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

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 *JAK2* V617F 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 *JAK2* V617F mutation screening in SVT patient evaluations has enabled the identification of MPN 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 *JAK2* V617F mutation, demonstrating an increased red cell mass (RCM) through isotopic methods was a key diagnostic criterion for polycythemia vera (PV). According to the 2008 World Health Organization (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 *JAK2* V617F-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 SVT, carrying the *JAK2* V617F 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 years, with females comprising 48% of the cohort. The median interval between SVT diagnosis and *JAK2* V617F

Table 1. Patient characteristics.

	Cohort N=71	Normal RCM N=31	Masked PV N=40	*P*
Median age in years	44	44	44	-
Female, N/N	34/71	15/31	19/40	-
Hemoglobin g/dL, mean (range)	14 (9.1-16.3)	13.8 (12.1-16)]	14,1 (9.1-16.3)	0.24
Hematocrit %, mean (range)	42 (34-47.4)	41.4 (34-47)	42.5 (29-47.4)	0.15
Platelets x10^9/L, mean (range)	310 (61-836)	324 (199-639)	304 (61-836)	0.42
Neutrophils x10^9/L, mean (range)	5.46 (1.7-13)	5.86 (2.4-13]	5.17 (1.7-11.4)	0.27
RCM %, mean (range)	135 (99-209)	110 (99-122)	149 (128-209)	<0.001
Plasma volume %, mean (range)	128 (88-236)	114 (88-180)	140 (104-236)	<0.001
Palpable splenomegaly, N/N	48/67	16/29	32/38	-
BM biopsy, hyperplasia, N/N 0-2 lineage 3 lineages	 22/32 10/32	 12/14 2/14	 10/18 8/18	 - -
EPO value IU/L, mean	5.9	7.5	5	0.27
JAK2 V617F allele burden %, mean (range)	20 (1-68)	15 (5-67)	24 (1-68)	0.08
Type of splanchnic vein thrombosis, N/N (%) 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)	 - - -

RCM: red cell mass ; PV: polycythemia vera; BM: bone marrow; EP: erythopoietin.

mutation detection was 1 month (range, 0-144). Coincident diagnosis of SVT and *JAK2* V617F mutation was observed in 64% of cases within a 3-month margin. The mutation was detected within 2 years post-SVT in 14 patients, beyond 2 years in nine 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% *versus* 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%. *JAK2* V617F 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 of 40 masked PV and 14 of 31 non-PV. Panmyelosis, the histology criterion of PV in the WHO 2016 classification, was found in eight of 18 masked PV patients and, interestingly, in two of 14 other *JAK2* V617F patients. The final diagnoses were masked PV in 40 patients, ET in ten, PMF in three, and MPN-U in 18 cases.

Upon diagnosis of SVT, therapeutic-dose anticoagulation was initiated for all but four patients. At the time the *JAK2* V617F 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 6 months from their SVT diagnosis (range, 0-239): treatment started within 1 year of thrombosis for 37 patients, and after 1 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.

