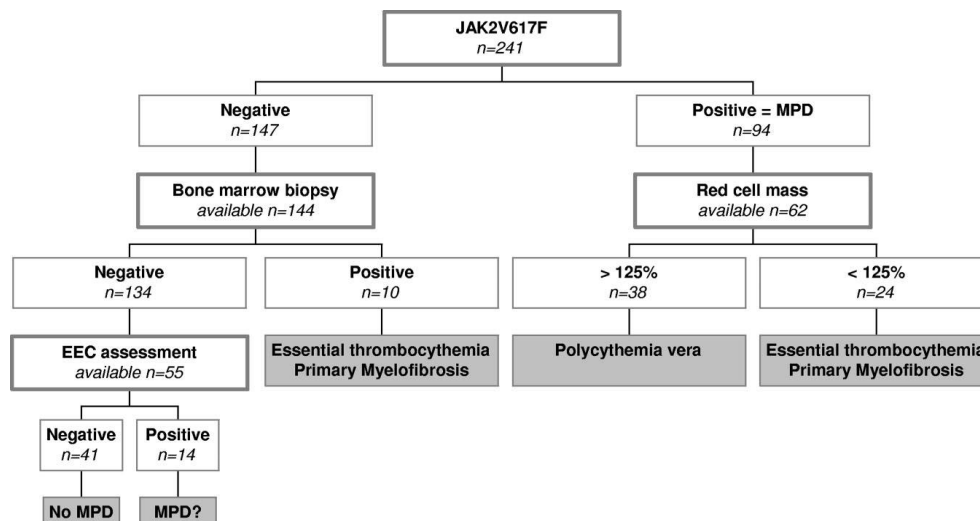
**Figure 4.** Age and gender male (M) vs female (F) distribution in Budd Chiari Syndrome (BCS, hepatic vein thrombosis, N=53) and Portal Vein Thrombosis (PVT, N=33) in the Mohonty study. Myeloproliferative disease and autoimmune disorders were excluded in the study. The involvement of Inferior Vena Cava (IVC) was observed in 65% of BCS. The prevalence of congenital thrombophilia in BCS and PVT (N=88) was FV Leiden 16 (18%), protein C deficiency 10 (11%), protein S deficiency 4 (4.5%), AT deficiency 2 (2.2%), anticardiolipin antibodies 6 (6.8%). Anticardiolipin antibodies were seen in concurrence with 5 cases of Protein C, 2 cases of Protein S deficiency and 2 cases of FV Leiden.

or IPV thrombosis was found in at least 70% and 36% of livers, respectively. The distribution of IHV lesions was patchy and largely confined to veins between 0.1 and 3 mm in diameter, suggesting multifocal origin in small veins. IPV lesions were more uniform throughout the liver. IHV lesions were associated with regions of confluent fibrosis (focal parenchymal extinction), and IPV lesions were associated with regional variation in the size of cirrhotic nodules and a history of bleeding varices. These observations suggest that microthrombosis of small to medium IPV and IHV is a frequent occurrence in cirrhosis, and that these events are important in causing progression of cirrhosis complicated by portal hypertension without or with PVT and extended SVT<sup>38</sup> (Figure 3 stage 2).

### The Concept of BCS Due to Intrahepatic Venoportal Obstructive Thrombosis/Cirrhosis

Here we propose the novel concept of intrahepatic venoportal obliterative microvascular disease related to venous thrombophilia, platelet-VWF arteriolar microvascular thrombosis in JAK2<sup>V617F</sup> thrombocythemia and ADAMTS13 deficiency as the three main underlying causes of BCS. Budd-Chiari syndrome (BCS: hepatic venous outflow obstruction) and splanchnic vein thrombosis (SVT) has been recognized by many investigators as a microvascular disease caused by a spectrum of underlying disorders including acquired JAK2<sup>V617F</sup> mutated thrombocythemia in various MPNs, congenital venous thrombophilia including FV Leiden and FII mutations, AT, PC and PS deficiencies, acquired coagulopathies including lupus

