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*JAK2*V617F mutational status and allele burden have little influence on clinical phenotype and prognosis in patients with post-polycythemia vera and post-essential thrombocythemia myelofibrosis

The *JAK2*V617F mutational status and mutated allele burden were evaluated in 65 patients with post-polycythemia vera or post-essential thrombocythemia myelofibrosis (PPV/PET-MF). All PPV-MF patients harbored the mutation as compared to 27% of PET-MF. The V617F allele burden was higher in PPV- than in PET-MF (72% *vs.* 50%); 78% of the patients had greater than 50% V617F allele burden, supporting an adverse role of highest allele burden for MF transformation. In cases of PET-MF, no meaningful difference between JAK2V617F mutated and unmutated patients could be ascertained. Patients in the highest quartile of V617F allele burden were significantly older and had higher leukocyte and $CD34^+$ cell count in peripheral blood than those in lower quartiles. There were 8 patients developing acute myeloid leukemia, who were equally distributed among *JAK2*V617F mutated or unmutated patients. We conclude that presence and burden of *JAK2*V617F mutation provide little clinically relevant information in patients with PPV/PET-MF.

Discovery of acquired recurrent molecular abnormalities in JAK2 (*JAK2*V617F mutation in exon 14 or mutations, insertions, deletions in exon 12) or MPL (mostly MPLW515L/K) has improved the diagnostic approach to the *classic* Philadelphia chromosome-negative chronic myeloproliferative disorders, defined as myeloproliferative neoplasms (MPNs) in the upcoming revised WHO classification.[1] Virtually all patients with polycythemia vera (PV) have a mutation in JAK2, which is represented by the V617F allele in greater than 95% of cases; frequency of *JAK2*V617F mutation is 60-70% in patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF), while 10% of PMF patients[2] and up to 8% of *JAK2*V617F unmutated ET[3,4] patients harbor MPLW515L/K mutation. Presence of these molecular abnormalities constitutes a major diagnostic criteria in the 2008 WHO classification.[1] A number of studies have addressed the relevance of V617F mutational status and of mutated allele burden on hematologic characteristics and clinical presentation. In patients with PV or ET, the amount of V617F allele measured in granulocytes was found to be positively associated with hemoglobin level and leukocyte count and inversely with platelet count; furthermore, the higher the mutational burden, the higher the risk of presenting aquagenic pruritus, of developing splenomegaly or suffering from cardiovascular events, and of requiring cytotoxic therapy.[5] In PMF, results have been conflicting. An association of *JAK2*V617F mutational status with poorer overall survival was reported by Campbell *et al.*,[6] but this has not been confirmed by others.[7,8] A greater risk of developing large splenomegaly and leukemia was found in *JAK2*V617F mutated PMF patients included in a large study,[8] while conversely a better overall and leukemia-free survival for patients presenting the highest V617F allele burden was reported in an analysis from the Mayo Clinic.[9]

Table 1. Main hematologic characteristics of PPV/PET-MF patients at the time of diagnosis.

	JAK2 WT	JAK2V617F		JAK2V617F allele burden		
		PPV-MF	PET-MF	1-50%	51-75%	76-100%
Patient N.	16	49		11	14	24
PPV/PET-MF	0/16	43/0	0/6	8/3	13/1	22/2
Age, years, median (range)	62 (33-77)	62 (36-78)	59 (49-70)	52 (36-70)	64 (52-78)	67 (48-77)[3]
Gender, female/male	11/5	20/23	4/2	7/4	6/8	11/13
N. patients with previous thrombosis history[1] (%)	0/9 (0)	10/28 (36)	1/6 (17)	3/10 (33)	2/8 (25)	6/16 (37)
N. patients with previous chemotherapy[1,2] (%)	4/9 (44)	6/25 (24)	0/6 (0)	1/6 (17)	4/10 (40)	1/15 (7)
*JAK2*V617F allele (%), median (range)	–	78 (10-100)	45 (24-100)	28 (10-50)	62 (51-73)	97 (78-100)
White blood cell count ($\times 10^9$/L,) median (range)	10.1 (5.0-53.9)	12 (3.4-48.9)	9.0 (2.5-15.5)	9.4 (2.5-17.2)	11.8 (6.2-48.9)	13 (4.4-47.7)[3]
Hemoglobin (g/dL), median (range)	11.9 (8.8-14.9)	14.2 (8.6-18)	12.0 (8.5-14.7)	13.9 (8.5-18)	13.6 (11-15.9)	13.5 (8.6-16.3)
Platelet count ($\times 10^9$/L), median (range)	722 (136-1,538)	408 (102-1,500)	496 (87-904)	337 (87-900)	384 (102-641)	541 (140-1,500)
N. patients with PB blasts >1%[1] (%)	2/9 (22)	2/33 (6)	0/6 (0)	1/11	1/14	0/14
LDH, U/L, median (range)	722 (426-1,723)	447 (223-1,500)	1,160 (745-1,525)	444 (240-1,525)	306 (223-859)	674 (286-1,500)
$CD34^+$ cell count (%), median (range)	0.98 (0-6.95)	0.19 (0.01-4.1)	1.0 (0.02-7.0)	1.3 (0.02-7)	0.12 (0.01-4.1)	0.36 (0.03-3.6)
$CD34^+$ cell count, ($\times 10^6$/L), median (range)	102.7 (0.7-2,000)	59.7 (1.4-3,000)	160.8 (2.1-417)	7.6 (3.3-417)	48 (1.4-309)	98 (3.1-3,000)[3]

[1]Referred to the number of patients for whom the information was available. [2]all patients treated with chemotherapy had received hydroxyurea alone or in association/sequence with busulphan; no patient had received interferon. [3]$p<0.05$ according to Spearman's rank test.