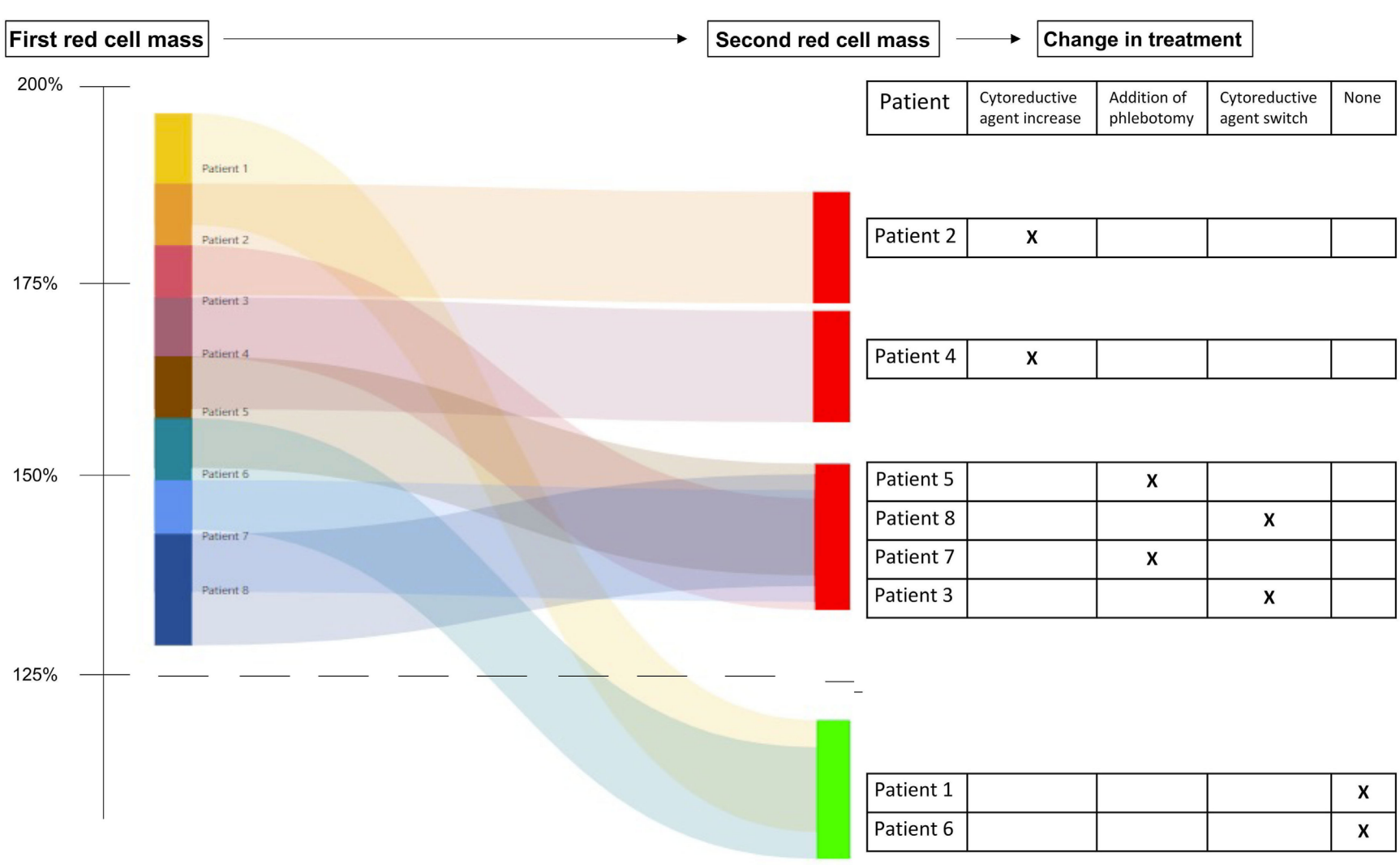
The average follow-up duration was 77 months, with a range from 3 to 358 months (interquartile range, 45-130 months). During this period, five patients initially diagnosed with conditions other than PV - two with ET and three with MPN-U - progressed to PV 1, 5, 9, and 15 years after their first diagnosis. 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 *JAK2* V617F 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 four anticoagulation therapy at the time of the thrombosis recurrence. Eight of 40 patients with a masked PV diagnosis underwent a second RCM assessment after at least 1 year of cytoreductive treatment. RCM levels remained stable in two patients, decreased but stayed above 125% in two others (1 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 eight exhibited panmyelosis, which is a major WHO 2016 criterion for PV. Bone marrow biopsies are particularly challenging for *JAK2* V617F 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 *JAK2* V617F-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 *JAK2* V617F 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 16.5 g/dL for men and 16 g/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 *JAK2* V617F-positive SVT, where thrombosis occurrence requires cytoreductive treatment and hemodilution is frequent.