anticoagulant (LAC), anticardiolipin (ACL) antibodies, paroxysmal nocturnal hemoglobinuria (PNH), and Behcet's disease, malignancy, surgery and infection<sup>13-15,37-39</sup>. BCS due to intrahepatic vein thrombosis is an end stage liver disease with severe congestion, fibrosis and cirrhosis. The histological type of fibrosis may range from bridging fibrosis between intrahepatic hepatic veins (IHV) without septal to portal fibrosis to more usual type of cirrhosis with septa bridging of IHV to portal tracts<sup>38</sup>. Tanaka and Wanless analysed the vascular pathology of 15 explanted whole livers with BCS and correlated histological patterns of cell loss and fibrosis with the distribution of IHV and intrahepatic portal vein (IPV) obstruction<sup>39</sup>. In the first stage of veno-centric cirrhosis or congestive cirrhosis show early lesions of severe cell loss near hepatic veins (IHV), whereas intrahepatic portal veins (IPV) were usually patent and dilated. (figure 3). The peculiar lesion of veno-centric cirrhosis is seldom seen in other diseases, and is seen in pure form in the minority of BCS livers that had not suffered from portal vein thrombosis (figure 3). In the second stage of old lesions, the portal tracts are incorporated into fibrous septa giving veno-portal cirrhosis with invisible hepatic veins and obliterated portal vein. Some explanted BCS livers had regions of both veno-centric and veno-portal cirrhosis. Areas of veno-centric fibrosis had less hepatic vein (IHV) disease and areas of vena-porta cirrhosis had more severe portal vein (IPV) disease. The large hepatic veins were normal in nearly all BCS patients, whereas bleeding varices before liver transplantation was associated with moderate to severe IPV disease (figure 3).



**Figure 5.** The JAK2<sup>V617F</sup> mutation as the clue to MPD in 241 patients with Splanchnic Vein Thrombosis: SVT of which 104 BCS and 137 PVT was found in 45% of BCS and 34% of PVT, but JAK2 exon 12 and MPL mutations were not detected in the study of Kiladjian et al *Blood* 2008;111:4922-4929. All 94 SVT cases with the JAK2<sup>V617F</sup> mutation had myeloproliferative disease (MPD) on bone marrow biopsy. Out of 147 JAK2<sup>V617F</sup> negative SVT cases only 10 cases had a positive BM and 14 had EEC suspicious for MPD.

The proposed two hit microvascular pathology hypothesis in BCS illustrates the full range and great heterogeneity of obstruction seen in microvascular obstructive disease and the co-existence of intrahepatic IHV and IPV thrombosis (figure 3)<sup>41</sup>. In addition, infarcts with contiguous necrosis involving periportal as well as perivenular tissue were noted in livers with combined intrahepatic IHV and IPV obstruction. Acute infarcts were seen in 3 cases, always in association with acute but healed IPV thrombosis, and may be indication for a platelet-mediated obstruction in the end-arterial liver circulation (figures 2 and 3). Based on the concept proposed by Tanaka and Wanless in figure 3, the main clinical manifestations of the intrahepatic venous outlet obstruction as a vascular liver disease in patients with congenital thrombophilia or acquired arteriolar thrombosis in myeloproliferative thrombocythemia are variable and include: 1) SVT the first presenting symptom without overt signs of BCS and complicated by BCS during long-term follow up, 2) various clinical and intrahepatic stages of BCS or intrahepatic venous outlet obstruction recently recognized as a microvascular liver disease complicated by varicosis of oesophageal veins and refractory ascites, but without SVT, and 3) BSC complicated by SVT (figure 3).

