Hydroxyurea does not appreciably reduce JAK2 V617F allele burden in patients with polycythemia vera or essential thrombocythemia

Variable results about the effects of hydroxyurea on the burden of mutant allele in patients with myeloproliferative neoplasms (MPN) harboring the *JAK2*^{V617F} mutation have been produced recently. In a retrospective single center study in 48 *JAK2*^{V617F}-positive patients with polycythemia vera (PV) or essential thrombocythemia (ET), the granulocyte V617F allele burden was found to remain stable over a median follow-up time of 34 and 23 months in polycythemia vera and essential thrombocythemia patients, respectively, independently of whether patients were already or not under hydroxyurea treatment at the time of first sampling. On the other hand, in 5 of 6 patients in whom hydroxyurea was started during the follow up, a statistically significant decrease of *JAK2*^{V617F} allele burden was observed at six months compared to baseline level; furthermore, the levels of mutant allele were found to increase again after stopping the treatment.[1] However, while in 9 essential thrombocythemia patients analyzed by Hussein *et al.* hydroxyurea treatment did not modify the V617F allele burden,[2] Girodon *et al.* reported a significant reduction of mutant allele in 36 patients with polycythemia vera or essential thrombocythemia from a median of 43% at diagnosis to 24% at a median follow up of 15 months after starting hydroxyurea.[3] Of note, a decrease in V617F allele burden greater than 30% of basal level was observed in 13 patients, and in 3 essential thrombocythemia patients the mutation was no longer detectable after 5-55 months of treatment. In the same study, the levels of V617 allele were also compared at a single time point between a cohort of newly-diagnosed polycythemia vera or essential thrombocythemia patients and a second cohort was comprised of patients who had been under hydroxyurea treatment for a median duration of 32 months. A lower amount of V617F allele was found in hydroxyurea-treated polycythemia vera patients (44% *vs*. 54% in PV patients analyzed at diagnosis) unlike in those with essential thrombocythemia in whom any difference was minimal. Interestingly, in both polycythemia vera and essential thrombocythemia, changes in V617F allele burden under hydroxyurea were more prominent in women than in males, suggesting females are more sensitive to the drug than males. A significant decrease in *JAK2*^{V617F} burden has been also documented in 72% of 18 patients (9 PV and 9 ET) studied by Ricksten *et al*; of note, only after four months of cytoreductive therapy with hydroxyurea were the levels of mutant allele significantly reduced compared to those pre-treatment.[4]

The aim of our study was to analyze changes in *JAK2*^{V617F} allele burden occurring during long-term follow up in patients with polycythemia vera or essential thrombocythemia and the relationships with hydroxyurea treatment. This two-center (Firenze and Bergamo, Italy) retrospective study concerned 172 *JAK2*^{V617F} mutated patients with a diagnosis of polycythemia vera or essential thrombocythemia according to the WHO 2008 criteria. We included patients for whom at least two sequential blood samples, drawn at an interval time of at least six months, were available. The *JAK2*^{V617F} allele load was measured by a sensitive quantitative real-time PCR in density-gradient purified granulocyte DNA according to the method of Lippert *et al*;[5] we used clone plasmids of wild-type and V617F mutated *JAK2* as standards. Differences between median values of *JAK2*^{V617F} allele burden were tested by Wilcoxon's matched-pairs signed-ranks test. Repeated measure test for *JAK2*^{V617F} mean change over time, irrespective of diagnosis, was also calculated to investigate a significant variance among ordered time measures. *JAK2 variance*^{MUT} (% *JAK2*^{V617F} in last sample - % *JAK2*^{V617F} in baseline sample) : (% *JAK2*^{V617F} in baseline sample x 100) was calculated as a measure of relative changes in *JAK2*^{V617F} allele burden between the baseline and follow-up sample.[1,2,6]

There were 104 patients with polycythemia vera and 68 with essential thrombocythemia (median age 56 years, range 15-84; 49% female). Clinical characteristics at diagnosis are reported in Table 1. The median follow-up time was 3.1 years (range 0.6-26.1); no evolution to myelofibrosis or acute leukemia occurred. The median time elapsing from diagnosis to first ("baseline") peripheral blood sample was nine months; six and 15 months in polycythemia vera or essential thrombocythemia, respectively. The median interval time between the baseline and the follow-up sample (considered the last in the case of multiple samples) in the whole patient population was 27 months (range 6-60); 28 and 23 months for polycythemia vera or essential thrombocythemia, respectively. According to previous reports, the mean V617F burden was significantly greater in polycythemia vera than in essential thrombocythemia patients (50±26% and 32±18%, respectively; P<0.0001).