In our patient group, 91% of those with masked PV had

A



B

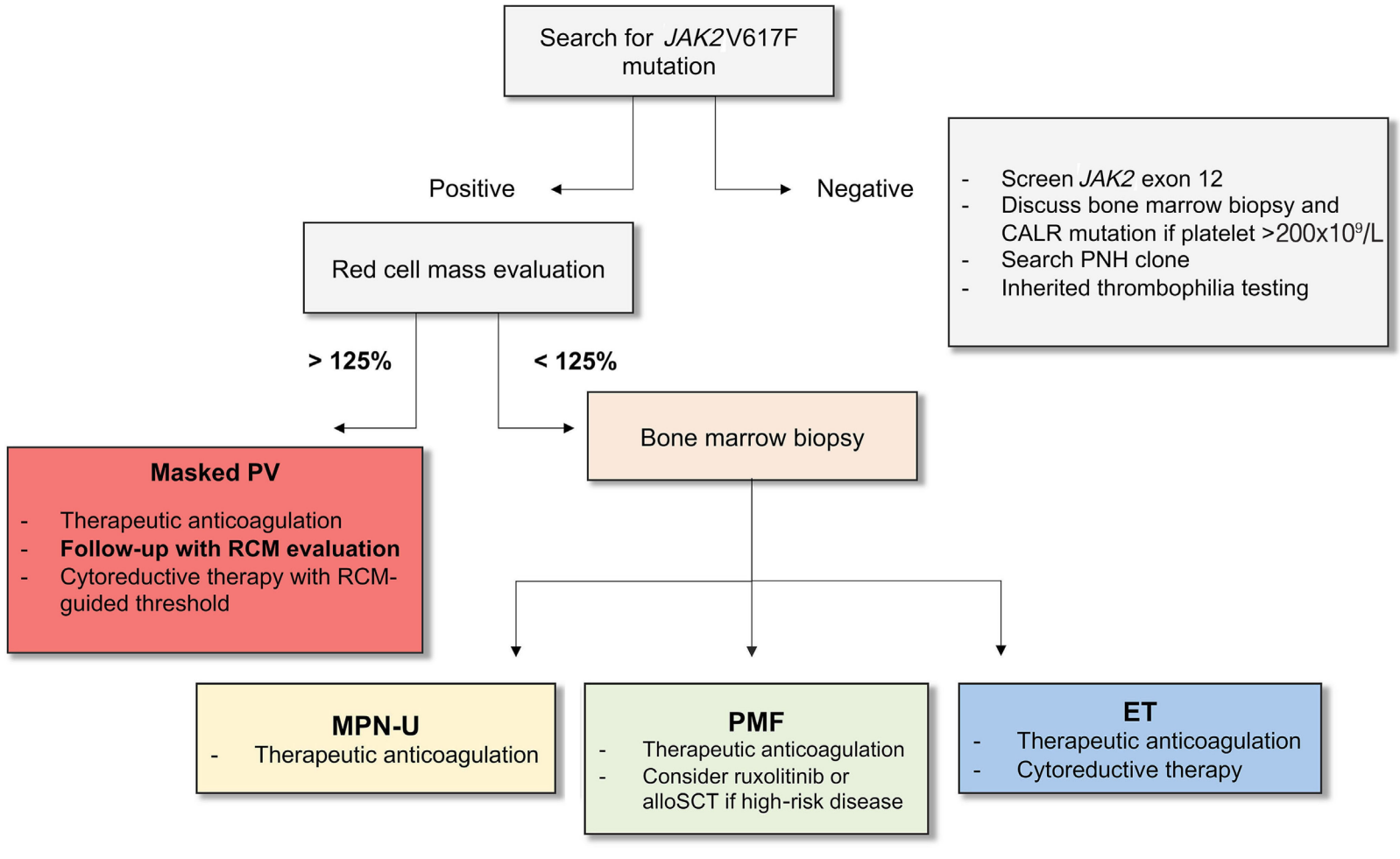


Figure 1. Role of red cell mass in myeloproliferative neoplasm with splanchnic vein thrombosis and normal red cell values. (A) Evolution of red cell mass (RCM) under cytoreductive therapy for 8 patients. The therapeutic adaptations that were made after second RCM evaluation are reported on the right. (B) Proposed strategy for diagnosis and follow-up of non-cirrhotic splanchnic vein thrombosis (SVT) with normal red cell value at diagnosis. ET: essential thrombocythemia; MPN-U: myeloproliferative neoplasm - undetermined; PMF: primary myelofibrosis; PV: polycythemia vera.

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 six of eight 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, three of five 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, three of four 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 *JAK2* V617F-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 MPN with normal blood counts (*clinicaltrials gov. identifier: NCT04539678*) are forthcoming.

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https://doi.org/10.3324/haematol.2023.284488

Received: October 27, 2023.
Accepted: January 26, 2024.
Early view: February 8, 2024.

Disclosures

No conflicts of intererest to disclose.

Contributions

JG, LD and YLB performed the research. JG and CJ designed the research study. JG analyzed the data. JG and CJ wrote the paper. All author made substantial contributions to research design and interpretation of data.

Acknowledgments

We thank the France Intergroupe des syndrome Myéloprolifératifs (FIM) for the support in the conception of the study.

Data-sharing statement

Data can be available on direct request to the corresponding author.

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