The demonstration in 17 BCS patients, with underlying thrombophilia in 5 cases, MPD in 11 cases or combined in 3, that pretransplant vascular imaging showed decreased portal perfusion in 16, and increase arterial perfusion in 9 and post-transplant obstructive intrahepatic portal venopathy and nodular regenerative hyperplasia in all are in line with the concept that intrahepatic BSC is a microvascular veno-portal obstructive liver disease<sup>40</sup>. In 282 BCS patients

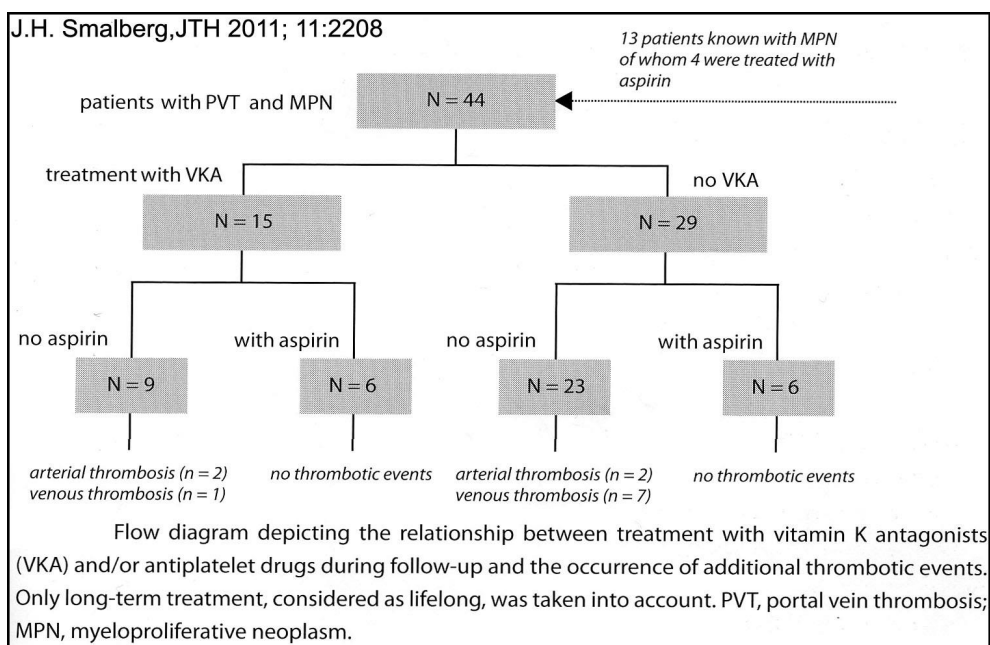
only 42 patients (15%) had combined intrahepatic BCS and portal vein thrombosis PVT<sup>41</sup>. BCS without PVT or SVT thrombosis is usually complicated by varicosis of oesophageal veins and ascites due to intrahepatic obstruction (figure 3). All patients with BCS-PVT had occlusion of hepatic and portal veins. Eighteen showed additional splenic vein (N=8), superior mesenteric vein (n-2) or both (N=8) thrombosis and 1 exhibited thrombosis of the inferior caval vein. The underlying disorders in 33 evaluable BCS-PVT patients were both myeloproliferative neoplasm (MPN) including PV, ET, MF and unclassifiable MPN in 9, as well as congenital thrombophilia in 15 and paroxysmal nocturnal hemoglobinuria (PNH), Behcet's disease or oral contraceptive use in 8 and support the concept of coagulation and platelet mediated etiology of BSC and SVT (Figures 1, 2 and 3)<sup>42</sup>. The number of etiological venous or arteriolar thrombophilic factors and eliciting local or other risk factors increased significantly with the extent of splanchnic vein thrombosis<sup>42</sup>. All these clinical data and liver pathology observations are in line with the two hit hypothesis of procoagulant-mediated fibrin and/or JAK2<sup>V617F</sup> mutated platelet-mediated arteriolar thrombosis in the liver microcirculation in patients with congenital and acquired procoagulant thrombophilia and combined procoagulant- and platelet-mediated microvascular in JAK2<sup>V617F</sup> mutated clonal thrombocythemia in ET and PV patients (Figures 1, 2 and 3). A search for the presence congenital and acquired thrombophilic risk factors for venous thrombosis, and JAK2<sup>V617F</sup> mutation screening as a specific clue for sticky platelets in clonal thrombocythemia is warranted in patients with a first episode of BCS and splanchnic vein thrombosis. Subsequent bone marrow