Sixty-four patients, 40 with polycythemia vera and 24 with essential thrombocythemia, remained chemotherapy-free during follow up (Group 1); polycythemia vera patients were managed with phlebotomies only plus low-dose aspirin while essential thrombocythemia patients received no drug at all or low-dose aspirin. One-hundred and eight patients (63%), accounting for 61% of polycythemia vera and 65% of essential thrombo-

Table 1. Characteristics of the 172 patients included in the study.

	All patients	PV	ET
N. patients	172	104	68
Male/Female	88/84	66/38	22/46
Disease duration, years[§] (range)	3.1 (0.6-26.1)	3.2 (0.6-26.1)	2.5 (0.6-24.5)
Age, years[§] (range)	56 (15-84)	57 (17-79)	52 (15-84)
White cell count (x10^9/L)°	10.0±3.2	10.3±3.2	9.4±3.1
Hemoglobin (gr/dL)°	16.5±2.4	17.9±1.9	14.4±1.4
Hematocrit (%)°	49.7±8.2	54.1±7.3	43.1±4.0
Platelet count (x10^9/L)°	636±262	533±197	795±271
Patients with thrombotic events (%)	51 (29%)	29 (28%)	22 (32%)
Patients with major hemorrhagic events (%)	11 (6%)	5 (5%)	6 (9%)
Cytoreductive therapy (%)	108 (63%)	64 (61%)	44 (65%)
Time between diagnosis and first sample, mo[§] (range)	9 (0-298)	6 (0-298)	15 (0-279)
Time between first and last sample, mo[§] (range)	27 (6-60)	28 (6-60)	23 (6-60)
JAK2^{V617F} Allele burden (%) °	-	50±26	32±18

[§]*Median value (range) is reported.* °*Mean value (±SD) is reported.*

cythemia patients, respectively, had received cytoreductive treatment with hydroxyurea. Of these, 44 patients were chemotherapy-naive and started therapy within two months after the basal blood sample was collected (Group 2) whereas 64 patients were already on treatment from a median of 57 (range 3-290) months at the time of first genotyping (Group 3).

