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by Jean Galtier, Louis Drevon, Yannick Le Bris, Stephane Giraudier, Mathieu Wemeau, Laurence Legros, Damien Luque Paz, François Girodon, Jean-Jacques Kiladjian, Charles Mesguich, Marie Parrens, Clemence Mediavilla, Lydia Roy, Alexandre Guy, Olivier Mansier, Jean-Christophe Ianotto, and Chloe James. Collaborative Groups: France Intergroupe des syndromes Myeloprolifératifs

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Role of red cell mass evaluation in myeloproliferative neoplasms with splanchnic vein thrombosis and normal hemoglobin value: a study of the France Intergroupe des Syndromes myeloprolifératifs

Jean Galtier[1], Louis Drevon[2], Yannick Le Bris[3], Stephane Giraudier[2], Mathieu Wemeau[4], Laurence Legros[5], Damien Luque Paz[6], François Girodon[7], Jean-Jacques Kiladjian[2], Charles Mesguich[8], Marie Parrens[9], Clémence Mediavilla[1], Lydia Roy[10], Alexandre Guy[11], Olivier Mansier[11,12], Jean-Christophe Ianotto[13], Chloe James[11,12]

[1] Service d'hématologie et thérapie cellulaire, CHU de Bordeaux, France

[2] Service d'hématologie clinique, Hôpital Saint-Louis, APHP, France

[3] Service d'hématologie biologique, CHU de Nantes, France

[4] Service d'hématologie clinique, CHRU de Lille, France

[5] Service d'hématologie clinique, APHP-Hôpital Bicêtre, France

[6] Service d'hématologie biologique, CHU d'Angers, France

[7] Service d'hématologie biologique, CHU de Dijon, France

[8] Service de médecine nucléaire, CHU de Bordeaux, France

[9] Service d'anatomie et cytologie pathologique, CHU de Bordeaux, France

[10] Service d'Hématologie Clinique, Hôpital Universitaire Henri Mondor, AP-HP & Faculté de Santé, UPEC, Créteil, France

[11] Service d'hématologie biologique, CHU de Bordeaux, France

[12] Univ. Bordeaux, INSERM, Biologie des maladies cardiovasculaires, U1034, F-33600 Pessac, France

[13] Service d'hématologie et d'hémostase clinique, CHU de Brest, France

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Correspondence :

Jean Galtier

Service d'hématologie clinique et de thérapie cellulaire,

Hôpital Haut Leveque, CHU de Bordeaux, France

Mail : jean.galtier@chu-bordeaux.fr

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KEY POINTS :

- JAK2V617F mutation is common in splanchnic vein thrombosis despite normal hemoglobin/hematocrit value, and precise definition of underlying myeloproliferative disorder is often challenging.

- Red cell mass evaluation allows for detection of true erythrocytosis defining masked polycythemia vera, and thus may be helpful for management and follow up of these patients.

Splanchnic vein thrombosis (SVT) include portal vein thrombosis (PVT), Budd-Chiari syndrome (BCS), and splenic and/or mesenteric vein thrombosis (S/MVT). They are frequently associated with Philadelphia-negative myeloproliferative neoplasms (MPN)[1], with up to 50% of idiopathic SVT cases exhibiting the JAK2V617F mutation [2]. Diagnosing MPN in the context of SVT is challenging because the resultant portal hypertension and subsequent splenomegaly and hypersplenism can lower blood cell counts, obscuring the elevated counts typical of MPN. The routine inclusion of JAK2V617F mutation screening in SVT patient evaluations has enabled the identification of MPNs that lack the usual hematological features, representing about 15% of BCS and PVT cases[2]. These patients, who otherwise would remain undiagnosed, are categorized as having unclassified MPN (MPN-U), leaving the decision to initiate cytoreductive therapy unresolved. Prior to the discovery of the JAK2V617F mutation, demonstrating an increased red cell mass (RCM) through isotopic methods was a key diagnostic criterion for polycythemia vera (PV). According to the 2008 WHO criteria, hemoglobin levels above 18.5 g/dL in males and 16.5 g/dL in females were considered indicative of increased RCM, leading to the discontinuation of RCM measurement in many facilities[3]. We hypothesized that for patients with JAK2V617F-positive SVT and normal hemoglobin levels, RCM measurement could facilitate the diagnosis of PV, thereby guiding the initiation and adjustment of cytoreductive therapy.