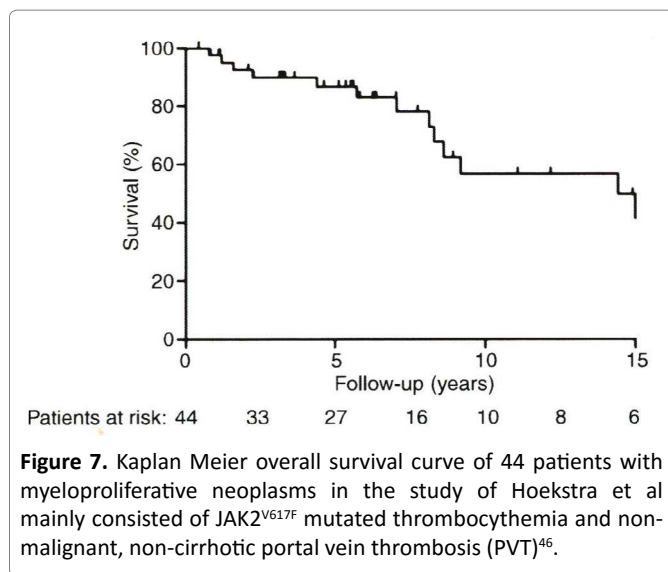
biopsy evaluation even in the absence of the JAK2<sup>V617F</sup> mutation is indicated to rule in or out latent, masked and overt stages of MPN into ET, PV and MF (table 1).

Low molecular weight heparin followed by vitamin K antagonist anticoagulation is the treatment of choice of splanchnic vein thrombosis (SVT). Variables of prognostic significance in a large study of 172 patients with portal vein thrombosis (PVT) were hepatic disorders in 30%, abdominal inflammation in 17%, malignancies in 24% abdominal intervention in 23% hypercoagulability in 27% and MNP in 14%<sup>43</sup>. In the absence of cancer, liver cirrhosis and mesenteric thrombosis, the survival of PVT without BCS was 95%, 89% and 81% after 1, 5 and 10 years follow-up<sup>43</sup>. The presence of MPN did not influence survival<sup>41</sup>. In a cohort of 136 non-malignant non-cirrhotic PVT patients, of whom 84 received anticoagulants the underlying causes of extrahepatic PVT were MPN in 31%, antiphospholipid syndrome in 17%, congenital thrombophilia in 165, septic phlebitis in 11%, other causes in 16% and no causes in 28%<sup>43</sup>. Thirty-eight thrombotic events in 26 patient occurred during 691 patient years follow-up (5.5 per 100 patient years) as DVT in 18, pulmonary embolism in 5, mesenteric thrombosis in 8 and arterial thrombosis in 5<sup>43</sup>. Underlying prothrombotic state including congenital venous thrombophilia and MPN (PV, ET or MF) and absence of anticoagulant treatment were independent predictors for thrombosis. The studies of Van Genderen et al and Landolfi et al produced good evidence that anticoagulation combined with low dose aspirin prevents arteriolar

microvascular thrombosis including BSC and PVT in MPN patients with symptomatic essential thrombocythemia (ET) or polycythemia vera (PV)<sup>44,45</sup>. Hoekstra et al from the Erasmus University Medical Center, Rotterdam studied 44 patients with PVT shown in figures 6 and 7 and searched for an underlying MPN (Chief JJ Michiels & F Leebeek 1985-2018)<sup>46</sup>. Based on clinical presentation and results of imaging 13 patients (30%) presented with acute PVT and 31 patients had already signs of portal hypertension (gastrointestinal varices, bleeding and splenomegaly) consistent with chronic PTV. In 31 patients (70%) PVT was the initial diagnosis of MPN. In the majority of these cases laboratory values around the time of PVT diagnosis were not suggestive for an underlying MPN according to WHO criteria, but MPN was confirmed by bone marrow histology in all. The JAK2<sup>V617F</sup> mutation was present in 26 of 29 tested MPN patients. The overall survival of 44 patients is shown in figure 7 of whom 31 were MPN cases, 20 had another venous thrombophilic factor and 5 cases had two risk thrombophilic factors for PVT. As shown in figure 6, twenty-three (52%) received standard Vitamin K antagonist (VKA) anticoagulation, and long term VKA anticoagulation was given in 15 cases after diagnosis of PVT. Anticoagulation was more frequently given in acute PVT as compared to chronic PVT (77% vs 42% respectively). A final retrospective analysis comparing low dose aspirin on top of VKA or no VKA was associated with no thrombotic events in 12 aspirin treated cases (Figure 6). There were 2 arterial and 1 venous thrombotic events in 9 cases not