On the other hand, information on patients with post-polycythemia vera (PPV-MF) or post-essential thrombocythemia myelofibrosis (PET-MF) is scanty, and limited to a few small series. Passamonti *et al.* found a significantly higher V617F allele burden in 16 patients with PPV-MF in comparison to both pre-fibrotic and fully-fibrotic PMF patients,[10] and allele burden was correlated to the number of circulating $CD34^+$ cells. Tefferi *et al.* reported that patients with PPV-MF were more frequently *JAK2*V617F mutated than those with PET-MF (91% *vs.* 38.9%) and more frequently had an allele burden greater than 50% (found in 18% *vs.* 11.1% of the patients respectively).[7] However, in these studies no attempt to correlate genotype with hematologic or clinical characteristics was made.

The aim of this study was to assess whether the *JAK2*V617F mutational status and/or allele burden had clinical and/or prognostic relevance for patients with PPV/PET-MF. Sixty-five patients, 43 with PPV-MF and 22 with PET-MF, were included; the diagnosis of evolution to MF from a pre-existing PV or ET was retrospectively verified in order to satisfy the recently adopted criteria from the IWG-MRT.[11] Retrospective evaluation of archived data was approved by the local Ethical Committee. All patients had a stored DNA sample from purified granulocytes collected within nine months of the diagnosis of evolution to MF; analysis of *JAK2*V617F mutational status and mutated allele burden was performed as described.[12]

The median time elapsed from original diagnosis of PV or ET to myelofibrosis was 112 months (range 30-317) for PPV-MF and 137 months (29-266) for PET/MF. Median follow-up from the diagnosis of myelofibrosis evolution was 39 months for the whole cohort of patients, and it was not dissimilar for PPV-MF (40 months, range 6-275) and PET-MF (39 months, range 10-165) patients. During this period, 7 out of 8 patients in whom acute myeloid leukemia (AML) developed died at a median of 26 months (range 1-40) after leukemia diagnosis. Mean survival from diagnosis of myelofibrosis was 96 months (95% CI, 81-111) for the entire cohort of patients, and 99 months (95% CI, 83-115) and 83 months (95 CI, 53-112) for PPV-MF and PET-MF patients respectively.

The hematologic characteristics of the patients at diagnosis are reported in Table 1. A total of 49 patients (75.3%) harbored the JAK2V617F mutation. In agreement with previous reports,[7,10] 100% of PPV-MF patients were *JAK2*V617F mutated. On the contrary, the frequency of *JAK2*V617F mutation was 27.2% among PET-MF patients, significantly lower than that observed in a control group of 260 ET patients from our Institution (63.4%; $p<0.01$).[13] This result argues for a dominant role of genetic mechanisms other than *JAK2*V617F in the MF transformation of ET. The median V617F allele burden in the entire population of patients was 73% ranging from 10 to 100%. The median value of V617F allele burden in PPV-MF was 72.0%, significantly higher than that observed in a group of 173 PV patients previously reported[12] who showed a mean allele burden of 52%, confirming a role for the

accumulation of mutated alleles in MF transformation of PV. Finally, in PET-MF the median allele burden was 57%, a value significantly greater than the 26% of a control group of ET patients from our Institution.[13] This result suggests that accumulation of mutant V617F alleles is also a mechanism of evolution toward MF in *JAK2*V617F mutated ET patients.

Apart from a significantly lower platelet count (p=0.04) in *JAK2*V617F mutated patients, no other meaningful correlation with *JAK2*V617F mutational status could be ascertained (Table 1). The V617F mutated or unmutated patients were also comparable in terms of spleen size, incidence of thrombosis, incidence of major hemorrhages (2 patients in each group, all from previous ET), and the rate of AML transformation which occurred in 2/16 JAK2 unmutated and 6/49 *JAK2*V617F mutated patients (12.5% and 12.2% respectively) (*data not shown*). The correlation between V617F allele burden and clinical presentation and prognosis was also studied by dividing patients into quartiles of V617F allele distribution. We grouped together the first two quartiles because of the low number of patients included (Table 1), and we found that V617F allele burden was positively correlated with age, white blood cell count and circulating $CD34^+$ cell count (all with a p value <0.05). However, there was no statistically significant correlation with clinical characteristics, i.e. spleen size, thrombosis, hemorrhages, nor with the rate of patients who evolved to AML (3 of whom were in the two first quartiles, one and 2 in the third and fourth quartile respectively).

To the best of our knowledge, this is the first study specifically addressing the clinical relevance of *JAK2*V617F mutation in patients who evolved to MF from a previous PV or ET. The data presented herein prompted us to conclude that neither the *JAK2*V617F mutational status nor the V617F allele burden seem to have relevance for disease phenotype or prognosis in this setting of patients. However, the relatively short follow-up might have prevented the discovery of correlations of *JAK2*V617F mutational status with clinical events occurring late in the history of these disorders, in particular for the evolution to AML. This will require larger patient series with a much longer observation.

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High-dose therapy and autologous stem cell transplantation versus conventional therapy for patients with advanced Hodgkin's lymphoma responding to front-line therapy: long-term results

The inclusion of high-dose therapy/autologous stem cell transplantation (HDT/ASCT) in the initial treatment plan for patients with unfavorable Hodgkin's lymphoma