A retrospective study was conducted on 71 patients diagnosed with splanchnic vein thrombosis (SVT), carrying the JAK2V617F mutation but presenting normal hemoglobin and hematocrit levels initially. Data were gathered from eight French medical centers. Patients gave written consent and the study was conducted in accordance with the Declaration of Helsinki. Key characteristics are detailed in **Table 1**. The median age at diagnosis was 44, with females comprising 48% of the cohort. The median interval between SVT diagnosis and JAK2V617F mutation detection was one month [ranges 0 - 144]. Coincident diagnosis of SVT and JAK2V617F mutation was observed in 64% of cases within a three-month margin. The mutation was detected within two years post-SVT in 14 patients, beyond two years in 9 patients, with one case's timeline unspecified. Increased RCM values over 125%, suggesting masked PV, were found in 56% of patients. Notably, average hemoglobin levels and hematocrits were comparable between the masked PV subgroup and non-PV patients. The total plasma volume value was available in 61 cases and was increased in 91% masked PV versus 46% non-PV, with a mean value of 140% vs. 114%, respectively ($p < 0.001$). Splenomegaly was palpable in a higher proportion of masked PV patients. PVT was the most common SVT, found in 78% of patients,

while BCS affected 17%, and porto-sinusoidal vascular disease was present in 4%. JAK2V617F allele burden at diagnosis and erythropoietin (EPO) levels were not significantly different between masked PV and non-PV patients. A bone marrow biopsy was performed and contributive in 32 patients (45%), i.e., 18/40 masked PV and 14/31 non-PV. Panmyelosis, the histology criterion of PV in the WHO 2016 classification, was found in 8/18 masked PV patients and, interestingly, in 2/14 other JAK2V617F patients. The final diagnoses were (masked) PV in 40 patients, ET in 10, PMF in 3, and MPN-U in 18 cases.

Upon diagnosis of SVT, therapeutic-dose anticoagulation was initiated for all but four patients. At the time the JAK2V617F mutation was identified, three patients were prescribed aspirin, and one patient with masked PV did not receive any antithrombotic treatment due to low platelet counts. After the diagnosis of MPN, 87% of patients began cytoreductive treatment after a median period of six months from their SVT diagnosis (ranges 0 – 239): Treatment started within one year of thrombosis for 37 patients, and after one year for 25 patients. The cytoreductive regimen included hydroxyurea for 43 patients, interferon for 18 patients, and ruxolitinib for one patient. Eight patients did not undergo any cytoreductive therapy; this group included two masked PV patients who were treated solely with phlebotomies. The treatment details for one patient remained undisclosed.

The average follow-up duration was 77 months, with a range from 3 to 358 months (interquartile range: 45 – 130 months). During this period, five patients initially diagnosed with conditions other than PV — two with ET and three with MPN-U — progressed to PV 1, 5, 9, and 15 years after their first diagnosis. In two cases, this progression was marked by increased red cell values, meeting the criteria for overt PV. The remaining three patients (two MPN-U and one ET) showed signs of evolving into masked PV as indicated by routine RCM assessments. Secondary myelofibrosis developed in two patients 17 and 19 years post-detection of the JAK2V617F mutation. Venous thrombosis recurred in five patients, involving the portal vein in three and the spleen in two, occurring at various times ranging from 1 to 21 years after their initial SVT diagnosis. At the time of these thrombotic events, three patients were diagnosed with masked PV with normal red cell values, one had progressed from MPN-U to overt PV, and another had MPN-U with normal red cell values. Three of them were receiving cytoreductive therapy, and 4 anticoagulation therapy at the time of the thrombosis reccurence. Eight out of 40 patients with a masked PV diagnosis underwent a second RCM assessment after at least a year of cytoreductive treatment. RCM levels remained stable in two patients, decreased but stayed

above 125% in two others (one of whom was re-evaluated due to SVT recurrence), and slightly increased in two. Only two patients achieved normal RCM levels. The total plasma volume remained elevated across the board (mean 139%, range 125 – 150%). Those with persistently high RCM were considered under-treated, prompting significant therapeutic adjustments, including the addition of phlebotomies for two patients, increased dosages of cytoreductive agents for another two, and a switch in cytoreductive agents for the remaining two. The progression of red cell values, RCM, and associated treatment modifications for these eight patients are detailed in **Figure 1A**.