**Figure 6.** Therapeutic implications according to Hoekstra et al<sup>46</sup> in 44 non-cirrhotic patients with portal vein thrombosis and myeloproliferative disease. Anticoagulation for venous thrombosis and thrombophilia in SVT (BCS & PVT) and anticoagulation combined with low-dose aspirin and proper treatment of the MPN is recommended in SVT patients (BCS & PVT) with the JAK2<sup>V617F</sup> mutation (Michiels et al, ANN Hematol 2007;86:793-800).



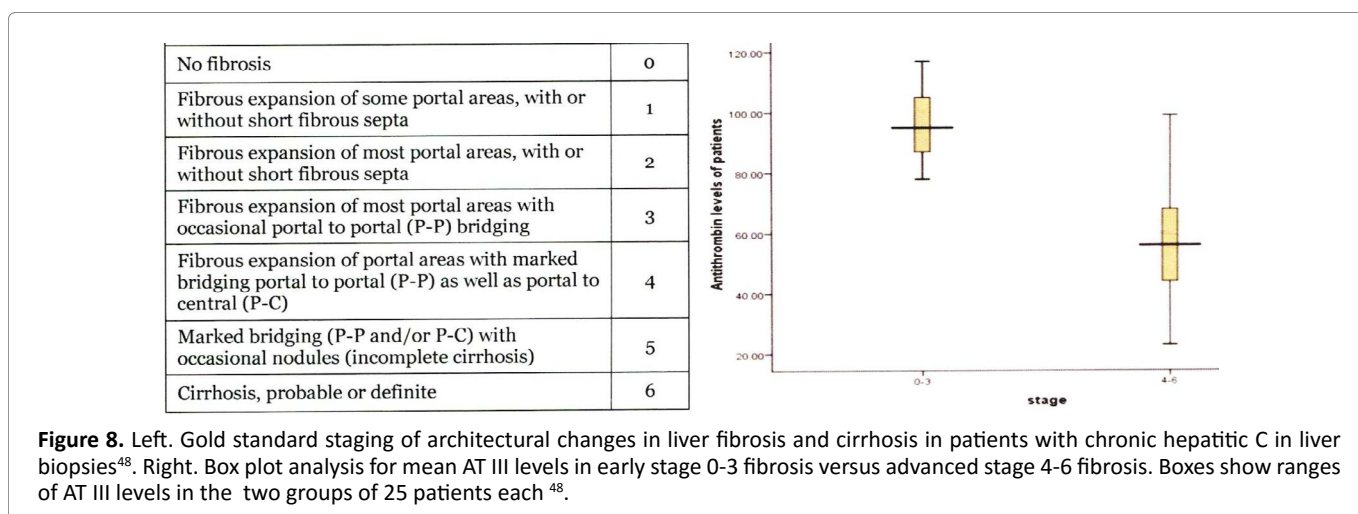
**Figure 7.** Kaplan Meier overall survival curve of 44 patients with myeloproliferative neoplasms in the study of Hoekstra et al mainly consisted of JAK2<sup>V617F</sup> mutated thrombocythemia and non-malignant, non-cirrhotic portal vein thrombosis (PVT)<sup>46</sup>.

on aspirin on top of VKA and 2 arterial and 7 venous thrombotic events not on aspirin and VKA (Figure 6). A total of five patients died from end-stage myelofibrosis (2 PV and 3 MF). Survival was not significantly different between patients treated without or with VKA and aspirin (Ascal 100 mg = aspirin 80 mg once daily) did not significantly influence survival (P=0.378) in this small cohort study. Treatment with low dose aspirin (80 mg once daily) seems to us of huge importance in the prevention of all variants of microvascular thrombosis in thrombocythemia of various molecular etiology (Figure 6), but this requires further prospective studies<sup>47</sup>. Life expectancy and mortality are primarily related to the early stage of underlying MPN and not to complications of PVT (Figure 7)<sup>46</sup>.