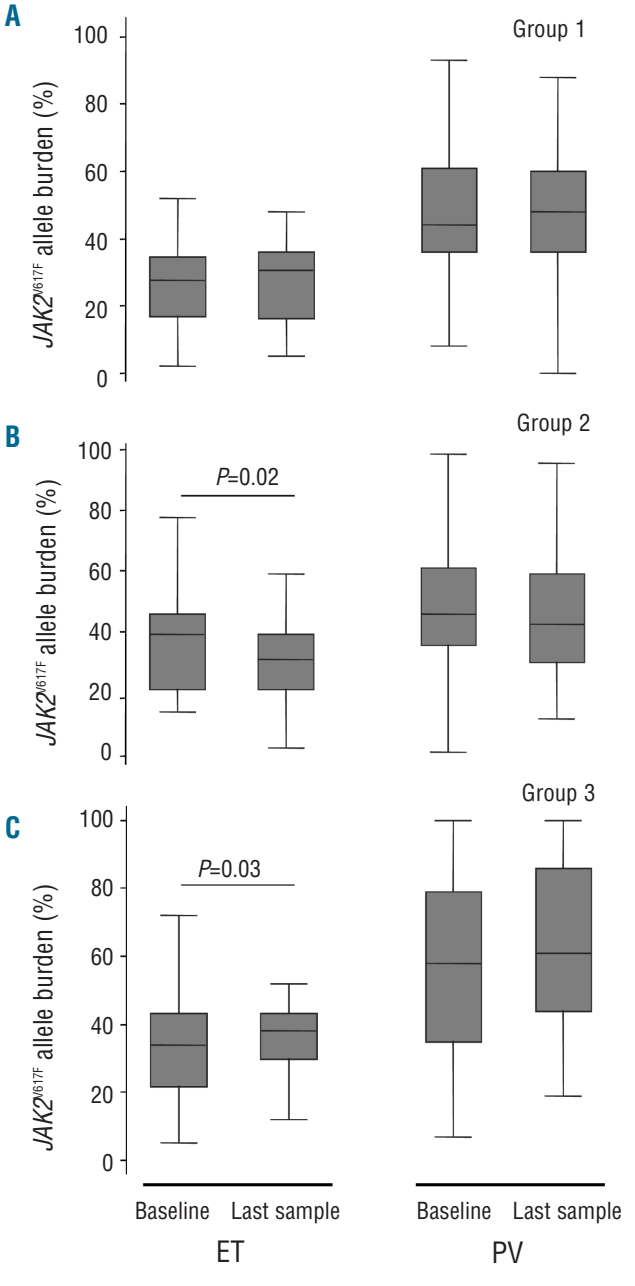

Figure 1. Boxplot representation of the *JAK2*V617F allele burden measured in a baseline and subsequent blood sample (last sample) in three group of patients with ET or PV: patients chemotherapy-free (Group 1, panel A), patients who were chemotherapy naïve at baseline sample and were on hydroxyurea at last sample (Group 2, panel B), and patients who were already on hydroxyurea at baseline samples and remained continuously under the drug up to the last sample analyzed (Group 3, panel C). Statistically significant differences, tested by the Wilcoxon matched-pairs signed-ranks test, between baseline and last samples, are reported in the figure.

Table 2. Individual *JAK2*V617F burden changes in Group 2 patients with essential thrombocythemia or polycythemia vera.

	% *JAK2* ^{V617F} baseline	% *JAK2* ^{617F} last sample	*JAK2* variance^{MUT}	Time between two assessments (mo)
ET patients				
01	41	29	-29	36
02	44	40	-9	38
03	50	35	-30	40
04	41	37	-10	21
05	52	56	+8	36
06	32	38	+19	21
07	73	67	-8	7
08	32	17	-47	14
09	38	37	-3	12
10	34	28	-18	57
11	20	25	+25	45
12	26	4	-85	13
13	41	35	-15	13
14	27	13	-52	43
15	26	16	-38	44
16	25	16	-36	15
17	37	35	-5	27
18	24	27	+13	27
19	38	43	+13	25
20	68	45	-34	9
PV patients				
01	59	57	-3	10
02	53	41	-23	10
03	40	41	+3	40
04	46	39	-15	34
05	47	26	-45	16
06	83	88	+6	33
07	39	21	-46	36
08	52	56	+8	34
09	71	50	-30	11
10	44	37	-16	25
11	35	23	-34	48
12	15	41	+173	19
13	3	30	+900	32
14	100	61	-39	26
15	40	56	+40	11
16	92	85	-8	12
17	15	45	+200	25
18	37	22	-41	19
19	93	43	-54	55
20	20	40	+100	37
21	14	42	+200	41
22	50	56	+12	17
23	98	89	-9	34
24	25	13	-48	41

JAK2 variance^{MUT} was calculated as (% JAK2V617F in last sample - % JAK2V617F in baseline sample) : (% JAK2V617F in baseline sample x100).