Our study presents the largest known cohort of patients with JAK2-mutated SVT evaluated for RCM. A key finding is that over half of these patients were diagnosed with masked PV, despite normal hemoglobin and hematocrit levels. Among 18 patients with masked PV who underwent bone marrow biopsy, only 8 exhibited panmyelosis, which is a major WHO 2016 criterion for PV. Bone marrow biopsies are particularly challenging for JAK2V617F patients with SVT due to the need for therapeutic-dose anticoagulation and possible thrombocytopenia from portal hypertension. Therefore, we advocate for routine RCM evaluation in JAK2V617F-mutated SVT patients to accurately classify the underlying MPN and to ensure appropriate cytoreductive treatment.

Concerns have been raised in several studies about the reliability of hemoglobin and hematocrit as indicators of RCM in PV[5–9]. The notion of masked PV emerged upon observing patients with JAK2V617F mutation, increased hematocrit and hemoglobin values below WHO 2008 criteria, and heightened RCM. Hemodilution, indicated by a total plasma volume exceeding 110%, is far more prevalent in masked PV than in overt PV[10]. Absence of cytoreductive therapy can lead to the undertreatment of masked PV, heightening thrombosis risks compared to overt PV[11]. These insights contributed to the revision of the WHO 2016 PV criteria, lowering the required hemoglobin levels to 16.5g/dl for men and 16g/dl for women[12]. However, data indicates that 25-50% of PV patients defined by an RCM greater than 125% still present with normal red cell counts, posing a risk of PV underdiagnosis and subsequent undertreatment[9,13.] This is especially concerning in JAK2V617F-positive SVT, where thrombosis occurrence requires cytoreductive treatment and hemodilution is frequent.

In our patient group, 91% of those with masked PV had increased plasma volume, significantly higher than that of non-PV patients, suggesting that hemodilution may play a key role in differentiating

between masked and overt PV post-SVT. This aligns with a previous cohort of JAK2-mutated SVT patients, where less than 10% had raised red cell counts per WHO 2008, yet about 60% had RCM over 125%[14]. In our follow-up, RCM remained high in 6 out of 8 evaluated masked PV patients despite cytoreductive therapy and normal red cell values, indicating that red cell counts alone may not suffice for adjusting cytoreductive therapy dosages. Furthermore, 3 of the 5 SVT recurrence cases occurred in patients with masked PV under anticoagulation therapy, who had normal red cell values at recurrence. Of these, one patient had subsequent RCM evaluation which exceeded the mean normal predicted value by 25%. Additionally, 3 of 4 patients with normal RCM at MPN diagnosis who had a second evaluation showed progression toward masked PV. Collectively, these findings emphasize that red cell values are not reliable indicators of RCM at diagnosis or during the follow-up of SVT-associated MPN, owing to the prevalent hemodilution. Current treatment guidelines, which do not recommend cytoreduction for patients with normal hemoglobin and advise keeping hematocrit below 45%, may inadvertently result in the undertreatment of masked PV within the SVT context[15]. A limitation of our study is the accessibility of isotopic RCM evaluation; however, the CO rebreathing technique, which shows promising correlation with isotopic methods, may broaden the feasibility of RCM assessment globally[16].

In summary, our findings advocate for routine RCM evaluation in patients diagnosed with JAK2V617F-mutated SVT who have normal red cell values to detect masked PV. Monitoring RCM during treatment may also inform more personalized hematocrit thresholds (as depicted in Figure 1B). Further research is necessary to solidify RCM's role in managing SVT. Results from an ongoing FIM study on the effects of cytoreductive agents in MPNs with normal blood counts (NCT04539678) are forthcoming.