AT III is a vitamin K independent protein synthesized by liver cells. Quantitative measurement of AT III levels using a chromogenic assay is the best objective marker of the degree of liver parenchymal insufficiency in liver cirrhosis patients<sup>2,4</sup>. Quantitatively deficient changes in AT III levels are very well known to be associated with the extent of liver cell

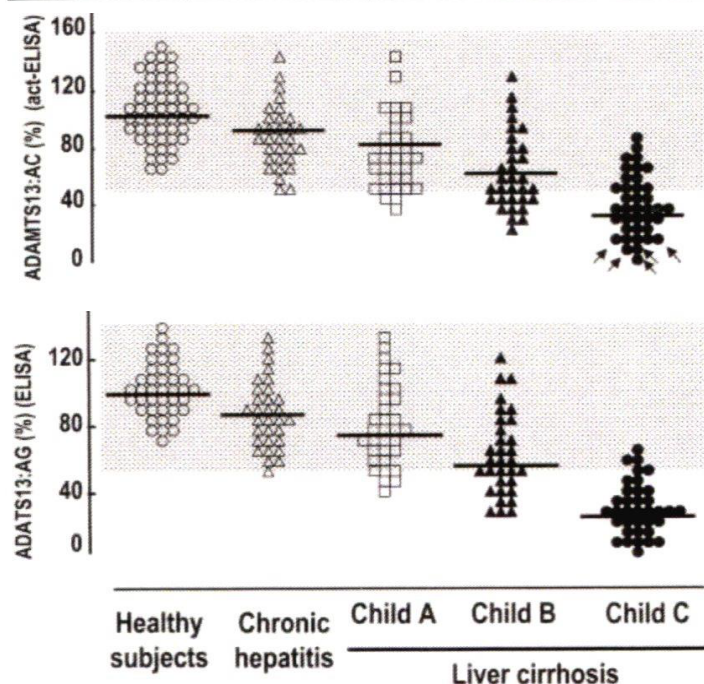
insufficiency in all variants of liver cirrhosis patients and not influenced by relative or true vitamin K deficiency<sup>2,4</sup>. Bhali et al studied the role of AT III levels as a noninvasive marker for the degree of histology proven cirrhosis in a well defined group of chronic hepatitis C patients<sup>48</sup>. The mean values of the AT III levels reflecting the liver parenchymal function in stage 0-3 liver fibrosis (N=25) versus stage 4-6 liver fibrosis (N=25) was 96.5 +12% (range 78-117) and 58.9 + 22% (range 23-111) respectively (p-value <0.001, Figure 8)<sup>50</sup>.

In the landmark study of Uemura et al the degree of both ADAMTS13 antigen (Ag) and ADAMTS13 Activity (AC depending on the method used) levels are strongly correlated to the degree of liver parenchymal dysfunction as measured by the Child A, B vs C classification (Figure 9)<sup>49,50,51</sup>. Severe ADAMTS13:AC (<3%) was measured in five liver cirrhosis patients with Child C by the VWFMM assay (Furlan method)<sup>52</sup>, but the ADAMTS13:AC-ELISA ranged from <5% to 15.9% in the ADAMTS13 AC-ELISA assay<sup>49</sup>. Interestingly, ADAMTS13 inhibitor was detected in all five liver cirrhosis patients with severe ADAMTS13:AC (Furlan method) (<3%), in 19 of 22 liver cirrhosis patients with moderate (3-25%) ADAMTS13 deficiency and in 4 of 22 liver cirrhosis with mild ADAMTS13 deficiency. In this study, Uemura et al produced good evidence that severe ADAMTS13 deficiency in advanced liver cirrhosis are decreased with increasing severity of cirrhosis due autoimmune antibodies against ADAMTS13 on top of a synthesis defect thereby explaining the more pronounced ADAMTS13 deficiency in advanced liver cirrhosis as compared to AT III synthesis deficiency in liver cirrhosis (Figure 9)<sup>49,50</sup>. Uemura et al found increased VWF:Ag and VWF:RCo levels, but decreased platelet counts coincidental with severely decreased ADAMTS13:AC, which suggest an additional mechanism for thrombocytopenia related to TTP-like hypercoagulability in patients with advanced liver cirrhosis. Yagita M, Uemura M, Nakamura T et al previously described a case of ADAMTS13 inhibitor development in a case of hepatitis C virus-related liver