In the group of untreated patients (Group 1) (Figure 1A) the median interval time between baseline and follow-up sample was 26 months (range 6-60) for polycythemia vera patients and 24 months (range 6-59) for essential thrombocythemia patients. The *JAK2*^{V617F} allele burden remained stable during the follow-up period: 44% and 49% in the baseline and follow-up sample, respectively, in polycythemia vera patients, and 27% and 31%, respectively, in essential thrombocythemia patients. Therefore, we found no evidence for a time-dependent increase of V617F allele burden in untreated polycythemia vera and essential thrombocythemia patients in a medium-term follow up (median 26 months). As a matter of fact, in a previous study in essential thrombocythemia patients we were able to document significantly raised levels of mutant allele compared to newly-diagnosed patients only in those who had a history of disease of at least ten years.[7]

Among patients belonging to Group 2, which included 24 polycythemia vera and 20 essential thrombocythemia patients, the median interval time between baseline and follow-up sample was 29 months (range 10-55) for polycythemia vera patients and 26 months (range 7-57) for essential thrombocythemia patients. The *JAK2*^{V617F} allele burden was 45% and 41% (P=0.7) in the baseline and follow-up sample in polycythemia vera patients, respectively, and 38% and 35% in that of essential thrombocythemia patients (P=0.024) (Figure 1B). The changes in individual *JAK2*^{V617F} allele burden at the second time point during continuous hydroxyurea treatment are reported in Table 2. By using the criteria for evaluating "molecular response" in polycythemia vera or essential thrombocythemia that have been recently published by a European LeukemiaNet consensus conference[8] we found that no patient with either disease had a "complete response", i.e. the reduction of *JAK2*^{V617F} allele burden to an undetectable level. A "partial response", i.e. a reduction of 50% or more from baseline in patients with less than 50% mutant allele burden at baseline, or a reduction of 25% or more in those with more than 50% at baseline, was observed in 3 essential thrombocythemia patients (15%) and 3 polycythemia vera patients (12%). On the contrary, in 5 essential thrombocythemia (25%) patients the burden of V617F allele increased by a median value of 13% (range 8-25%) while in 10 polycythemia vera (41%) patients the mutant allele burden increased by 70% (range 3-900%).

In patients in Group 3 (Figure 1C), which included 40 polycythemia vera and 24 essential thrombocythemia patients, the median time interval between baseline and follow-up sample was 28 months for both polycythemia vera (range 6-54) and essential thrombocythemia patients (range 6-60). The *JAK2*^{V617F} allele burden was 58% and 61% in the baseline and follow-up sample in polycythemia vera patients, respectively, and 34% and 38% that of essential thrombocythemia patients (P=0.039). Considering polycythemia vera and essential thrombocythemia patients all together, there was a statistically significant increase in the median value of V617F allele burden over time from 36% to 41% in the basal and the follow-up sample, respectively (P=0.023, repeated measure test).

As a whole, when we considered patients receiving hydroxyurea, we observed a low (less than 10%), yet statistically significant, reduction in V617F allele burden only in the group of newly treated essential thrombocythemia patients; this is in accordance with another study.[1] When considering individual cases, a modification of mutated allele burden consistent with the definition of "partial response" according to the European LeukemiaNet criteria involved 15% of essential thrombocythemia patients and 12% of polycythemia vera patients in our series. Furthermore, the fact that the amount of V617F allele showed a tendency towards raising levels in essential thrombocythemia and polycythemia vera patients who were already under hydroxyurea at the time of baseline evaluation and remained continuously under drug for a median of 28 months (patients in Group 3) suggests that the small changes in allele burden measured in Group 2 patients may be of little significance. Since only 6 patients showed a molecular response, we did not attempt to correlate it with clinical parameters.

In summary, we conclude that in untreated patients with polycythemia vera and essential thrombocythemia, changes in V617F allele burden occur very smoothly over years. This means that the routine serial quantitative determinations of V617F allele do not provide clinically useful information and are not cost-effective. However, in cases where the modified phenotype suggests evolution to post-PV/post-ET myelofibrosis, a comparison of allele burden with that measured at diagnosis might be worthwhile since an increased load of mutated *JAK2* burden has been implicated in myelofibrotic transformation.[9,10] Finally, we confirmed previous reports that hydroxyurea can reduce the *JAK2*^{V617F} allele burden in subsets of patients.[1,3,4] However, we suggest that serial determination of V617F allele burden in patients receiving hydroxyurea should be confined to clinical studies since its role in routine patient management remains unclear.

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