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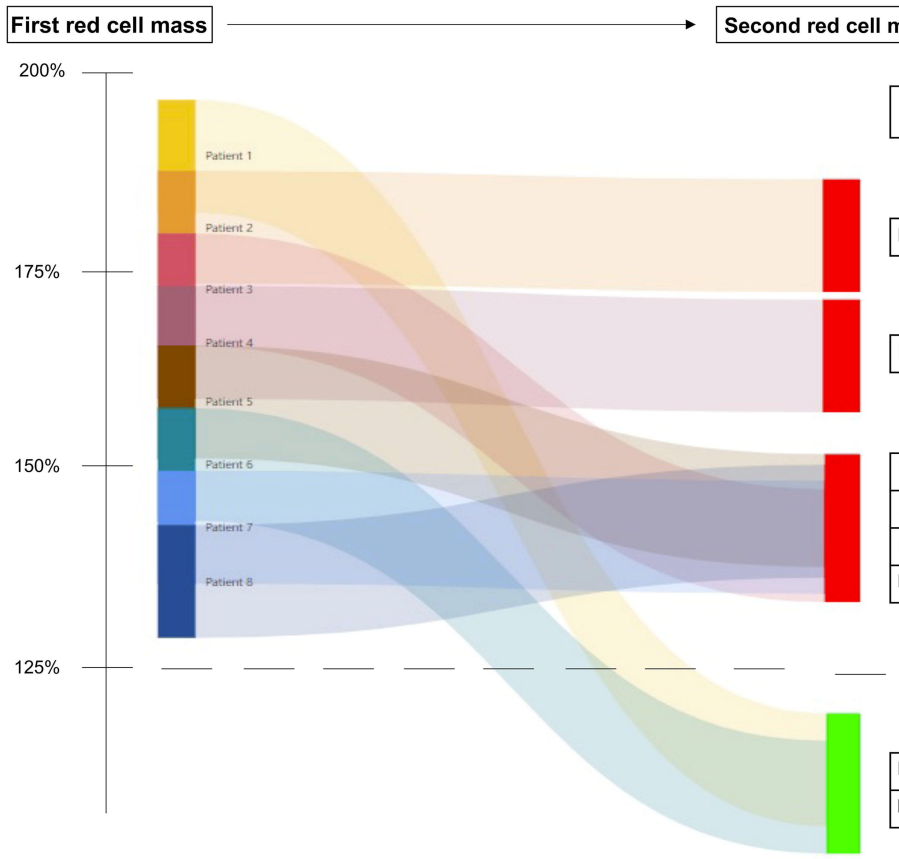
TABLE 1 : patients characteristics. RCM : red cell mass ; PV : polycythemia vera.

	Cohort (n = 71)	Normal RCM (n = 31)	Masked PV (n = 40)	*p* value
Median age	44	44	44	.
Female	34/71	15/31	19/40	.
Hemoglobin (g/dl), mean (ranges)	14 [9.1 – 16.3]	13.8 [12.1 – 16]	14,1 [9.1 – 16.3]	0.24
Hematocrit, mean (ranges)	42% [34 – 47.4]	41.4% [34 – 47]	42.5% [29 – 47.4]	0.15
Platelets (G/L), mean (ranges)	310 [61 – 836]	324 [199 - 639]	304 [61 – 836]	0.42
Neutrophils (G/L), mean (ranges)	5.46 [1.7 – 13]	5.86 [2.4 – 13]	5.17 [1.7 – 11.4]	0.27
RCM, mean (ranges)	135% [99 – 209]	110% [99 – 122]	149% [128 – 209]	< 0.001
Plasma volume, mean (ranges)	128% [88 – 236]	114% [88 – 180]	140% [104 – 236]	< 0.001
Palpable splenomegaly	48/67	16/29	32/38	.
BM biopsy, hyperplasia 0-2 lineage 3 lineages	 22/32 10/32	 12/14 2/14	 10/18 8/18	
EPO value, mean	5.9	7.5	5	0.27
*JAK2*V617F allele burden, mean (ranges)	20% [1 – 68]	15% [5 – 67]	24% [1 – 68]	0.08
Type of splanchnic vein thrombosis: - Portal - Budd-Chiari - Porto-sinusoidal vascular disease	 55/70 (78%) 12/70 (17%) 3/70 (4%)	 24/31 (77%) 7/31 (23%) 0/31 (0%)	 31/39 (80%) 5/39 (12%) 3/39 (8%)	 . . . .

FIGURE 1: Role of RCM in MPN with SVT and normal red cell values **A:** Evolution of red cell mass under cytoreductive therapy for eight patients. The therapeutic adaptations that were made after second red cell mass evaluation are reported on the right **B:** Proposed strategy for diagnosis and follow-up of non-cirrhotic SVT with normal red cell value at diagnosis.

ET: essential thrombocythemia; MPN-U: myeloproliferative neoplasm – undetermined; PMF: primary myelofibrosis; PV: polycythemia vera; RCM: Red cell mass; SVT : splanchnic vein thrombosis

A.



Patient	Cytoreductive agent increase	Addition of phlebotomy	Cytoreductive agent switch	None
Patient 2	X			
Patient 4	X			
Patient 5		X		
Patient 8			X	
Patient 7		X		
Patient 3			X	
Patient 1				X
Patient 6				X

B.