**Figure 8.** Left. Gold standard staging of architectural changes in liver fibrosis and cirrhosis in patients with chronic hepatitis C in liver biopsies<sup>48</sup>. Right. Box plot analysis for mean AT III levels in early stage 0-3 fibrosis versus advanced stage 4-6 fibrosis. Boxes show ranges of AT III levels in the two groups of 25 patients each<sup>48</sup>.

Measure	1 point	2 points	3 points
Total bilirubin, $\mu\text{mol/L}$ (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8–3.5	<2.8
Prothrombin time, prolongation (s) OR INR	<4.0 <1.7	4.0–6.0 1.7-2.3	> 6.0 >2.3
Ascites	None	Mild (or suppressed with medication)	Moderate to severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV



**Figure 9.** Table Child criteria to assess the prognosis of chronic liver disease, mainly cirrhosis. Each of the 5 measures is scored 1-3<sup>49,50</sup>. Child A, 5-6 points; Child B 7-9; Child C points 10-15 reflecting two years survival of 85%, 60% and 35% respectively in the study of Cholangitis et al<sup>50</sup>. Figure Results of ADAMTS13:Ag-ELISA and ADAMTS13:AC-ELISA measurements in healthy subjects, chronic hepatitis and liver cirrhosis Child A, B and C in the study of Uemura et al<sup>49</sup>.

corrhosis as the cause of thrombotic thrombocytopenic purpura (TTP)<sup>51</sup> consistent with the classical diagnosis of TTP (Furlan method)<sup>52</sup>. ADAMTS13:AC in the case of Yagita et al was extremely low and the inhibitor was positive in both heated plasma and purified IgG consistent with the diagnosis of TTP<sup>51</sup>. An imbalance between the severely decreased ADAMTS13:AC level and its substrate ADAMTS13:Ag may indeed reflect the predisposing state for platelet-VWF mediated platelet thrombi in the complex liver microcirculation in patients with advanced liver cirrhosis similar as has been observed for anticoagulant responsive congenital and acquired venous thrombophilia and aspirin responsive platelet-VWF mediated peripheral, ocular, cerebral and coronary microvascular thrombosis in JAK2<sup>V617F</sup> mutated thrombocythemia<sup>7-11</sup> very likely also play an important etiological role as causative risk factors of intrahepatic microvascular obstructive diseases in BCS followed by SVT<sup>49-51</sup>. Targeted well designed prospective clinical and basic research and follow-up studies in newly

diagnosed BCS, SVT and PVT patients are warranted within a novel setting of clinical, laboratory, pharmacological and molecular biology in nature medicine.

### Declaration of interest

The first author founded in 2000 the Goodheart Institute & Foundation in Nature Medicine & Health, Rotterdam, The Netherlands, Freedom of Science and Education, European Free University Network. JJ Michiels is co-founder of the Central European Vascular Forum (CEVF) and serves as consultant professor in the Bloodcoagulation, Hemostasis Research Laboratory (co-founder VWF-VWD and MPN research programs) at the department of Hematology University Hospital, Antwerp; as consultant to the Dutch Society of Internal Medicine and Ministry of Public Health; consultant of R&D quality driven Industrial and Pharmaceutical Medicine; as an editor of 3 Medical Journals and as a guest editor on request and by self initiation.

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